DIVERSITY AND POPULATION BIOLOGY OF WILD MUSHROOMS FROM SOUTHWESTERN CHINA

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ABSTRACT

Wild mushrooms have been a part of the human diet and an important source of essential nutrients and medicine for centuries. While some wild edible mushrooms are artificially cultivable in human-made environments, the majority has resisted human domestication attempts. Southwestern China is a hotbed for wild edible mushrooms. Unfortunately, with increasing consumer demands and changes in environmental conditions, the genetic resources of many indigenous wild mushrooms are diminishing, with some populations on the brink of extinction. We have recently started a large-scale investigation of the diversity and population biology of wild edible mushrooms in this region. Our results indicate extensive diversity and reveal evidence of cryptic speciation, genetic differentiation and geographic structuring. In this paper, we present some preliminary results from our recent surveys.

Keywords: Wild edible mushroom; biodiversity; cryptic speciation; gene flow

INTRODUCTION

Fungi are among the most specious organisms on Earth. Morphologically, they are extremely diverse, from unicellular yeasts, to filamentous hyphal forms and a huge variety of fruiting body structures. They play significant roles in human health, forestry, agriculture, industry, food and the environment. They are an integral component of nutrient and elemental cycling of the global ecosystem. In natural environments, many fungi grow conspicuous fruiting bodies for reproduction and dispersal. Some of these fruiting bodies (mushrooms) have been collected for food and food supplements for hundreds to thousands of years. Over the millennia, humans have developed special preferences towards many of these wild mushrooms, some as expensive delicacies and others as potent medicine. Though different societies showed different preferences, strong relationships between people and wild mushrooms are found across all human racial, ethnical and geographic populations. For example, southern Europeans from France and Italy have a distinctive preference for truffles; the Japanese for the matsutake mushrooms, and those from southwestern China for ganbajun. However, rapid globalization in recent years has brought people from very different backgrounds into close contact with each other, enhancing the exchange and sharing of material goods, cultures, foods and medicine. Consequently, regional wild edible mushrooms are becoming global commodities and regional

resources are susceptible to global demands and exploitations. Such changes are leading to increasing problems for the management of these wild mushrooms, with potentially far-reaching implications for the survival of such resources and for the livelihood of the indigenous people who depend on these resources.

In this presentation and paper, we will describe our recent and current efforts in trying to understand the genetic resources of wild gourmet mushrooms from southwestern China. This region is geographically and ecologically extremely diverse and is one of the world's 34 biodiversity hotspots. For example, over 600 out of 2,000 edible fungal species worldwide occur in this region [1]. Some of the economically important mushrooms from this region include *Thelephora ganbajun* M. Zang, *Tricholoma matsutake* (S. Ito & S. Imai) Singer, and *Russula* spp. Our preliminary analyses of these wild mushrooms identified high and novel phylogenetic and population genetic diversities. In the following sections, we highlight some of our findings and discuss the implications of our results for conservation and management of these genetic resources.

MATERIALS AND METHODS

Since 2006, we have been collecting wild edible mushrooms from forests and farmers' markets in many parts of southwestern China, with a focus on Yunnan Province. Specimens were recorded, dried, brought into the lab, and preserved for taxonomic, systematic, population genetic, and molecular ecological studies. Our collection efforts were primarily on mushrooms with relatively high consumption levels, high economic values, and/or broad geographic distributions.

To study the phylogenetic diversity of wild edible mushrooms in southwestern China, we rely almost completely on DNA sequence information from the collected specimens. To obtain those DNA sequences, we first extracted their DNA and then amplified the inter-transcribed spacer (ITS) regions of the ribosomal RNA gene cluster using the fungal universal primers. The DNA sequences were then compared with each other and with those in databases.

To study the genetic variation among strains and populations from different geographic and/or ecological areas within individual species, whenever possible, species-specific and/or single gene markers were used for genotyping strains. The data were then analyzed for ecological and population genetic patterns using various computer software programs that incorporate ecological, geographical, and other relevant information [2].

RESULTS AND DISCUSSION

Below we summarize our preliminary understanding of the ecology, diversity, and population biology of three selected wild edible mushrooms. When possible, our data are compared with those from other geographic areas and or closely related species.

Ganbajun: diversity, geographic structuring, cryptic speciation. *Thelephora ganbajun* is a gourmet mushroom that forms ectomycorrhizae with pine trees, predominantly *Pinus yunnanensis* endemic to Yunnan Province in southwestern China [3,4]. The mature mushroom produces a unique and attractive aroma and is well liked by locals. In Yunnan, freshly collected mature fruiting bodies of this mushroom are priced similarly to or higher than most other wild mushrooms. Because of consumer demand and our inability to cultivate this mushroom

artificially, there has been noticeable overexploitation and consequent decline of local populations of this species in many areas in Yunnan. However, despite its economic and potential ecological importance, very little is known about its ecology and genetics. In a recent study, DNA sequence variation among strains and populations were analyzed within the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster [5]. The ITS regions were chosen here because there are universal fungal PCR primers for this region that could be used for amplification and sequencing [6]. In addition, the ITS is among the most commonly used gene regions for analyzing relationships among strains within as well as between closely related fungal species [6-8], and it's the recommended DNA fragment for fungal barcoding. Furthermore, no gene sequence information (not even ITS) about this organism was available before the study, thus no primers were known to exist that could allow us to amplify specific gene sequences from this organism. In total, the ITS sequences were obtained from 156 fruiting bodies obtained from 23 sites in nine regions of Yunnan province (Table 1). These regions covered about 600 km from east to west and over 300 km from south to north.

Table 1. ITS sequence types and their distribution among geographic populations of Ganbajun from Yunnan, southwestern China (modified from Sha et al. [5])

Region/ District	County/ Community	Sample size	ITS sequence type (# of isolates in each type)[1]	ITS diversity
Baoshan	Changning	4	1(2); 2(1); 3(1)	0.625
	Baoshan	9	1(8); 3(1)	0.198
Chuxiong	Chuxiong	8	1(4); 7(1); (22(1); 24(1)	0.688
	Nanhua	10	1(3); 7(3); 13(1); 17(1); 20(1); 23(1)	0.780
	Wuding	8	1(2); 7(4); 28(1); 31(1)	0.656
	Lufeng	6	1(4); 21(1); 31(1)	0.500
Dali	Midu	5	1(2); 25(2); 31(1)	0.640
	Wenxian	4	1(4)	0.000
	Dizi	4	2(1); 7(2); 31(1)	0.625
	Fengyi	5	1(2); 13(1); 7(1); 26(1)	0.720
Honghe	Shiping	4	1(1); 5(1); 6(1); 7(1)	0.750
Kunming	Jingning	1	1(1)	N/A
	Anning	1	1(1)	N/A
	Yiliang	5	1(1); 8(1); 9(1); 10(1); 11(1)	0.800
	Songming	9	1(6); 15(1); 25(1); 31(1)	0.519
	Luquan	6	1(2); 7(2); 20(1); 25(1)	0.722
	Xundian	9	1(2); 12(1); 14(3); 16(1); 25(2)	0.765
Pu'er	Pu'er	20	8(13); 18(1); 19(2); 34(4)	0.525
Qujing	Malong	2	1(1); 2(1)	0.500
Wenshan	Guangman	4	1(2); 4(1); 7(1)	0.625
Yuxi	Tonghai	4	1(3); 7(1)	0.375
	E'shan	11	1(5); 2(4); 25(1); 32(1)	0.645
	Yimeng	17	1(12); 25(1); 27(1); 30(1); 31(1); 33(1)	0.484

[1,] The ITS sequence types correspond to those in Figure 1.

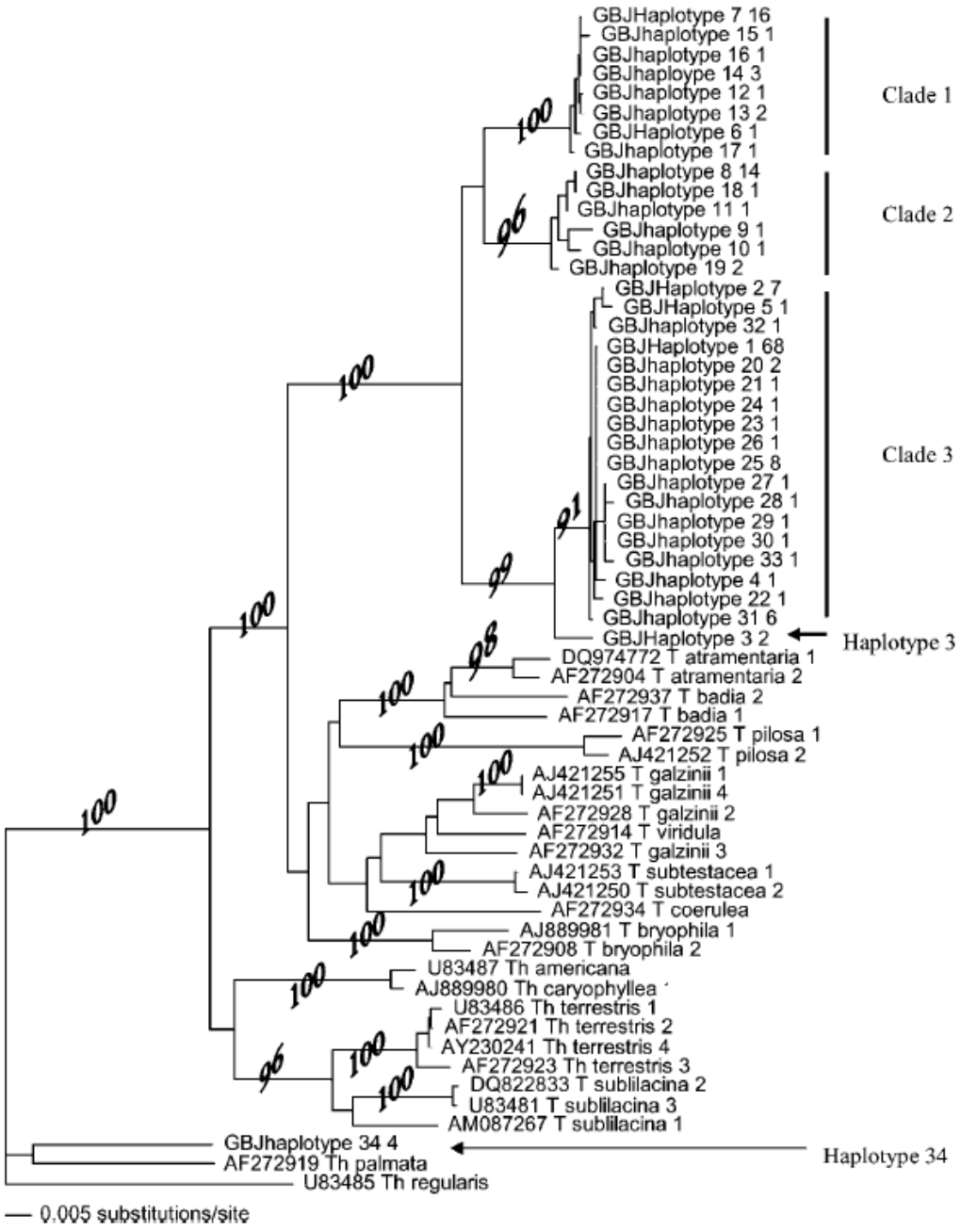

Figure 1. Phylogenetic relationships among ITS sequences of 34 haplotypes of *Thelephora ganbajun* and representative sequences from closely related species of two genera *Tomentella* and *Thelephora*.

For each ITS haplotype of Gan-Ba-Jun (GBJ), the first number represents the haplotype assignment corresponding to those in Table 1; the second number represents the total number of strains belonging to the specific haplotype. The 27 reference strains are each represented by its GenBank accession number, the genus abbreviation (T for *Tomentella* and Th for *Thelephora*), the species name and when multiple strains from the same species were available, the strain code (1, 2, 3, or 4). Numbers across branches are bootstrap values greater than 90% obtained from 1000 replicates. Gaps were treated as missing data. Branch lengths are proportional to the amount of sequence divergence. Tree length = 670; consistency index = 0.610; retention index = 0.882. [Modified from ref. 5]

The 156 aligned sequences were 671 nucleotides long and contained 138 variable sites. Each of the variable sites contained only two alleles. These variable sites included 35 insertions/deletions, 85 transitional substitutions, and 18 transversional substitutions. Among these variable sites, 130 were phylogenetically informative and the remaining 8 sites were phylogenetically uninformative. The analyses of the aligned ITS sequences of all 156 specimens identified 34 unique sequence types (Figure 1). The distributions of these ITS sequence types among the 23 local populations are presented in Table 1. Among the 34 ITS sequence types, 22 were represented by one specimen each and the remaining 12 were each shared by two or more specimens. The most common type, haplotype 1, contained 68 specimens that were distributed in 21 of the 23 sites (Table 1). Similarly, haplotype 7 was also widely distributed – it contained 16 specimens collected from eight of the 23 local populations (Table 1). However, the other major shared halpotype 8 was found in only two regions. The number of ITS haplotypes for each local population ranged between one and seven. Aside from the two local populations where only one specimen each was available for analysis, 20 of the remaining 21 local populations had more than one ITS genotype. Of these 21 local populations, 13 were found to contain unique ITS haplotypes not found in other local populations.

Based on BLAST searches against the GenBank database, 27 representative ITS sequences with species identifications and with high levels of sequence identity (>90 %) to our sequences were included for comparative analyses. These sequences were found belonging to either *Thelephora* or *Tomentella* genera. These sequences were retrieved from the GenBank based on: (i) their high sequence identity to our samples, (ii) their comparable sequence lengths to ours, (iii) their representative phylogenetic positions, and (iv) in several cases, the availability of two or more strains for the same species. The selections of multiple strains from the same species were to compare the potential divergence within other species to that within *Th. ganbajun*. These 27 ITS sequences from GenBank represented 14 species total, nine in *Tomentella* and five in *Thelephora*. The analyses of our sequences and the 27 representative GenBank sequences revealed five phylogenetically distinct clades for the ganbajun samples from Yunnan (Figure 1). Clade 1 contained eight ITS haplotypes represented by 22 strains; clade 2 contained six haplotypes represented by 20 strains; clade 3 contained 18 haplotypes represented by 106 strains; clade 4 contained one haplotype (haplotype 3) represented by two strains; and clade 5 contained one haplotype (haplotype 34) represented by four strains. Our results showed that strains in clades 1-4 had closer evolutionary relationships to representative species from the genus *Tomentella* than to those from *Thelephora*. In contrast, clade 5 was more similar to ITS sequences of in *Thelephora*, with the closest relative in *Th. palmata* and *Th. regularis*.

Taken together, the ITS sequence analyses suggest that the wild collected gourmet mushroom ganbajun in Yunnan are highly heterogeneous, belonging to two different genera *Thelephora* (the minority of our isolates) and *Tomentella* (the majority of our isolates) and contained at least five divergent evolutionary lineages. These lineages showed sequence divergences from each other similar to or greater than those between several known sister species pairs in these two genera. In addition, there are significant differences in the distribution of the sequence types and lineages. While a couple of the ITS sequence types were geographically broadly distributed, the majority of the sequence types were unique to specific local populations. The lack of significant gene flow among local populations suggests that targeted efforts should be made to preserve the significant genetic diversity within individual populations of ganbajun.

The "Big Red Mushroom": diversity, cryptic speciation, and population genetics. The genus *Russula* contains a highly diverse group of ectomycorrhizal fungi that includes over 700 reported species [9]. In natural forests, this genus contributes a significant amount of the ectomycorrhizal biomass with a broad distribution ranging from the tropics to subtropics, temperate regions, and even arctic zones [10]. Except a few species such as *R. emetica* and *R. subnigricans* that are not edible, many species in this genus are enjoyed by humans and mushrooms in this genus are among the most commonly found along roadside markets in southwestern China. While this genus contains species with very diverse morphological features, distinguishing closely related species is often very difficult due to the large number of species, extensive phenotypic plasticity among strains within individual species, and the lack of macro-morphological features to separate them. Consequently, molecular information has been increasingly used to help define and identify species boundaries, especially between morphologically ambiguous species pairs. However, most such studies have focused on European and North American samples [e.g. 11]. The diversity and genetic structure of *Russula* species in other parts of the world, including southwestern China, remain poorly understood.

Among the *Russula* mushrooms collected and traded in southern China, one called 'Dahongjun' [literary means ''Big Red or Bright Red Mushroom''] is probably the most prominent. This mushroom has been harvested and traded in local, national and international markets for over 20 years. Like many other pricey gourmet wild mushrooms such as matsutake, ganbajun and chanterelles, *Russula* cannot be artificially cultivated because of their dependency on a living plant host. Therefore natural populations in the forests are the only sources for the consumer market. Similar to ganbajun discussed above and the matsutakes discussed below, the significant profits, uncontrolled harvesting practices in recent years, human disturbances of the forest ecosystem, and loss of habitats are threatening the wild populations of Dahongjun in the regions.

Historically, mushroom enthusiasts and mycologists have regarded Dahongjun in southern China as *R. vinosa* Lindblad, which was originally described in Europe [12]. Recently, a population of Dahongjun from southern Yunnan was found to have ITS sequences very different from those of the typical *R. vinosa* from Europe, and this group was described as a new species *R. griseocarnosa* X. H. Wang et al. [13].

We recently investigated the diversity of Dahongjun from southern China [14]. A total of 122 samples were collected from five local populations representing the known distribution ranges of this mushroom in southern China (Table 2). We analyzed the genetic diversity and geographic structure of this mushroom using sequences from four DNA fragments: the ITS, the nuclear large subunit of the ribosomal RNA gene (nucLSU rRNA), the mitochondrial small subunit of the ribosomal RNA gene (mtSSU rRNA), and the second largest subunit of the nuclear RNA polymerase enzyme II (RPB2).

Among the 683 aligned nucleotide sites for the ITS regions, 82 were variable with 54 base substitutions and 28 insertion/deletions. All the indels were found between strains and none was found within any of the 122 strains. Our analyses of these ITS sequences suggested that our samples contained at least three phylogenetically distinct lineages (Figure 2). Lineage 1 contained 85 strains from the Ailaoshan (AL) region in central Yunnan and 5 strains from Cangwu (CW) in eastern Guangxi (GX). This lineage included a total of 42 ITS genotypes with 37 from AL and 5 from CW. Lineage 2 contained 17 ITS sequence types and 27 isolates, including 9 strains from Mengla (ML), 8 from Jinuo (JN), 8 from Dadugang (DDG), and 2 from CW. Lineage 3 contained 3 strains from DDG and 2 strains from CW. The ITS genotype

distribution within and among the 5 local populations are presented in Table 2. The analyses of sequences from the three other additional DNA fragments confirmed the existence of three divergent lineages within our Dahongjun samples.

To analyze the relationships between our samples and those of other closely related *Russula* spp., the sequences representing our 63 ITS genotypes identified above were used as queries to retrieve similar sequences in GenBank through BLAST searches. We retrieved 17 sequences with a comparable length to ours and that showed an overall sequence identity ≥90% to our Dahongjun sequences. These included three ITS sequences of *R. griseocarnosa* recently identified based on three specimens of Dahongjun in southern Yunnan [13] and 12 ITS sequences representing 5 closely related species in *Russula* (*R. vinosa, R. claroflava, R. occidentalis, R. decolorans, R. xerampelina*; Figure 2).

Table 2: Geographic distribution and ITS sequence diversity of Dahongjun samples collected from Yunnan and Guangxi provinces in southern China (Modified from Li et al. 2010).

Region/ District	County/ Community	Sample size	ITS sequence type[1] (# isolates in each type)	ITS diversity[2]
Central Yunnan	Ailaoshan (AL)	85	1(2); 2(4); 3(1) 4(1); 5(1); 6(1) 7(2); 8(1); 9(1) 10(1); 11(2); 12(1) 13(3); 14(1); 15(9) 16(1); 17(1); 18(1) 19(1); 20(16); 21(2) 22(1); 23(1); 24(1); 25(5); 26(1); 27 (4); 28(5); 29(1); 30 (2); 31(1); 32(2); 33(1); 34(4); 35(1); 36(1); 37(1)	0.945
Southern Yunnan	Mengla (ML)	9	38(1); 39(1); 41(1); 42(1); 43(1); 44(1); 45(1); 46(1); 54(1);	1.000
	Jinuo (JN)	8	39(1); 43(1); 49(1); 50(1); 47(2); 48(1); 51(1)	0.964
	Dadugang (DDG)	11	38(2); 40(2); 49(1); 50(2); 51(1); 55 (1); 56(1); 57(1);	0.945
Eastern Guangxi	Cangwu (CW)	9	58(2); 53(1); 52(1); 59(1); 60(1); 61(1); 62(1); 63(1)	0.972

[1,] The ITS genotype code correspond to those in Figure 2.

[2,] Genotypic diversity is defined as the probability that two individuals taken at random have different genotypes. It's calculated as $(1-\sum p_i^2)\bullet n/(n-1)$, where p_i is the frequency of the *i*th genotype and n is the number of individuals in the sample.

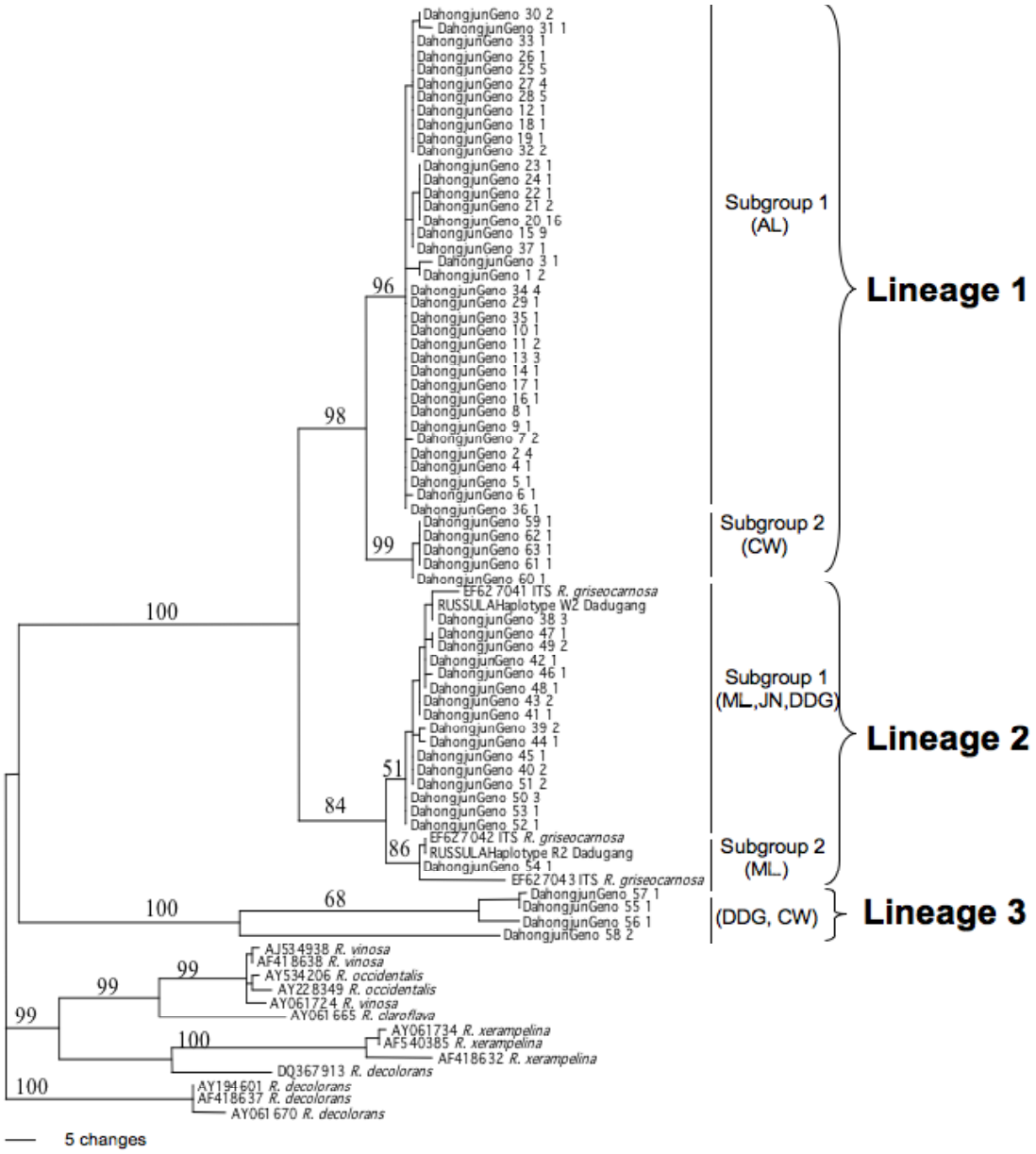

Figure 2: Phylogenetic relationships among ITS sequences from 122 isolates of Dahongjun from southern China and from closely related reference sequences from GenBank.
Each strain label contains its geographic location information [See text and Table 2 for details], followed by a field isolation number. Numbers along branches are bootstrap values greater than 90% obtained from 1000 replicates. Reference sequences contain species identification (when available), followed by the specific GenBank accession number. Sequences of non-Dahongjun samples are used as outgroups. Tree length=368, Consistency index=0.766, Retention index=0.943. [Modified from ref. 14].

The joint analyses of our sequences and those from the GenBank confirmed the distinctiveness of the three lineages within Dahongjun from southern China. One lineage (Lineage 2) corresponded to the known species, *R. griseocarnosa* recently identified from southern Yunnan [13]. The separation of these three lineages was supported by >98% bootstrap

values. Similarly, the separation of the three lineages of Dahongjun from a closely related species *R. vinosa* had 100% bootstrap support, including large phylogenetic distances among them. The divergences among the three lineages were comparable to or greater than those among the closely related known *Russula* species (Figure 2). While these lineages were prominently structured geographically based on ITS sequences, evidence for ancient and/or recent gene flow was also identified within individual lineages. In addition, as expected, the local population of Lineage 1 from Ailaoshan in central Yunnan Province where 85 of our 122 specimens came from showed clear evidence of recombination, consistent with the important roles of sexual spores and sexual reproduction in the ecology and population biology of Dahongjun.

Matsutake: diversity, ecology, population genetics, and counterfeiting. The matsutake (or Pine-mushroom) is among the most revered and valuable mushrooms in the world, especially in Japan. Similar to Ganbajun and Dahongju, high consumer demand, high price, limited natural production areas, and low productivity are threatening its genetic resources. However, unlike Ganbajun and Dahongjun where the consumer pressure is mostly from local communities in southern China, the pressures on matsutake are from both domestic and international sources. These pressures have called for a concerted effort to develop effective management and conservation programs in southwestern China. Understanding the ecology, reproductive biology, and population relationships of the organism would be essential for developing such a strategy.

Broadly speaking, matsutake refers to a loosely defined species complex in the genus *Tricholoma.* Like most mushrooms, the major biomass of matsutake is underground in the soil in which their mycelia form an extensive network. Its mycelia form symbiotic relationships with the roots of conifer and broadleaf trees. However, due to the lack of distinct morphological features, it has been difficult to separate the different species within the *T. matsutake* species complex. Based on DNA sequence information at the ITS, the European specimens of *T. nauseosum* and the Asian *T. matsutake* are considered con-specific and belong to the "true matsutake" (15; Figure 3). Though also consumed in Japan and North America, *Tricholoma magnivelare* is considered a "matsutake-ally", similar to *Tricholoma caligatum* and *Tricholoma fulvocastaneum*. On the other hand, though morphologically similar to *T. matsutake*, a more distantly related species *Tricholoma bakamatsutake* is not typically consumed and is commonly called the "fool's matsutake".

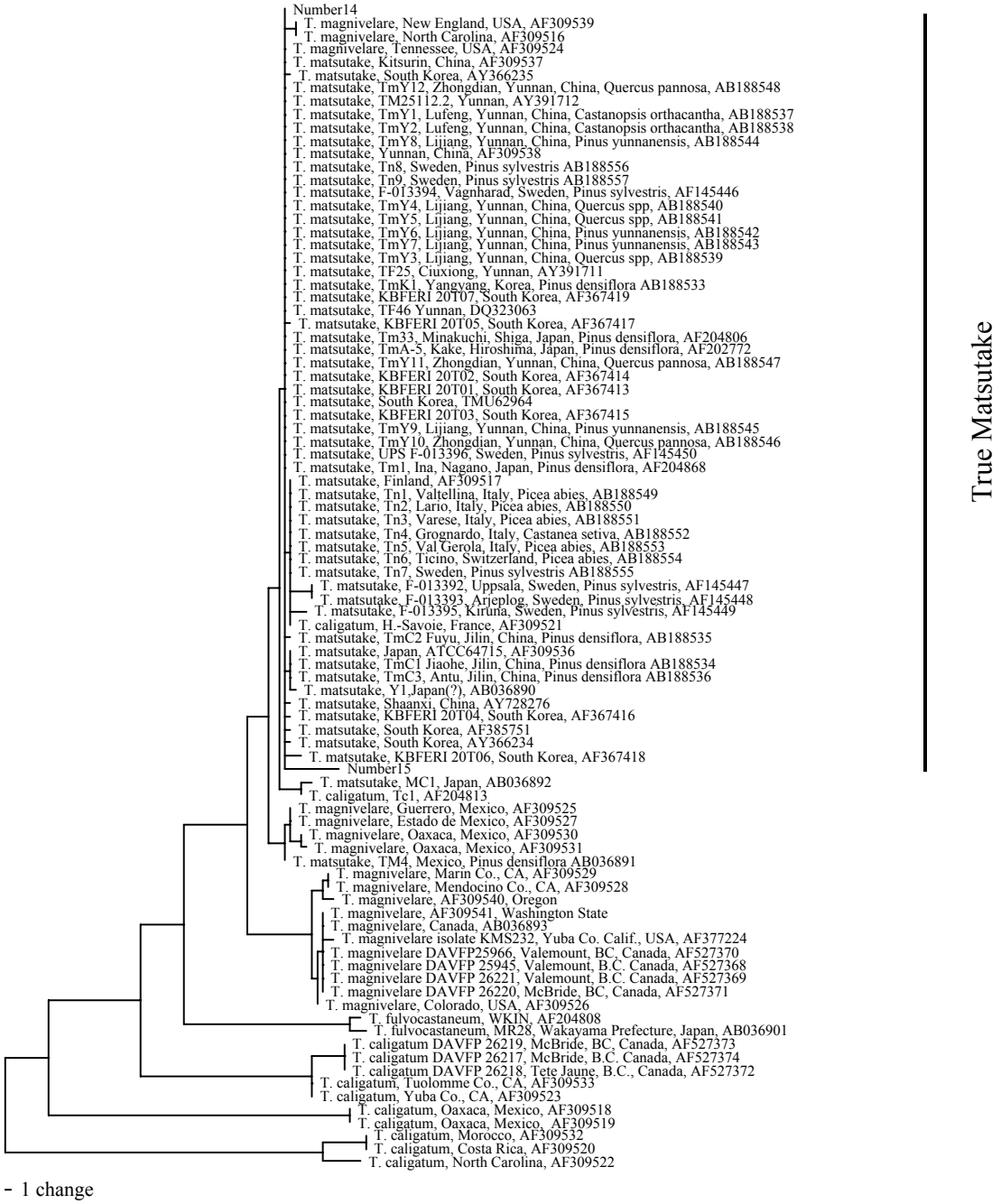

Figure 3: Phylogenetic relationships among strains of *Tricholoma matsutake* and its close allies based on ITS sequences. Each entry contains the species name, followed by strain name, geographic location, likely associated plant host, and the GenBank accession number. Strains labeled "Number 14" and "Number 15" are two strains from Yunnan associated with *Pinus yunnensis* and *Quercus spp* respectively. Note the limited or no ITS sequence variation pattern among populations of *T. matsutake* from different geographic areas. [Modified from ref. 16]

Geographically, the *T. matsutake* species complex has been reported from northern and highland Europe, northwestern Africa, southeastern-Himalaya, the Far East, the Pacific Rim in North America, the Great Lakes, the east coast of the United States, and Mexico. In general, the range of matsutake coincides approximately the distribution of coniferous genera such as *Pinus, Pseudotsuga, Tsuga, Picea, Cedrus* and *Abies*. However, matsutake is often found associated with oak (*Quercus* spp) in southwestern China; and *T. magnivelare* associated with *Lithocarpus densiflora* (tanoak) in the Pacific Northwest of North America. *T. bakamatsutake,* the "fool's matsutake" sympatric with *T. matsutake,* is believed to be associated with *Castanopsis, Fagus, Pasania* and *Quercus* spp., despite its occurrence in forests where conifers also are present [16]. The matsutake-ally *T. caligatum* from the US and Mexico is also suspected to be associated with angiosperm hosts.

Japan is the world's preeminent consumer market for the matsutakes, most prominently for the "true-matsutakes". Matsutakes collected and imported from different parts of the world are priced very differently in Japan, from less than US$100 to over US$4000/kg of fresh fruiting bodies [16,17]. The significant price differences for matsutakes from different parts of the world create conditions for counterfeiting. Two types of counterfeiting are possible. In the first, "matsutake-ally" and "false matsutake" are marketed as "true matsutake". A "trained eye" in morphological identifications and ITS sequencing or PCR-RFLP of the ITS using universal primers can reliably distinguish the "true matsutake" from the "matsutake-ally" and "false matsutake". However, as shown from the ITS phylogeny of the matsutake species complex, ITS sequencing has very limited discriminating power to distinguish different geographic populations of the "true matsutakes" from different regions (Figure 3). At present, there is relatively little information about the genetic and phenotypic differences among the "true matsutakes" from different parts of the world (i.e. northern Europe, southeastern Himalaya, northeastern China, Korea, Japan, and eastern north America). In our analyses of local and regional samples of *T. matsutake* from southwestern China (the main component of the southeastern Himalaya matsutake population) based on 14 single nucleotide polymorphisms, we found plenty of genetic variation within individual samples but very limited genetic differentiation among the 17 analyzed geographic populations [18,19]. On the other hand, comparisons of PCR-fingerprinting profiles using a pair of transposable element-based primers identified that *T. matsutake* from the Far East (Japan, Korea and northeastern China) were heterogeneous and showed consistent difference from those from southwestern China [16]. Below we describe how the simple difference in DNA fingerprinting profile allowed us to identify counterfeit *T. matsutake* in China [20].

Evidence for counterfeiting was found in our analyses of matsutakes from two major natural production and trading regions in China, the northeast (NE) and the southwest (SW) China [20]. In this analysis, we obtained DNA profiles of *T. matsutake* fruiting bodies from matsutake trading companies and compared them with known authentic wild-collected specimens from NE and SW China. The commercially purchased matsutake included 107 fruiting bodies from four companies in NE China and 45 fruiting bodies from three companies in SW China. The geographically authentic matsutake samples included 38 mushrooms from four local populations in Jilin and Heilongjiang in NE China, and 183 samples from 18 local populations in Yunnan, Tibet, and Sichuan provinces in SW China [19]. Our analyses showed that 67% commercial matsutake claimed to be from the northeast were in fact genetically identical at this marker to those from southwest China but different from authentic northeast

Chinese samples [Figure 4]. Such analyses highlight the importance of accurate identification of matsutake mushrooms, not only at the species level but also at population and strain level. Our analysis is similar to those found for counterfeited fish in New York City, USA [21] (Wong and Hanner 2008). Our finding suggests that caution should be applied to authenticate commercial matsutake from northeast China. Similar concerns about the authenticity of other gourmet mushrooms such as truffles, morels, and tubers have also been raised but the identification system is yet to be finalized.

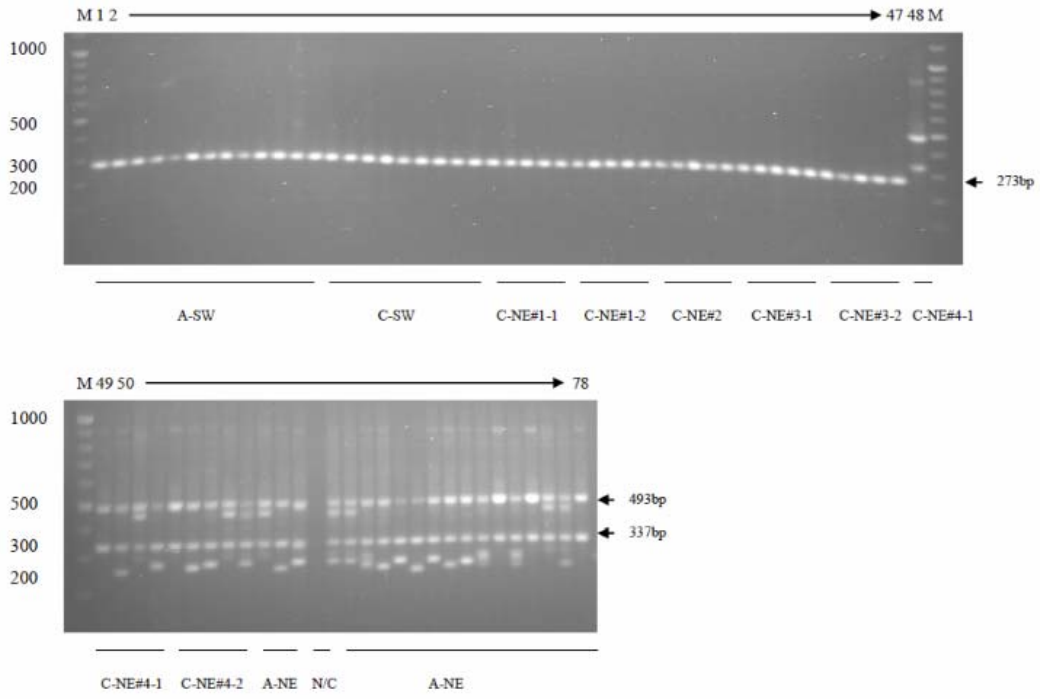

Figure 4: PCR fingerprinting profiles of representative Chinese isolates of *Tricholoma matsutake* based on the pDGSL313-1/pS48 primer typing system.
The lane numbers are indicated at the top and the origins of specimens are given at the bottom of gels: A-SW, authentic southwestern matsutake (lanes 1-13); C-SW, commercial southwestern matsutake (lanes 14-22); C-NE, commercial samples claimed from the northeastern (lanes 23-57), with different sets from different trading companies; A-NE, authentic northeast matsutake (lanes 58-60, 62-78); N/C, negative control (water, no sample DNA). Lanes M, molecular markers (200 to 1000 bp). Right-hand labels indicate signature DNA fragments corresponding to SW (273bp) and NE (493bp and 337bp) Chinese matsutake samples. [Modified from ref. 20].

CONCLUSIONS

The three commercially harvested wild mushrooms share several features: all are ectomycorrhizae, not cultivable, of significant importance to the local and regional economy, and with very limited knowledge about their biology. Our analyses identified that each of these commercial mushrooms contained significant genetic diversities, including multiple evolutionary divergent lineages that likely correspond to several phylogenetic species. These lineages typically show different geographic distribution patterns, with some lineages broadly distributed

while others more limited. Whether such patterns are representative of the wild edible ectomycorhizal mushrooms or wild mushrooms in general in southwestern China remain to be determined. Our results based on the ganbajun and dahongjun samples indicate that the number of edible mushroom species in southwestern China may be underestimated by 3-5 folds. The genetic information obtained here should enhance our ability to develop strategies for effective conservation and management programs of these genetic resources.

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