Mycosphere 13(2): 56–85 (2022) www.mycosphere.org ISSN 2077 7019
Article – Special issue
Doi 10.5943/mycosphere/si/1f/3

Evolutionary relationship and a novel method of efficient identification of *Lentinula edodes* cultivars in China

Ling YY[1,2#], Zhang MZ[1,2#], Ling ZL[1], Cao B[1], Wu XP[5], Peng H[4], Wang ZR[3*] and Zhao RL[1,2*]

[1]*State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, PR China*
[2]*College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, PR China*
[3]*Institute of Cash Crops, Hubei Academy of Agricultural Sciences, Wuhan, 430064, PR China*
[4]*Institute for Systems Biology, Jianghan University, Wuhan, Hubei 430056, PR China*
[5]*Mycological Research Center, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, PR China*

Ling YY, Zhang MZ, Ling ZL, Cao B, Wu XP, Peng H, Wang ZR, Zhao RL 2022 – Evolutionary relationship and a novel method of efficient identification of *Lentinula edodes* cultivars in China. Mycosphere 13(2), 56–85, Doi 10.5943/mycosphere/si/1f/3

Abstract

Lentinula edodes (shiitake in Japanese, xiang-gu in Chinese) is the most productive mushroom in China, and there are more than 500 cultivars names. However, the traditional identification of mushroom cultivars can be challenging due to the problems of the heavy workload, insufficient accuracy and low reproducibility, which greatly affect the industrial development and germplasm innovation process. In this study, we developed and illustrated a methodological and technical system for the identification of *L. edodes* cultivars and for detecting their evolutionary relationship based on the multiple nucleotide polymorphism marker (MNP) sequences. Specifically, 501 universal MNP markers were screened based on the whole-genome resequencing data of 188 *L. edodes* strains mainly from China (25 of which were self-tested). The following analysis of detection rate, repeatability rate and accuracy rate showed those MNP markers are satisfactory with 94.4%, 99.92%, and 99.96% respectively. This method of multiplex PCR amplification and high-sequencing resulted in the construction of an MNP sequence library of *L. edodes*. On this basis, 187 MNP markers were further screened as core MNP markers. An ML phylogenetic tree of 162 wild strains and 78 cultivars was generated by using core MNP sequences, revealing all cultivars can be separated into 24 distinct evolutionary pedigrees. The genetic similarity (*GS*) value between different pedigrees and all cultivars tested showed that a broad library of pedigrees had *GS* values less than or equal to 94%, and the different cultivars within the same pedigrees had *GS* values range of 94-99.5%. The combination of all results indicated that most cultivars of *L. edodes* were originally from the Northeast Asia except the only cultivar *L. edodes* from south China, but there has a serious homogenization of *L. edodes* germplasms in China. In this study, we established a genealogical map of *L. edodes* cultivars in China, a standard library of MNP markers, and a rapid experimental procedure for genealogical and cultivar identification, which lays out a highly reliable platform for encouraging original innovation and modified cultivar innovation. Finally, a brief prospect for MNP markers identification of mushroom cultivars is addressed.

Keywords – edible mushroom – evolution – molecular marker – multiple nucleotide polymorphism – shiitake

Submitted 24 February 2022, Accepted 18 July 2022, Published online 24 August 2022, Published 29 December 2022
Corresponding Author: Rui-Lin Zhao – e-mail – zhaorl@im.ac.cn,
Zhuo-Ren Wang – e-mail – 843073895@qq.com

Introduction

The edible fungi industry is remarkably important for circular agriculture and the income of farmers. China is the world's largest producer of edible fungi, with 35 million tons produced per year, contributing to 3/4 of the world's total edible fungi (Royse et al. 2017). In China, the production of *L. edodes* is produced in greater quantity than any other individual edible mushroom, and reached to 1.188 million tons in 2020, accounting for 29% of total production, and it also has been widely cultivated and consumed worldwide (China Edible Fungi Association 2022).

With the rapid development of the edible fungi industry, artificial selective breeding, and germplasm exchange have also developed rapidly. The newly established and named cultivars have increased rapidly. For example, the names of cultivars of shiitake increased from 120-150 to 500 within seven years in China alone (Bian 2008, Song et al. 2015b). Because of the lack of an efficient and accurate method to identify them, the phenomenon of different names for the same cultivars or the same names for different cultivars is common for many cultivated mushrooms (Bian 2008). The confusion of cultivar (strain) names not only brings unnecessary difficulties for edible fungi production but also creates serious obstacles to germplasm innovation. Accurately identifying cultivars is therefore critical to the industry as a whole.

However, the accurate and efficient determination of cultivars is a quite challenging process as many cultivars have a very close genetic relationship, and the phenomenology often shows a great diversity under different cultivation conditions. Traditional morphological, cytological, and biochemical methods are susceptible to environmental factors and subjective factors of the detector (Worrall 1997). Molecular markers developed based on DNA polymorphisms have shown great advantages, including the numerous and high availability of markers, the codominance of molecular markers that can effectively distinguish between homozygous and heterozygous genotypes, and the identification of molecular markers that are not affected by environmental factors or developmental stage (Kaur et al. 2015). Various molecular methods have been developed, such as Restriction Fragment Length Polymorphism (RFLP) (Kulkarni 1991), Random Amplified Polymorphic DNA (RAPD) (Park et al. 1997, Sun & Lin 2003, Fu et al. 2010), Amplified Fragment Length Polymorphism (AFLP) (Terashima et al. 2002), Inter Simple Sequence Repeat (ISSR) (Fu et al. 2010), and Sequence Characterized Amplified Regions (SCAR) (Wu et al. 2005). However, the results of these molecular markers usually depend on specific DNA fragments generated by restriction endonuclease digestion or oligonucleotide DNA primer amplification, analyzed via agarose gel electrophoresis, and therefore the reproducibility and accuracy of genotyping can be relatively low and can be slow and laborious, relying on a high workload for technicians.

Currently, Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) markers are used for the identification of edible fungi, including *L. edodes* (Liu et al. 2010, Wang et al. 2013, Kim et al. 2015, Lee et al. 2017, Saito et al. 2019). SSR are essentially non-coding regions that have remained conserved during evolution. This approach consists of tandem repeats in the genome, and the different number of repeats and degree of repetition leads to high variability in SSR length, resulting in SSR markers or SSLP markers that can be distinguishable between cultivars (Ellegren 2014). SSR markers can be detected by a polymerase chain reaction followed by gel electrophoresis (Varshney et al. 2005), and have been widely used for cultivar identification (Liu et al. 2010, Lee et al. 2017, Saito et al. 2019), construction of linkage mapping (Dong et al. 2019), as well as population structure and genetic diversity analysis of *L. edodes* (Xiang et al. 2016, Moon et al. 2017, Lee et al. 2020). However, the duplicate SSRs in a sample can often cause DNA polymerase slippage during PCR amplification, thereby artificially extending or shortening the length of the duplicated elements, reducing the accuracy of SSR genotyping (Ellegren 2004). On the other hand, SSRs the direct result of this method is based on the length of the target fragment (Pourabed et al. 2015). If there is a little or none difference in the fragment length, it is difficult to make an accurate judgment by electrophoresis. In addition, the electrophoretic detection of SSRs is inefficient, and only a small number of SSRs can be processed at a time.

The SNP method is another widely used molecular marker approach, which involves DNA sequence polymorphisms resulting from variation in a single base in the genomic DNA sequence with a frequency of 1% or higher (Vignal et al. 2002). The SNP marker method obtains specific base sequences, and genotyping assays do not require electrophoresis, which is more accurate than those of the SSR marker method. High-throughput genotyping methods such as microarrays or whole-genome sequencing (WGS) are often used to detect large numbers of SNPs (Huang et al. 2010). However, the WGS cost limitations have led to only a few SNP markers of functional genes that have been used to identify *L. edodes* (Wang et al. 2013, Kim et al. 2015). Microarray technology has been used to detect SNPs, which has drawbacks including missing data, low sensitivity, and high false-positive rates (Allen et al. 2017). The higher manufacturing cost of microarrays and the relatively complex analytical instrumentation make it a high-cost analysis method (Jehan & Lakhanpaul 2006).

Insertions or deletions of more than one base sequence at the nucleic acid level (Indel) represent another major type of DNA variation. The distribution and density of Indel markers are higher than SSR markers but lower than SNP markers. Recently, some researchers have used the Indel molecular marker method for variety identification of *L. edodes* (Xiang et al. 2016, Shen et al. 2021). Unfortunately, Indel markers in the field of edible mushrooms have lagged significantly behind SNPs, and relatively few Indels have been identified (Shen et al. 2020). The accuracy of Indel identification in genomic studies is also affected by several factors, such as structural genomic features, repetitive sequences, short interspersed elements, the presence of dimers, and the quality of the Indel detection method. Therefore, the development and use of this method can be challenging (Bennett et al. 2020).

The multiple nucleotide polymorphism (MNP) marker method has recently been developed based on single nucleotide polymorphisms (Xu et al. 2020). At present, MNP markers have been successfully applied to variety identification of rice, soybean, rapeseed, eggplant, maize, tomato, and other crops (Zhang et al. 2019). The basic principle is that there are multiple SNPs in the genome, and examining a combination of all alleles with distinct SNPs can be used to distinguish between different individuals. The advantage of the MNP method is that it is more accurate and easier to perform than other molecular marker methods at present.

In this study, the MNP marker method was first applied to fungi using *L. edodes* as a sample. Based on 188 genomic sequences from *L. edodes* strains, we developed 501 MNP markers, and further selecting 186 core MNP marker sequences, we carried out a phylogenetic analysis to reveal their evolutionary relationship. Finally, we developed an accurate method to calculate the genetic similarities between different lineages and cultivars, which can be used for identification of *L. edodes* cultivars.

Materials & Methods

Samples collection

A total of 167 cultivars and wild strains of *L. edodes* were collected from China, of which 78 representative strains were selected for further study (Table 1), including 25 cultivars approved by the National Variety Committee of China, 7 cultivars approved (identified and recorded) by Locally Variety Committee, other cultivars with representative traits or typically biological characteristics, and several wild strains from different regions.

DNA extraction and whole-genome resequencing

The 78 representative strains of *L. edodes* were chosen (Table 1) and cultured in PDA solid medium covered with cellophane at 25℃ until the mycelia were fully grown. Genomic DNA was extracted from mycelia using the CTAB method. The concentration and integrity of the DNA solution were assayed via Nanodrop and 1.0% agarose gel electrophoresis and finally stored at -20℃. Eighteen commercial strains and seven wild strains were chosen as whole genomic resequencing materials (Table 1). The genomic DNA solutions (volume>2 μg,

concentration>50 ng/ μL, indicating no pigment contamination, as well as no RNA, protein, and other impurity contamination) were sent to Beijing Bemac Biotechnology Co.

Table 1 The cultivars and wild stains of *L. edodes* used in this study.

NO.	Cultivar Name	MNP sequence code	Appraisal Situation	Pedigree
1	L952	XG0104-11	National variety 2008006	Introduced from Japan and systematic breeding
2	**Guangxiang-51**	**XG0083-11**	**National variety 2008003**	**Domestication from a wild strain**
3	**L135**	**XG0084-11**	**National variety 2007005**	**Introduced from Japan and systematic breeding**
4	L9319	XG0156-11	National variety 2008008	Derived from *L. edode* tissue isolation in Lishui
5	**241-4**	**XG0085-11**	**National variety 2007010**	**Derived from 241 by tissue isolation and systematic breeding**
6	Ganxiang-1	XG0011-11	National variety 2007012	**1303×HO3, single spore cross-breeding**
7	Qinke-20	LH20-1	National variety 2010003	Nature variation from Qingyuan-9015 and systematic breeding
8	**Wuxiang-1**	**XG0086-11**	**National variety 2007011**	**Introduced from Japan and systematic breeding**
9	**L808**	**XG0088-11**	**National variety 2008009**	**Derived from basswood *L. edode* tissue isolation in Lanzhou**
10	Shenxiang-8	XG0114-11	National variety 2007001	70(wild strain) × Suxiang, single spore cross-breeding
11	Cr62	XG0157-11	National variety 2007007	7917(from Japan) × L21(wild strain from Jiangxi), single spore cross-breeding
12	Junxing-8	XG0161-11	National variety 2008007	Systematic breeding from a wild strain
13	Senyuan-1	SRR3504456	National variety 2007014	856(from Japan) × 8404(wild strain), single spore cross-breeding
14	Huaxiang-8	SRR3504451	National variety 2008004	Cultivars from Hubei
15	Shenxiang-10	LH36	National variety 2007002	L26(from Japan) × Suxiang, protoplast monokaryon non-symmetry crossing

Table 1 Continued.

NO.	Cultivar Name	MNP sequence code	Appraisal Situation	Pedigree
16	Minfeng-1	XG0121-11	National variety 2007006	L12(from Japan) × L34 (from Ninghua), single spore cross-breeding
17	Shenxiang-12	XG0033-11	National variety 2007003	69 (wild strain) × Suxiang, protoplast monokaryon non-symmetry crossing
18	**Cr02**	**XG0091-11**	**National variety 2007004**	**7402(from Japan) × Lc-01(wild strain from Fujian), single spore cross-breeding**
19	**Hunong-1**	**SRR3504449**	**National variety 2010004**	**Introduced from Japan**
20	**Senyuan-8404**	**XG0093-11**	**National variety 2007016**	**Domestication from wild strain in Hubei**
21	**L9015**	**XG0095-11**	**National variety 2007009/**	**241(from Japan) × 8210×Rifeng-34, cross-breeding**
22	Xiangza-26	XG0039-11	National variety 2008002	NO.8(wild strain) × NO.40, cross-breeding
23	Jindexianggu	LH46-1	National variety 2007013	L939×Junxing135, protoplast monokaryon non-symmetry crossing
24	**Xiangjiu**	**XG0098-11**	**National variety 2008001**	**Domestication from a wild strain**
25	Cr04	SRR3504459	National variety 2007008	7917(from Japan) × L21(wild strain from Jiangxi), single spore cross-breeding
26	Jiuxiang-4	XG0001-11	Approved by Shanghai in 2015	Unknown
27	**7401**	**SRR8954575**	–	**Introduced from Japan**
28	Xiaxiang-18	LH6	–	Unknown
29	939	XG0105-11	–	Domestication from a wild strain
30	Hunong-3	SRR3504449	–	Unknown
31	Qiuxiang-607	XG0108-11	Approved by Shanghai in 2015	Systematic breeding from Qiuzai-6
32	L241	XG0164-11	–	Introduced from Japan
33	L856	SRR3504443	–	Systematic breeding from L03 (from Japan)
34	Senyuan-2	SRR3504457	–	8404(wild strain) × L856(from Japan), single spore cross-breeding
35	Baihua-2	XG0018-11	–	Unknown
36	**L12**	**R20**	–	**Introduced from Japan**
37	**L26**	**XG0089-11**	–	**Introduced from Japan**

Table 1 Continued.

NO.	Cultivar Name	MNP sequence code	Appraisal Situation	Pedigree
38	Qinke-20 variety	XG0163-11	–	Variety from Qinke-20
39	Shenxiang-4	XG0167-11	Approved by Shanghai in 2004	Introduced from Fujian and systematic breeding
40	**L087**	**XG0094-11**	–	**Systematic breeding from L27**
41	L109	SRR8954573	–	Unknown
42	**Suxiang**	**XG0096-11**	–	**Unknown**
43	Senyuan-8	XG0124-11	–	Unknown
44	908	SRR3504553	–	Introduced from Japan
45	Shenxiang-6	SRR8954576	–	Domestication from wild strain in Jiangxi
46	**Jiuxiangqiu-7**	**XG0099-11**	**Approved by Hubei in 2015**	**Unknown**
47	Shenxiang-2	XG0127-11	Approved by Shanghai in 2004	Introduced from Fujian and systematic breeding
48	**Dachun-1**	**XG0100-11**	–	**Unknown**
49	Chunzai-208	XG0128-11	–	Unknown
50	**Aihua-3**	**XG0101-11**	–	**Unknown**
51	218	XG0129-11	–	Unknown
52	9608-WY	XG0130-11	–	**Systematic breeding from 9608**
53	Xiangnong-49	XG0148-11	–	Unknown
54	Senyuan-16	XG0131-11	–	Unknown
55	7402	XG0132-11	–	Introduced from Japan
56	807(Zhongxiang-36)	XG0133-11	–	Unknown
57	Cr33	XG0134-11	–	7917 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding
58	Cr52	XG0154-11	–	7917 (from Japan) × Xiangjiu, cross-breeding
59	Cr66	XG0136-11	–	7917 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding
60	L03(Zhongxiang-37)	XG0155-11	–	Introduced from Japan
61	L11	XG0137-11	–	Cr02×L456, systematic breeding
62	L27	XG0065-11	–	Introduced from abroad
63	L42	XG0138-11	–	Systematic breeding from Cr04
64	Shenxiang-93	XG0141-11	–	Introduced from Jiangxi and systematic breeding
65	Shenxiang-9	XG0068-11	Approved by Shanghai in 2004	Introduced from Jiangxi and systematic breeding

Table 1 Continued.

NO.	Cultivar Name	MNP sequence code	Appraisal Situation	Pedigree
66	868	SRR3504589	–	Unknown
67	238	XG0070-11	–	Introduced from Japan and systematic breeding
68	Yuhua-2	SRR3491419	–	Unknown
69	922	XG0162-11	–	Unknown
70	Lingxian-1	XG0073-11	–	Unknown
71	Taiyin	XG0144-11	–	Unknown
72	931	XG0146-11	–	Unknown
73	Junxing-68	XG0142-11	–	Unknown
74	Shenxiang-215	XG0077-11	Approved by Shanghai in 2014	Systematic breeding from L808
75	Shenxiang-16	XG0143-11	Approved by Shanghai in 2009	939×135, protoplast monokaryon non-symmetry crossing
76	Liaofu-10	XG0079-11	–	Unknown
77	Gaoxiang-1	XG0080-11	–	Unknown
78	Xiangnong-49	XG0148-11	–	Unknown
79	**GX20170368**		–	**Wild**
80	**ZRL20180417**		–	**Wild**
81	**ZRL20180817**		–	**Wild**
82	**5.0439**		–	**Wild**
83	**Xiang24**		–	**Wild**
84	**5.0491**		–	**Wild**
85	**GX20170702**		–	**Wild**

Note: Strains in bold were chosen for genomic re-sequenced strains in this study.

Screening and primer design for universal MNP markers in *L. edodes*

In total, 188 whole-genome resequencing data of *L. edodes* were analyzed, including 25 obtained from this study (Table 1) and 162 which were accessed from NCBI (Appendix 4). Firstly, the sequencing data were mapped to the reference genome of *L. edodes* (genome version: GCA_001562095.1) with Bowtie2 (Langmead & Salzberg 2012). Next, all SNPs were identified with Samtools-mpileup (Danecek et al. 2021). A sliding window of 125 base pairs was used to scan all SNP-containing genome segments with an increment of 5 bp. The discriminative power (DP) of a window was defined as (*c(N,2)*: the number of pairs among N samples; t: the number of each pair that had two dispersed SNPs within a window at least.):

$$\frac{t}{c(N,2)}$$

The windows with no SSRs, no successive SNPs, and DP≥0.2 were chosen for the target region. Primers were designed for the target region using GenoBaits probe software (Guo et al. 2019). The principles of primer design included conservative amplification regions, single copy, high polymorphism, low missing rates in different samples, etc.; ensuring consistent annealing temperatures for each primer, no primer dimer formation, and specific amplification of the genome. Finally, primers were synthesized and tested to complete the development of the MNP marker combinations.

Library construction and high-throughput sequencing of MNP

All primers were diluted to 100 μM, then 5 μL of each primer was pipetted into the primer mix pool. The multi-PCR reaction system consisted of 12 μL Template DNA, 5 μL Primer Mix, 5 μL 10×Multi HotStart Buffer, 4 μL Super Pure dNTPs, 1 μL Multi HotStart DNA Polymerase, and 27 μL ddH_2O. The total volume of each reaction mixture was 50 μL. The PCR reactions were performed as follows: 95℃ for 15 min; followed by 15 cycles at 94℃ for 30 sec, 58℃ for 90 sec, and 72℃ for 90 sec; followed by elongation at 72℃ for 10 min and finally cooling to 4℃. After the reaction was completed, the PCR products were purified using the paramagnetic particle method. The concentration and integrity of the library were assayed using Qubit dsDNA HS Assay Kit and agarose gel electrophoresis. Qualified libraries were mixed at equal mass (100 ng) and sequenced by the Illumina MiSeq platform from Beijing Novozymes. The sequencing data volume for each strain was set at 500 M.

Analysis of the reproducibility and accuracy

The 15 strains were randomly chosen from all tested strains of *L. edodes* for reproducibility and accuracy of experiments. The same DNA extracted from each strain was used to construct two separate libraries, the two libraries were sequenced twice at different times, and the number of differential markers were compared between two batches of libraries (the first batch and the second batch). The reproducibility of each sample was calculated according to the formula (*n*: Numbers of reproducible genotype pairs compared, *N*: Numbers of all genotype pairs compared, *r*: reproducibility):

$$r = \frac{n}{N}$$

Reproducible genotypes in both experiments were taken as correct and the accuracy of each sample was calculated according to the formula:

$$1 - \frac{(1-r)}{4} = 0.5 + 0.5r$$

Determination of pedigree and core MNP markers

From all the MNP markers, the markers with 100% amplification rate for all tested strains were chosen as the core MNP markers for *L. edodes*. Based on the amplified sequences of these core markers, a phylogenetic tree was constructed using the ML method which was used to further differentiate the pedigree of all commercial cultivars.

Analysis of genetic similarity (*GS*) between different pedigrees and cultivars

The multiplex PCR sequencing and resequencing results of each sample were mapped to the reference genome and the consensus sequence was obtained. All MNP sequences of all samples were extracted based on the location information of the core MNP markers. After removing the sequence which (contained) the null value “N”, the MNP sequences of all samples were performed with pairwise comparison. Each of the paired samples with identical sequences at the same MNP locus (degenerate bases at the heterozygous site are considered identical to the normal bases) were

considered to have the same genotype at that locus (This locus is recorded as the same locus), otherwise, they are different genotypes (This locus is noted as a different locus).
The genetic similarity (*GS*) between the two cultivars was calculated according to the formula (N_{ij}: Number of common non-null MNP sequences between two strains; n_{ij}: Number of identical MNP sequences):

$$GS = \frac{n_{ij}}{N_{ij}} \times 100\%$$

The range of genetic similarity between cultivars, similar cultivars, and highly similar cultivars was analyzed on a case-by-case basis.

Results

Genome resequencing, screening of universal MNP markers, and the construction of a MNP sequences library of *L. edodes*

A total of 25 cultivars and strains were genome re-sequenced (Table 1), and about 93.3 Gb of clean data were obtained. After assessment of the quality of the sequencing data, the mean Q20 value was 98.16%, the mean Q30 value was 94.53%, and the GC-content ranged from 42.62% to 46.33%. Due to the sufficient amount of data and normal GC value distribution, this sequencing data was determined to be usable for further data analysis. In addition, the 162 genome resequencing data of *L. edodes* were downloaded from NCBI (those sequence names start with SRR in Fig. 2) for the screening of MNP markers.

A total of 501 universal MNP markers of *L. edodes* were selected based on genomic screening, and 501 pairs of primers were designed and synthesized. Those primers and genomic DNAs from 78 cultivars were subjected to multiplex PCR amplification and sequencing. Finally, a total of 37,830 markers were detected, with an average sequencing coverage of 3464.904-fold per strain. All of these results contributed to the construction of the MNP sequences library of *L. edodes* (The basic library information is listed in Appendix 1).

Evaluation of universal MNP markers

Among all 78 tested cultivars, cultivars had a marker detection ranging from 250-497 from a total of 501 MNP markers, with an average of 472.96 MNP markers per cultivar, so the detection rate was 94.4%. The distribution of the number of MNP markers detected by each cultivar is shown in Fig. 1, Appendix 1.

Two batches of libraries were constructed from the same DNA of 15 randomly selected cultivars, and the number of differential markers from these two batches was counted. The statistical results are shown in Table 2. In total, the number of genotype pairs in this study was 7607 and the number of reproducible genotype pairs was 7601, so the reproducibility rate was 99.92% and the accuracy rate was 99.96%.

Construction of phylogenetic topology using core MNP sequences

187 MNP markers from 501 universal MNP markers were successfully detected in all cultivars in this study, and these markers were chosen as core MNP markers. These core MNP sequences from each of 240 *L. edodes* strains were used to build a ML phylogenetic tree (Fig. 2) which also included 78 cultivars in this study, and 162 independent genomic sequences downloaded from NCBI. Generally, there are two main clades in the phylogenetic tree, one (Clade A in Fig. 2) is composed of wild strains which are mainly from the southern China; while another (Clade B in Fig. 2) is composed of almost all cultivars and mixed with several wild strains. Of note, those wild strains mainly come from northern China. All cultivars could be recognized as part of one of 24 lineages, 23 of which are from Clade B, and only one from Clade A (Fig. 2).

Table 2 Statistical results of reproducibility and accuracy rates based on the genotype pairs of MNP markers on 15 randomly selected cultivars.

Cultivar Name	Library		Number of common markers	Number of different markers	Number of identical markers	Reproducibility rate	Accuracy rate
	First batch	Second batch					
7401	XG0082-11	XG0082-12	470	0	470	100%	100%
L135	XG0084-11	XG0084-12	470	0	470	100%	100%
241-4	XG0085-11	XG0085-12	460	0	460	100%	100%
Wuxiang-1	XG0086-11	XG0086-12	494	0	494	100%	100%
L12	XG0087-11	XG0087-12	479	0	479	100%	100%
Cr02	XG0091-11	XG0091-12	497	0	497	100%	100%
Hunong-1	XG0092-11	XG0092-12	469	0	469	100%	100%
Suxiang	XG0096-11	XG0096-12	488	0	488	100%	100%
Shenxiang-15	XG0077-11	XG0145-11	480	0	480	100%	100%
L808	XG0088-11	XG0088-12	474	1	473	99.79%	99.89%
L26	XG0089-11	XG0089-12	492	1	491	99.8%	99.9%
L087	XG0094-11	XG0094-12	478	1	477	99.79%	99.9%
L9015	XG0095-11	XG0095-12	461	1	460	99.78%	99.89%
Senyuan-8404	XG0093-11	XG0093-12	469	2	467	99.57%	99.79%
Total	–	–	7601	6	7595	99.92%	99.96%

The *GS* values between different lineages and cultivars

The *GS* values between all pairwise lineages and each pair of cultivars were calculated. The results showed that the *GS* values in the lineage level were in the range of 22.40%-94.12% (Appendix 2), and we recognized them as 24 pedigrees based on the combination of data regarding their phylogenetic relationship. The *GS* values among different cultivars were all higher than 94.54%, and some cultivars showed a *GS* value of 100% (Fig. 3, Appendix 3).

Discussion

Based on 188 whole-genomic sequence data of which 25 were self-sequenced in this study, 501 universal MNP markers were screened. A total of 78 tested cultivars were all subjected to multiplex PCR amplification and high-throughput sequencing using the 501 pairs of MNP primers. The following analysis of detection rate, repeatability rate and accuracy rate showed those MNP markers are satisfactory with 94.4%, 99.92%, and 99.96% respectively. Then a MNP sequence library of *L. edodes* cultivars was constructed. 187 MNP markers from 501 universal MNP markers were chosen as core MNP markers, and their sequences from each of 240 *L. edodes* strains (including 78 tested cultivars and 162 independent genomic sequences downloaded from NCBI) were used to build the phylogenetic tree (Fig. 2), and the evolutionary relationships among cultivars and wide strains, pedigrees of all cultivars were discussed.

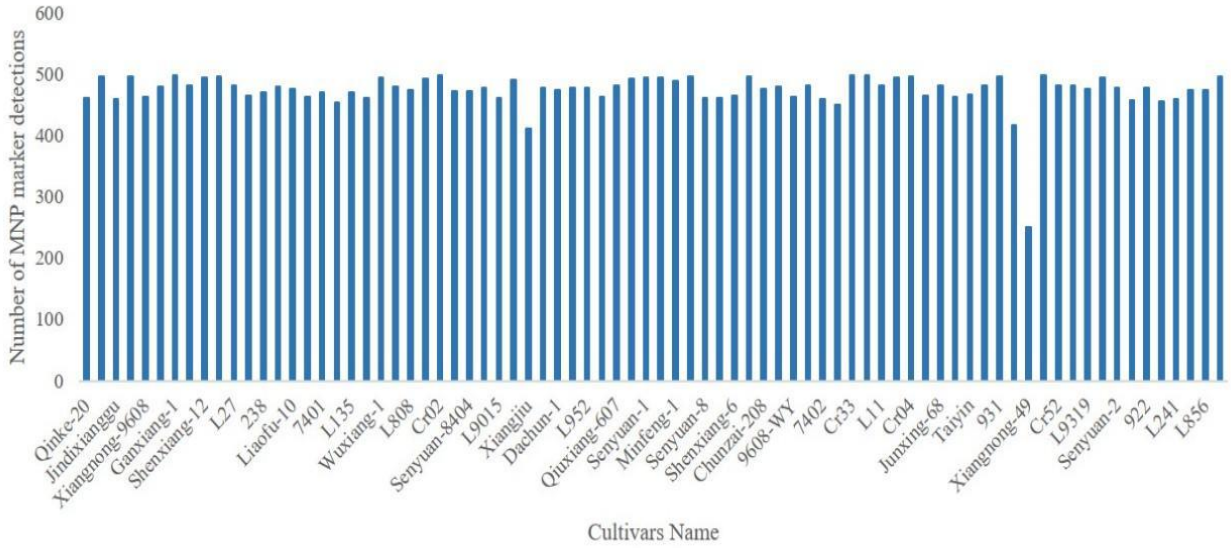


Figure 1 – Distribution of the number of detected MNP markers in *L. edodes* cultivars used 501 universal MNP makers.

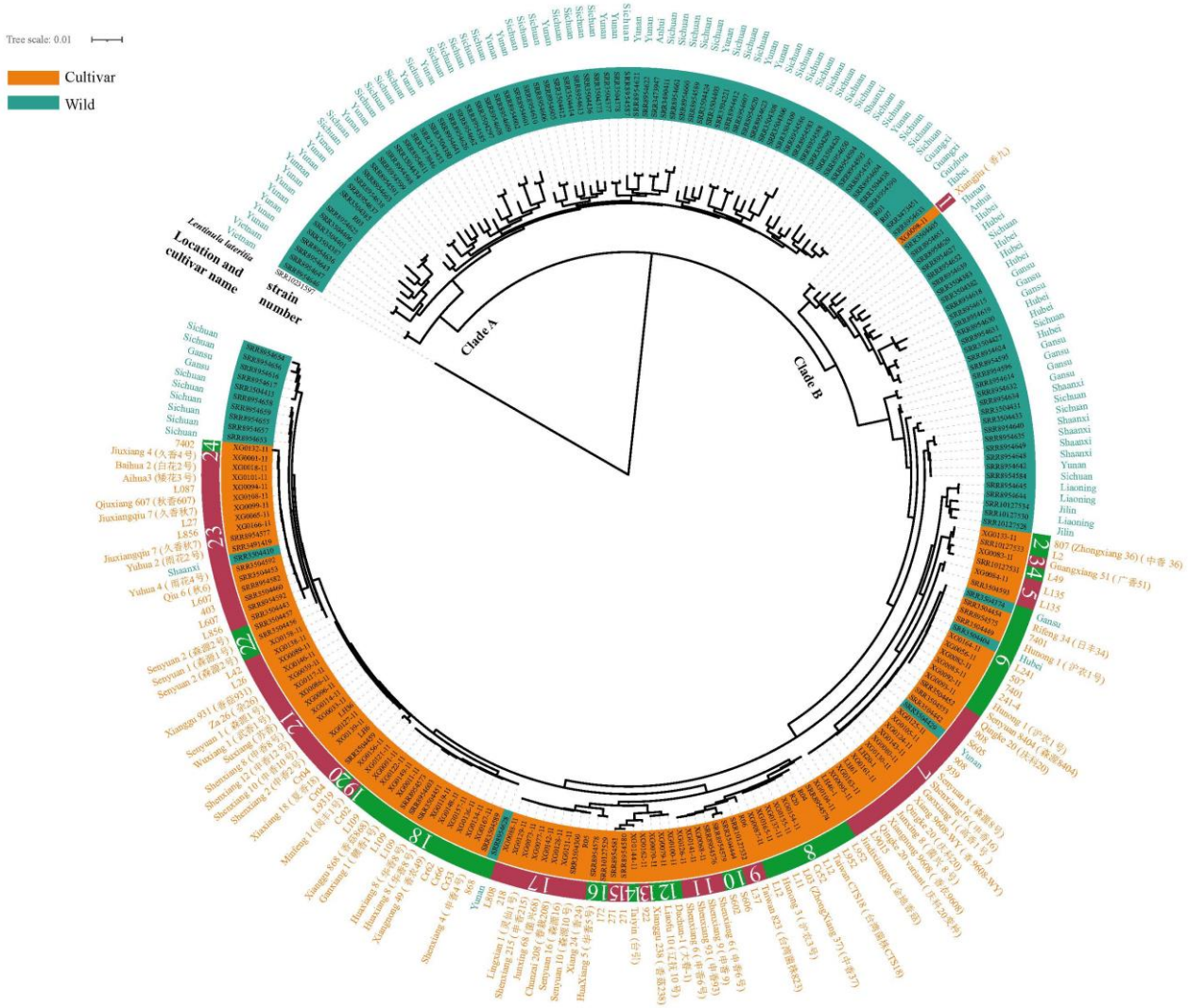


Figure 2 – ML phylogenetic tree of *L. edodes* based on 187 core MNP sequences from 240 *L. edodes* cultivars/strains.

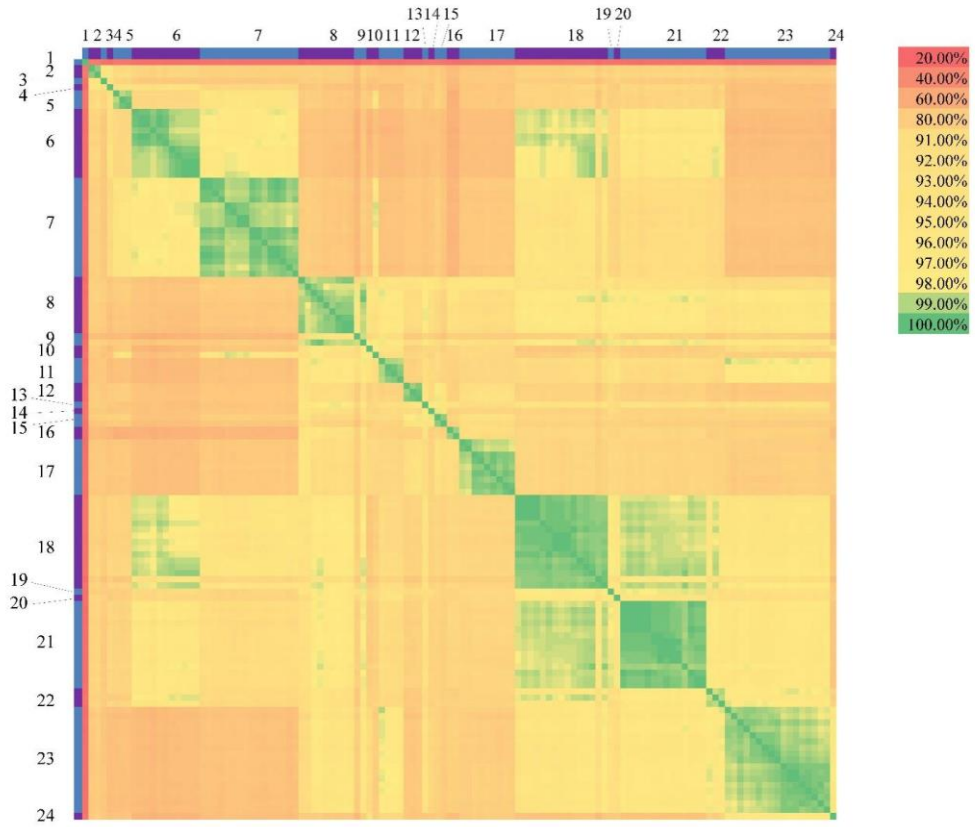


Figure 3 – The heat map of the *GS* values of pairwise comparison between 24 pedigrees and all cultivars.

The evolutionary relationship of cultivars and wide strains of *L. edodes*

Generally, the wide stains and cultivars of *L. edodes* are separated clearly in the phylogeny used 187 core MNP marker sequences from 240 samples (Fig. 2), and our phylogeny is widely consistent with the use of 49,172 SNPs at fourfold degenerate sites (Zhang et al. 2022). There are two main evolution clades: one is composed of only wild strains mainly from the southwest China (Yunnan and Sichuan provinces, Clade A in Fig. 2); while another (Clade B in Fig. 2) is composed of almost all cultivars and mixed with wild strains which mainly come from northern China. The recently research had showed *L. edodes* belongs to the Asian-Australasian clade in a multigene sequences analysis (Menolli et al. 2022), and compared with the cultivation history of *L. edodes* in China, we could conclude that most cultivars of China are the origin of Northeast Asia. All cultivars except cultivar Xiangjiu have closely evolutionary relationships.

The identification of cultivars and its pedigree of *L. edodes*

Based on the phylogenetic tree using these187 core MNP marker sequences from each of 240 *L. edodes* wild strains and cultivars (Fig. 2). All cultivars of *L. edodes* can be clearly separated into 24 distinct pedigrees (named as pedigrees 1-24 respectively, Fig. 3, Appendix 2). The following genetic similarity values between pedigrees and each cultivar showed that these pedigrees can be distinguished by a *GS* value of less than or equal to 94%; and the *GS* value between cultivars (strains) with the range of 94%-99.5%, they could be identified as the different cultivars but in the same pedigree; while the cultivars take as the same cultivars when their *GS* value higher than 99.5% (Fig. 3, Appendix 2, 3).

We compared the MNP molecular phylogeny relationship with other molecular marker-based studies and found the results of this study are generally consistent with previous studies. We examined in detail the complex pedigrees (pedigree 6, 7, 18, 21) as an example of this dataset.

In this study, the cultivars 7401, Hunong-1, L241, 241-4, Rifeng-34, and Senyuan-8404 from pedigree 6; cultivars 908, L9015, Qingke-20, 939, Shenxiang-16, Jindixianggu, and 9608 from pedigree 7; cultivars Cr62, Huaxiang-8, Ganxiang-1, 868, and L109 from pedigree 18; and cultivars L26, Xiangza-26, Wuxiang-1, Shenxiang-8, Suxiang, Shenxiang-10, Shenxiang-12, Cr04, and Xiangza-26 from pedigree 21 showed a similar evolutionary relationship, a finding which was confirmed using other molecular markers (Indel markers in Shen et al. 2021, the fourfold degenerate SNPs in Zhang et al. 2022, SSR markers in Wu 2014, Dong et al. 2017, ISSR markers in Xiao et al. 2018; ISSR, SRAP, and TRAP markers in Wang et al. 2019). However, when using MNP markers, we can further distinguish more pedigrees, such as pedigree 5 (L135 and Senyuan-8404), and pedigree 6 (L241 and L241-4), which Indel markers failed to distinguish (Shen et al. 2021). Therefore, the MNP marker method is more specific and accurate for the identification of cultivars in *L. edodes*.

Genetic background analysis of cultivars of *L. edodes* in China

In this study, we designated the earliest identified or earliest widely used cultivar in each pedigree as the original, while other cultivars within the pedigree were suspected be developed from those originals (Table 3). We found among the totally 24 pedigrees distinguished in this study, the original cultivars of thirteen pedigrees were from Japan (Table 3), five pedigrees from China (pedigree 1, 3, 15, 17 from mainland of China, and pedigree 12 from Taiwan), and three pedigrees of currently unknown origin (pedigree 2, 9, 13). These results indicated there has been a serious homogenization of *L. edodes* germplasms in China, and the mostly cultivars of *L. edodes* have a likely origin in the Northeast Asia.

The *GS* values of 100 for some cultivars in this study indicates that those cultivars have almost identical genetic background, but they were given distinct names: cultivars Qingke20, L9015, Gaoxiang1 and Shenxiang16 from Pedigree 7 are same; cultivars L12 and Hunong3 from Pedigree 8 are same; in Pedigree 18, cultivars Cr66, Cr33, Cr62 and Shenxiang-4 are same; Cultivars 868, L109 and Cr02 are same; in Pedigree 21, cultivars Zaxiang-26, Wuxiang-1, Shengxiang-8, Suxiang, and Senyuan-1 are same; cultivars Cr04, Shenxiang-10, Shenxiang-2 and Xiaxiang-18 are same; in Pedigree 23 cultivars L27 and L856 are same too. Whether they are the same cultivars will require further cultivation experiments to determine their agronomic traits.

Prospects for MNP markers in the identification of edible fungi cultivars

The results presented in this work strongly suggest that using MNP markers can be advantageous compared to other methods. Firstly, the method avoids error-prone sequences such as simple repeats and successive SNPs when screening target regions; secondly, the high-throughput sequencing-based detection of MNP marker overcomes the uncertainty of SSR amplification length displayed on gel electrophoresis and the microarray hybridization noise for SNP markers; the amplicons of MNP markers were often sequenced thousands of times, reproducibility and accuracy were therefore greatly improved. The SSR and SNP marker method typically require 30 or more PCR amplification cycles to produce enough signals for adequate detection, and a large amount of non-linear PCR amplification may distort the actual proportions of marker alleles, resulting in inaccurate genotyping. In contrast, the MNP marker used in this study requires only about 15 cycles in the PCR amplification phase, and combined with high-throughput sequencing can detect the bases of each amplified sequence, greatly improving the accuracy of genotyping.

Once the species-specific MNP marker library has been constructed, the cultivar identification is relatively straightforward to determine. It is only necessary to amplify the tested DNA sample using mixed MNP marker primers in a single PCR system, so the experimental operation of the assay is very convenient. For the SNP marker method, it requires whole-genome resequencing data of DNA to screen different SNP markers according to different samples, which is more demanding in terms of experimental operation and time.

MNP marker technology has been used with remarkable success in plant variety identification (Zhang et al. 2019). However, the size of plant genomes is significantly larger than

fungal genomes, the criteria and precision required for differentiation in edible fungi species are stricter. In this study, we have made a series of optimizations based on the MNP marker method for plant varieties, to make it suitable for the practice of edible fungi variety identification, and further developed a suitable experimental procedure for edible fungi variety identification, including the screening of the core MNP markers from universal MNP markers in *L. edodes*, a more precise set of principles for genotype identification, and criteria for differentiating genetic similarity between cultivars and pedigrees in edible fungi. The efficient identification of cultivars and their evolutionary origins are not only of benefit to *L. edodes* cultivation but also provides an important basis for differentiating original and innovative breeding from modified breeding.

Table 3 The speculation of the original and developed cultivars of *L. edodes* in China based on the MNP phylogeny and *GS* values.

Pedigree	Cultivars/strain type	Cultivars/strain name	Appraisal Situation	Sources of Cultivars	References
1	Original	Xiangjiu	National variety 2008001	Domestication from a wild strain	Yang (1988)
	Developed	–	–	–	–
2	Original	807 (Zhongxiang-36)	–	–	–
	Developed	L2	–	–	–
3	Original	Guangxiang-51	National variety 2008003	Domestication from a wild strain	Luo & Liu (1993)
	Developed	–	–	–	–
4	Original	L49	National variety 2008003	Domestication from a wild strain	–
	Developed	–	–	–	–
5	Original	L135	National variety 2007005	Introduced from Japan and systematic breeding	Xie et al. (2004)
	Developed	–	–	–	–
6	Original	7401	–	Introduced from Japan	Applied Fungal Research Unit, Department of Plant Protection (1984)
	Developed	L241	–	Introduced from Japan	Yao & Zhang (1997)
		241-4	National variety 2007010	Derived from L241 by tissue isolation and systematic breeding	Wu et al. (1994)
		Hunong-1	National variety 2010004	Introduced from Japan	Wang et al. (1990)
		Senyuan-8404	National variety 2007016	Domestication from wild strain in Hubei	Song et al. (2015b)
		507	–	Introduced from Japan	Qian (1993)
		Rifeng-34	–	–	–
7	Original	908	–	Introduced from Japan	Yao & Zhang (1997)
	Developed	L9015	National variety 2007009	Introduced from Japan	Xie et al. (2004)
		Qinke-20	National variety 2010003	Nature variation from Qingyuan-9015 and systematic breeding	Chen et al. (2005)
		Qinke-20 variety	–	Variety from Qinke-20	–
		939	–	Systematic breeding from L9015 (from Japan)	Xie et al. (2004)

Table 3 Continued.

Pedigree	Cultivars/strain type	Cultivars/strain name	Appraisal Situation	Sources of Cultivars	References
		Shenxiang-16	–	L939×135, protoplast monokaryon non-symmetry crossing	Song et al. (2010)
		Jindixianggu	National variety 2007013	L939×Junxing-135, protoplast monokaryon non-symmetry crossing	Wu et al. (1994)
		Junxing-8	National variety 2008007	Systematic breeding from a wild strain	Wu et al. (2005)
		Gaoxiang-1	–	–	–
		Senyuan-8	–	–	–
		S605	–	–	–
		9608	–	Systematic breeding from L9015 (from Japan)	Wu et al. (1994)
		9608-WY		Systematic breeding from 9608	
8	Original	L952	National variety 2008006	Introduced from Japan and systematic breeding	Song et al. (2015b)
	Developed	Taiwan strain-CTS18	–	–	–
		L12	–	Introduced from Japan	Song et al. (2015b)
		Cr52	–	7917 (from Japan) × Xiangjiu, cross-breeding	Xie et al. (2004)
		L11	–	Cr02×L456, systematic breeding	Wang et al. (1992)
		Hunong-3	–	–	–
		L03 (Zhongxiang-37)	–	Introduced from Japan	–
		Shenxiang-8	National variety 2007001	70 (wild strain) × Suxiang, single spore cross-breeding	Wu et al. (1994)
9	Original	Taiwan strain-823	–	–	–
	Developed	L37	–	–	–
10	Original	S606	–	–	–
	Developed	S602	–	–	–
11	Original	Shenxiang-6	–	Domestication from wild strain in Jiangxi	–
	Developed	Shenxiang-9	Approved by Shanghai in 2004	Domestication from wild strain in Jiangxi, different offsprings shared the same parents with Shenxiang-6	Liu et al. (2011)
		Shenxiang-93	–	Domestication from wild strain in Jiangxi, different offsprings shared the same parents with Shenxiang-6	Liu et al. (2011)
12	Original	238	–	Introduced from Japan and systematic breeding	–

Table 3 Continued.

Pedigree	Cultivars/strain type	Cultivars/strain name	Appraisal Situation	Sources of Cultivars	References
	Developed	Dachun-1	–	–	–
		Liaofu-10	–	–	–
13	Original	922	–	Introduced from Japan	–
	Developed	–	–	–	–
14	Original	Taiyin	–	Introduced from Taiwan	–
	Developed	–	–	–	–
15	Original	271	–	–	–
	Developed	–	–	–	–
16	Original	172	–	Introduced from Japan	–
	Developed	Huaxiang-5	National variety 2008005	Introduced from Germany and systematic breeding	Song et al. (2015b)
17	Original	L808	National variety 2008009	Derived from basswood *L. edode* tissue isolation in Lanzhou	Song et al. (2015b)
	Developed	Shenxiang-215	Approved by Shanghai in 2014	Systematic breeding from L808	Song et al. (2015a)
		Senyuan-10	–	8404×135, single spore cross-breeding	Song et al. (2015b)
		Chunzai-208	–	–	–
		Senyuan-16	–	–	–
		Junxing-68	–	–	–
		Lingxian-1	–	–	–
		218	–	–	–
		Xiang24			
18	Original	Cr02	National variety 2007004	7402 (from Japan) × Lc-01 (wild strain from Fujian), single spore cross-breeding	Microbiology Society of Fujian Province (1983)
	Developed	Cr62	National variety 2007007	7917 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding	Huang & Wu 1997
		Cr66	–	7918 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding	Xie et al. (2004)
		Cr33	–	7919 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding	Xie et al. (2004)
		Xiangnong-49	–	–	–
		Huaxiang-8	National variety 2008004	Cultivar from Hubei	Song et al. (2015b)

Table 3 Continued.

Pedigree	Cultivars/strain type	Cultivars/strain name	Appraisal Situation	Sources of Cultivars	References
		Ganxiang-1	National variety 2007012	1303(from Japan) × HO3, single spore cross-breeding	Wu et al. (1994)
		Shenxiang-4	Approved by Shanghai in 2004	Introduced from Fujian and systematic breeding	Song et al. (2015b)
		868	–	–	–
		L109	–	–	–
19	Original	Minfeng-1	National variety 2007006	L12(from Japan) × L34 (from Ninghua), single spore cross-breeding	Xie et al. (2004)
	Developed	–	–	–	–
20	Original	L9319	National variety 2008008	Derived from *L. edode* tissue isolation in Lishui	Wu (2014)
	Developed	–	–	–	–
21	Original	L26	–	Introduced from aboard	Zeng & Wu (1993)
	Developed	Xiangza-26	National variety 2008002	NO.8(wild strain) × NO.40, cross-breeding	Kang et al. (1994)
		Wuxiang-1	National variety 2007011	Introduced from Japan and systematic breeding	Li et al. (1998)
		Cr04	National variety 2007008	7917 (from Japan) × L21 (wild strain from Jiangxi), single spore cross-breeding	Xie et al. (2004)
		Suxiang	–	–	–
		Shenxiang-2	Approved by Shanghai in 2004	Introduced from Fujian and systematic breeding	Gong & Zhu (1993)
		Shenxiang-12	National variety 2007003	69 (wild strain) × Suxiang, protoplast monokaryon non-symmetry crossing	Wu (2014)
		Shenxiang-10	National variety 2007002	L26(from Japan) × Suxiang, protoplast monokaryon non-symmetry crossing	Tan et al. (2000)
		L42	–	Systematic breeding from Cr04	Xie et al. (2004)
		931	–	–	–
		Xiaxiang-18	–	–	–
22	Original	Senyuan-1	National variety 2007014	856(from Japan) × 8404(wild strain), single spore cross-breeding	Wang & Xu (1998)
	Developed	Senyuan-2	–	856(from Japan) × 8404(wild strain), single spore cross-breeding	Wang & Xu (1998)
23	Original	L27	–	Introduced from Japan	Xie et al. (2004)

Table 3 Continued.

Pedigree	Cultivars/strain type	Cultivars/strain name	Appraisal Situation	Sources of Cultivars	References
	Developed	L087	–	Systematic breeding from L27 (from Japan)	Xie et al. (2004)
		Jiuxiangqiu-7	Approved by Hubei in 2015	Systematic breeding from L205	–
		Jiuxiang-4	Approved by Shanghai in 2015	–	–
		L856	–	Systematic breeding from L03 (from Japan)	Song (1989)
		Qiuxiang-607	Approved by Shanghai in 2015	Systematic breeding from Qiuzai-6	–
		L607	–	–	–
		Baihua-2	–	–	–
		Aihua-3	–	–	–
		403	–	–	–
		Qiu-6	–	–	–
		Yuhua-4	–	–	–
		Yuhua-2	–	–	–
24	Original	7402	–	Introduced from Japan	Huang (1980)
	Developed	–	–	–	–

Acknowledgements

This work was financed by the National Natural Science Foundation of China (Project ID: 31961143010, 31970010, 31470152), Beijing Innovative Consortium of Agriculture Research System (Project ID: BAIC05-2021), CAS Engineering Laboratory for Advanced Microbial Technology of Agriculture KFJ-PTXM-016, Henan Province key research and development project “Precise breeding and directional development of important edible fungi germplasm” (Project ID: 221111110600).

References

Allen AM, Winfield MO, Burridge AJ, Downie RC et al. 2017 – Characterization of a wheat breeders’ array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (*Triticum aestivum*). Plant Biotechnology Journal 15, 390401.

Applied Fungal Research Unit, Department of Plant Protection. 1984 – Briefing on the domestication trials of foreign introduced shiitake cultivars. Journal of Huazhong Agricultural College 3, 96–97.

Bennett EP, Petersen BL, Johansen IE, Niu YY et al. 2020 – INDEL detection, the ‘Achilles heel’ of precise genome editing: a survey of methods for accurate profiling of gene editing induced indels. Nucleic Acids Research, 48(21): 11958-11981.

Bian YB. 2008 – Germplasm resources of shiitake mushrooms in China and construction of germplasm resource information base. Edible and Medicinal Mushrooms 1, 12–15.
Chen QB, Wu YM, Ye CW. 2005 – Report on the selection and breeding of a new *Lentinula edodes* strain “Qingke 20”. Acta Edulis Fungi.
China Edible Fungi Association. 2022 – Analysis of national edible fungi statistical survey results in 2020. Edible Fungi of China 41(1), 85–91
Danecek P, Bonfield JK, Liddle J, Marshall J et al. 2021 – Twelve years of SAMtools and BCFtools. Gigascience 10(2).
Dong H, Shang XD, Zhao XY, Yu HL et al. 2019 – Construction of a genetic linkage map of *Lentinula edodes* based on SSR, SRAP and TRAP markers. Breeding Science.
Dong H, Zhang LJ, Zhang MY, Jiang N et al. 2017 – Genetic diversity and fingerprint profiles of Chinese major *Lentinula edodes* cultivars based on SSR markers. Microbiology China 44(06): 1427–1436.
Ellegren H. 2004 – Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5(6), 435–445.
Fu LZ, Zhang HY, Wu XQ, Li HB et al. 2010 – Evaluation of genetic diversity in *Lentinula edodes* strains using RAPD, ISSR and SRAP markers. World Journal of Microbiology and Biotechnology 26(4), 709–716.
Gong SP, Zhu MC. 1993 – Characteristics and cultivation points of Shenxiang-2. Edible Fungi 15(6), 2.
Guo ZW, Huang GY, Zhu W, Liu H et al. 2019 – Mechanism and suppression of the effect of axial rare faction waves on the eccentric initiation effect. International Journal of Impact Engineering 124, 37–47.
Huang XH, Wei XH, Sang T, Zhao Q et al. 2010 – Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics 42(11), 961–967.
Huang XX. 1980 – Mushroom cultivation: *Lentinula edodes* and *Pleurotus ostreatus*. Shanghai Scientific & Technical Publishers.
Huang XZ, Wu JL. 1997 – A brief report on the breeding of *Lentinula edodes* strain Cr-20 and Cr-62. Acta Edulis Fungi 03, 51–54.
Jehan T, Lakhanpaul S. 2006 – Single nucleotide polymorphism (SNP) – Methods and applications in plant genetics: A review. Indian Journal of Biotechnology 5, 4.
Kang YN, Lian MZ, Zhong YJ, Lin YN et al. 1994 – Studies on selection of *Lentinus edodes* “Xiangza-26”. Acta Edulis Fungi 1(02), 6.
Kaur S, Panesar PS, Bera MB, Kaur V. 2015 – Simple sequence repeat markers in genetic divergence and marker-assisted selection of rice cultivars: a review. Critical Reviews in Food Science and Nutrition 55(01), 41–49.
Kim KH, Ka KH, Kang JH, Kim S et al. 2015 – Identification of single nucleotide polymorphism markers in the laccase gene of shiitake mushrooms (*Lentinula edodes*). Mycobiology 43(01), 75–80.
Kulkarni RK. 1991 – DNA polymorphisms in *Lentinula edodes*, the shiitake mushroom. Applied and Environmental Microbiology 57(06), 1735–1739.
Langmead B, Salzberg SL. 2012 – Fast gapped-read alignment with Bowtie2. Nature Methods 9(4), 357–359
Lee HY, Moon S, Ro HS, Chung JW et al. 2020 – Analysis of genetic diversity and population structure of wild strains and cultivars using genomic SSR markers in *Lentinula edodes*. Mycobiology 48(02), 115–121.
Lee HY, Moon S, Shim D, Hong CP et al. 2017 – Development of 44 novel polymorphic SSR markers for determination of shiitake mushroom (*Lentinula edodes*) cultivars. Genes 8(04), 109.
Li MY, Zhu HZ, Guo MY. 1998 – Characteristics of *Lentinula edodes* strain Wuxiang-1. Edible Fungi 5, 13–14.
Liu C, Li NY, Zhang YQ. 2010 – Development of expressed sequence tag-simple sequence repeat

(EST-SSR) markers in *Lentinula edodes*. Acta Edulis Fungi.
Liu JY, Song XG, Ye X, Chen AH et al. 2011 – Differentiation of *Lentinula edodes* strains using ISSR, RAPD and SRAP markers. Acta Edulis Fungi 18(03), 1–8. Doi 10.16488/j.cnki.1005-9873.2011.03.014.
Luo KH, Liu SE. 1993 – Research on the selection and breeding of *Lentinula edodes* strains "Guangxiang-51". Edible Fungi of China 12, 3, 3.
Menolli JN, Sánchez-Ramírez S, Sánchez-García M, Wang CQ et al. 2022 – Global phylogeny of the shiitake mushroom and related *Lentinula* species uncovers novel diversity and suggests an origin in the Neotropics. Molecular Phylogenetics and Evolution 173: 107494.
Microbiology Society of Fujian Province. 1983 – Sanming Institute of Fungal Research developed new strains of shiitake mushrooms: Cr-01 and Cr-02. Edible Fungi Technology 2(3), 49.
Moon S, Lee HY, Shim D, Kim M et al. 2017 – Development and molecular characterization of novel polymorphic genomic DNA SSR markers in *Lentinula edodes*. Mycobiology 45(02), 105–109.
Park WM, Ko HG, Park RJ, Hong KS. 1997 – Differentiation of *Lentinus edodes* isolates in Korea by isozyme polymorphisms and random amplified polymorphic DNA (RAPD) analysis. The Korean Journal of Mycology 25(03), 176–190.
Pourabed E, Noushabadi MRJ, Jamali SH, Alipour NM et al. 2015 – Identification and DUS testing of rice varieties through microsatellite markers. International Journal of Plant Genomics.
Qian YF. 1993 – Practical shiitake cultivation. Xueyuan Publishing House 46.
Royse DJ, Baars J, Tan Q. 2017 – Current overview of mushroom production in the world. Edible and Medicinal Mushrooms: Technology and Applications 5–13.
Saito T, Sakuta G, Kobayashi H, Ouchi K et al. 2019 – Genetically independent tetranucleotide to hexanucleotide core motif SSR markers for identifying *Lentinula edodes* cultivars. Mycobiology 47(04), 466–472.
Shen XF, Zhang LJ, Zhang MY, Shang XD et al. 2020 – InDel marker and its application and prospection in research of edible fungi. Acta Agriculturae Shanghai 36(3): 136–140.
Shen XF, Zhang LJ, Zhang MY, Zhang D et al. 2021 – Genetic diversity and population structure of *Lentinula edodes* analyzed by InDel markers. Mycosystema 40(09), 16.
Song CY, Liu DY, Shang XD, Tan Q et al. 2010 – A new hybrid cultivar "Shenxiang-16" in *Lentinula edodes*. Acta Horticulturae Sinica. (11): 1887-1888.
Song CY, Zhang LJ, Yu HL, Zhang MY et al. 2015a – A New *Lentinula edodes* cultivar "Shenxiang-215" 42(S2): 2929-2930.
Song XX, Li CH, Tan Q, Li QZ et al. 2015b – The summary of resources of some cultivated strains of *Lentinula edodes* in China. Journal of Fungal Research 13(03), 146–154.
Song YH. 1989 – Comparative test of ten *Lentinula edodes* strains. Edible Fungi 5, 12.
Tan Q, Pan YJ, Huang WY. 2000 – The development process of *Lentinula edodes* breeding in China. Acta Edulis Fungi 4, 48–52.
Terashima K, Matsumoto T, Hayashi E, Fukumasa-Nakai Y et al. 2002 – A genetic linkage map of *Lentinula edodes* (shiitake) based on AFLP markers. Mycological Research 106(08), 911–917.
Varshney RK, Graner A, Sorrells ME. 2005 – Genic microsatellite markers in plants: features and applications. TRENDS in Biotechnology 23(01), 48–55.
Vignal A, Milan D, SanCristobal M, Eggen A. 2002 – A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution 34(03), 275–305.
Wang DL, Zhang JX, Bian YB, Chen Q et al. 2013 – SNP analysis of the fragments of three functional genes of the mushroom *Lentinula edodes*. Mycosystem 32(01), 81–88.
Wang H, Niu RC, Rong CB, Liu Y et al. 2019 – Genetic diversity analysis of 19 *Lentinus edodes* strains based on ISSR, SRAP and TRAP markers. Jiangsu Agricultural Sciences 47(17).
Wang L, Yang XX, Li YY, Wang Q. 1992 – Study on the single-spore hybridization of *Lentinus edodes*. Journal of the Hebei Academy of Sciences 02, 71–76.

Wang Q, Xu NS. 1998 – Senyuan-1,2 *Lentinula edodes* cross strain's selection and popularization. Journal of Jilin Agricultural University S1, 185.
Wang ZY, Wang YY, Gong SP, Pan YJ et al. 1990 – Selection and breeding of *Lentinula edodes* strain "Huanong-1". Edible Fungi 12(02), 10–11.
Worrall JJ. 1997 – Somatic incompatibility in basidiomycetes. Mycologia 89(01), 24–36.
Wu KM, Wu XQ, Zhou ZQ, Tao XS. 1994 – Selection and breeding of a new shiitake strain "241-4". Edible Fungi of Zhejiang 5, 13–16.
Wu P. 2014 – Genetic diversity and fingerprinting of *Lentinula edodes* strains cultivated in China using simple sequence repeat (SSR) markers. Nanjing Agricultural University.
Wu QX, Li HB, Wei HL, Fu LZ et al. 2005 – Application of SCAR molecular marker technology in identification of *Lentinula edodes*. Mycosystema 02, 259–266.
Xiang XJ, Li C, Li L, Bian YB et al. 2016 – Genetic diversity and population structure of Chinese *Lentinula edodes* revealed by InDel and SSR markers. Mycological Progress 15(4), 1–13.
Xiao DL, Zhang D, Lin YQ, Yang J. 2018 – Evaluation on 20 *Lentinula edodes* strains using ISSR and F-MSAP markers. Fujian Journal of Agricultural Sciences. 33(08): 794–798.
Xie FQ, Xie BG, Yi H. 2004 – Determination of mating types of *Lentinula edodes* and its application in strain identification of main cultivating strains in Fujian Province. Journal of Fujian Agriculture and Forestry University (Natural Science Edition) 33(4): 521–52
Xu YB, Yang QN, Zheng HJ, Xu YF et al. 2020 – Genotyping by target sequencing (GBTS) and its applications. Scientia Agricultura Sinica 53(15), 6–27.
Yang XM. 1988 – Edible fungi cultivation of China. Agriculture Press.
Yao SX, Zhang SC. 1997 – New technology for mushroom cultivation. China Agriculture Press.
Sun Y, Lin FC. 2003 – Analysis of genetic diversity in natural germplasm of *Lentinula edodes* in China using RAPD technique. Mycosystema 22(3), 387–393.
Zeng JF, Wu SZ. 1993 – Biological characteristics and cultivation points of *Lentinula edodes* strains L26 and Mindou-1. Fujian Agricultural Science and Technology 5, 2.
Zhang JC, Shen N, Li C, Xiang XJ et al. 2022 – Population genomics provides insights into the genetic basis of adaptive evolution in the mushroom-forming fungus *Lentinula edodes*. Journal of Advanced Research. 38: 91-106.
Zhang Y, Peng H, Dong YH, Li TT et al. 2019 – Application of an accurate identification technology MNP marker method in the identification of Qiai. China Association for Acupuncture and Moxibustion.

Appendix 1 The basic information of MNP marker library of *L. edodes.*

NO.	Cultivars Name	Number of reads	Average coverage ploidy of MNP markers	Number of MNP marker detections	Detection rate
1	Qinke-20	3132205	6251.91	461	92.02%
2	Shenxiang-10	6353545	12681.73	496	99.00%
3	Jindixianggu	1537753	3069.37	459	91.62%
4	Xiaxiang-18	5640470	11258.42	496	99.00%
5	Xiangnong-9608	5973329	11922.81	462	92.22%
6	Jiuxiang-4	1072767	2141.25	479	95.61%
7	Ganxiang-1	1281591	2558.07	497	99.20%
8	Baihua-2	1607357	3208.3	480	95.81%
9	Shenxiang-12	1457113	2908.41	494	98.60%
10	Xiangza-26	1326433	2647.57	495	98.80%
11	L27	1494883	2983.8	480	95.81%
12	Shenxiang-9	1338947	2672.55	464	92.61%
13	238	1152295	2299.99	469	93.61%
14	Lingxiang-1	1184276	2363.82	479	95.61%
15	Liaofu-10	1523625	3041.17	475	94.81%
16	Gaoxiang-1	1469908	2933.95	462	92.22%
17	7401	1687298	3367.86	470	93.81%
18	Guangxiang-51	2185975	4363.22	453	90.42%
19	L135	1516488	3026.92	470	93.81%
20	241-4	1548866	3091.55	460	91.82%
21	Wuxiang-1	2140084	4271.62	494	98.60%
22	L12	2520267	5030.47	479	95.61%
23	L808	2038096	4068.06	474	94.61%
24	L26	1511153	3016.27	492	98.20%
25	Cr02	2112789	4217.14	497	99.20%
26	Hunong-1	1263723	2522.4	471	94.01%
27	Senyuan-8404	1068551	2132.84	471	94.01%
28	L087	1824676	3642.07	478	95.41%
29	L9015	1664199	3321.75	461	92.02%
30	Suxiang	2647509	5284.45	491	98.00%
31	Xiangjiu	1502328	2998.66	411	82.04%
32	Jiuxiangqiu-7	1536272	3066.41	478	95.41%
33	Dachun-1	1328543	2651.78	473	94.41%
34	Aihua-3	1726521	3446.15	478	95.41%

Appendix 1 Continued.

NO.	Cultivars Name	Number of reads	Average coverage ploidy of MNP markers	Number of MNP marker detections	Detection rate
35	L952	1570723	3135.18	477	95.21%
36	939	1478688	2951.47	462	92.22%
37	Qiuxiang-607	1581888	3157.46	481	96.01%
38	Shenxiang-8	1641065	3275.58	492	98.20%
39	Senyuan-1	1988477	3969.02	493	98.40%
40	Huaxiang-8	2037079	4066.03	493	98.40%
41	Minfeng-1	1544825	3083.48	489	97.60%
42	L109	1529647	3053.19	496	99.00%
43	Senyuan-8	1848395	3689.41	460	91.82%
44	908	1886780	3766.03	460	91.82%
45	Shenxiang-6	1399301	2793.02	465	92.81%
46	Shenxiang-2	1699262	3391.74	496	99.00%
47	Chunzai-208	1330953	2656.59	475	94.81%
48	218	1749552	3492.12	479	95.61%
49	9608-WY	1918037	3828.42	462	92.22%
50	Senyuan-16	1883305	3759.09	480	95.81%
51	7402	1795473	3583.78	459	91.62%
52	807 (Zhongxiang36)	1467047	2928.24	449	89.62%
53	Cr33	1812786	3618.34	497	99.20%
54	Cr66	1781955	3556.8	498	99.40%
55	L11	1580592	3154.87	480	95.81%
56	L42	1394512	2783.46	493	98.40%
57	Cr04	1698782	3390.78	496	99.00%
58	Shenxiang-93	1502415	2998.83	464	92.61%
59	Junxing-68	1570818	3135.37	480	95.81%
60	Shenxiang-16	1286098	2567.06	462	92.22%
61	Taiyin	1077065	2149.83	467	93.21%
62	Shenxiang-215	1608783	3211.14	480	95.81%
63	931	1743825	3480.69	496	99.00%
64	Yuhua-2	232326	463.72	416	83.03%
65	Xiangnong-49	170318	339.96	250	49.90%
66	868	2052197	4096.2	498	99.40%
67	Cr52	1536540	3066.95	480	95.81%
68	L03 (Zhongxiang-37)	1756082	3505.15	480	95.81%

Appendix 1 Continued.

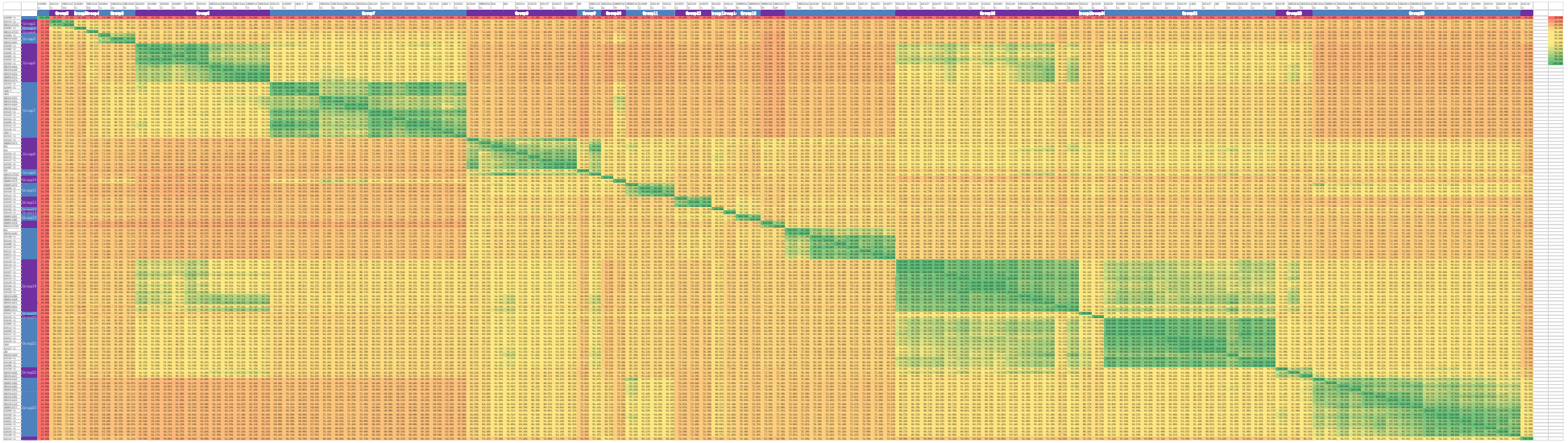
NO.	Cultivars Name	Number of reads	Average coverage ploidy of MNP markers	Number of MNP marker detections	Detection rate
69	L9319	973885	1943.88	476	95.01%
70	Cr62	2083093	4157.87	493	98.40%
71	Senyuan-2	1213888	2422.93	478	95.41%
72	Junxing-8	2277948	4546.8	456	91.02%
73	922	780176	1557.24	478	95.41%
74	Qinke-20 variety	2026782	4045.47	455	90.82%
75	L241	1080085	2155.86	459	91.62%
76	Hunong-3	592243	1182.12	474	94.61%
77	L856	522171	1042.26	473	94.41%
78	Shenxiang-4	1683173	3359.63	496	99.00%

Appendix 2 Genetic similarity of 24 pedigrees.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24
G1	100.00%	22.40%	16.39%	15.47%	20.04%	15.29%	16.87%	15.14%	13.24%	17.62%	14.24%	16.45%	19.13%	20.77%	18.65%	15.57%	16.02%	18.03%	16.94%	16.94%	15.03%	17.36%	15.04%	20.22%
G2	22.40%	98.40%	91.44%	91.62%	85.25%	81.09%	78.42%	77.87%	73.21%	75.14%	71.83%	75.67%	77.27%	73.53%	74.09%	66.31%	73.58%	79.95%	77.54%	82.35%	81.25%	80.06%	74.79%	76.20%
G3	16.39%	91.44%	100.00%	89.19%	81.11%	73.10%	73.12%	75.69%	70.78%	70.82%	72.24%	73.35%	75.94%	74.33%	74.36%	63.90%	73.61%	74.98%	74.87%	80.21%	74.64%	75.85%	71.31%	72.73%
G4	15.47%	91.62%	89.19%	100.00%	90.00%	88.97%	83.08%	70.72%	68.82%	69.39%	66.49%	74.50%	72.43%	69.73%	76.02%	61.62%	71.63%	80.67%	75.68%	74.05%	82.81%	80.66%	67.52%	69.73%
G5	20.04%	85.25%	81.11%	90.00%	98.01%	82.40%	90.13%	74.22%	70.67%	82.01%	69.39%	76.44%	71.40%	73.02%	77.38%	57.37%	72.98%	79.63%	76.08%	77.52%	80.42%	79.03%	68.61%	71.58%
G6	15.29%	81.09%	73.10%	88.97%	82.40%	98.02%	93.40%	69.57%	64.88%	65.22%	62.91%	69.19%	67.83%	69.01%	68.41%	57.97%	67.02%	95.13%	84.21%	82.11%	91.70%	92.28%	65.22%	60.14%
G7	16.87%	78.42%	73.12%	83.08%	90.13%	93.40%	98.05%	73.72%	68.94%	81.67%	69.58%	72.64%	70.94%	72.48%	73.95%	61.30%	72.93%	89.26%	81.35%	80.35%	85.33%	83.43%	69.27%	66.76%
G8	15.14%	77.87%	75.69%	70.72%	74.22%	69.57%	73.72%	97.62%	93.96%	89.84%	92.22%	86.10%	94.27%	88.47%	82.64%	89.67%	86.80%	91.99%	93.07%	91.22%	92.16%	88.50%	89.54%	74.55%
G9	13.24%	73.21%	70.78%	68.82%	70.67%	64.88%	68.94%	93.96%	96.20%	89.34%	90.53%	80.55%	86.72%	82.67%	77.95%	83.75%	81.81%	85.93%	92.42%	88.35%	86.47%	81.80%	84.50%	70.50%
G10	17.62%	75.14%	70.82%	69.39%	82.01%	65.22%	81.67%	89.84%	89.34%	94.54%	88.47%	80.12%	83.24%	80.81%	81.55%	80.41%	83.39%	74.90%	82.70%	81.89%	76.63%	79.49%	80.67%	83.28%
G11	14.24%	71.83%	72.24%	66.49%	69.39%	62.91%	69.58%	92.22%	90.53%	88.47%	98.01%	79.58%	86.52%	83.96%	82.21%	78.51%	82.21%	84.22%	88.95%	91.24%	84.80%	83.78%	93.65%	83.02%
G12	16.45%	75.67%	73.35%	74.50%	76.44%	69.19%	72.64%	86.10%	80.55%	80.12%	79.58%	98.74%	90.52%	84.08%	92.61%	78.44%	88.32%	79.06%	85.69%	81.57%	77.72%	74.10%	73.02%	71.92%
G13	19.13%	77.27%	75.94%	72.43%	71.40%	67.83%	70.94%	94.27%	86.72%	83.24%	86.52%	90.52%	100.00%	94.12%	91.81%	89.30%	85.34%	90.05%	87.17%	85.56%	88.93%	85.15%	89.16%	73.26%
G14	20.77%	73.53%	74.33%	69.73%	73.02%	69.01%	72.48%	88.47%	82.67%	80.81%	83.96%	84.08%	94.12%	100.00%	93.45%	77.81%	80.13%	79.68%	86.10%	83.42%	79.51%	77.82%	81.83%	74.33%
G15	18.65%	74.09%	74.36%	76.02%	77.38%	68.41%	73.95%	82.64%	77.95%	81.55%	82.21%	92.61%	91.81%	93.45%	98.35%	80.51%	86.91%	80.24%	81.19%	79.83%	78.51%	78.22%	76.87%	74.37%
G16	15.57%	66.31%	63.90%	61.62%	57.37%	57.97%	61.30%	89.67%	83.75%	80.41%	78.51%	78.44%	89.30%	77.81%	80.51%	97.86%	86.09%	82.30%	79.95%	80.21%	81.77%	77.37%	77.69%	66.58%
G17	16.02%	73.58%	73.61%	71.63%	72.98%	67.02%	72.93%	86.80%	81.81%	83.39%	82.21%	88.32%	85.34%	80.13%	86.91%	86.09%	97.89%	80.63%	83.54%	82.35%	80.31%	79.47%	76.11%	75.58%
G18	18.03%	79.95%	74.98%	80.67%	79.63%	95.13%	89.26%	91.99%	85.93%	74.90%	84.22%	79.06%	90.05%	79.68%	80.24%	82.30%	80.63%	98.96%	93.43%	91.63%	96.16%	92.70%	90.56%	70.28%
G19	16.94%	77.54%	74.87%	75.68%	76.08%	84.21%	81.35%	93.07%	92.42%	82.70%	88.95%	85.69%	87.17%	86.10%	81.19%	79.95%	83.54%	93.43%	100.00%	93.58%	95.14%	86.76%	88.31%	72.73%
G20	16.94%	82.35%	80.21%	74.05%	77.52%	82.11%	80.35%	91.22%	88.35%	81.89%	91.24%	81.57%	85.56%	83.42%	79.83%	80.21%	82.35%	91.63%	93.58%	100.00%	90.58%	87.65%	86.60%	75.94%
G21	15.03%	81.25%	74.64%	82.81%	80.42%	91.70%	85.33%	92.16%	86.47%	76.63%	84.80%	77.72%	88.93%	79.51%	78.51%	81.77%	80.31%	96.16%	95.14%	90.58%	99.15%	92.49%	91.51%	70.62%
G22	17.36%	80.06%	75.85%	80.66%	79.03%	92.28%	83.43%	88.50%	81.80%	79.49%	83.78%	74.10%	85.15%	77.82%	78.22%	77.37%	79.47%	92.70%	86.76%	87.65%	92.49%	96.77%	93.54%	78.72%
G23	15.04%	74.79%	71.31%	67.52%	68.61%	65.22%	69.27%	89.54%	84.50%	80.67%	93.65%	73.02%	89.16%	81.83%	76.87%	77.69%	76.11%	90.56%	88.31%	86.60%	91.51%	93.54%	97.71%	92.92%
G24	20.22%	76.20%	72.73%	69.73%	71.58%	60.14%	66.76%	74.55%	70.50%	83.28%	83.02%	71.92%	73.26%	74.33%	74.37%	66.58%	75.58%	70.28%	72.73%	75.94%	70.62%	78.72%	92.92%	100.00%

Note: The average *GS* values within each group are marked in blue, and the average *GS* values between two adjacent groups are marked in yellow.

Appendix 3 Genetic similarity of all cultivars.



Appendix 4 The genome sequencing data of *L. edodes* from NCBI.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
1	WGS	PRJNA625026	*Lentinula edodes*	SRR11534230	SRP256131	CV57	China:Zhejiang
2	WGS	PRJNA625026	*Lentinula edodes*	SRR11534232	SRP256131	CV54	China:Zhejiang
3	WGS	PRJNA625026	*Lentinula edodes*	SRR11534233	SRP256131	CV53	China:Zhejiang
4	WGS	PRJNA625026	*Lentinula edodes*	SRR11534234	SRP256131	CV52	China:Zhejiang
5	WGS	PRJNA625026	*Lentinula edodes*	SRR11534235	SRP256131	CV51	China:Zhejiang
6	WGS	PRJNA625026	*Lentinula edodes*	SRR11534237	SRP256131	CV444	Australia
7	WGS	PRJNA625026	*Lentinula edodes*	SRR11534238	SRP256131	CV443	Australia
8	WGS	PRJNA625026	*Lentinula edodes*	SRR11534239	SRP256131	CV442	Australia
9	WGS	PRJNA625026	*Lentinula edodes*	SRR11534241	SRP256131	CV439	Australia
10	WGS	PRJNA625026	*Lentinula edodes*	SRR11534242	SRP256131	CV43	China:Shandong
11	WGS	PRJNA625026	*Lentinula edodes*	SRR11534244	SRP256131	CV410	Australia
12	WGS	PRJNA625026	*Lentinula edodes*	SRR11534245	SRP256131	CV41	China:Shandong
13	WGS	PRJNA625026	*Lentinula edodes*	SRR11534246	SRP256131	CV39	China:Shanghai
14	WGS	PRJNA625026	*Lentinula edodes*	SRR11534247	SRP256131	CV38	China:Shanghai

Appendix 4 Continued.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
15	WGS	PRJNA625026	*Lentinula edodes*	SRR11534248	SRP256131	CV35	China:Shanghai
16	WGS	PRJNA625026	*Lentinula edodes*	SRR11534250	SRP256131	CV32	China:Shanghai
17	WGS	PRJNA625026	*Lentinula edodes*	SRR11534252	SRP256131	CV301	China:Hongkong
18	WGS	PRJNA625026	*Lentinula edodes*	SRR11534253	SRP256131	CV30	China:Jiangsu
19	WGS	PRJNA625026	*Lentinula edodes*	SRR11534254	SRP256131	CV29	China:Hebei
20	WGS	PRJNA625026	*Lentinula edodes*	SRR11534255	SRP256131	CV28	China:Hubei
21	WGS	PRJNA625026	*Lentinula edodes*	SRR11534256	SRP256131	CV27	China:Hubei
22	WGS	PRJNA625026	*Lentinula edodes*	SRR11534257	SRP256131	CV26	China:Hubei
23	WGS	PRJNA625026	*Lentinula edodes*	SRR11534258	SRP256131	CV24	China:Fujian
24	WGS	PRJNA625026	*Lentinula edodes*	SRR11534259	SRP256131	CV22	China:Hubei
25	WGS	PRJNA625026	*Lentinula edodes*	SRR11534260	SRP256131	CV21	China:Zhejiang
26	WGS	PRJNA625026	*Lentinula edodes*	SRR11534261	SRP256131	CV203	China:Taiwan
27	WGS	PRJNA625026	*Lentinula edodes*	SRR11534263	SRP256131	CV201	China:Taiwan
28	WGS	PRJNA625026	*Lentinula edodes*	SRR11534264	SRP256131	CV20	China:Hubei
29	WGS	PRJNA625026	*Lentinula edodes*	SRR11534265	SRP256131	CV19	China:Hubei
30	WGS	PRJNA625026	*Lentinula edodes*	SRR11534266	SRP256131	CV17	China:Jiangxi
31	WGS	PRJNA625026	*Lentinula edodes*	SRR11534267	SRP256131	CV16	China:Fujian
32	WGS	PRJNA625026	*Lentinula edodes*	SRR11534268	SRP256131	CV14	China:Guangdong
33	WGS	PRJNA625026	*Lentinula edodes*	SRR11534269	SRP256131	CV13	China:Guangdong
34	WGS	PRJNA625026	*Lentinula edodes*	SRR11534270	SRP256131	CV12	China:Guangdong
35	WGS	PRJNA625026	*Lentinula edodes*	SRR11534271	SRP256131	CV11	China:Zhejiang
36	WGS	PRJNA625026	*Lentinula edodes*	SRR11534272	SRP256131	CV108	Japan
37	WGS	PRJNA625026	*Lentinula edodes*	SRR11534274	SRP256131	CV105	Japan
38	WGS	PRJNA625026	*Lentinula edodes*	SRR11534275	SRP256131	CV102	Japan
39	WGS	PRJNA625026	*Lentinula edodes*	SRR11534276	SRP256131	CV101	Japan
40	WGS	PRJNA625026	*Lentinula edodes*	SRR11534277	SRP256131	CV09	China:Fujian
41	WGS	PRJNA625026	*Lentinula edodes*	SRR11534278	SRP256131	CV08	China:Fujian
42	WGS	PRJNA625026	*Lentinula edodes*	SRR11534279	SRP256131	CV07	China:Zhejiang
43	WGS	PRJNA625026	*Lentinula edodes*	SRR11534280	SRP256131	CV06	China:Fujian
44	WGS	PRJNA625026	*Lentinula edodes*	SRR11534281	SRP256131	CV05	China:Shanghai
45	WGS	PRJNA625026	*Lentinula edodes*	SRR11534282	SRP256131	CV04	China:Shanghai
46	WGS	PRJNA625026	*Lentinula edodes*	SRR11534283	SRP256131	CV03	China:Shanghai
47	WGS	PRJNA625026	*Lentinula edodes*	SRR11534285	SRP256131	CV02	China:Shanghai
48	WGS	PRJNA625026	*Lentinula edodes*	SRR11534286	SRP256131	WD6201	China:Gansu
49	WGS	PRJNA625026	*Lentinula edodes*	SRR11534287	SRP256131	WD6101	China:Shaanxi

Appendix 4 Continued.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
50	WGS	PRJNA625026	*Lentinula edodes*	SRR11534288	SRP256131	WD5348	China:Yunnan
51	WGS	PRJNA625026	*Lentinula edodes*	SRR11534289	SRP256131	WD5321	China:Yunnan
52	WGS	PRJNA625026	*Lentinula edodes*	SRR11534290	SRP256131	WD5301	China:Shaanxi
53	WGS	PRJNA625026	*Lentinula edodes*	SRR11534291	SRP256131	WD5203	China:Guizhou
54	WGS	PRJNA625026	*Lentinula edodes*	SRR11534292	SRP256131	WD5140	China:Sichuan
55	WGS	PRJNA625026	*Lentinula edodes*	SRR11534293	SRP256131	WD5134	China:Sichuan
56	WGS	PRJNA625026	*Lentinula edodes*	SRR11534294	SRP256131	WD5132	China:Sichuan
57	WGS	PRJNA625026	*Lentinula edodes*	SRR11534296	SRP256131	WD5123	China:Sichuan
58	WGS	PRJNA625026	*Lentinula edodes*	SRR11534297	SRP256131	WD5115	China:Sichuan
59	WGS	PRJNA625026	*Lentinula edodes*	SRR11534298	SRP256131	WD5114	China:Sichuan
60	WGS	PRJNA625026	*Lentinula edodes*	SRR11534299	SRP256131	WD5101	China:Sichuan
61	WGS	PRJNA625026	*Lentinula edodes*	SRR11534300	SRP256131	WD4322	China:Hunan
62	WGS	PRJNA625026	*Lentinula edodes*	SRR11534301	SRP256131	WD4311	China:Hunan
63	WGS	PRJNA625026	*Lentinula edodes*	SRR11534302	SRP256131	WD4301	China:Hunan
64	WGS	PRJNA625026	*Lentinula edodes*	SRR11534303	SRP256131	WD4250	China:Hubei
65	WGS	PRJNA625026	*Lentinula edodes*	SRR11534304	SRP256131	CV58	China:Zhejiang
66	WGS	PRJNA625026	*Lentinula edodes*	SRR11534305	SRP256131	WD4232	China:Hubei
67	WGA	PRJNA625026	*Lentinula edodes*	SRR11534307	SRP256131	L808pm-1_2	China:Shanghai
68	WGS	PRJNA625026	*Lentinula edodes*	SRR11534308	SRP256131	L808pm-1_1	China:Shanghai
69	WGS	PRJNA625026	*Lentinula edodes*	SRR11534309	SRP256131	WD4214	China:Hubei
70	WGS	PRJNA625026	*Lentinula edodes*	SRR11534311	SRP256131	WD4204	China:Hubei
71	WGS	PRJNA625026	*Lentinula edodes*	SRR11534312	SRP256131	WD4202	China:Hubei
72	WGS	PRJNA625026	*Lentinula edodes*	SRR11534313	SRP256131	WD3615	China:Jiangxi
73	WGS	PRJNA625026	*Lentinula edodes*	SRR11534314	SRP256131	WD3604	China:Jiangxi
74	WGS	PRJNA625026	*Lentinula edodes*	SRR11534315	SRP256131	WD3409	China:Anhui
75	WGS	PRJNA625026	*Lentinula edodes*	SRR11534316	SRP256131	WD3406	China:Anhui
76	WGS	PRJNA625026	*Lentinula edodes*	SRR11534317	SRP256131	WD3302	China:Zhejiang
77	WGS	PRJNA625026	*Lentinula edodes*	SRR11534318	SRP256131	WD2207	China:Jilin
78	WGS	PRJNA625026	*Lentinula edodes*	SRR11534319	SRP256131	WD2206	China:Jilin
79	WGS	PRJNA625026	*Lentinula edodes*	SRR11534320	SRP256131	WD2205	China:Jilin
80	WGS	PRJNA625026	*Lentinula edodes*	SRR11534321	SRP256131	L808pm-13	China:Shanghai
81	WGS	PRJNA625026	*Lentinula edodes*	SRR11534322	SRP256131	WD2203	China:Jilin
82	WGS	PRJNA625026	*Lentinula edodes*	SRR11534323	SRP256131	WD2202	China:Jilin
83	WGS	PRJNA625026	*Lentinula edodes*	SRR11534324	SRP256131	WD2201	China:Jilin
84	WGS	PRJNA625026	*Lentinula edodes*	SRR11534325	SRP256131	WD2102	China:Liaoning

Appendix 4 Continued.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
85	WGS	PRJNA625026	*Lentinula edodes*	SRR11534326	SRP256131	WD2101	China:Liaoning
86	WGS	PRJNA625026	*Lentinula edodes*	SRR11534330	SRP256131	CV75	Japan
87	WGS	PRJNA625026	*Lentinula edodes*	SRR11534331	SRP256131	CV73	China:Henan
88	WGS	PRJNA625026	*Lentinula edodes*	SRR11534332	SRP256131	CV72	China:Henan
89	WGS	PRJNA625026	*Lentinula edodes*	SRR11534333	SRP256131	CV71	China:Zhejiang
90	WGS	PRJNA625026	*Lentinula edodes*	SRR11534335	SRP256131	CV70	China:Zhejiang
91	WGS	PRJNA625026	*Lentinula edodes*	SRR11534336	SRP256131	CV69	China:Zhejiang
92	WGS	PRJNA625026	*Lentinula edodes*	SRR11534337	SRP256131	CV68	China:Zhejiang
93	WGS	PRJNA625026	*Lentinula edodes*	SRR11534338	SRP256131	CV67	China:Zhejiang
94	WGS	PRJNA625026	*Lentinula edodes*	SRR11534339	SRP256131	CV66	China:Sichuan
95	WGS	PRJNA625026	*Lentinula edodes*	SRR11534340	SRP256131	CV65	China:Zhejiang
96	WGS	PRJNA625026	*Lentinula edodes*	SRR11534341	SRP256131	CV61	China:Henan
97	WGS	PRJNA625026	*Lentinula edodes*	SRR11534342	SRP256131	CV60	China:Zhejiang
98	WGS	PRJNA625026	*Lentinula edodes*	SRR11534343	SRP256131	CV59	China:Zhejiang
99	WGS	PRJNA320211	*Lentinula edodes*	SRR3473451	SRP074229	YS1	missing
100	WGS	PRJNA320211	*Lentinula edodes*	SRR3473453	SRP074229	YS100	missing
101	WGS	PRJNA320211	*Lentinula edodes*	SRR3473946	SRP074229	YS104	missing
102	WGS	PRJNA320211	*Lentinula edodes*	SRR3473947	SRP074229	YS11	missing
103	WGS	PRJNA320211	*Lentinula edodes*	SRR3490411	SRP074229	YS111	missing
104	WGS	PRJNA320211	*Lentinula edodes*	SRR3491419	SRP074229	ZP87	missing
105	WGS	PRJNA320211	*Lentinula edodes*	SRR3504221	SRP074229	YS110	missing
106	WGS	PRJNA320211	*Lentinula edodes*	SRR3504295	SRP074229	YS113	missing
107	WGS	PRJNA320211	*Lentinula edodes*	SRR3504297	SRP074229	YS115	missing
108	WGS	PRJNA320211	*Lentinula edodes*	SRR3504300	SRP074229	ZP49	missing
109	WGS	PRJNA320211	*Lentinula edodes*	SRR3504306	SRP074229	YS118	missing
110	WGS	PRJNA320211	*Lentinula edodes*	SRR3504308	SRP074229	YS119	missing
111	WGS	PRJNA320211	*Lentinula edodes*	SRR3504309	SRP074229	YS120	missing
112	WGS	PRJNA320211	*Lentinula edodes*	SRR3504373	SRP074229	YS121	missing
113	WGS	PRJNA320211	*Lentinula edodes*	SRR3504374	SRP074229	YS14	missing
114	WGS	PRJNA320211	*Lentinula edodes*	SRR3504377	SRP074229	YS1515	missing
115	WGS	PRJNA320211	*Lentinula edodes*	SRR3504378	SRP074229	YS1518	missing
116	WGS	PRJNA320211	*Lentinula edodes*	SRR3504380	SRP074229	YS234	missing
117	WGS	PRJNA320211	*Lentinula edodes*	SRR3504382	SRP074229	YS29	missing
118	WGS	PRJNA320211	*Lentinula edodes*	SRR3504383	SRP074229	YS30	missing
119	WGS	PRJNA320211	*Lentinula edodes*	SRR3504385	SRP074229	YS3334	missing

Appendix 4 Continued.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
120	WGS	PRJNA320211	*Lentinula edodes*	SRR3504387	SRP074229	YS3353	missing
121	WGS	PRJNA320211	*Lentinula edodes*	SRR3504401	SRP074229	YS358	missing
122	WGS	PRJNA320211	*Lentinula edodes*	SRR3504403	SRP074229	YS366	missing
123	WGS	PRJNA320211	*Lentinula edodes*	SRR3504404	SRP074229	YS37	missing
124	WGA	PRJNA320211	*Lentinula edodes*	SRR3504405	SRP074229	YS39	missing
125	WGS	PRJNA320211	*Lentinula edodes*	SRR3504406	SRP074229	YS5	missing
126	WGS	PRJNA320211	*Lentinula edodes*	SRR3504410	SRP074229	YS51	missing
127	WGS	PRJNA320211	*Lentinula edodes*	SRR3504411	SRP074229	YS55	missing
128	WGS	PRJNA320211	*Lentinula edodes*	SRR3504412	SRP074229	YS7	missing
129	WGS	PRJNA320211	*Lentinula edodes*	SRR3504413	SRP074229	YS70	missing
130	WGS	PRJNA320211	*Lentinula edodes*	SRR3504414	SRP074229	YS73	missing
131	WGS	PRJNA320211	*Lentinula edodes*	SRR3504420	SRP074229	YS76	missing
132	WGS	PRJNA320211	*Lentinula edodes*	SRR3504424	SRP074229	YS78	missing
133	WGS	PRJNA320211	*Lentinula edodes*	SRR3504427	SRP074229	YS79	missing
134	WGS	PRJNA320211	*Lentinula edodes*	SRR3504429	SRP074229	YS8	missing
135	WGS	PRJNA320211	*Lentinula edodes*	SRR3504431	SRP074229	YS84	missing
136	WGS	PRJNA320211	*Lentinula edodes*	SRR3504433	SRP074229	YS88	missing
137	WGS	PRJNA320211	*Lentinula edodes*	SRR3504434	SRP074229	YS89	missing
138	WGS	PRJNA320211	*Lentinula edodes*	SRR3504436	SRP074229	YS91	missing
139	WGS	PRJNA320211	*Lentinula edodes*	SRR3504438	SRP074229	YS94	missing
140	WGS	PRJNA320211	*Lentinula edodes*	SRR3504440	SRP074229	ZP10	missing
141	WGS	PRJNA320211	*Lentinula edodes*	SRR3504442	SRP074229	ZP2	missing
142	WGS	PRJNA320211	*Lentinula edodes*	SRR3504443	SRP074229	ZP20	missing
143	WGS	PRJNA320211	*Lentinula edodes*	SRR3504444	SRP074229	ZP23	missing
144	WGS	PRJNA320211	*Lentinula edodes*	SRR3504447	SRP074229	ZP27	missing
145	WGS	PRJNA320211	*Lentinula edodes*	SRR3504449	SRP074229	ZP28	missing
146	WGS	PRJNA320211	*Lentinula edodes*	SRR3504451	SRP074229	ZP31	missing
147	WGS	PRJNA320211	*Lentinula edodes*	SRR3504452	SRP074229	ZP42	missing
148	WGS	PRJNA320211	*Lentinula edodes*	SRR3504453	SRP074229	ZP47	missing
149	WGS	PRJNA320211	*Lentinula edodes*	SRR3504454	SRP074229	ZP48	missing
150	WGS	PRJNA320211	*Lentinula edodes*	SRR3504456	SRP074229	ZP50	missing
151	WGS	PRJNA320211	*Lentinula edodes*	SRR3504457	SRP074229	ZP51	missing
152	WGS	PRJNA320211	*Lentinula edodes*	SRR3504459	SRP074229	ZP6	missing
153	WGS	PRJNA320211	*Lentinula edodes*	SRR3504460	SRP074229	ZP64	missing
154	WGS	PRJNA320211	*Lentinula edodes*	SRR3504553	SRP074229	ZP67	missing

Appendix 4 Continued.

No.	Assay Type	Bio Project	Organism	Run	SRA_Study	Sample_Name	geo_loc_name
155	WGS	PRJNA320211	*Lentinula edodes*	SRR3504589	SRP074229	ZP82	missing
156	WGS	PRJNA320211	*Lentinula edodes*	SRR3504590	SRP074229	ZP85	missing
157	WGS	PRJNA320211	*Lentinula edodes*	SRR3504592	SRP074229	ZP88	missing
158	WGS	PRJNA320211	*Lentinula edodes*	SRR3504593	SRP074229	ZP9	missing
159	WGS	PRJNA625026	*Lentinula edodes*	SRR11534249	SRP256131	CV33	China:Shanghai
160	WGS	PRJNA625026	*Lentinula edodes*	SRR11534243	SRP256131	CV42	China:Shandong
161	WGS	PRJNA625026	*Lentinula edodes*	SRR11534236	SRP256131	CV501	USA
162	WGS	PRJNA625026	*Lentinula edodes*	SRR11534231	SRP256131	CV55	China:Zhejiang