J Genet Resour2021;7(2): 166-173 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2021.21055.1242*

J|G|R

Genetic Diversity and Structure of Chanterelles (*Cantharellus* spp.) in Hyrcanian Forest Using SSR Markers

Fariba Abdolrahmani[1], Hamed Yousefzadeh[1*] and Ghasem Ali Parad[2]

[1] Department of Environmental science, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Mazandaran, Iran
[2] Department of Forestry, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Mazandaran, Iran

ARTICLE INFO

Article history:
Received 12 October 2020
Accepted 15 December 2020
Available online 10 Jan 2021

Keywords:
Conservation genetic
Ectomycorrhizae
Edible fungus
Genetic diversity
Hyrcanian forest

Corresponding authors:
✉ H. Yousefzadeh
h.yousefzadeh@modares.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Cantharellus is one of the most widely used ectomycorrhizal fungus in the world, which forms mycorrhizal communities with various plant species and plays a major ecological role in forest ecosystems. The present study is the first report to know the extent of genetic diversity and differentiation of Zarde-Kija mushroom populations using microsatellite markers within the internal transcribed spacer (ITS). In this study, 70 fungal samples were collected from six populations of this species in the Hyrcanian Forest (Gorgan, Neka, Sari, Noor, Chalous, and Rasht populations) and its genetic diversity and structure were measured. The number of observed alleles ranged from 5 to 7 and the expected heterozygosity ranged from 0.320 to 0.864. AMOVA analysis showed that the largest portion of the genetic variation was among individuals within the population (95%), whereas 5% was among populations. The mean coefficient of differentiation (F_{ST}) was 0.096, which indicates the medium genetic differentiation between the populations under study. The mean inbreeding value (F_{IS}) and gene flow (N_M) were 0.853, 2.509, respectively. Most loci showed deviation from Hardy–Weinberg equilibrium due to the excess of heterozygotes in all populations. The genetic landscape shape plot revealed that high pairwise genetic distances among individuals in central populations of the Hyrcanian forest. This study revealed that the effective population size of Zarde-Kija has been decreased in the Hyrcanian forest and emphasizes the need to develop an effective strategy for the conservation of this species. However, it is suggested that research on this species be repeated using stronger markers and more populations to increase the accuracy of the results.

Please cite this paper as: Abdorahmani F, Yousefzadeh H, Parad G. 2021. Genetic diversity and structure of chanterelles (*Cantharellus* spp.) in Hyrcanian forest using SSR markers. *J Genet Resour* 7(2): 166-173. doi: 10.22080/jgr.2021.21055.1242

Introduction

Mushrooms play a very important role in the functioning of an ecosystem because of their importance in the nutrient cycle of plants and animals, forest diseases, as well as the coexistence of two different organisms (Smith and Read, 2008). Most mushrooms form mycorrhizal communities with various plant species (Jany *et al*., 2006). Communities of symbiosis formed between mycorrhizal mushrooms (EM) and most forest trees are one of the most common forms of symbiosis in forest ecosystems (Molina *et al*., 2001). This group of mushrooms develops into symbiotic communities with the micro-roots of trees that form ectomycorrhizae (EM) (Koch and Aime, 2018). Their existence is essential to the survival and exploitation of forestry (Pilz *et al*., 2003).
As a result, forest management activities and the overexploitation of fungi may negatively affect production continuity (Lu *et al*., 2018).

Zarde-Kija (*Cantharellus* spp.) is one of the most widely used ectomycorrhizal fungus in the world, found in nearly all continents that have adapted host trees, and is considered a bio-indicator of forest health (Parad *et al*., 2018). Concerns about the survival and sustainability of Zarde-Kija populations in the Hyrcanian forests, due to the increasing trend towards deforestation and the need to prioritize important areas is increasing and requires careful planning to secure its genetic diversity. Many studies are carried out on species of the genus Zarde-Kija worldwide, primarily to identify its various species using molecular markers (Bergemann and Largent, 2000). Some studies have been conducted on the genetic diversity of species in this genus using microsatellite markers (c). Dunham *et al.* (2003 a, b) studied the genetic diversity of *Cantharellus formosus* in 6,400 hectares of Oregon's forests with five microsatellite markers to provide an appropriate management plan to protect threats. The results showed that the observed heterozygous ranged from 23 to 70 percent and was expected to range from 54 to 83 percent.

However, at a higher level, forest managers should have sufficient information about spatial patterns associated with intragroup genetic diversity, because these patterns can provide important clues about the evolution of the environment and population events in the past and present, especially for species that are not easily visible during their lifetime (Wang *et al*., 2019). Therefore, exploring the genetic variation especially in the macro-fungi population is an important phase in conservation ecology to find a reasonable conservation strategy.

The main purposes of this study were to understand the genetic diversity and population structure of *Cantharellus* spp. using SSR markers, and relationships of this species in different ecological groups. This study provides valuable information on the genetic parameters of Zarde-Kija for the first time.

Materials and Methods

Sampling and SSR amplification

Six populations of Zarde-Kija were chosen along with the geographic ranges of Hyrcanian forests (Fig. 1). To avoid a genetic relationship among individuals, the samples were taken at least 20 meters' distances from each other as the protocol was described by Rochon *et al*. (2011).

Total DNA was extracted from the internal parts of the basidiomycetes using a K protein protocol (0.8 M Tris-HCl, 0.2 M $(NH_4)_2SO_4$, 0.2% w/v Tween-20; Solis BioDyne, Tartu, Estonia) in a lysing pad (Parad *et al*., 2018; Loit *et al*., 2019). Five SSR markers with high polymorphism and successful amplification were chosen based on Dunham *et al*. (2003a) (Table 1). PCR amplifications were performed in a total volume of 15µL containing 0.5µL of genomic template DNA, 0.2 µL of 10 pmol forward primer with a fluorescence dye, 0.2 µL of 10 pmol reverse primer, 1.0 µL (with 2.5 mM $MgCl_2$) of PCR buffer, 0.2 µL (each 10 mM) of dNTPs, and 0.05 µL (5 U/µL) of *Taq* DNA polymerase (Incline Biotech, Gyeonggi-do, Korea). PCR conditions were an initial denaturation at 95°C for 3 min; followed by 35 cycles at 95°C for 45 seconds (initial denaturation), annealing at 56°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR amplicons were visualized on 8% acrylamide gel and allele size was scored using GeneMapper ver. 5.0.

Statistical analysis

The presence of null alleles at each locus was calculated by the expectation-maximization algorithm (Dempster *et al*., 1977) using FreeNA (Chapuis and Estoup, 2007). The genetic diversity parameter number of alleles (N_A), the effective number of alleles (N_E), observed heterozygosity (H_O), the expected heterozygosity (H_E), Fixation Index (F value), and the inbreeding coefficient (F_{IS}) measured GenAlEx 6.5 (Peakall and Smouse, 2012).

Inbreeding coefficients (F_{IS}) for each population over all loci, and each locus across all populations were calculated using FSTAT 2.9.3.2 (Goudet, 2001) with 1000 permutations and *p* values were adjusted using sequential Bonferroni corrections. Departures from Hardy-Weinberg equilibrium at each locus were assessed by GenAlEx 6.5 (Peakall and Smouse, 2012). Pairwise F_{ST} values, gene flow (N_M), and analysis of molecular variance (AMOVA) were computed using GenAlEx 6.5 (Peakall and Smouse, 2012).

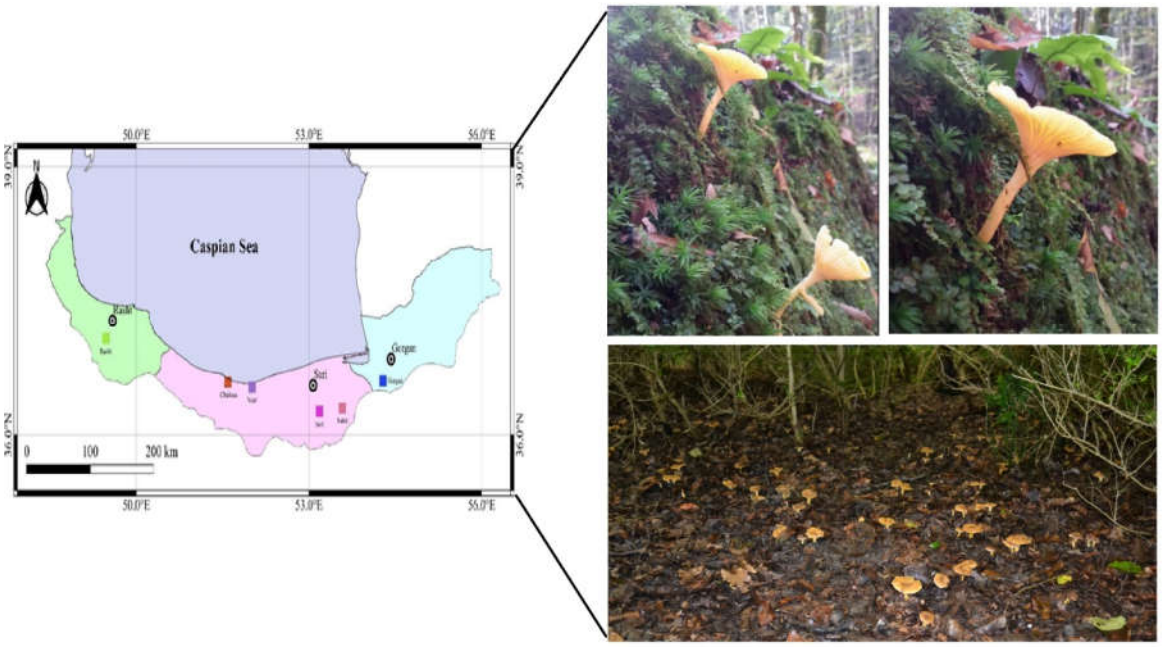

Fig. 1. Geographic sample points of the *Cantharellus* sp. populations under study (left) and the fungi in natural habitats (right).

Table 1. Characteristics of Zarde-Kija microsatellites.

Primer	Name	Primer sequence (5′→3′)	Annealing temperature (˚C)	Allele size range
1	Cf339F	GAATCGGGTTCGCAGAAG	55	173-182
	Cf339R	CTTAGCCCAAGCGTCTTC	55	
2	Cf126F	GCTTGTGCGGGATGACGG	55	221-231
	Cf126R	CGAACACGCCCTACTGCC	55	
3	Cf113F	CGATCTCGCTGTTATTGGAG	55	96-120
	Cf113R	GCTAGAGCGACAATAACCTC	55	
4	Cf642F	CTATGCAAATCCGCCAGC	55	275-279
	Cf642R	GATACGTTTAGGCGGTCG	55	
5	Cf145F	GTACGAAACTGATTGGATGG	55	342-373
	Cf145R	CATGCTTTGACTAACCTACC	55	

Results

Genetic diversity analysis

From the five SSR markers understudy, four markers showed a polymorphic pattern. The genetic parameters of the investigated loci are shown in Table 2. The number of alleles per primer ranged from a minimum of 2 (CF339 and CF640 markers, Sari and Chalous populations) to a maximum of 10 (CF339 marker, Neka population) with an average count of 10.5 alleles per primer. The inbreeding coefficient among populations (F_{IT}) values ranged from 0.707 (CF126 primer) to 1.000 (CF640), with a mean of 0.853 (Table 3). The values for H_O ranged from 0.000 (CF113, CF339, CF640 markers from Nour population, CF339, CF126 and CF640 from Sari population, CF640 marker from Neka and Chalous population, CF113, CF339 and CF640 markers from Rasht population, CF113 and CF640 markers from Gorgan population) to 0.571 (CF126 markers from Rasht population), with an overall mean of 0.113, while the values of H_E ranged from 0.320 (CF339 marker from Sari population) to 0.864 (CF339 marker from Neka population), with a general mean of 0.728. In all studied locus, all populations showed excess homozygosity. The average number of migrants per generation (N_M) in the whole population and across all the loci was found to be 2.50 (Table 3). In all populations under study, lower than 5% of the loci did not differ significantly ($p > 0.05$) departure from the HWE (Table 2).

Structure analysis

The pairwise Nei's genetic distances (DS) among populations ranged from 0.171 (Noor and Neka populations) to 0.747 (Chalous and Noor populations) (Table 4, above the diagonal). The highest and lowest pairwise F_{ST} were observed between Nour and Chalous population (0.103) and Neka and Nour population (0.025), respectively (Table 4, below the diagonal). AMOVA analysis showed that the largest portion of the genetic variation was among individuals within the population (95%), whereas 5% was among populations (Table 5).

Table 2. Genetic variability of microsatellite loci in six populations of Zarde-Kija.

		CF113	**CF339**	**CF126**	**CF640**	**Mean**
Gorgan	N_A	4	7	6	4	5
	N_E	3.240	5.765	4.378	2.909	4.073
	H_O	0.000	0.143	0.111	0.000	0.031
	H_E	0.691	0.827	0.772	0.656	0.737
	(F value)	1.000	0.827	0.856	1.000	0.921
	pHw	***	***	*	***	-
Neka	N_A	6	10	8	4	7
	N_E	4.737	7.377	6.081	3.073	5.317
	H_O	0.133	0.133	0.200	0.000	0.117
	H_E	0.789	0.864	0.836	0.675	0.791
	(F value)	0.831	0.846	0.761	1.000	0.859
	pHw	***	*	***	*	-
Sari	N_A	7	2	6	4	5
	N_E	4.900	1.471	4.829	3.571	3.693
	H_O	0.143	0.000	0.000	0.000	0.036
	H_E	0.796	0.320	0.793	0.720	0.657
	(F value)	0.821	1.000	1.000	1.000	0.955
	pHw	***	***	***	***	-
Nour	N_A	6	6	8	3	6
	N_E	3.273	3.667	5.647	2.880	3.867
	H_O	0.000	0.000	0.417	0.000	0.104
	H_E	0.694	0.727	0.823	0.653	0.724
	(F value)	1.000	1.000	0.494	1.000	0.873
	pHw	**	**	***	***	-
Chalous	N_A	7	6	6	2	5
	N_E	5.042	4.629	4.082	1.658	3.853
	H_O	0.545	0.222	0.100	0.000	0.217
	H_E	0.802	0.784	0.755	0.397	0.684
	(F value)	0.320	0.717	0.868	1.000	0.726
	pHw	***	***	ns	***	-
Rasht	N_A	6	5	6	4	5
	N_E	5.000	4.571	4.900	3.556	4.507
	H_O	0.000	0.000	0.571	0.000	0.143
	H_E	0.800	0.781	0.796	0.719	0.774
	(F value)	1.000	1.000	0.282	1.000	0.821
	pHw	***	**	***	***	-

Table 3. N_M, F_{ST} and F_{IS} index of four microsatellite loci in six populations for Zarde-Kija.

Locus	CF113	CF339	CF126	CF640	Mean
N_M	2.664	1.898	3.619	1.855	2.509
F_{ST}	0.089	0.116	0.065	0.119	0.096
F_{IS}	0.820	0.884	0.707	1.000	0.853

Table 4. Pairwise Nei's (above the diagonal) and Fst (below the diagonal) genetic distances between geographical populations of Zarde-Kija.

Population	Gorgan	Neka	Sari	Nour	Chalous	Rasht
Gorgan	-	0.200	0.314	0.365	0.612	0.400
Neka	0.028	-	0.352	0.171	0.566	0.343
Sari	0.059	0.060	-	0.321	0.473	0.445
Nour	0.052	0.025	0.060	-	0.747	0.517
Chalous	0.091	0.079	0.091	0.103	-	0.305
Rasht	0.051	0.039	0.072	0.063	0.050	-

Table 5. Summary of the AMOVA results for 71 specimens of Zarde-Kija.

Source of variation	DF	SS	MS	VC	POV
Between populations	5	50.510	10.102	0.345	5%
Within populations	65	393.251	6.050	6.050	95%
Total	70	443.761		6.395	100%

DF: Degree of freedom; SS: Sum of squares; MS: Mean of squares; VC: Variance component; POV: Percentage of variation.

Genetic landscape shape plot showing patterns of spatial genetic distance for 6 populations of *Cantharellus* sp (Fig. 2). X and Y axes correspond to geographic coordinates and the Z-axis (height) corresponds to the genetic distance between individuals. Peaks are indicative of areas with high pairwise genetic distances and valleys are indicative of areas with low pairwise genetic distances.

Discussion

The present study is the first genetic analysis of *Cantharellus* along a latitudinal gradient in the southern Caspian Sea. Based on the economic and nutritional importance of *Cantharellus* species and its ecological value in Hyrcanian forests, we evaluated the genetic diversity of this ectomycorrhizal mushroom in six different geographic habitats. Our results indicated that the Neka population had the highest alleles average (7 alleles) and effective alleles (N_E) values (5.317). The mean observed and expected heterozygosity (H_O and H_E) in all Zarde-Kija mushroom populations is 0.108 and 0.728, respectively. This value for Ho is very low, even in comparison with the amount of observed and expected heterozygosity in *Cantharellus formosus*, reported by Dunham *et al*. 2003. Temperature and humidity along with the type of vegetation are the main factors for the presence of Zarde-Kija in the Hyrcanian forest. These conditions are more available in the central and eastern part of the Hyrcanian forest (Except for some microhabitats in the western part) and due to this reason, it is expected to have habitats with high density and possibly high diversity on this side of the Hyrcanian forest.

To date, most of the forest area dominated by Zarde-Kija especially in the plain region in the southern Caspian Sea is under severe human impacts. Although Zarde-Kija is used by the villagers in all areas of the Hyrcanian forest, its use is much greater in the central and eastern parts of the Hyrcanian forest. Therefore, human disturbances and the mating system could be the major causes for the lower degree of genetic diversity of Zarde-Kija in the Hyrcanian forest.

Deviation from the Hardy-Weinberg disequilibrium for the understudy species could be happened by a Wahlund effect, presence of null alleles, a propensity to autogamy, or any mix of these reasons (Fiore-Donno and Martin, 2001; Dunham *et al*., 2003a; Wadud *et al*., 2006; Roy *et al*., 2008; Shirmohammadli *et al*., 2018). Another reason explained by Dunham et al. (2003a) and Jany *et al*. (2006) is the low efficiency of ITS microsatellite markers in ectomycorrhizal fungi due to the lack of repetitive sequences at the genome level of these species.

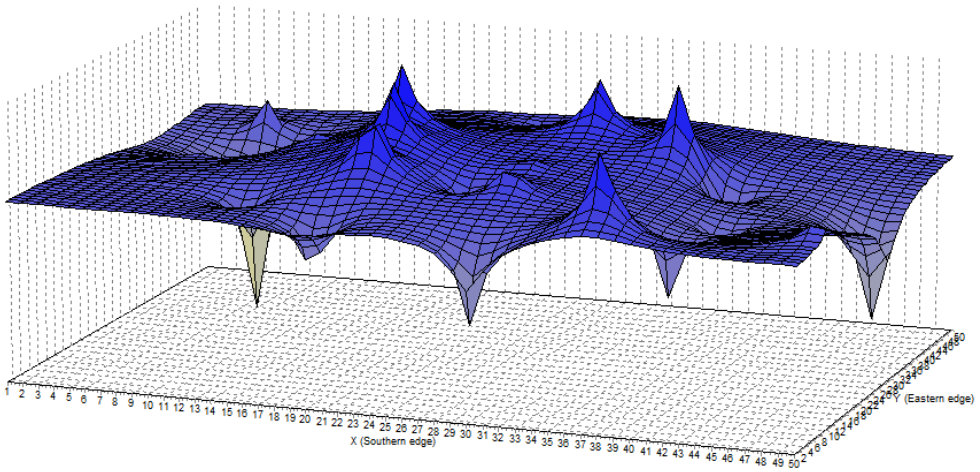

Fig. 2. Genetic landscape shape plot showing patterns of spatial genetic distance for 6 understudy populations.

The results indicate an excess of homozygotes and thus high inbreeding depression in Zarde-Kija populations. Self-fertilizes or mates with a relative are often made homozygous, and thus an inbreeding depression occurs in an outcrossing species (Gao and Gao, 2016). Also, inbreeding and thus excessive homozygotes could be a result of the rapidly decreased population sizes in the wild population (Gao, 2003).

Inbreeding cause reduces heterozygosity, resistance to diseases, survival, and ultimately weakens the local population (Lu *et al*., 2018). In most basidiomycetes, a change in Hardy-Weinberg equilibrium is rare (10-20%) (Wang *et al*., 2015; Mi *et al*., 2016; Koch and Aime, 2018). However, in all populations of the present study, a shift in balance was observed. The heterozygote deficiency suggests a deviation from Hardy–Weinberg Equilibrium, which is observed under non-random mating and in the absence of selection and migration (Mi *et al.,* 2016). In our study, the presence of high null alleles, the closeness of sampling areas in each population, or crossbreeding could be the main reason for deviation in Hardy-Weinberg equilibrium (Danham *et al*., 2003; Mi *et al*., 2016). In line with our study findings, results of Xu *et al*. (1997), Mi *et al*. (2016), Tsykun *et al*. (2017), and Lu *et al*. (2018), respectively on *Agaricus bisporus*, *Trogia venenata*, *Armillaria cepistipes,* and *Leucocalocybe mongolica* showed deviation on Hardy-Weinberg equilibrium in some loci.

Conclusion

Due to the favorable climatic conditions in the north of Iran and the existence of different ectomycorrhizal hosts species like *Fagus orientalis*, *Quercus castaneifolia*, *Carpinus betulus*, *Alnus subcordata,* and *Alnus glutinosa*, we expected the spread, abundance, and genetic diversity of Zarde-Kija mushroom should be in a better situation, but unfortunately, in recent decades, its habitats severely damaged due to deforestation, illegal logging, wood smuggling, the presence of livestock and recreational activities which influence genetic diversity and survival of this species at different regions of Hyrcanian forests.

The present study is the first report to know the extent of genetic diversity and differentiation of Zarde-Kija mushroom populations using ITS microsatellite markers. Despite the different habitat conditions and geographical distance of the studied populations, the level of genetic differentiation between the populations was low. The main reasons for the reduction of genetic diversity of fungal populations are the degradation of habitat, the same climatic conditions, the same seas, and as well as long-distance spore and mycelium distribution (Zhao Meng-ran *et al*., 2016). On the other hand, low genetic differentiation among Zarde-Kija populations could be due to the high genetic exchange between the populations, especially due to human activities. However, to obtain more accurate information about the genetic

diversity of this fungus, it is suggested that in future research, markers of other gene regions along with larger populations of this fungus be used.

Conflicts of interest

The authors have no conflicting interests.

References

Bergemann SE, Douhan GW, Garbelotto M, Miller SL. 2006. No evidence of population structure across three isolated subpopulations of *Russula brevipes* in an oak/pine woodland. *New Phytol* 170: 177-184.

Bergemann SE, Largent DL. 2000. The site-specific variables that correlate with the distribution of the Pacific Golden Chanterelle, Cantharellus formosus. *Forest Ecol Manag* 130: 99-107.

Chapuis M, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Mol Biol Evol* 24:621-631.

Dempster AP, Laird NM, Rubin DB. 1977. Maximum likelihood from incomplete data via the EM algorithm. *J R Stat Soc B (Methodological)* 39(1): 1-22.

Dunham S, Kretzer A, Pfrender M. 2003a. Characterization of pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. *Mol Ecol* 12: 1607-1618.

Dunham SM, O'dell TE, Molina, R. 2003b. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. *Mycol Res* 107: 1163-1177.

Fiore-Donno AM, Martin F. 2001. Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. *New Phytol* 152: 533-542.

Gao LZ, Gao CW. 2016. Lowered diversity and increased inbreeding depression within peripheral populations of wild rice *Oryza rufipogon*. *Plos One* 11: e0150468.

Gao LZ. 2003. The conservation of Chinese rice biodiversity: genetic erosion, ethnobotany and prospects. *Genet Resour Crop Ev* 50: 17-32.

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9. 3. http://www2. unil. ch/popgen/softwares/fstat. htm.

Jany JL, Bousquet J, Gagné A, Khasa DP. 2006. Simple sequence repeat (SSR) markers in the ectomycorrhizal fungus *Laccaria bicolor* for environmental monitoring of introduced strains and molecular ecology applications. *Mycol Res* 110: 51-59.

Koch RA, Aime MC. 2018. Population structure of *Guyanagaster necrorhizus* supports termite dispersal for this enigmatic fungus. *Mol Ecol* 27: 2667-2679.

Loit K, Adamson K, Bahram M, Puusepp R, Anslan S, Kiiker R, Drenkhan R, Tedersoo L. 2019. Relative performance of MinION (Oxford Nanopore Technologies) versus Sequel (Pacific Biosciences) thirdgeneration sequencing instruments in identification of agricultural and forest fungal pathogens. *Appl Environ Microbiol* 85: e01368-19.

Lu Q, Li H, Hong Y, Zhang G, Wen S, Li X, Zhou G, Li S, Liu H, Liu H. 2018. Genome sequencing and analysis of the peanut B-genome progenitor (*Arachis ipaensis*). *Front Plant Sci* 9: 604.

Lu T, Bao H, Bau T. 2018. Genetic diversity and population structure of endemic mushroom *Leucocalocybe mongolica* in Mongolian Plateau uncovered by EST-SSR markers. *Biotechnol Biotechnol Eq* 32: 1195-1204.

Mi F, Zhang Y, Yang D, Tang X, Wang P, He X, Zhang Y, Dong J, Cao Y, Liu C. 2016. Evidence for inbreeding and genetic differentiation among geographic populations of the saprophytic mushroom *Trogia venenata* from southwestern China. *PloS One* 11: e0149507.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach TO, Dell T, Castellano M. 2001. Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In: Fungal Conservation (eds Moore D, Nauta M, Rotheroe M) Cambridge University Press, Cambridge.

Parad GA, Ghobad-Nejhad M, Tabari M, Yousefzadeh H, Esmaeilzadeh O, Tedersoo L, Buyck, B. 2018. *Cantharellus alborufescens* and *C. ferruginascen*s

(Cantharellaceae, Basidiomycota) new to Iran. *Crypto Mycol* 39: 299-310.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinf* 28: 2537-2539.

Pilz D, Norvell L, Danell E, Molina R. 2003. Ecology and management of commercially harvested chanterelle mushrooms. United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, General Technical Report.

Rochon C, Pare D, Pelardy N, Khasa DP, Fortin JA. 2011. Ecology and productivity of *Cantharellus cibarius* var. *roseocanus* in two eastern Canadian jack pine stands. *Botany* 89: 663-675.

Roy M, Dubois MP, Proffit M, Vincenot L, Desmarais E, Selosse MA. 2008. Evidence from population genetics that the ectomycorrhizal basidiomycete Laccaria amethystina is an actual multihost symbiont. *Mol Ecol* 17: 2825-2838.

Shirmohammadli S, Sabouri H, Ahangar L, Ebadi AA, Sajjadi SJ. 2018. Genetic diversity and association analysis of rice genotypes for grain physical quality using iPBS, IRAP, and ISSR markers. *J Genet Resour* 4: 122-129.

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. 3rd Edition, Academic Press, 800.

Tsykun T, Rellstab C, Dutech C, Sipos G, Prospero S. 2017. Comparative assessment of SSR and SNP markers for inferring the population genetic structure of the common fungus *Armillaria cepistipes*. *Heredity* 119: 371-380.

Wadud MA, Lian CL, Nara K, Ishida TA, Hogetsu T. 2006a. Development of microsatellite markers from an ectomycorrhizal fungus, *Laccaria amethystina*, by a dual-suppression-PCR technique. *Mol Ecol Notes* 6: 130-132.

Wang Ln, Wei G, Wang Qy, QU Jb, Zhang Jx, Huang Cy. 2019. Identification of commercial cultivars of *Agaricus bisporus* in China using genome-wide microsatellite markers. *J Integr Agr* 18: 580-589.

Wang P, Zhang Y, Mi F, Tang X. He X, Cao Y, Liu C, Yang D, Dong J, Zhang K. 2015. Recent advances in population genetics of ectomycorrhizal mushrooms *Russula* spp. *Mycology* 6: 110-120.

Zhao MR, Huang Cy, Wu Xl, Qiang C, Qu Jb, LI Yc, Wei G, Zhang Jx. 2016. Genetic variation and population structure of the mushroom *Pleurotus ferulae* in China inferred from nuclear DNA analysis. *J Integr Agr* 15: 2237-2246.