ORIGINAL ARTICLE

Genetic diversity of eight wild populations of *Pampus argenteus* along the coast of China inferred from fifteen polymorphic microsatellite markers

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ABSTRACT

Pampus argenteus (Perciformes: Stromateidae) is widely distributed along the coast of China, Indian Ocean, Arabian Gulf and North Sea. Due to overfishing and environmental degradation, its resources reduced year after year. Thus, new management strategies are urgently needed for the sustainable growth and utilization of this species. Characterization of the genetic variation of this fish species is essential for conserving the genetic resource and restraining the population decline. Therefore, it is necessary to have a clear understanding of the intraspecific genetic diversity and population structure of the species. In this study, we assess the genetic diversity and population structure of *P. argenteus* by using microsatellites. We genotyped 240 *P. argenteus* individuals from eight wild populations collected from Shidao (SD), Lianyungang (LYG), Lvsi (LS), Zhoushan (ZS), Dongtou (DT), Xiapu (XP), Haikou (HK), and Beibuwan (BBW) along the coast of China using fifteen polymorphic microsatellites. A total of 139 alleles were determined at 15 loci across the eight populations, and a relatively high level of genetic diversity was observed, with observed heterozygosity (Ho) and expected heterozygosity (He) ranging from 0.100 to 1.000, and from 0.669 to 0.934 per locus-location combination, respectively. LS had the highest average allele (number of alleles, A=15.200), and HK the lowest (A=13.000). Hos of *P. argenteus* are less than Hes, indicating lack of heterozygote within populations. Analysis of molecular variance (AMOVA) showed that most variation (95.66%) occurred within populations, suggesting that this is the main source of total variance. This study will provide useful information for conservation and sustainable exploitation of this important fishery resource.

Descriptors: *Pampus argenteus*, microsatellite, genetic diversity, population structure.

INTRODUCTION

Pampus argenteus (Perciformes: Stromateidae) is an economically important benthopelagic fish, which is widely distributed along the coast of China, Indian Ocean, Arabian Gulf and North Sea (Davis and Wheeler, 1985; Liu et al., 2002). Due to overfishing and environmental degradation, its numbers reduced year after year (Jin et al., 2005; Zhang et al., 2007). Thus, new management strategies are urgently needed for the sustainable growth and utilization of this species. For effective fishery management, we need a mass of biological and evolutionary data. Characterization of the genetic variation of this fish species is essential for conserving the genetic resource and restraining the population decline. Therefore, it is necessary to have a clear understanding of the intraspecific genetic diversity and population structure of the species.

Genetic diversity and population structure have vital importance in understanding and managing populations (Palumbi, 2003; Muths et al., 2009). So far, several studies

on genetic diversity and population structure of *P. argenteus* have been reported (Xu et al., 2008; Peng et al., 2010; Meng et al., 2009; Wu et al., 2012; Zhao et al., 2011a). The markers used were mitochondrial DNA COI, D-loop, RAPD, and isozyme. No studies based on microsatellites of *P. argenteus* is yet available.

Microsatellites or simple sequence repeats (SSR) are short (1-6 bp) repetitive DNA sequences that are highly abundant and almost evenly distributed in genomes. They are generally neutral, highly polymorphic, co-dominant and easily scored with PCR (Goldstein and Schlotterer, 1999). As a more variable marker than RFLP and RAPD, microsatellites have been widely used for population genetics, genome mapping, ecology, and evolution of animals including fishery species for several decades (Goldstein and Schlotterer, 1999; Herwerden et al., 1999; Liu and Cordes, 2004; Vargas-Caro et al., 2017; Kiper et al., 2018).

Population structure is an important factor that should be taken into account by the appropriate regulatory authorities when considering sustainable applications of fish. The aim of this research was to examine the genetic diversity and population structure of *P. argenteus* along the coast of China to provide useful genetic information for the management of this species. In the present study, we used fifteen polymorphic microsatellite markers to assess the genetic diversity and population structure of eight wild populations of *P. argenteus* along the coast of China. This study provide useful information for better understanding the evolution potential and population structure of P. argenteus, and is good for the protection and reasonable utilization of this species.

MATERIALS AND METHODS

SAMPLING AND DNA EXTRACTION

From June to August, 2013, a total of 240 wild *P. argenteus* individuals, i.e., 30 individuals of each population, were collected from eight locations along the coast of China (Figure 1). The eight locations are Shidao (SD) of Shandong Province, Lianyungang (LYG) and Lvsi (LS) of Jiangsu Province, Zhoushan (ZS) and Dongtou (DT) of Zhejiang Province, Xiapu (XP) of Fujian Province, Haikou (HK) of Hainan Province, and Beibuwan (BBW) of Guangxi Zhuang Autonomous Region, respectively. The vouchers of the sampled population were deposited in the herbarium of East China Sea Fisheries Research Institute. All individuals were transferred to the laboratory in dry ice and then stored at -80°C before DNA extraction.
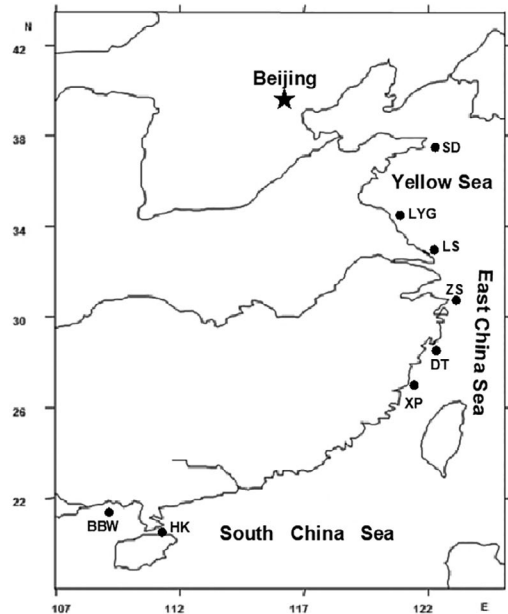

Figure 1. Map of the P. argenteus sampled locations (indicated by circles). The abbreviations correspond to sampled location names, as indicated in text.

Total genomic DNA was extracted from muscle tissue of *P. argenteus* using the standard proteinase K/phenol/chloroform procedure (Sambrook et al., 1989). The quality of the extracted DNA was checked using 0.8% agarose gel electrophoresis, then stored at -20°C for PCR amplification.

MICROSATELLITE GENOTYPING

Twenty-six pairs of novel polymorphic microsatellite markers for *P. argenteus* were isolated using combined biotin capture method. Fifteen of them, i.e., YC79, YC90, YC140, YC275, YC339, YC353-1, YC353-2, YC459, YC687, YC705, YC731, YC742, YC754, YC764, and YC792 (Table 1), were used for genotyping all the 240 *P. argenteus* individuals. Polymerase chain reaction (PCR) amplification was performed in a 15 μL reaction volume containing approximately 10-50 ng of genomic DNA, 0.6 μM of each primer, 7.5 μL 2 ×Taq PCR MasterMix (TIANGEN), with the following thermal cycling conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at primer-specific annealing temperature (T_a, see Table 1) for 30 s, elongation at 72°C for 30 s, followed by a final extension at 72°C for 7 min. The final PCR products were separated by electrophoresis in denaturing 6% polyacrylamide gels and visualized by silver staining. The allele sizes

Table 1. Characterization of fifteen microsatellite loci used in this study.

Locus	Repeat motif	Primer sequence (5'-3')	annealing temperature Ta (°C)	GenBank accession number
YC79	$(CT)_8T(TC)_8$	F: ACAAGCATAGGATGACTT R: TACAGGTGGAGATGAGAA	50	KR091917
YC90	$(CA)_{11}$	F: GGCTAGGGATGAAACTCG R: GGGAGGGAATCGGAATAG	55	KR091918
YC140	$(AC)_{12}$	F: GAATAAGCAAACAAACAC R: ATACAACACTGACAACCT	48	KR091921
YC275	$(AC)_{23}$	F: AAGTTATGCTCGACCTGA R: GTCCTTTTATCCCCTGAA	46	KR091925
YC339	$(CA)_8...(CA)_5$	F: CTCAGCAGTTCCTCTTTG R: ATGTAGCGAGTCATTGTG	53	KR091926
YC353-1	$(AC)_{10}...(CT)_{15}$	F: GATTCGCATAAACAGATAAGT R: AAGGGGAGGGGAGTAAGT	50	KR091927
YC353-2	$(AC)_{13}$	F: ATGGCTTCCAATCCTACA R: ACTGTGCTGCCTGAAATA	50	KR091928
YC459	$(CA)_6$	F: CACGCTGGTGCTAATGGA R: GTCACAGGGGCAAGTAGA	55	KR091930
YC687	$(GT)_{10}(AT)_6$	F: AATAGCATTCAGACAGTGG R: CTACAAAGACAGAGACGC	53	KR091931
YC705	$(GT)_6$	F: TGGGGAGAGTGTGATGTG R: TAGCAGGAGAAGTGTTTTT	48	KR091932
YC731	$(TC)_5C(CA)_8$	F: GTTCAGATAAAGGGAGAT R: GACCCAGAATAAGTGTAA	50	KR091935
YC742	$(TG)_5$	F: ACAACCGCTCTGTCATAC R: TACAAACAAGCAAATACACT	48	KR091936
YC754	$(CA)_{10}$	F: GCGGAGTTCTGCCCTTAT R: ACTGAGTCCCCATTACCC	55	KR091937
YC764	$(TG)_9$	F: AGAAAGACCCTTATCTCCA R: CTTCACCTCATTGCCCTC	53	KR091939
YC792	$(GT)_8...(GT)_7$	F: GCTGCATCACATCTTGAC R: GACGTGGATCGACTCTTA	53	KR091941

were estimated with a 10 bp DNA ladder (Invitrogen) as reference.

STATISTICAL ANALYSIS

MEASUREMENTS OF GENETIC DIVERSITY

The number of alleles (A), number of effective alleles (Ae), observed heterozygosity (Ho), expected heterozygosity (He), and the inbreeding coefficient (F_{IS}) for each locus from each population were obtained using GenAlEx 6.0 (Peakall and Smouse, 2006). The diversity level of each genetic locus was evaluated with polymorphic information content (PIC) using the CERVUS version 3.0.3 (Marshall et al., 1998). GENEPOP on the Web (http://genepop.curtin.edu.au/, Raymond and Rousset, 1995) was used to check the deviations from Hardy-Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) of each locus within each site. HWE and LD tests were performed using the Markov Chain method (10,000 dememorization steps, 100 batches, 5000 iterations).

MEASUREMENTS OF POPULATION DIFFERENTIATION

To study population structure, population-level pairwise F_{ST} was analyzed using a permutation with 10,000 replicates. Analysis of molecular variance (AMOVA) were calculated using software ARLEQUIN 3.1 (Excoffier et al., 2005). To examine the genetic relationships among populations, the matrix of Nei's genetic distance of pairwise locations (Nei, 1978) was calculated using the GenAlEx 6.0 (Peakall and Smouse, 2006). Then an unweighted pair-group mean analysis (UPGMA) tree was constructed based on Nei's genetic distance matrices

using MEGA version 5.0 software (Tamura et al., 2011). In order to provide a visual representation of population subdivision, Principal Component Analysis (PCA) was performed in GenAlEx 6.0 (Peakall and Smouse, 2006).

Demographical Bottleneck

We detect recent population declines by computing the heterozygosity excess statistic using the computer program BOTTLENECK with two methods (Cornuet and Luikart, 1996). The first method basing on the principle of heterozygosity excess was executed under two different mutation models: two-phase mutation model (TPM) and stepwise mutation model (SMM), where 95% and 90% single-step mutations, and 5% and 10% multiple steps mutations with 1,000 simulation iterations were set as recommended by Piry et al. (1999). SMM was used because microsatellite loci appear to evolve under a mutation model that is more similar to the SMM than the infinite allele model (IAM) (Valdes et al., 1993; Shriver et al., 1993). The second method, mode-shift test (Luikart and Cornuet, 1998), was used to detect a potential bottlenecked population using an L-shaped distribution of allele frequency as mutation-drift equilibrium.

RESULTS

Genetic diversity

All the 15 microsatellite markers were well amplified in the eight populations of *P. argenteus*. Of the 120 HWE tests across all 15 loci, 38 were conformed to HWE (Table Sup) within each sample location after applying sequential Bonferroni corrections (minimum adjusted alpha = 0.00050) (Rice, 1989). In addition, significant genotypic LD for multiple comparisons of 15 loci within populations was detected in 18 out of 1680 tests (minimum adjusted alpha = 0.00015) (Rice, 1989) after sequential Bonferroni correction. Thus, we think that most microsatellite loci could be deemed as genetically independent for further analysis.

All 15 loci were polymorphic in all of the eight studied populations of *P. argenteus* with high allelic diversity and heterozygosity (Table Sup.). A total of 139 alleles were detected at the 15 microsatellite loci across the eight populations. The number of private alleles for the eight populations are 9 (SD), 10 (LYG), 7 (LS), 6 (ZS), 7 (DT), 18 (XP), 18 (HK), and 23 (BBW), respectively. For A, LS was the highest population with A=15.200, while HK was the least variable one with A=13.000. For Ae, DT was

the highest with Ae=10.222, while HK was the least with Ae=8.340.

For the eight populations, the average polymorphism information content (PIC) ranged from 0.854 (HK) to 0.879 (LS) per location. The average inbreeding coefficient (F_{IS}) ranged from -0.200 (YC459-XP) to 0.870 (YC687-HK) with an average value 0.363 per locus, and ranged between 0.290 (DT) and 0.426 (HK) for populations (Table Sup.), suggesting that there is high inbreeding in these eight *P. argenteus* populations. Observed and expected heterozygosity (H_o and H_e) ranged from 0.100 to 1.000 and from 0.669 to 0.934 per locus-location combination, while from 0.504 (HK) to 0.627 (DT) and from 0.867 (HK) to 0.889 (LS) per location, respectively (Table Sup.). Within sampling locations, the mean expected heterozygosities (H_e) were consistently higher than the observed one (H_o) across all loci, which revealed a deficit of heterozygosity among the samples.

Population structure

Significant genetic heterozygosity among the eight populations was indicated by AMOVA analysis. The results showed that 4.34% of total genetic variation came from among population variation, while the within population variation explained 95.66% of total variation. Genetic differentiation between populations was analyzed using F_{ST}. The overall F_{ST} value over all locations and loci was statistically different from zero (Fixation Index F_{ST}=0.0434, *p*<0.001) (Table 2). Pairwise F_{ST} comparing population pairs ranged from 0.022 (DT and ZS) to 0.074 (HK and XP) (Table 3). The highest genetic differentiation between populations using F_{ST} was between the populations HK and XP, while the lowest differentiation was between DT and ZS (Table 3). The results of F_{ST} indicated that there was low but statistically significant genetic differentiation among the populations.

UPGMA phylogenetic trees (Figure 2) were constructed on the basis of Nei's genetic distance matrix (Table 3). Eight populations were divided into two main clusters: HK and BBW populations formed Cluster I; ClusterIIincludes the remaining six populations. Cluster II is consisted of three subgroups, i.e., subgroup 1 includes XP, subgroup 2 includes LS, and subgroup 3 includes two SD, LYG, ZS, and DT (Figure 3). Mantel Test showed that there are significant linear correlation between geographic distances and genetic distances with R^2=0.8012 (data not shown), which could imply isolation by distance. Furthermore, the PCA analysis also showed the HK and BBW populations

Table 2. AMOVA analysis of eight populations of *P. argenteus*.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation (%)	Fixation Index (FST)	*p*
Among populations	7	193.265	0.30572 V[a]	4.34	0.0434	0.0000
within populations	232	2149.650	2.51932 V[b]	95.66		
Total	239	2343	2.82504			

Table 3. Estimates of pairwise FST values (below diagonal) and Nei's Genetic Distance (above diagonal) between eight populations of *P. argenteus* detected by 15 microsatellites.

Population	SD	LYG	LS	ZS	DT	XP	HK	BBW
SD	-	0.450	0.560	0.440	0.490	0.535	1.141	1.162
LYG	0.029***	-	0.501	0.496	0.412	0.641	1.115	0.948
LS	0.036***	0.032***	-	0.519	0.463	0.628	0.976	0.975
ZS	0.028***	0.033***	0.033***	-	0.359	0.494	1.084	1.025
DT	0.033***	0.027***	0.030***	0.022***	-	0.507	1.027	0.987
XP	0.039***	0.047***	0.044***	0.036***	0.038***	-	1.042	1.068
HK	0.072***	0.072***	0.064***	0.071***	0.069***	0.074***	-	0.579
BBW	0.071***	0.063***	0.062***	0.066***	0.066***	0.073***	0.046***	-
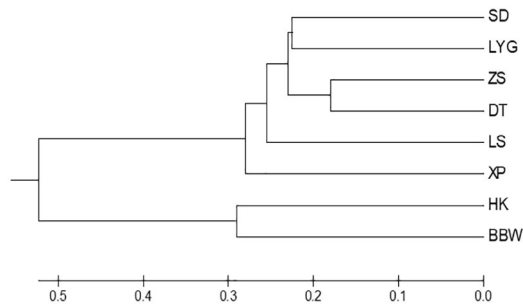
Figure 2. Unweighted Pair-group Method with Arithmetic Means tree (UPGMA) of eight populations of *P. argenteus* based on Nei's genetic distance.
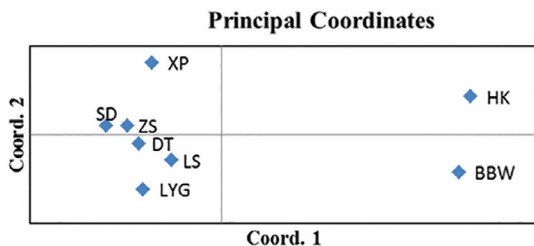
Figure 3. Principal component analysis (PCA) of eight populations of *P. argenteus*.

to be genetically distinct from the other six locations (Figure 3). These results are consistent with the F$_{ST}$ and Nei's distance tests.

DEMOGRAPHIC BOTTLENECK

Analysis of recent population declines of *P. argenteus* was detected using the Wilcoxon signed-rank test in BOTTLENECK under two models of microsatellite evolution (SMM and TPM)(Table 4). Excepted TPM (90%) for SD and DT, the probability values of the bottleneck test all above 0.05 (Table 4), suggesting no significant excess of heterozygosity in most populations. It indicated that there was no genetic bottleneck in most of the eight populations due to mutation-drift equilibrium. In addition, the mode-shift test showed that all populations were in normal L-shaped pattern of the allele frequency distributions (Table 4), revealing the lack of population declines in the recent history of *P. argenteus*.

DISCUSSION

In this study, we examined the genetic diversity and population structure of eight wild populations of *P. argenteus* using 15 highly polymorphic microsatellites. We observed low but significant genetic differences in the spatial distribution of *P. argenteus* along the coast of China. The results suggested a high level of population genetic diversity of *P. argenteus* (A:13.000-15.200, Ho:0.504-0.627, He: 0.867-0.889, and PIC:0.854-0.879), which is in line with the previous studies that showed a high level of genetic diversity in *P. argenteus* using RAPD, AFLP and mtDNA (Meng et al., 2009; Zhao et al., 2011a; Peng et al.,

Table 4. *p*-values of bottleneck tests for detecting the recent population declines of *P. argenteus* using stepwise mutation model (SMM), two phased mutation model (TPM), and mode shift indicator.

Population	Heterozygosity excess (*p* value)				Mode-shift
	SMM (90%)	SMM (95%)	TPM (90%)	TPM (95%)	
SD	0.27686	0.30280	0.00336**	0.07300	normal
LYG	0.76154	0.80396	0.25238	0.42120	normal
LS	0.71973	0.97797	0.18762	0.42120	normal
ZS	0.71973	0.67877	0.05536	0.38940	normal
DT	0.33026	0.27686	0.02557*	0.15143	normal
XP	0.10699	0.15143	0.35913	0.42120	normal
HK	0.84692	0.89038	0.10699	0.48871	normal
BBW	0.84692	0.76154	0.30280	0.52448	normal

*, Signification *p* value <0.05; **, Extremely signification *p* value <0.01. *, significant evidence of a population decline from bottleneck.

2009a; Wu et al., 2012). Genetic diversity is not only the consequence of evolution, but also represented the evolution potential of animals. In order to be fit for survival, animals accumulated more genetic diversity to ensure their increase of the fitness for many kinds of environmental pressures (Liu et al., 2010). *P. argenteus* may use a relative high genetic diversity to adopt its rigorous habitats during its evolution.

The amount of effective alleles (Ae) was obviously less than that of the observed alleles (A) (Table Sup.), indicating that some alleles were lost between the populations studied in this study. The amount of alleles is very important to maintain populations because it provides the necessary spectrum of genotypes for adaptive response to changing environments (Thai et al., 2007). The loss of genetic diversity will prevent future improvements via selection of the species to a certain extent. The most likely explanation for the loss of alleles may be that the wild population was under stress from overfishing and deterioration in the environment resulting in less recruitment (Alam and Islam, 2005). In order to prevent the alleles from losing and to conserve the genetic resource, we could strengthen the conservation and supervision of wild resources and forbid overfishing in the natural waters.

Generally, marine species are capable of migration over long distances, showing little or no genetic differentiation in geographic scales because of long distance pelagic dispersal potential during planktonic egg, larval, or adult history stages coupled with an absence of physical barriers to movement between ocean basins or adjacent continental margins (Hewitt, 2000). The small but highly significant values of pairwise F_{ST} in the present study indicated that the *P. argenteus* have genetic differentiation among populations, and more than 95.66% of the total

genetic variation was among individuals within populations (Table 2). UPGMA and PCA analysis indicated that the population of eight *P. argenteus* can divided into two major groups: one group including populations of *P. argenteus* in the South China Sea (SCS) and the other group consisting populations of *P. argenteus* in the Yellow Sea (YS) and the East China Sea (ECS). Earlier studies using RAPD and mtDNA (Meng et al., 2009; Peng et al., 2009) did support the idea that the populations from the South China Sea are genetically different from other populations. In all, these results revealed that there are different isolation patterns between two major groups and significant population differentiation throughout the examined range of sampled locations. Populations of *P. argenteus* seem to show high levels of gene flow between ECS and YS groups. A complex system of surface currents is likely the main factor influencing gene flow. The China Coastal Current and Yellow Sea Warm Current, including outflow of water from the Yellow Sea to the East China Sea along the China coast and inflow from the East China Sea to the Yellow Sea along the west coast of Korea (Li et al., 2000), respectively, could contribute to mixing of YS and ECS groups. The gene flow between populations from the South China Sea and other populations may be limited by some factors such as oceanographic characteristics and life history.

Zhao et al. (2011b) indicates that LYG and ZS samples have close relationship. However, our study results show that the normalized Euclidean distance between ZS and DT samples was shorter than all other pairwise distances. In general, morphological differences of *P. argenteus* among Chinese coastal waters can be discerned by multivariate morphometrics methods. Our study with microsatellite as marker showed significant genetic differentiation

in *P. argenteus*, which were to some extend different from the results based on mitochondrial DNA gene sequences (Peng et al., 2009b; Wu et al., 2012). The reasons leading to this divergence may include intrinsic variability of the population, sample size, spatial replication, and the number and characteristics of the marker loci used (Curley and Gillings, 2009). More samples should be collected from all over Chinese coastal waters including Bohai Sea, Yellow Sea, East China Sea and South China Sea to allow for a more comprehensive picture of genetic variation of this species.

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SUPPLEMENTARY TABLE

Table Sup. Statistics for genetic variation at 15 microsatellite loci in eight populations of *P. argenteus*.

Locus	Population	Parameters						
		A	Ae	Ho	He	PIC	PHWE	Fis
YC79	SD	17	13.333	0.567	0.925	0.920	0.0000 *	0.387
	LYG	14	9.524	0.767	0.895	0.886	0.0019	0.143
	LS	17	13.636	0.767	0.927	0.922	0.0040	0.173
	ZS	16	12.950	0.667	0.923	0.917	0.0004	0.278
	DT	16	12.245	0.533	0.918	0.913	0.0000 *	0.419
	XP	16	10.169	0.767	0.902	0.894	0.0526	0.150
	HK	10	7.531	0.433	0.867	0.853	0.0000 *	0.500
	BBW	12	9.278	0.433	0.892	0.882	0.0000 *	0.514
YC90	SD	15	12.500	0.333	0.920	0.914	0.0000 *	0.638
	LYG	15	9.836	0.333	0.898	0.890	0.0000*	0.629
	LS	14	11.613	0.533	0.914	0.907	0.0000*	0.416
	ZS	15	10.588	0.400	0.906	0.898	0.0000*	0.558
	DT	14	11.111	0.667	0.910	0.903	0.0025	0.267
	XP	16	10.227	0.533	0.902	0.894	0.0000 *	0.409
	HK	12	8.571	0.367	0.883	0.872	0.0000*	0.585
	BBW	11	8.451	0.300	0.882	0.870	0.0000*	0.660
YC140	SD	16	9.184	0.733	0.891	0.882	0.0008	0.177
	LYG	19	14.516	0.867	0.931	0.927	0.0042	0.069
	LS	19	13.433	0.833	0.926	0.921	0.0001	0.100
	ZS	17	10.843	0.800	0.908	0.901	0.0621	0.119
	DT	19	12.766	0.833	0.922	0.916	0.3156	0.096
	XP	13	8.000	0.433	0.875	0.863	0.0000 *	0.505
	HK	12	4.972	0.533	0.799	0.781	0.0000 *	0.332
	BBW	12	4.700	0.533	0.787	0.761	0.0000*	0.323
YC275	SD	18	11.921	0.800	0.916	0.910	0.0000*	0.127
	LYG	20	15.126	0.833	0.934	0.930	0.0000*	0.108
	LS	21	13.043	0.867	0.923	0.918	0.1694	0.061
	ZS	17	13.534	0.833	0.926	0.921	0.0593	0.100
	DT	20	14.876	0.900	0.933	0.929	0.0226	0.035
	XP	15	7.531	0.733	0.867	0.856	0.0000*	0.154
	HK	18	13.235	1.000	0.924	0.919	0.0000 *	-0.082
	BBW	18	10.843	0.933	0.908	0.901	0.0000 *	-0.028
YC339	SD	11	6.844	0.300	0.854	0.838	0.0000 *	0.649
	LYG	12	7.895	0.600	0.873	0.862	0.0000*	0.313
	LS	12	8.612	0.333	0.884	0.873	0.0000*	0.623
	ZS	11	8.867	0.533	0.887	0.877	0.0000*	0.399
	DT	13	7.759	0.467	0.871	0.858	0.0000 *	0.464
	XP	11	8.333	0.633	0.880	0.868	0.0002	0.280
	HK	11	6.475	0.267	0.846	0.829	0.0000*	0.685
	BBW	12	7.792	0.400	0.872	0.859	0.0000*	0.541
YC353-1	SD	18	12.245	0.733	0.918	0.913	0.0230	0.201
	LYG	19	13.534	0.733	0.926	0.921	0.0001	0.208

	LS	21	14.754	0.833	0.932	0.928	0.0077	0.106
	ZS	19	12.857	0.633	0.922	0.917	0.0001	0.313
	DT	19	13.433	0.633	0.926	0.921	0.0000 *	0.316
	XP	13	7.792	1.000	0.872	0.859	0.0110	-0.147
	HK	14	7.895	0.767	0.873	0.861	0.0005	0.122
	BBW	16	9.783	0.900	0.898	0.889	0.4368	-0.002
YC353-2	SD	11	6.642	0.467	0.849	0.833	0.0000 *	0.451
	LYG	11	8.295	0.733	0.879	0.868	0.0089	0.166
	LS	14	8.696	0.433	0.885	0.875	0.0000 *	0.510
	ZS	18	10.345	0.600	0.903	0.897	0.0000*	0.336
	DT	16	10.465	0.467	0.904	0.897	0.0000*	0.484
	XP	11	6.569	0.733	0.848	0.831	0.0002	0.135
	HK	11	6.923	0.667	0.856	0.841	0.0006	0.221
	BBW	12	8.295	0.800	0.879	0.868	0.0938	0.090
YC459	SD	10	5.902	0.400	0.831	0.810	0.0000 *	0.518
	LYG	9	3.766	0.367	0.734	0.698	0.0000 *	0.501
	LS	12	5.085	0.500	0.803	0.787	0.0000*	0.378
	ZS	7	3.358	0.267	0.702	0.654	0.0000*	0.620
	DT	6	3.025	0.367	0.669	0.618	0.0000*	0.452
	XP	10	6.000	1.000	0.833	0.813	0.0000*	-0.200
	HK	15	8.451	0.167	0.882	0.871	0.0000*	0.811
	BBW	12	5.248	0.267	0.809	0.792	0.0000*	0.671
YC687	SD	14	10.588	0.233	0.906	0.898	0.0000 *	0.742
	LYG	18	9.677	0.300	0.897	0.888	0.0000 *	0.665
	LS	14	11.250	0.400	0.911	0.904	0.0000*	0.561
	ZS	18	12.000	0.467	0.917	0.911	0.0000*	0.491
	DT	18	11.250	0.567	0.911	0.905	0.0000*	0.378
	XP	20	11.921	0.633	0.916	0.910	0.0000*	0.309
	HK	6	4.327	0.100	0.769	0.733	0.0000*	0.870
	BBW	9	5.696	0.200	0.824	0.803	0.0000*	0.757
YC705	SD	18	13.043	0.633	0.923	0.918	0.0000*	0.314
	LYG	18	10.976	0.633	0.909	0.902	0.0000*	0.303
	LS	19	13.433	0.633	0.926	0.921	0.0000*	0.316
	ZS	18	13.235	0.733	0.924	0.919	0.0002	0.207
	DT	17	14.876	0.800	0.933	0.929	0.1004	0.142
	XP	20	14.063	0.633	0.929	0.924	0.0000 *	0.318
	HK	20	14.400	0.700	0.931	0.926	0.0000 *	0.248
	BBW	22	14.754	0.833	0.932	0.928	0.0067	0.106
YC731	SD	13	9.890	0.600	0.899	0.890	0.0000 *	0.333
	LYG	13	9.626	0.200	0.896	0.887	0.0000 *	0.777
	LS	13	8.531	0.433	0.883	0.872	0.0000*	0.509
	ZS	15	10.112	0.533	0.901	0.893	0.0000*	0.408
	DT	11	8.612	0.467	0.884	0.873	0.0000*	0.472
	XP	10	5.625	0.400	0.822	0.801	0.0000*	0.514
	HK	8	6.186	0.167	0.838	0.819	0.0000 *	0.801
	BBW	13	8.295	0.267	0.879	0.867	0.0000*	0.697

YC742	SD	9	4.523	0.233	0.779	0.754	0.0000*	0.700
	LYG	9	6.767	0.300	0.852	0.835	0.0000*	0.648
	LS	10	5.751	0.633	0.826	0.804	0.0000*	0.233
	ZS	12	6.792	0.700	0.853	0.836	0.0009	0.179
	DT	9	5.471	0.700	0.817	0.792	0.0343	0.143
	XP	9	3.956	0.167	0.747	0.717	0.0000 *	0.777
	HK	12	6.792	0.533	0.853	0.837	0.0000*	0.375
	BBW	12	6.250	0.433	0.840	0.821	0.0000*	0.484
YC754	SD	9	6.818	0.633	0.853	0.837	0.0007	0.258
	LYG	12	6.383	0.300	0.843	0.826	0.0000 *	0.644
	LS	11	5.202	0.567	0.808	0.788	0.0000*	0.298
	ZS	10	5.263	0.500	0.810	0.788	0.0000*	0.383
	DT	10	4.775	0.700	0.791	0.761	0.1399	0.115
	XP	11	7.059	0.433	0.858	0.842	0.0000*	0.495
	HK	16	10.526	0.633	0.905	0.898	0.0000*	0.300
	BBW	12	8.451	0.700	0.882	0.871	0.0502	0.206
YC764	SD	21	15.652	0.767	0.936	0.932	0.0058	0.181
	LYG	19	12.500	0.633	0.920	0.915	0.0000 *	0.312
	LS	18	9.890	0.600	0.899	0.891	0.0000*	0.333
	ZS	15	10.651	0.400	0.906	0.899	0.0000*	0.559
	DT	18	13.043	0.667	0.923	0.918	0.0000*	0.278
	XP	17	12.000	0.700	0.917	0.911	0.0000*	0.236
	HK	14	8.036	0.533	0.876	0.864	0.0002	0.391
	BBW	13	9.375	0.500	0.893	0.884	0.0000*	0.440
YC792	SD	14	8.451	0.533	0.882	0.872	0.0000*	0.395
	LYG	12	7.115	0.400	0.859	0.844	0.0000*	0.535
	LS	13	8.911	0.533	0.888	0.877	0.0000*	0.399
	ZS	13	7.500	0.467	0.867	0.854	0.0000*	0.462
	DT	15	9.626	0.633	0.896	0.888	0.0003	0.293
	XP	13	8.036	0.267	0.876	0.864	0.0000*	0.695
	HK	16	10.778	0.700	0.907	0.900	0.0011	0.228
	BBW	16	11.538	0.700	0.913	0.907	0.0001	0.234
All locus	SD	14.267	9.836	0.531	0.885	0.875	0.0038	0.405
	LYG	14.667	9.702	0.533	0.883	0.872	0.0024	0.401
	LS	15.200	10.123	0.593	0.889	0.879	0.0139	0.334
	ZS	14.733	9.926	0.569	0.884	0.872	0.0165	0.361
	DT	14.733	10.222	0.627	0.881	0.868	0.0441	0.290
	XP	13.667	8.485	0.604	0.870	0.857	0.0057	0.309
	HK	13.000	8.340	0.504	0.867	0.854	0.0004	0.426w
	BBW	13.467	8.583	0.547	0.873	0.860	0.0430	0.379

A = the number of alleles per locus, **Ae** = the number of effective alleles per locus, PIC = polymorphism information content, Ho = observed heterozygosity, He = expected heterozygosity, FIS = the inbreeding coefficient (*, significantly value of HWE greater than zero after sequential Bonferroni corrections).