# Microsatellite analysis of variation among wild, domesticated, and genetically improved populations of blunt snout bream (*Megalobrama amblycephala*)

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**Abstract:** In the present study, the genetic diversity of one selected strain (Pujiang No. 1), two domesticated populations (GA and HX) and four wild populations (LZ, YN, SS and JL) of blunt snout bream (*Megalobrama amblycephala*) was analyzed using 17 microsatellite markers. The results showed that an average of 4.88-7.65 number of alleles (*A*); an average of 3.20−5.33 effective alleles ($N_e$); average observed heterozygosity ($H_o$) of 0.6985−0.9044; average expected heterozygosity ($H_e$) of 0.6501−0.7805; and the average polymorphism information content (*PIC*) at 0.5706−0.7226. Pairwise $F_{ST}$ value between populations ranged from 0.0307−0.1451, and Nei's standard genetic distance between populations was 0.0938−0.4524. The expected heterozygosities in the domesticated populations (GA and HX) were significantly lower than those found in three wild populations (LZ, SS and JL), but no difference was detected when compared with the wild YN population. Likewise, no difference was found between the four wild populations or two domesticated populations. The expected heterozygosity in Pujiang No. 1 was higher than the two domesticated populations and lower than the four wild populations. Regarding pairwise $F_{ST}$ value between populations, permutation test *P*-values were significant between the GA, HX and PJ populations, but not between the four wild populations. These results showed that the expected heterozygosity in the selected strain of blunt snout bream, after seven generations of selective breeding, was lower than that of wild populations, but this strain retains higher levels of genetic diversity than domesticated populations. The genetic differences and differentiation amongst wild populations, domesticated populations and the genetically improved strain of blunt snout bream will provide important conservation criteria and guide the utilization of germplasm resources.

**Keywords:** Blunt snout bream; Genetic variation; Microsatellite; Wild population; Domesticated population; Genetically improved strain

The herbivorous blunt snout bream (*Megalobrama amblycephala*), known as the Wuchang fish in China, is distributed in a few medium and large-sized lakes of the middle reaches of the Yangtze River. It was first discovered and named in Lake Liangzi in Hubei province in the 1950s (Yi, 1955). This species has since become a major freshwater aquaculture commodity in China because of its herbivorous diet, general hardiness, resistance to disease, good seinability and reproductive performance (Ke, 1965). After domestication, the aquaculture performance of many populations of *M. amblycephala* deteriorated and slower growth rates, earlier maturity and thin and longer bodies emerged. Poor management of broodstocks and inbreeding depression were thought to be the major causes for this deterioration (Li et al, 1991a, b; Li & Yang, 1996). In view of this, a systematic selection program was started in 1986 based on an original founder population from Lake Yuni and a good breed (selected strain $F_6$) called Pujiang No. 1 was successfully selected by mass selection. The growth rate improved by 29% and body depth improved also (Li and Cai, 2000, 2003). The Chinese blunt snout bream industry has benefited from the spread and utilization of Pujiang No. 1; however, germplasm resources of blunt

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snout bream in China still face serious threats. For example, crossbreeding of domesticated populations with natural populations has occurred because of artificial breeding and release and the survival and evolution of the gene pool is threatened by water pollution. To ensure the sustainable development and utilization of the germplasm resources of this highly valuable species, an assessment of genetic diversity is urgently required.

Microsatellites have emerged as a popular molecular marker because of their codominance, neutrality, high variability and ease of identification and application. Microsatellites have been successfully used for estimating genetic variation of wild and hatchery stocks in many fishes (Aung et al, 2010; Liu et al, 2011; Šegvić-Bubić et al, 2011; Wachirachaikarn et al, 2011). Intraspecific variation and population diversity of blunt snout bream have been investigated using enzymes, random amplified polymorphic DNA (RAPD) and mtDNA markers (Bian et al, 2007; Li et al, 1991b; Li & Yang, 1996; Li et al, 2005; Zhang, 2001). Here, we used a larger set of 17 polymorphic microsatellite markers to estimate genetic diversity across four wild populations, two domesticated populations and one genetically improved strain of blunt snout bream.

## MATERIALS AND METHODS

### Sample collection and DNA extraction

One genetically improved population and four wild and two domesticated populations of *M. amblycephala* (Table 1, Figure 1) were surveyed. Samples were collected from four locations: Lake Liangzi, Ezhou city, Hubei (LZ; 32 individuals); Lake Yuni, Gongan city, Hubei (YN; 32 individuals); the middle reaches of the Yangtze River in Shishou city, Hubei (SS; 32 individuals); and the middle reaches of the Yangtze River in Jianli city, Hubei (JL; 32 individuals). Samples of domesticated *M. amblycephala* were collected from two independent fish farms in Gongan city (GA; 32 individuals) and Tianjin municipality (HX; 32 individuals). No details regarding the founding and maintenance of the Gongan population were available; the Tianjin (HX) population was established from animals from Lake Liangzi in the 1970s. Samples of genetically improved


Figure 1 Locations and the abbreviated names of seven focal blunt snout bream samples (●)

1: YN; 2: GA; 3: SS; 4: JL; 5: LZ; 6: PJ; 7: HX.

**Table 1 List of the seven focal populations of blunt snout bream**

| Population name | Remarks | Sample size |
|---|---|---|
| LZ | Wild population from Lake Liangzi in Ezhou city, Hubei | 32 |
| YN | Wild population from Lake Yuni in Gongan city, Hubei | 32 |
| SS | Wild population from Lake Laohe in Shishou city, Hubei | 32 |
| JL | Wild population from Lake Laojianghe in Jianli city, Hubei | 32 |
| GA | Domesticated population from one fish farm in Gongan city, Hubei | 32 |
| HX | Domesticated population from one fish farm in Huanxin in Tianjin municipality | 32 |
| PJ | Genetically improved population (Pujiang No. 1 selected strain $F_7$) from the Fish Germplasm Experimental Station, Shanghai Ocean University | 32 |

blunt snout bream (Pujiang No. 1 selected strain $F_7$) were collected from the Fish Germplasm Experimental Station of Shanghai Ocean University (PJ; 32 individuals). A small piece of caudal fin from each fish was clipped and stored in 95% ethanol until DNA extraction. Genomic DNA was extracted using the phenol-chloroform procedure following Sambrook & Russell (2001).

**Microsatellite analysis**

Genetic variation was assayed in all populations using 17 microsatellite loci. Details of PCR conditions and references for all microsatellite loci are provided in Table 2. PCR amplifications were performed in a final reaction volume of 10 μL containing 30 ng of genomic DNA, 1 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl, 50 mmol/L KCl,0.2 mmol/L of each dNTPs, 0.35 μmol/L of each forward and reverse primers, and 1.25 U *Taq* DNA Polymerase (Promega). Loci were amplified using a thermal cycler (Eppendorf Mastercycler). The conditions were as follows: a pre-denaturation at 94°C for 5 min followed by 40 cycles of 30 s at 94°C, 30 s at the appropriate annealing temperatures listed in Table 2, 30 s extension at 72°C, and concluded with a 10 min final extension at 72°C. PCR products were then electrophoresed on an 8% nondenaturing polyacrylamide gel and visualized by the silver staining method of Sambrook & Russell (2001) with minor modifications. A pBR322 DNA/*Msp*I Marker (Tiangen) was used as a size standard for polyacrylamide gel.

**Statistical analyses**

For each population and locus, the number of alleles ($A$), effective number of alleles ($N_e$), expected heterozygosity ($H_e$) and observed heterozygosity ($H_o$) were calculated using PopGene v1.32 (Yeh et al, 1997). A nonparametric analysis of variance (Mann-Whitney U test) (Sokal & Rohlf, 1995) was used to test for differences between measures of genetic diversity ($H_e$) between domesticated and wild populations. Genetic differentiation between populations was evaluated by pairwise estimates of $F_{ST}$ values (Weir & Cockerham, 1984) and their significance tested through bootstrapping analysis (1000 replicates) using ARLEQUIN v.3.0 (Excoffier et al, 2005). To examine genetic relationships among populations, a matrix of pairwise $D_A$ distances (Nei et al, 1983) was calculated using DISPAN (Ota, 1993) and allele frequencies as input data. The $D_A$ distance measure was chosen because it is independent of mutation models (Nei, 1987) and superior to other distance measures in correct tree topology construction using microsatellites (Takezaki & Nei, 1996). A phylogenetic tree topology based on the $D_A$ distance was constructed using the neighbor-joining method (Saitou & Nei, 1987). This procedure was done using MEGA v3.1 (Kumar et al, 2004). Polymorphism information content (*PIC*) was calculated according to the following formula (Botstein et al, 1980):

$$PIC = 1 - \left( \sum_{i=1}^{n} P_i^2 \right) - \left( \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2 \right)$$

where, $n$ is the number of alleles at one locus; and $P_i$ and $P_j$ are the frequencies of the $i$th and $j$th alleles at one locus, $j=i+1$.

## RESULTS

**Genetic variability**

Genetic variability indices for the wild, domesticated and genetically improved populations of *M. amblycephala* are summarized in Table 3. The number of alleles per locus varied from 2−17, and the effective number of alleles per locus ranged from 1.28−12.19 across all populations. The mean effective number of alleles per locus for LZ was 5.33, YN 4.77, PJ 4.60, JL 4.59, SS 4.36, GA 3.68 and HX 3.20.

**Table 2 The 17 microsatellite markers used in this study**

| Locus | Repeat motif | Primer sequence (5′-3′) | Annealing temperature (°C) | Allele size range (bp) | Reference/GenBank Accession no. |
|---|---|---|---|---|---|
| TTF01* | $(CA)_{21}$ | F: TGGAGATGAAAGCTGAAGGAA<br>R: ATGCACGAACTGCCACATAA | 55.9 | 238−328 | FJ168686 |
| TTF02* | $(CA)_5(CT)_{21}$ | F: AAACAGCTGCTACCCTTGGA<br>R: TTTGCCAGAAGAGCAAATCA | 55.9 | 196−228 | FJ168687 |
| TTF03* | $(TC)_{27}$ | F: AAGACGCCACGGAAACTTTA<br>R: CTGACCGGATAGCAAAGTGA | 56.4 | 214−266 | FJ168688 |
| TTF04* | $(CA)_{14}$ | F: GACTGGAGTCGTCAGGCTTC<br>R: TGCCCCACATTGTTAGACTG | 60.5 | 182−228 | FJ168689 |
| TTF05* | $(CA)_{15}$ | F: CTAGTGGGTAGGTGGCAGGT<br>R: TGACTGGGAGAGACAGAGGAG | 60.5 | 164−188 | FJ168690 |
| TTF06* | $(GA)_{13}$ | F: GGCAGGTCAGGCACATTTAT<br>R: TCTCTACCTCACATTCTCTCATTCT | 60.5 | 186−218 | FJ168691 |
| TTF07* | $(GT)_{13}$ | F: ATGGGTAAGCCGATGGATTC<br>R: GTGTCAGCATTCCAGCTCCT | 60.5 | 285−331 | FJ168692 |
| TTF08* | $(GT)_{18}$ | F: GGGGAAATAAAGGGAGAAAGTG<br>R: TTTCTCCTGATCCGTTGACC | 60.5 | 178−224 | FJ168693 |
| TTF09* | $(TC)_{19}$ | F: AAGACGCCACGGAAACTTTA<br>R: GAGGTGGGACTGTGTGGAAT | 56.9 | 269−319 | FJ168694 |
| TTF10* | $(TC)_6(TG)_5$ | F: AAACAGGCTCGCCAATTTC<br>R: TCACCCACACACTCTTATTCTCTC | 55.9 | 255−291 | FJ168695 |
| MAC21HLJ# | $(CA)_{47}$ | F: TTTCCCAGTTCAGTCGGT<br>R: CAAGCAAATCAAGCCATC | 53 | 145−251 | Li et al, 2006 |
| MAC31HLJ# | $(GT)_{26}$ | F: GCA TCGGTAACAGTCAAA<br>R: CAGGGATAATGTAGGAAGAA | 50 | 174−242 | Li et al, 2006 |
| MAC46HLJ# | $(GT)_{12}$ | F: TACAAGAGCAGGTAAGCA<br>R: CAGCCACTGACTGAACAT | 48 | 176−204 | Li et al, 2006 |
| MAC50HLJ# | $(GT)_{16}$ | F: GGTATCGTGCTTGCTTGT<br>R: TTCCATTTAGAGCTACGG | 51 | 176−200 | Li et al, 2006 |
| MAC53HLJ# | $(GT)_{28}$ | F: AGCGGGTCTGTGCTAATC<br>R: CGGCCAGTTCCAAAGAGT | 54 | 179−251 | Li et al, 2006 |
| MAC56HLJ# | $(GT)_{29}$ | F: TTCAACGGCGAGACTCAA<br>R: CGACTACACCTGGAACCTG | 56 | 102−158 | Li et al, 2006 |
| Mam25† | $(AC)_5(AC)_{14}$ | F: TCACACCAACAACACCGAAT<br>R: CCTTGTTTTCTCCAGGCATC | 62 | 174−212 | DQ996307 |

*, Loci developed by Tang et al, 2009; #, Loci developed by Li et al, 2006; †, Loci developed by Li et al, 2007.

Pairwise *P* values from expected heterozygosity and effective number of alleles test were calculated for all pairs of populations in Table 4. There was no difference in the effective number of alleles per locus between the four wild populations (all $P>0.05$) or between the two domesticated populations ($P=0.333$). The effective number of alleles for the two domesticated populations (GA and HX) was lower than the wild populations LZ, YN and JL and the genetically improved population (PJ). No difference in the effective number of alleles was detected between wild (LZ, YN, JL and SS) and genetically improved populations (PJ) (all $P>0.05$).

Mean expected heterozygosity values were 0.6501 and 0.6596 in the two domesticated populations, respectively, and ranged from 0.7330−0.7805 in the four wild populations. The $H_e$ value was 0.7185 in the genetically improved population. Compared with the wild populations LZ, SS and JL, expected heterozygosities were reduced in both domesticated populations ($P<0.05$). No differences were found between the four wild populations or the two domesticated populations (all $P>0.05$). The $H_e$ value of Pujiang No. 1 was higher than the two domesticated populations and lower than all four wild populations.

**Genetic differentiation**

Matrices of pairwise multilocus $F_{ST}$ estimates are provided in Table 5. Most genetic differentiation was distributed between the domesticated populations and wild populations ($F_{ST}$ range 0.1123−0.1206), except for

**Table 3 Genetic diversity of wild, domesticated and genetically improved populations of blunt snout bream**

| Locus | Parameter | Wild populations | | | | Domesticated populations | | Genetically improved strain |
|---|---|---|---|---|---|---|---|---|
| | | LZ | YN | JL | SS | GA | HX | PJ |
| TTF01 | *A* | 15 | 13 | 11 | 16 | 10 | 6 | 13 |
| | $N_e$ | 10.24 | 10.67 | 7.88 | 10.67 | 8.26 | 3.41 | 10.67 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.7500 | 1.0000 |
| | $H_e$ | 0.9315 | 0.9355 | 0.9012 | 0.9355 | 0.9073 | 0.7298 | 0.9355 |
| | *PIC* | 0.8945 | 0.8987 | 0.8602 | 0.8992 | 0.8665 | 0.6718 | 0.8987 |
| TTF02 | *A* | 12 | 9 | 4 | 3 | 8 | 5 | 9 |
| | $N_e$ | 9.85 | 5.22 | 2.64 | 2.33 | 5.39 | 3.18 | 5.22 |
| | $H_o$ | 1.0000 | 1.0000 | 0.7500 | 0.7500 | 1.0000 | 0.7500 | 1.0000 |
| | $H_e$ | 0.9274 | 0.8347 | 0.6411 | 0.5887 | 0.8407 | 0.7077 | 0.8347 |
| | *PIC* | 0.8895 | 0.7856 | 0.5637 | 0.4956 | 0.7897 | 0.6503 | 0.7856 |
| TTF03 | *A* | 11 | 10 | 4 | 7 | 3 | 2 | 10 |
| | $N_e$ | 7.88 | 6.83 | 2.96 | 2.37 | 1.55 | 1.68 | 6.83 |
| | $H_o$ | 0.5625 | 0.6250 | 0.6875 | 0.3750 | 0.2500 | 0.0625 | 0.6250 |
| | $H_e$ | 0.9012 | 0.8810 | 0.6835 | 0.5968 | 0.3649 | 0.4173 | 0.8810 |
| | *PIC* | 0.8602 | 0.8370 | 0.6136 | 0.5531 | 0.3094 | 0.3225 | 0.8370 |
| TTF04 | *A* | 7 | 8 | 9 | 8 | 4 | 8 | 8 |
| | $N_e$ | 5.02 | 5.33 | 8.00 | 5.75 | 3.20 | 3.32 | 5.02 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.7500 | 1.0000 |
| | $H_e$ | 0.8266 | 0.8387 | 0.9032 | 0.8528 | 0.7097 | 0.7218 | 0.8266 |
| | *PIC* | 0.7748 | 0.7874 | 0.8619 | 0.8026 | 0.6299 | 0.6550 | 0.7742 |
| TTF05 | *A* | 8 | 5 | 11 | 8 | 4 | 4 | 4 |
| | $N_e$ | 3.63 | 4.03 | 7.01 | 4.38 | 3.20 | 3.20 | 2.88 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| | $H_e$ | 0.7480 | 0.7762 | 0.8851 | 0.7964 | 0.7097 | 0.7097 | 0.6734 |
| | *PIC* | 0.6826 | 0.7087 | 0.8424 | 0.7398 | 0.6299 | 0.6299 | 0.5862 |
| TTF06 | *A* | 6 | 3 | 4 | 3 | 3 | 3 | 3 |
| | $N_e$ | 3.71 | 1.76 | 3.22 | 2.52 | 2.18 | 1.86 | 1.76 |
| | $H_o$ | 0.5625 | 0.4375 | 0.6875 | 0.8750 | 0.5625 | 0.1250 | 0.4375 |
| | $H_e$ | 0.7540 | 0.4456 | 0.7117 | 0.6230 | 0.5585 | 0.4758 | 0.4456 |
| | *PIC* | 0.6836 | 0.3777 | 0.6305 | 0.5336 | 0.4642 | 0.3977 | 0.3777 |
| TTF07 | *A* | 3 | 3 | 3 | 5 | 3 | 5 | 3 |
| | $N_e$ | 2.28 | 2.93 | 2.61 | 3.08 | 2.65 | 3.74 | 2.61 |
| | $H_o$ | 0.8125 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.8125 | 1.0000 |
| | $H_e$ | 0.5786 | 0.6794 | 0.6371 | 0.6976 | 0.6431 | 0.7560 | 0.6371 |
| | *PIC* | 0.4962 | 0.5845 | 0.5439 | 0.6255 | 0.5520 | 0.6882 | 0.5439 |
| TTF08 | *A* | 5 | 4 | 5 | 4 | 3 | 3 | 4 |
| | $N_e$ | 3.88 | 3.74 | 3.66 | 3.48 | 2.12 | 2.12 | 2.68 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.9375 |
| | $H_e$ | 0.7661 | 0.7560 | 0.7500 | 0.7359 | 0.5464 | 0.5464 | 0.6472 |
| | *PIC* | 0.6999 | 0.6835 | 0.6816 | 0.6635 | 0.4185 | 0.4185 | 0.5559 |
| TTF09 | *A* | 7 | 6 | 5 | 4 | 2 | 6 | 6 |
| | $N_e$ | 4.65 | 2.64 | 3.10 | 2.89 | 1.28 | 2.12 | 2.64 |
| | $H_o$ | 0.7500 | 0.6875 | 0.7500 | 0.6875 | 0.0000 | 0.2500 | 0.6875 |
| | $H_e$ | 0.8105 | 0.6411 | 0.6996 | 0.6754 | 0.2258 | 0.5444 | 0.6411 |
| | *PIC* | 0.7602 | 0.5795 | 0.6383 | 0.6019 | 0.1948 | 0.4908 | 0.5795 |

(continued)

| Locus | Parameter | Wild populations | | | | Domesticated populations | | Genetically improved strain |
|---|---|---|---|---|---|---|---|---|
| | | LZ | YN | JL | SS | GA | HX | PJ |
| TTF10 | *A* | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | $N_e$ | 2.50 | 1.89 | 2.50 | 2.26 | 2.77 | 1.99 | 1.89 |
| | $H_o$ | 0.8125 | 0.5000 | 0.8125 | 0.5625 | 0.9375 | 0.5000 | 0.5000 |
| | $H_e$ | 0.6190 | 0.4859 | 0.6190 | 0.5746 | 0.6593 | 0.5141 | 0.4859 |
| | *PIC* | 0.5151 | 0.4163 | 0.5151 | 0.4565 | 0.5665 | 0.4356 | 0.4163 |
| MAC21HLJ | *A* | 17 | 15 | 14 | 11 | 17 | 9 | 15 |
| | $N_e$ | 11.13 | 12.19 | 10.45 | 7.64 | 10.67 | 7.01 | 12.19 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 0.8750 | 1.0000 | 0.4375 | 1.0000 |
| | $H_e$ | 0.9395 | 0.9476 | 0.9335 | 0.8972 | 0.9355 | 0.8851 | 0.9476 |
| | *PIC* | 0.9037 | 0.9120 | 0.8966 | 0.8560 | 0.8995 | 0.8413 | 0.9120 |
| MAC31HLJ | *A* | 6 | 6 | 8 | 8 | 6 | 7 | 6 |
| | $N_e$ | 5.39 | 5.95 | 6.10 | 6.17 | 3.71 | 5.75 | 5.95 |
| | $H_o$ | 0.8125 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.5000 | 1.0000 |
| | $H_e$ | 0.8407 | 0.8589 | 0.8629 | 0.8649 | 0.7540 | 0.8528 | 0.8589 |
| | *PIC* | 0.7873 | 0.8088 | 0.8149 | 0.8177 | 0.6862 | 0.8029 | 0.8088 |
| MAC46HLJ | *A* | 5 | 5 | 5 | 7 | 2 | 5 | 5 |
| | $N_e$ | 3.76 | 4.57 | 2.91 | 3.44 | 2.00 | 3.74 | 4.57 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.9375 | 1.0000 |
| | $H_e$ | 0.7581 | 0.8065 | 0.6774 | 0.7319 | 0.5161 | 0.7560 | 0.8065 |
| | *PIC* | 0.6947 | 0.7455 | 0.5965 | 0.6655 | 0.3750 | 0.6919 | 0.7455 |
| MAC50HLJ | *A* | 3 | 2 | 3 | 2 | 3 | 3 | 2 |
| | $N_e$ | 1.91 | 1.52 | 2.40 | 1.88 | 2.25 | 2.12 | 1.52 |
| | $H_o$ | 0.6250 | 0.4375 | 0.8750 | 0.7500 | 1.0000 | 1.0000 | 0.4375 |
| | $H_e$ | 0.4919 | 0.3528 | 0.6028 | 0.4839 | 0.5726 | 0.5464 | 0.3528 |
| | *PIC* | 0.4275 | 0.2834 | 0.5048 | 0.3589 | 0.4555 | 0.4185 | 0.2834 |
| MAC53HLJ | *A* | 6 | 4 | 5 | 5 | 3 | 4 | 4 |
| | $N_e$ | 2.84 | 2.50 | 2.78 | 2.93 | 2.46 | 2.83 | 2.50 |
| | $H_o$ | 0.7500 | 0.8750 | 0.8750 | 0.9375 | 1.0000 | 1.0000 | 0.8750 |
| | $H_e$ | 0.6694 | 0.6190 | 0.6613 | 0.6794 | 0.6129 | 0.6673 | 0.6190 |
| | *PIC* | 0.6071 | 0.5429 | 0.5941 | 0.6084 | 0.5112 | 0.5805 | 0.5429 |
| MAC56HLJ | *A* | 10 | 9 | 9 | 8 | 9 | 4 | 9 |
| | $N_e$ | 7.11 | 4.49 | 7.31 | 6.40 | 6.24 | 2.56 | 4.49 |
| | $H_o$ | 0.9375 | 1.0000 | 0.9375 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| | $H_e$ | 0.8871 | 0.8024 | 0.8911 | 0.8710 | 0.8669 | 0.6290 | 0.8024 |
| | *PIC* | 0.8440 | 0.7471 | 0.8481 | 0.8250 | 0.8221 | 0.5301 | 0.7471 |
| Mam25 | *A* | 6 | 6 | 4 | 6 | 4 | 6 | 6 |
| | $N_e$ | 4.83 | 4.83 | 2.56 | 5.89 | 2.56 | 3.71 | 4.83 |
| | $H_o$ | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| | $H_e$ | 0.8185 | 0.8185 | 0.6290 | 0.8569 | 0.6290 | 0.7540 | 0.8185 |
| | *PIC* | 0.7633 | 0.7633 | 0.5301 | 0.8064 | 0.5301 | 0.6862 | 0.7633 |
| Average | *A* | 7.65 | 6.53 | 6.29 | 6.35 | 5.12 | 4.88 | 6.47 |
| | $N_e$ | 5.33 | 4.77 | 4.59 | 4.36 | 3.68 | 3.20 | 4.60 |
| | $H_o$ | 0.8603 | 0.8566 | 0.904 | 0.8713 | 0.8676 | 0.6985 | 0.8529 |
| | $H_e$ | 0.7805 | 0.7341 | 0.7464 | 0.7330 | 0.6501 | 0.6596 | 0.7185 |
| | *PIC* | 0.7226 | 0.6742 | 0.6786 | 0.6652 | 0.5706 | 0.5830 | 0.6564 |

A, number of alleles; $N_e$, effective number of alleles; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity; *PIC*, polymorphism information content.

**Table 4 *P*-values from expected heterozygosity (below diagonal) and effective number of alleles (above diagonal) tests between wild (LZ, YN, JL and SS), domesticated (GA and HX) and genetically improved (PJ) populations of blunt snout bream**

| Populations | LZ | YN | SS | JL | GA | HX | PJ |
|---|---|---|---|---|---|---|---|
| LZ | | 0.141 | 0.114 | 0.248 | 0.001** | 0.004** | 0.054 |
| YN | 0.072 | | 0.367 | 0.728 | 0.018* | 0.011* | 0.080 |
| SS | 0.122 | 0.971 | | 0.550 | 0.130 | 0.027* | 0.605 |
| JL | 0.237 | 0.720 | 0.504 | | 0.043* | 0.013* | 0.986 |
| GA | 0.012* | 0.096 | 0.048* | 0.021* | | 0.333 | 0.043* |
| HX | 0.006** | 0.051 | 0.011* | 0.016* | 0.792 | | 0.025* |
| PJ | 0.015* | 0.088 | 0.630 | 0.442 | 0.169 | 0.117 | |

*$P < 0.05$; **$P < 0.01$.

**Table 5 Pairwise $F_{ST}$ values (above diagonal) and $D_A$ distance (below diagonal) among wild (LZ, YN, JL and SS), domesticated (GA and HX) and genetically improved (PJ) populations of blunt snout bream**

| population | LZ | YN | SS | JL | GA | HX | PJ |
|---|---|---|---|---|---|---|---|
| LZ | | 0.0749 | 0.0565 | 0.0536 | 0.1127* | 0.1161* | 0.1136* |
| YN | 0.1888 | | 0.0405 | 0.0487 | 0.0329 | 0.1206* | 0.1229* |
| SS | 0.1633 | 0.1237 | | 0.0307 | 0.1123* | 0.1179* | 0.1350* |
| JL | 0.1565 | 0.1358 | 0.0938 | | 0.1158* | 0.1144* | 0.1451* |
| GA | 0.3241 | 0.1136 | 0.3168 | 0.3453 | | 0.1185* | 0.1196* |
| HX | 0.3369 | 0.3583 | 0.3482 | 0.3278 | 0.3495 | | 0.1107* |
| PJ | 0.3295 | 0.3694 | 0.4479 | 0.4524 | 0.3532 | 0.3128 | |

*$P<0.05$.

YN and GA ($F_{ST}$=0.0329). Differentiation between the four wild populations was relatively low ($F_{ST}$ range 0.0307−0.0749). Estimated $F_{ST}$ values between PJ and other population pairs deviated from zero ($P<0.05$). Nonsignificant $F_{ST}$ values were found among the four wild populations (all $P>0.05$). Pairwise comparison of domesticated populations revealed significant $F_{ST}$ values ($P<0.05$). Significant $F_{ST}$ values were detected between two domesticated populations and the genetically improved population. All estimates of relative genetic differentiation (pairwise $F_{ST}$) between wild and domesticated populations were significant except for YN vs GA ($F_{ST}$=0.0329).

Genetic distances ($D_A$) were calculated for all possible pairs of populations (Table 5) and $D_A$ measures ranged from 0.0938−0.4524. The smallest estimate for $D_A$ was between JL and SS, and the highest estimate was between JL and PJ. The neighbor-joining tree generated from the $D_A$ values is shown in Figure 2. The seven populations fell into two clusters: one cluster comprising the domesticated (HX) and genetically improved (PJ) populations, and another cluster comprising all wild and one domesticated (GA) population.

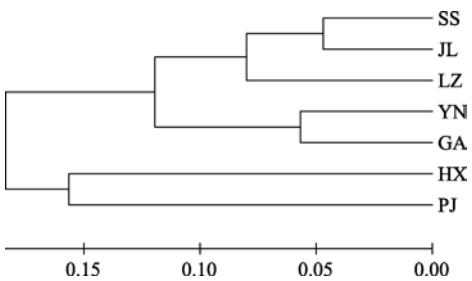


Figure 2 NJ phylogenetic dendrograms based on $D_A$ distance in wild (LZ, YN, JL and SS), domesticated (GA and HX) and genetically improved (PJ) populations of blunt snout bream

## DISCUSSION

Blunt snout bream are distributed in relatively narrow natural areas and prior to the 1960s were endemic to a few medium and large-sized lakes along the middle reaches of the Yangtze River. Since 1964, blunt snout bream have been transplanted to many parts of the country for aquaculture purposes and after domestication, many closed breeding groups were formed, including one genetically improved population produced by mass selection. Developments in production technology and

biotechnology have altered the genetic composition of blunt snout bream and resulted in a small number of excellent individuals on one hand, and a loss of genetic diversity and a unique genetic resource on the other. Given this important juncture in blunt snout bream aquaculture, a survey of the relationships between domesticated and genetically improved animals and the conservation and management of genetic resources was overdue.

Here, the population genetic structure of four wild populations, two domesticated populations and a genetically improved population was analyzed using 17 microsatellite markers. The results show that human intervention has had a strong impact on the genetic structure of these fishes. There was no difference in the heterozygosity and genetic differentiation index ($F_{ST}$ values) among the four wild populations. One reason for this may be that Lakes Liangzi, Yuni, Laohe and Laojianghe are subsidiary lakes of the middle reaches of the Yangtze River, and distances between each of them are no greater than 300 km. Historically, these lakes were connected to the Yangtze River by channels and we cannot exclude the possibility that gene flow between the four wild populations has occurred via migration or that these wild populations originated from the same population. An alternative explanation is that despite the fact that these lakes have undergone great change, the genetic structure of wild populations has been readjusted through natural selection in the last 50 years.

We did detect genetic differentiation between wild populations and domesticated populations of blunt snout bream. Artificial and natural selection in the aquaculture environment may have changed the overall allelic composition of domesticated fish relative to wild fish (Eknath & Doyle, 1985). It is well known that heterozygosity reflects genetic variation (Nei et al, 1975) and it is the primary parameter used in the genetic management of populations (Leberg, 1992). Typically, the higher heterozygosity one population contains, the stronger its capacity to adapt to and tolerate environmental stressors and variation. The expected heterozygosities in the domesticated populations (GA and HX) were lower than the wild populations LZ, SS and JL. Reduced heterozygosity of microsatellites in domesticated animals have been reported in many species, including grass carp (*Ctenopharyngodon idella*) (Zhang et al, 2006), turbot (*Scophthalmus maximus*) (Coughlan et al, 1998), common carp (*Cyprinus carpio*) (Desvignes et al, 2001), Atlantic salmon (*Salmo salar*)(Norris et al, 1999) and Pacific abalone (*Haliotis discus hannai*)(Li et al, 2004), attributable to population bottlenecks due to a small number of effective parents. The reason why heterozygosity in the GA domesticated population was not lower than the YN wild population may be because domesticated individuals have escaped from fish farms and reproduced with wild individuals in Lake Yuni and assuming that GA domesticated individuals entering the YN wild population would reduce the latter's genetic variability (see Clifford et al, 1998). Reduced genetic variability in natural populations reduces the long-term adaptability of individuals and species and thereby lowers the buffering capacity against environmental change (Utter et al, 1993). Erosion of genetic variability could also bring about an increase in homozygous individuals carrying deleterious alleles that lead to a reduction in fitness (Caughley, 1994).

Genetic differences between the two domesticated populations were also found and may have resulted from different founder populations and selection procedures. The HX domesticated population resulted from wild individuals introduced from Lake Liangzi and has undergone seven generations of closed artificial breeding in the Tianjin Huanxin Fish Breeding Farm since the 1970s. The GA domesticated population is the progeny of wild individuals from Lake Yuni, but the course of artificial propagation for this population remains unknown. Of note is that the GA fish farm is adjacent to Lake Yuni and it is likely that domesticated individuals have escaped into the lake or have been released into the lake; wild individuals also have a chance to be introduced into the fish farm. In addition to founder effects, different artificial and natural selection pressures in the different culture environments may have changed the overall allelic composition of the GA and HX domesticated populations.

The fact that the average expected heterozygosity of the genetically improved strain Pujiang No. 1 was lower than the four wild populations, but higher than the domesticated populations, is due to the following three principles applied during the selection process. First, a large base population (>500 individuals) was used to avoid the genetic bottlenecks. Second, a larger effective breeding population (>200 individuals) was created to minimize the inbreeding coefficient (one-thousandth or less). Third, the overall selection ratio was 0.04% of offspring whereby the high-intensity selection from fry

to adult fish ensures the retention of the best individuals (Li, 1988). Two of the most important characteristics of the genetically improved population after 15 years of mass selection are that crucial economic traits such as growth rate and body shape have been significantly improved and relatively high levels of genetic diversity have been maintained for further selective breeding.

Genetic variation among late-selected strains and wild populations of blunt snout bream have previously been analyzed by inter-simple sequence repeat (ISSR) markers (Zhao et al, 2009). Our results further demonstrate that the genetically improved population has undergone significant genetic differentiation from the wild population and comprises fairly stable hereditary characteristics because of long-term selection of over nine generations. However, there remains distance from the plateau of selection. As with the management of crop, livestock and poultry genetic resources, the main purpose of fish genetic resource management is to maintain genetic variation of wild, domesticated and genetically improved populations for the later establishment of new strains. The existing strains must adapt to new conditions to meet the changing demands of consumption and production.

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