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# GENETIC DIVERSITY OF THE CAPTIVE CHINESE POND TURTLE (*MAUREMYS REEVESII*) POPULATIONS IN CHINA ASSESSED BY MICROSATELLITE MARKERS

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## ABSTRACT

The Chinese pond turtle (*Mauremysreevesii*) is one of the most important commercial and medicinal chelonians in China. In this study, 87Chinese pond turtle individuals were collected to represent five captive populations of this species in China. Twelve polymorphic microsatellite markers were used to evaluate the genetic diversity of five cultured populations, and it revealed that the level of genetic diversity was relatively high in these cultured populations. Except for Mclw03, Mclw06 and Mmu1 in the GZ population, all microsatellite loci of the other four populations did not deviated from Hardy–Weinberg equilibrium. The analysis of molecular variance (AMOVA) revealed that 80.7% of the genetic variation within populations and 19.3% among populations. Pair wise $F_{ST}$ further showed that there was moderate to high degree of genetic differentiation between populations. Phylogenetic analysis separated the Chinese pond turtle samples in the present study into two clusters, with the first group included Guangzhou, Nanning and Haikou populations and the second group consist of individuals from two populations from Wuhu. Our results demonstrated that different degrees of inbreeding existed in five captive populations and it is urgent to raise a new superior breeding population with high genetic diversity using turtles from different captive populations.

**Keywords:** Chinese pond turtle; *Mauremysreevesii*; Microsatellite; Genetic diversity; Genetic variation.

## INTRODUCTION

Most of Asian turtles are facing extinction nowadays because of destruction of their natural habitat and over-hunting; it is imperative to take effective conservation strategies and captive breeding program to protect the endangered and threatened turtles (Fong *et al*., 2007; Bu *et al*., 2014; Gong *et al*., 2018; Lee *et al*., 2019).The Chinese pond turtle (*Mauremysreevesii*) is mainly distributed in China, Japan and Korea (Zhou and Li, 2013). This species has been recorded as resources for food, the pet trade and traditional Chinese medicine in different regions of the world. Every last part of the Chinese pond turtle has medicinal value, including their meat, heads, eggs, shells and even their blood, bile, etc. In particular, turtle shells (gui ban) have a long history of application in traditional Chinese medicine, which had been recorded in ancient medical books such as shennong's herbal and compendium of materiamedica (Lan *et al*., 2012).However, the population size, particular the wild populations of Chinese pond turtle has been largely shortened because of long-term over-exploitation and habitat destruction (Xu *et al*., 2014). It has been listed as an endangered species in the IUCN Red List as of 2010 (http://www.iucnredlist.org) and the China Red Data Book of Endangered Animals (Zhao, 1998).

At present, it is difficult to find the Chinese pond turtle in the wild. While the wild populations of Chinese pond turtle are becoming scarce, many turtle farms have been built rapidly in China. At the same time, the genetic pedigree does not be constructed in many captive populations of *Mauremysreevesii*, which easily leads to inbreeding. Currently, the studies on Chinese pond turtle mainly focused on nutrition, reproductive behavior, artificial hatching and toxicology, etc. (Yang *et al*., 2004; Xu *et al*., 2014; Huo *et al*., 2017; Nakamuta *et al*., 2018; Xiong *et al*., 2019). However, little is known about the genetic diversity and gene flow of cultured Chinese pond turtles. Zhu *et al*. (2005) have observed a high genetic diversity of wild Chinese pond turtle population from Hubei province using the RAPD technique.

In the present study, twelve stable and effective microsatellite markers were screened out from the 31 microsatellite markers of the Chinese pond turtle that have been developed, thereby the 12 microsatellite markers were used to investigate the genetic diversity and population differentiation of five captive populations (from the east and south China). The results of population genetic diversity in this study are conducive to scientific guidance on genetic pedigree construction for the Chinese pond turtle captive populations. In addition, it could also be helpful in the selecting of proper individuals for the reintroduction program of *Mauremysreevesii* in the future.

## MATERIALS AND METHODS

***M. reevesii* samples and DNA extraction:** A total of 87Chinese pond turtle individuals were collected from Guangzhou (GZ, Guangdong province, n = 22), Nanning (NN, Guangxi province, n = 8), Haikou (HK, Hainan province, n = 15), Wuhu1 (LT, Anhui province, n = 20) and Wuhu2 (LH, Anhui province, n = 22) (Figure 1). The isolation of total genomic DNA from tail muscle tissues was performed using a Genomic DNA Extraction Kit (TIANGEN, China).All animal experiments in this study were approved by the Animal Ethics Committee of Anhui Normal University (approval number: # 20170515).

**PCR amplification and genotyping:** The genetic diversity of the five captive populations was assessed using 12 microsatellite markers. Among them, six pairs of primers (Cre22, Cre37, Cre46, Cre107, Mmu1, Mmu2) were retrieved from Ye *et al*. (2009), and the remaining six pairs of primers (Mclw01, Mclw02, Mclw03, Mclw04, Mclw06, Mclw08) were newly developed by this study (Table 1). Forward primers were further labeled with a fluorescent dye (TAMRA, HEX or FAM) at its 5' end. The PCR system and thermal cycling conditions were performed according to Bu *et al*. (2017). The amplified fragments were separated and analyzed using an ABI PRISM 3730 Genetic Analyzer. Microsatellite alleles were precisely sized employing the GeneMarker1.65 (Applied Biosystems).

**Microsatellite data analysis:** Popgene 1.32 (Yeh and Boyle 1997) was used to estimate the number of alleles ($N_A$) and effective number of alleles ($N_E$) , and CERVUS 3.0 (Kalinowski *et al.,* 2007) was employed to calculate the polymorphic information content (PIC), expected heterozygosity ($H_E$) and observed heterozygosity ($H_O$). GENETIX 4.05 (Belkhir *et al*., 2004) was used to assess Inbreeding coefficients ($F_{IS}$), Fixation index ($F_{ST}$) and Gene flow (*N*m)were estimated using. GENEPOP 4.2 (Raymond and Rousset, 1995) was employed to estimate Potential deviation from Hardy–Weinberg equilibrium (HWE).

To test the genetic differentiation among and within populations, Arlequin 3.5 (Excoffier and Lischer, 2010) was employed to perform an analysis of molecular variance (AMOVA). Populations 1.2.31 (http://bioinformatics.org/~tryphon/populations/) was employed to construct two un-weighted pair group method with arithmetic mean (UPGMA) dendrograms. The phylogenetic tree of Chinese pond turtles was built according to the Nei's genetic distance ($D_A$) (Nei *et al*., 1983) with 1000 bootstraps.

## RESULTS

**Genetic diversity of five captive populations:** The genetic diversity of the five cultured populations was assessed using 12 polymorphic microsatellite loci (Table 2).A total of 138 alleles were detected in 87 individuals, with the average number of alleles per locus ($N_A$) was 11.5 (range: 4-23) and the effective number of alleles ($N_E$) ranged from 2.3731 to 6.8747 (average 4.0220). The average observed heterozygosity (*H*o) ranged from 0.391 to 0.678 (average 0.497), which are comparative lower than the expected heterozygosity ($H_E$) that ranged from 0.582 to 0.860 (with an average of 0.725). Except for Mclw03, Mclw06 and Mmu1 in the GZ population, all microsatellite loci were consistent with Hardy–Weinberg equilibrium in the other four populations.

**phylogenetic analysis and Population genetic differentiation:** The pair wise $F_{ST}$ values ranged between 0.0950 and 0.2766 (Table 3, below the diagonal), and all of the captive populations showed significant genetic differences between each other ($F_{ST} > 0.05$). The smallest genetic differentiation was observed between the GZ and NN populations ($F_{ST} = 0.0950$), which had reached a moderate differentiation level ($0.05 \leq F_{ST} \leq 0.15$). The highest genetic differentiation was found between the HK and LT populations ($F_{ST} = 0.2766$). Consistent with the observed high pairwise $F_{ST,}$ the pairwise *N*m values varied from 0.6538 to 2.3805 (Table 3, above the diagonal), suggesting that there is limited gene flow among populations.

The results of the AMOVA indicated that 19.3% of the genetic variation was between the populations, with 80.7% within populations (Table 4). This indicated that genetic variation among the five captive populations of Chinese pond turtle was significant ($F_{ST} = 0.193$, $p \leq 0.05$), showing that these populations are differentiating.

The genetic distances ($D_A$) obtained among populations showed that the minimum $D_A$ value (0.2762) was found between LT and LH populations, whereas the maximum $D_A$ value (0.5308) was identified between HK and LT populations (Table 5).

The UPGMA tree showed that the 87Chinese pond turtles formed different two groups (Figure 2), with the first cluster formed by LT and LH populations, and the another cluster included individuals from the GZ, NN and HK populations(Figure 3), which coincides with the sampling geography of the five Chinese pond turtle populations.

**Intra-population heterozygosity and analysis of inbreeding coefficient:** $F_{IS}$ and heterozygosity of Chinese pond turtle were estimated for each population by12 microsatellite loci (table 6). The average expected heterozygosity ($H_E$) per population was higher than its average observed heterozygosity ($H_O$), implying a loss of heterozygosity in the captive populations. The $F_{IS}$ values (ranged from 0.119 to 0.265), which measure the deficiency of heterozygotes within populations, further suggested that various degrees of inbreeding occurred in

the five captive populations ($H_E > H_O$, $F_{IS} > 0$).

**Table 1. The information of twelve polymorphic microsatellite loci**

| Locus | Repeat motif | Primer sequence(5′-3′) | Ta(ºC) | Allele size(bp) | GenBank accession No. |
|---|---|---|---|---|---|
| Mclw01 | $(GT)_{10}$ | TCCTATACCCAGTGGGACATGAAGTTTCACCCATCCATCAGC | 52.9 | 165-185 | JF712880 |
| Mclw02 | $(CA)_{13}$ | ACTGTGCCGGGTCGTGATGGGGTGGTGTCTGAGTGTTCTTGC | 58 | 255-265 | JF712881 |
| Mclw03 | $(TG)_{11}$ | GTGGTGGCACAGAGGTAGTTGCTCACATTTTCAGTTTGGTTA | 57.5 | 216-290 | JF712882 |
| Mclw04 | $(TG)_{11}$ | GAAAGGCTGCTGCTCACCACGACTGACCCAACCCTCCCTGCT | 57.5 | 194-228 | JF712883 |
| Mclw06 | $(GT)_{17}$ | GGGCTATTTCATTGCTGTCGTCCTTCAAATGCCACC | 57.5 | 284-322 | JF712885 |
| Mclw08 | $(TG)_{13}$ | AGCTCCTCCAGGAACTAAAAAAACCAAAGTCTTTCCAACC | 57.5 | 239-303 | JF712887 |
| Cre22 | $(CA)_{8}$ | GTCCGTGGGTCACATACTAGAGACGCCATTCCTTTA | 52.5 | 388-424 | EU825727 |
| Cre37 | $(CT)_{7}G(AC)_{9}$ | GCTGGTTGTGTCTCACTTGACCCTGCCTTTGCTTATTC | 52 | 414-448 | EU825728 |
| Cre46 | $(AC)_{11}$ | ACATACAACTTACACAAGCGACAAAATGCAGACTACA | 55 | 296-314 | EU825730 |
| Cre107 | $(TG)_{19}$ | AGCAACAGCAAAATGAAGAGAGGGATAAGGCAAAGA | 50 | 377-393 | EU825732 |
| Mmu1 | $(CA)_{12}$ | CCCCACTTTTTACCAGCCCCAAATGTTGCCACAATCTA | 59 | 261-287 | EU062888 |
| Mmu2 | $(AC)_{14}$ | GCTACTGCCAAGAATACCAGTGTTTTCCTTCCCCCTC | 52 | 260-280 | EU062889 |

**Table 2. Genetic diversity at 12 microsatellite loci in five captive populations.**

| | Mclw01 | Mclw02 | Mclw03 | Mclw04 | Mclw06 | Mclw08 | Cre22 | Cre37 | Cre46 | Cre107 | Mmu1 | Mmu2 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GZ | | | | | | | | | | | | | |
| $N_A$ | 8 | 3 | 14 | 6 | 9 | 10 | 7 | 14 | 4 | 3 | 6 | 11 | 7.9 |
| $N_E$ | 2.4261 | 1.6269 | 6.7692 | 2.5340 | 5.0681 | 5.6608 | 2.6376 | 8.5664 | 3.0345 | 2.4694 | 3.7812 | 6.2857 | 4.2383 |
| $H_O$ | 0.545 | 0.318 | 0.682 | 0.409 | 0.364 | 0.727 | 0.591 | 0.864 | 0.409 | 0.727 | 0.409 | 0.773 | 0.568 |
| $H_E$ | 0.601 | 0.394 | 0.872 | 0.619 | 0.821 | 0.842 | 0.635 | 0.904 | 0.686 | 0.609 | 0.753 | 0.860 | 0.717 |
| *PIC* | 0.560 | 0.327 | 0.839 | 0.558 | 0.776 | 0.804 | 0.583 | 0.872 | 0.608 | 0.526 | 0.689 | 0.824 | 0.664 |
| *P* | 0.5899 | 0.6431 | 0.0009 | 0.0102 | 0.0000 | 0.1110 | 0.7241 | 0.0979 | 0.0044 | 0.0062 | 0.0022 | 0.7174 | 0.2423 |
| NN | | | | | | | | | | | | | |
| $N_A$ | 5 | 2 | 9 | 3 | 9 | 3 | 3 | 3 | 2 | 2 | 5 | 4 | 4.2 |
| $N_E$ | 2.7234 | 2.000 | 7.1111 | 2.6122 | 6.7368 | 1.4713 | 1.9104 | 2.7234 | 1.9692 | 1.8824 | 2.9091 | 3.2821 | 3.1110 |
| $H_O$ | 0.625 | 0.500 | 0.875 | 0.250 | 0.750 | 0.125 | 0.375 | 0.375 | 0.375 | 0.250 | 0.500 | 0.750 | 0.479 |
| $H_E$ | 0.675 | 0.533 | 0.917 | 0.658 | 0.908 | 0.342 | 0.508 | 0.675 | 0.525 | 0.500 | 0.700 | 0.742 | 0.640 |
| *PIC* | 0.599 | 0.375 | 0.845 | 0.544 | 0.835 | 0.294 | 0.427 | 0.556 | 0.371 | 0.359 | 0.595 | 0.645 | 0.537 |
| *P* | 0.5129 | 1.0000 | 0.2155 | 0.0428 | 0.3000 | 0.0707 | 0.2818 | 0.1993 | 0.5302 | 0.2140 | 0.1177 | 0.8544 | 0.3616 |
| HK | | | | | | | | | | | | | |
| $N_A$ | 7 | 2 | 6 | 2 | 2 | 4 | 2 | 3 | 3 | 2 | 3 | 5 | 3.4 |
| $N_E$ | 2.9605 | 1.9912 | 2.4064 | 1.9912 | 1.9231 | 2.3077 | 1.8000 | 2.3810 | 2.1127 | 1.7241 | 2.0179 | 3.3088 | 2.2437 |
| $H_O$ | 0.600 | 0.667 | 0.667 | 0.267 | 0.400 | 0.667 | 0.267 | 0.600 | 0.333 | 0.200 | 0.400 | 0.467 | 0.461 |
| $H_E$ | 0.685 | 0.515 | 0.605 | 0.515 | 0.497 | 0.586 | 0.460 | 0.600 | 0.545 | 0.434 | 0.522 | 0.722 | 0.557 |
| *PIC* | 0.630 | 0.374 | 0.523 | 0.374 | 0.365 | 0.512 | 0.346 | 0.513 | 0.419 | 0.332 | 0.407 | 0.643 | 0.453 |
| *P* | 0.6021 | 0.3277 | 0.2055 | 0.1167 | 0.6003 | 0.7420 | 0.2287 | 0.4280 | 0.1210 | 0.0566 | 0.7433 | 0.0737 | 0.3538 |
| LT | | | | | | | | | | | | | |
| $N_A$ | 11 | 4 | 13 | 7 | 2 | 2 | 2 | 4 | 5 | 2 | 4 | 2 | 4.8 |
| $N_E$ | 4.8193 | 3.1873 | 6.5041 | 1.9465 | 1.9560 | 1.9950 | 1.9802 | 2.0672 | 2.1978 | 1.9550 | 2.1918 | 1.9950 | 2.7363 |
| $H_O$ | 0.750 | 0.600 | 0.800 | 0.600 | 0.350 | 0.450 | 0.400 | 0.450 | 0.400 | 0.550 | 0.450 | 0.450 | 0.521 |
| $H_E$ | 0.813 | 0.704 | 0.868 | 0.499 | 0.501 | 0.512 | 0.508 | 0.529 | 0.559 | 0.512 | 0.558 | 0.512 | 0.589 |
| *PIC* | 0.776 | 0.628 | 0.830 | 0.463 | 0.369 | 0.374 | 0.372 | 0.427 | 0.462 | 0.374 | 0.460 | 0.374 | 0.493 |
| *P* | 0.2644 | 0.1123 | 0.5431 | 1.0000 | 0.2071 | 0.6709 | 0.3965 | 0.0559 | 0.1697 | 1.0000 | 0.4196 | 0.6698 | 0.4591 |
| LH | | | | | | | | | | | | | |
| $N_A$ | 10 | 2 | 2 | 5 | 2 | 2 | 3 | 3 | 4 | 2 | 2 | 6 | 3.6 |
| $N_E$ | 3.0440 | 1.9360 | 1.9360 | 1.7286 | 1.9959 | 1.9635 | 2.6233 | 2.0379 | 2.1851 | 1.9836 | 1.5414 | 3.8566 | 2.2360 |
| $H_O$ | 0.818 | 0.273 | 0.273 | 0.409 | 0.318 | 0.409 | 0.455 | 0.545 | 0.409 | 0.364 | 0.364 | 0.591 | 0.436 |
| $H_E$ | 0.687 | 0.495 | 0.495 | 0.431 | 0.511 | 0.502 | 0.633 | 0.521 | 0.555 | 0.507 | 0.359 | 0.758 | 0.538 |
| *PIC* | 0.652 | 0.367 | 0.367 | 0.403 | 0.374 | 0.370 | 0.539 | 0.401 | 0.455 | 0.373 | 0.290 | 0.705 | 0.441 |
| *P* | 0.9061 | 0.0714 | 0.0705 | 0.2162 | 0.0984 | 0.4176 | 0.0619 | 0.6420 | 0.0508 | 0.2181 | 1.0000 | 0.0915 | 0.3204 |

**Note:** $N_A$, number of alleles; $N_E$, effective number of alleles; $H_o$, observed heterozygosity; $H_E$, expected heterozygosity; *PIC*, polymorphism information content; *P*, values for exact tests of Hardy–Weinberg.

**Table 3. Pair wise estimation of Fixation index ($F_{ST}$, below the diagonal) and gene flow ($Nm$, above the diagonal) among five captive Chinese pond turtle populations based on the twelve microsatellite loci.**

| Population | GZ | NN | HK | LT | LH |
|---|---|---|---|---|---|
| GZ | | 2.3805 | 1.6325 | 0.9595 | 1.1191 |
| NN | 0.0950 | | 1.7468 | 0.8582 | 0.9112 |
| HK | 0.1328 | 0.1252 | | 0.6538 | 0.7262 |
| LT | 0.2067 | 0.2256 | 0.2766 | | 1.3028 |
| LH | 0.1826 | 0.2153 | 0.2561 | 0.1610 | |

**Table 4. Hierarchical AMOVA analysis of fivecaptive populations of Chinese pond turtle.**

| Source of variation | Sum of squares | Variance components | Percentage variation |
|---|---|---|---|
| Among populations | 136.55 | 0.87674 | 19.3 |
| Within populations | 616.12 | 3.66589 | 80.7 |
| Total | 752.67 | 4.54263 | 100 |

**Table 5. Genetic distance ($D_A$) of five captive populations of Chinese pond turtle**

| Population | GZ | NN | HK | LT | LH |
|---|---|---|---|---|---|
| GZ | | | | | |
| NN | 0.3376 | | | | |
| HK | 0.3152 | 0.2999 | | | |
| LT | 0.4772 | 0.5087 | 0.5308 | | |
| LH | 0.3737 | 0.3992 | 0.4104 | 0.2762 | |

**Table 6. $F_{IS}$ and heterozygosity calculated for each population from twelve microsatellite loci in Chinese pond turtle.**

| Population | $N$ | $H_O$ | $H_E$ | $F_{IS}$ | 95% CI |
|---|---|---|---|---|---|
| GZ | 22 | 0.568 | 0.717 | 0.211 | 0.108-0.268 |
| NN | 8 | 0.479 | 0.640 | 0.265 | -0.010-0.338 |
| HK | 15 | 0.461 | 0.557 | 0.177 | -0.008-0.259 |
| LT | 20 | 0.521 | 0.589 | 0.119 | 0.016-0.173 |
| LH | 22 | 0.436 | 0.538 | 0.194 | 0.079-0.259 |

**Note:** $N$, population size; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $F_{IS}$, inbreeding coefficient; CI, Confidence interval.

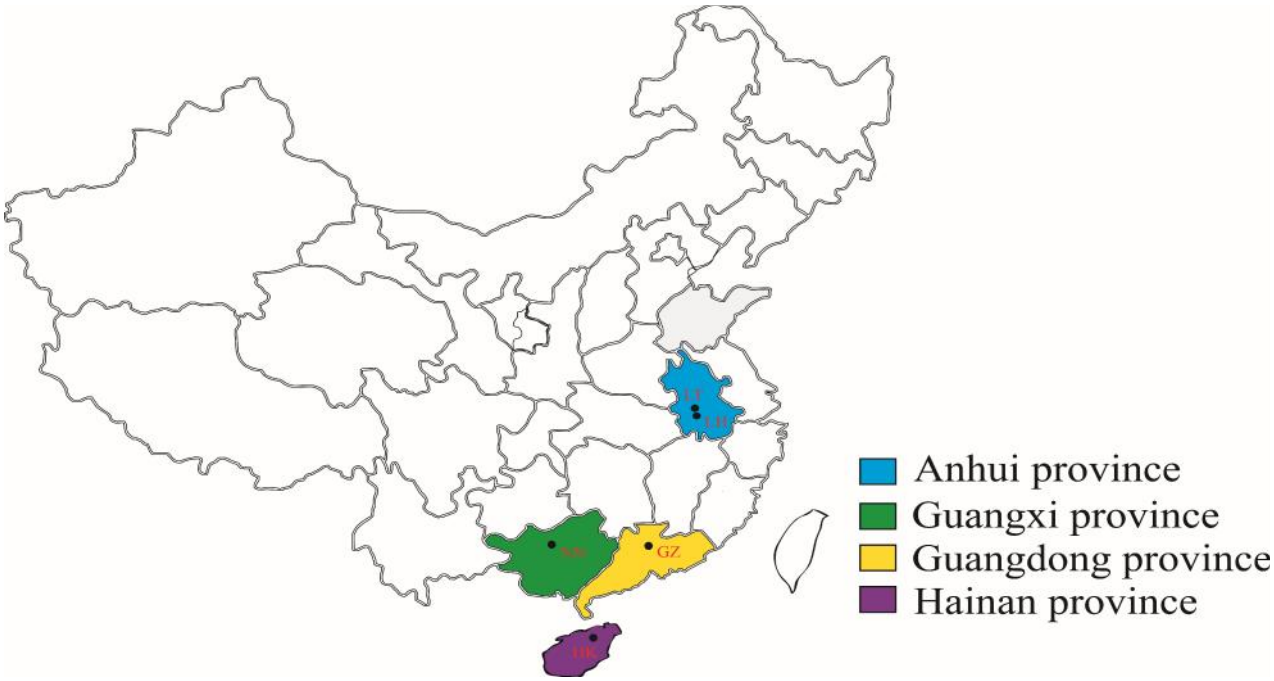


**Figure 1. Sampling locations of captive Chinese pond turtle populations used in the present study. GZ: Guangzhou; NN: Nanning; HK: Haikou; LT: Wuhu1; LH: Wuhu2.**

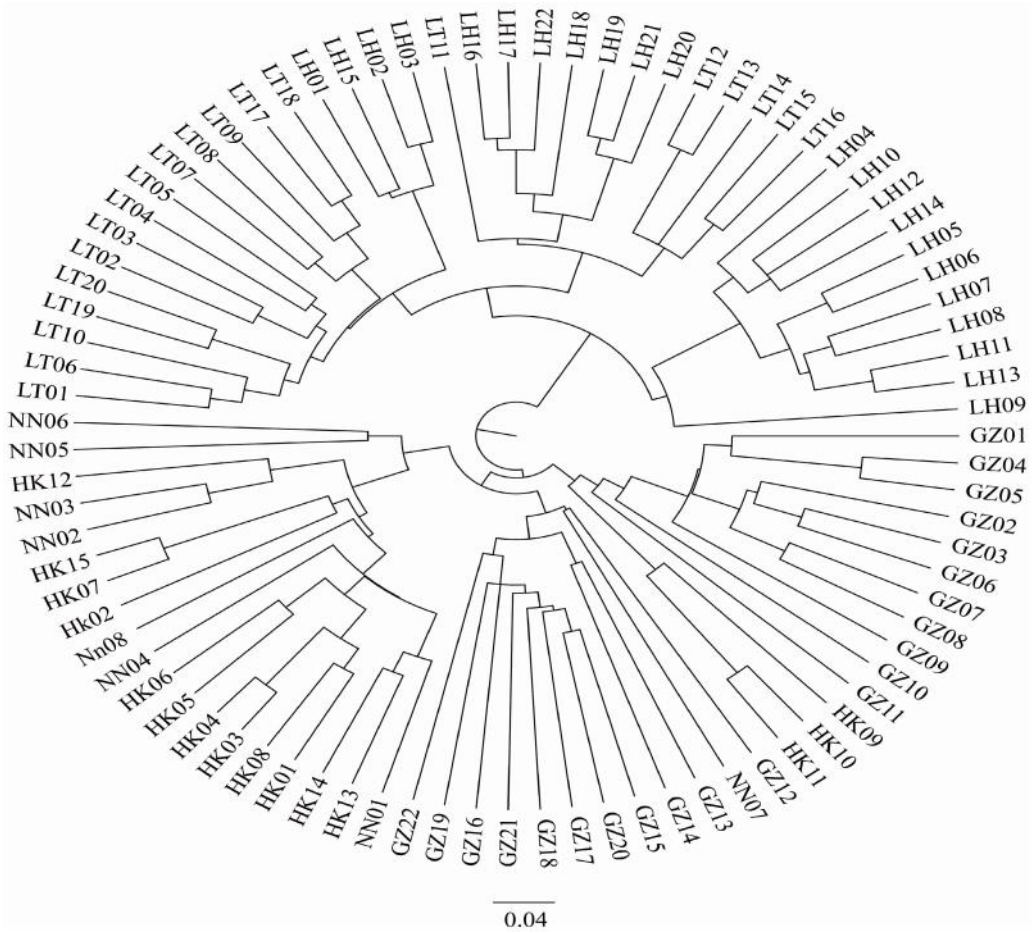


**Figure 2.Thedendrogram of the 87 Chinesepond turtles constructed according to the $D_A$.**

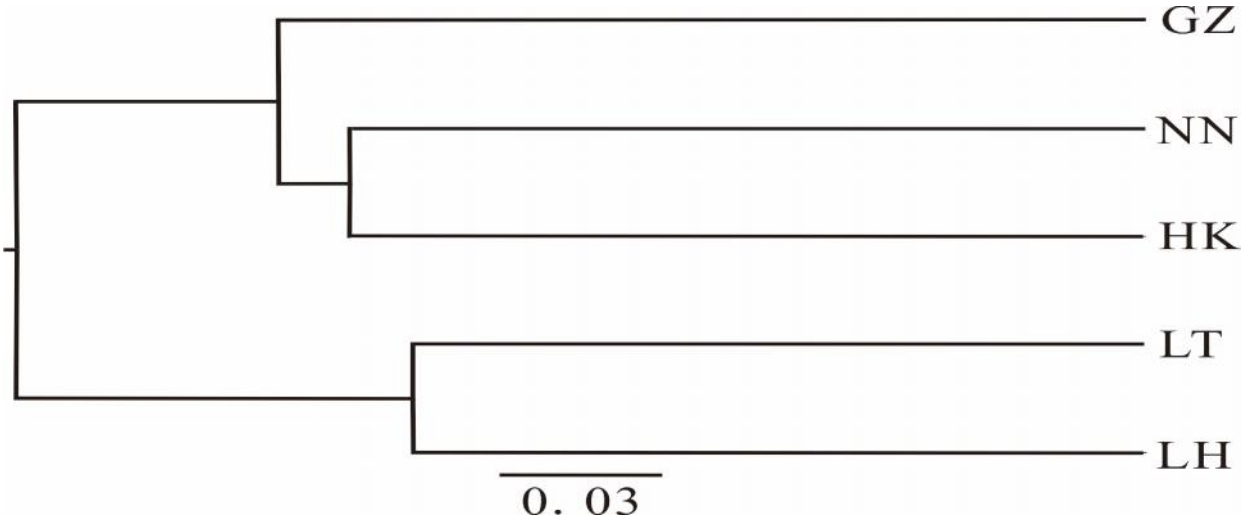


**Figure3. The phylogenetic tree of the five captive populations of Chinese pond turtle. GZ: Guangzhou population; NN: Nanning population; HK: Haikou population; LT: Wuhu1population; LH: Wuhu2 population.**

## DISCUSSION

Microsatellites are often used as an effective molecular marker in the study of genetic structure and diversity of animal and plant populations because of their co-dominant inheritance, high polymorphism, wide distribution in the genome and easy detection, etc. (Bu *et al*., 2014; Sun *et al*., 2015; Butiuc-Keul1 *et al*., 2018). Furthermore, the results of this study also indicate that a set of microsatellite markers can effectively assess the genetic structure of the Chinese pond turtle populations. In the study of population genetic structure, the PIC is one of the key indicators to measure the genetic variation and polymorphism of a marker locus in a population. The informativeness level of the locus is high (PIC > 0.5), moderate (0.25 < PIC < 0.5), and low (PIC < 0.25) (Botstein *et al*., 1980). In this research, the PIC values estimated in each captive population were highly polymorphic (0.517- 0.838), suggesting the 12 loci were sufficient for the evaluating genetic diversity in the cultured Chinese pond turtle populations.

Generally, the sample size has a strong influence on the observed heterozygosity (*Ho*), while the expected heterozygosity ($H_E$) was not sensitive to sample size variation and can more accurately estimate population genetic diversity (Bao *et al*., 2007). In this research, the average $H_E$ of five captive populations was 0.608, indicating that all five captive populations had relatively high level of genetic diversity. In previous studies, different molecular markers (microsatellite and RAPD) were used to evaluate the genetic diversity in captive populations of the Chinese pond turtle and found that they had higher levels of genetic diversity, which is consistent with our findings (Zhu *et al*.,2005; Zhang *et al*., 2010; Zhu,2011). However, the estimated heterozygosity of Chinese pond turtle in our study is lower than that ($H_E$, 0.74) of Zhang *et al*. (2010), suggesting that the genetic diversity of the population may have decreased along with the culture time. Our results also revealed that inbreeding has occurred in captive populations of Chinese pond turtle. Such inbreeding could lead to the heterozygote deficiency of population, the lower genetic diversity and the lower ability of adaptation (Edwards *et al*., 2014).The level of genetic diversity is an important reference indicator for the ability of organisms to adapt to the environment, especially for the sustainable development and utilization of cultured resources. Moreover, higher levels of genetic diversity among farmed populations facilitate to enhance their ability dealing with environmental change, artificial selection, etc (Min *et al*., 2015).Therefore, turtle farmers should pay more attention to the information of heterozygosity and genetic diversity in their cultured populations, and try to maintain the high level of heterozygosity and genetic diversity by choosing optimal breeding pairs from different captive populations.

Previous studies have shown that there was no or low genetic differentiation between populations for the Chinese pond turtle (Zhang *et al*., 2010; Zhu, 2011).However, our study revealed significant population differentiation among the five captive populations. In fact, all the captive populations have been selectively bred for many years. Long-term artificial selection could have caused the differentiation among these five captive populations, meanwhile, founder effect, geographical isolation, environmental condition and management patterns could also have contributed to this significant differentiation (Edwards *et al*., 2014; Yang *et al*., 2015; Wang *et al*., 2016).

In conclusion, our results indicated that long-term artificial propagation has reduced the genetic diversity of Chinese pond turtle and led to significant population

differentiation. Therefore, it is imperative to take effective strategies to restore the genetic diversity in the captive population. Our results provided valuable information for the conservation and management of the Chinese pond turtle in future.

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