Characterization of Single Nucleotide Polymorphism Marker in the Chinese Giant Salamander Andrias davidianus

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ABSTRACT

Background: Chinese giant salamander Andrias davidianus is called “baby fish” in China. Due to its palatability and nutritional value, it was widely cultured in China. The current study was to evaluate the genetic diversity of the farmed Andrias davidianus.

Methods: 30 salamanders were collected from farm and DNA was extracted. Thirty contigs potentially containing SNPs were selected from the previously developed RAD-seq library. The primer was designed and PCR was performed, than the products was sequenced from different direction. At last the sequences were analyzed using the vector NTI 10.

Result: 5824bp high-quality sequences embodied in 16 contigs were sequenced and eighty SNP loci were identified which each SNP locus exhibited bi-alleles. The effective allele number from 1.03 to 2.00 and the minor allele frequency was 0.017 to 0.500. The expected heterozygosity was from 0.0333 to 0.5091 and the observed heterozygosity was 0.0333 to 1.00 00. Sixty-six of the 80 loci significantly deviated from the Hardy-Weinberg equilibrium (P < 0.05). The results provide useful genomic resources to evaluate the genetic diversity of Andrias davidianus for conservation and aquaculture.

Key words: Andrias davidianus, Amphibian, Genetic diversity, SNP marker.

INTRODUCTION

Chinese giant salamander Andrias davidianus was widely distributed in China in history, but because of habitat destruction and harvesting, the wild population has sharply decreased (Zhang et al., 2002). Since the 1980s, International Union for Conservation of Nature classified Chinese giant salamander as the endangered species. Artificial propagation for conservation and, because of its high nutritional value, for commercial purposes has increased (Li et al., 2012). Recent studies of have focused on Andrias davidianus development and the immune system (Hu et al., 2017; Pei et al., 2018), but research on genetics is limited to studies of a few microsatellites, amplified fragment length polymorphisms (AFLP) and mitochondrial sequences (Murphy et al., 2000; Yang et al., 2011; Meng et al., 2012). Single nucleotide polymorphism (SNP) markers result from nucleotide substitutions distributed throughout the genome. The SNP marker can detect the polymorphisms which cannot be found by other molecular markers (Liu and Cordes, 2004). Consequently, the SNP marker was widely employed in analysis of genetic diversity (Visser et al., 2016; Ba et al., 2017; Bhaladhare et al., 2020; Praneeth et al., 2020). In Atlantic salmon, SNP markers were investigated by whole genome sequencing to analyze the associations between genotype and phenotype (Yañez et al., 2016). An SNP chip technology was used to analyze the genetic variation in Angora goat populations from different areas (Visser et al., 2016).

The purpose of present study is to analyze the genetic structure and genetic dynamics of the farmed Andrias davidianus using an SNP marker developed in an earlier study. The results expand information on the population structure to aid breeding aimed at conserving the species.

MATERIALS AND METHODS

Animal population

Thirty salamanders which were apparently healthy, were collected from Zhejiang Yongqiang Chinese Giant Salamander Limited Company. The salamanders with age ranging from 1 to 3 years and weighing 40-300g were selected for this study. The salamanders used in this study was narcotized by MS222 and then killed according to Yangtze River Fisheries Research Institute Care Committee (no.2013001). The muscle sample from each individual was collected and immediately preserved in absolute ethanol for DNA extraction. Genomic DNA was extracted using TIANamp Genomic DNA kit (Tiangen Biotech, Beijing, China) following manufacturer’s instructions. The DNA quality and concentration were identified by 1% agarose gel and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).
SNP identification and genotyping

Thirty contigs potentially containing SNPs were selected from the previously developed RAD-seq library and Primer Premier 5.0 software was employed to designed the primers according to the sequences (Table S1). Polymerase chain reaction (PCR) was carried out on ABI Veriti 96 well Thermal Cycl with a volume of 25 μL including 10 μL doubled-distilled H₂O, 0.5 μL 10 uM forward/reverse primer, 13 μL 2×PCR Mix (Dongsheng Biotech, Guangdong, China) and 1 μL (50–100 ng/μL) genomic DNA. PCR reaction was performed according the following programs: 94°C 5 min; 32 cycles of 94°C 30 s, primer-specific temperature for 30 s, 72°C 45 s; at last 72°C 5 min. To determine the quality of the product, 5ul were analyzed by agarose gel electrophoresis. When the product exhibited a specific and clear band, the product was sequenced from different directions using the ABI 3730 automated DNA sequencer. The sequences from the same individual were assembled using software Vector NTI 10. The genotype per locus was identified by the peak and the colors. If the locus had double peaks with different color, the locus was considered as SNP. The POPGENE 1.31 software was used to evaluate the parameters minor allele frequency, observed heterozygosity, expected heterozygosity, observed number of alleles, effective number of alleles and Chi square tests for Hardy-Weinberg equilibrium (Yeh et al. 1999).

RESULTS AND DISCUSSION

Population variation played an important role in genetic diversity. It played important role for species to adapt the various environments. In present study, 80 SNP loci were validated (Fig 1) and distributed in seventeen contigs containing 5824 bp and the SNP density was 73 bp per locus (Table 1). In Rana dalmatina, a single SNP locus of 100 bp was confirmed from transcriptome sequence (Vences et al., 2013). In Portunus pelagicus, one SNP locus was observed per 93 bp (Miao et al. 2017) and in Scylla paramamosain, every 146 bp of genomic DNA contains one SNP locus (Feng et al., 2014). In the previous study andrias davidianus reported containing a complex genome of about 50G (Geng et al., 2017). Recently study showed that salamander consists of at least five species-level lineages in China (Yan et al., 2018). Twenty years ago, the farm was established and the parent salamanders were collected from Zhejiang, Hebei, Guizhou and Shanxi provinces. Taken together, the offspring of the salamanders exhibited high SNP density.

In this study, 80 SNP loci which 59 substitutions were transition type containing 29 A/G and 30 C/T and 21 substitutions were transversion type containing 5 A/C, 6 A/T, 3 C/G and 7 T/G were collected and bi-allelic polymorphism was observed in total of 160 alleles in the 30 specimens (Table 1). Genetic diversity of each locus was analyzed with the observed heterozygosity was 0.0333 to 1.0000 and the expected heterozygosity was 0.0333 to 0.5091. From previous study using a microsatellite marker, the observed heterozygosity was 0.250 to 1.000 and the expected heterozygosity was 0.553 to 0.784 (Meng et al., 2012). The reason for the different results might be the type of marker used and the limited number of specimens. Sixty-six out of eighty loci showed that these loci significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$). Similarly, in P. pelagicus, 44 of 91 SNP loci exhibited that significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$), possibly due to a limited number of specimens and small sample collection area (Vences et al., 2013). The present study sharply increased the genetic information of the endangered Chinese giant salamander. Our results were also useful for evaluating the genetic structure of the farmed and wild salamander population, Furthermore, the SNP data could be used for development of conservation and breeding plans.

![Fig 1: View of SNP genetic type. A: T/C SNP genotype, B: no SNP genotype.](image-url)
## Table 1: Diversity value of the 80 SNP loci of *Andrias davidianus* from 30 individuals.

| SNP_ID         | PCR primer                                | PCR product size(bp) | SNP position | Allele | MAF      | No/Ne   | $H_o$ | $H_e$ | HW  |
|----------------|-------------------------------------------|----------------------|--------------|--------|----------|---------|-------|-------|-----|
| scaffold4071   | GCCAGCATGCTGTCAGACCA                      | 238                  | 107          | C/T    | 0.150    | 2.00/1.34 | 0.3000 | 0.2593 | 0.366 |
|                | CACCAAGTTCAGTGCAAGTCGAA                   |                      |              |        |          |         |       |       |      |
| scaffold607    | CCAACAACCATACATACATACACACAA               | 402                  | 229          | C/T    | 0.0167   | 2.00/1.03 | 0.0333 | 0.0333 | 1.000 |
|                | TCTTGGTCACCTGCGGACAT                    |                      |              |        |          |         |       |       |      |
| scaffold2614   | CAGAAGGGATCCAAACATCTTCTCA                | 221                  | 119          | C/G    | 0.250    | 2.00/1.60 | 0.3667 | 0.3814 | 0.828 |
|                | TGCTTTATAGAGCGCATGCTGTGCTGCT            |                      |              |        |          |         |       |       |      |
| scaffold2614   | CAGAAGGGATCCAAACATCTTCTCA                | 221                  | 129          | C/T    | 0.017    | 2.00/1.03 | 0.0333 | 0.0333 | 1.000 |
|                | TGCTTTATAGAGCGCATGCTGTGCTGCT            |                      |              |        |          |         |       |       |      |
| scaffold2614   | CAGAAGGGATCCAAACATCTTCTCA                | 221                  | 130          | T/G    | 0.017    | 2.00/1.03 | 0.0333 | 0.0333 | 1.000 |
|                | TGCTTTATAGAGCGCATGCTGTGCTGCT            |                      |              |        |          |         |       |       |      |
| RAD_209        | TAAGTCAGAGGGACCTTACCAG                   | 324                  | 270          | A/G    | 0.383    | 2.00/1.90 | 0.5667 | 0.4808 | 0.318 |
|                | GGTATCATTCCCTATCAGCATCT                  |                      |              |        |          |         |       |       |      |
| RAD_209        | TAAGTCAGAGGGACCTTACCAG                   | 324                  | 272          | A/G    | 0.450    | 2.00/1.98 | 0.5667 | 0.5034 | 0.483 |
|                | GGTATCATTCCCTATCAGCATCT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TCATTGTCCACACTGACCACAAT                  | 424                  | 134          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TAAAGCATGCCCGCGAGCTCA                    |                      |              |        |          |         |       |       |      |
| RAD_003        | TCATTGTCCACACTGACCACAAT                  | 424                  | 140          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TAAAGCATGCCCGCGAGCTCA                    |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 164          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 207          | A/G    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 231          | T/G    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 243          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 252          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5091 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 263          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 264          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 273          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |
| RAD_003        | TAAGCATGGCCCGACGTCA                      | 424                  | 274          | C/T    | 0.500    | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
|                | TCATTGTCCACACTGACCACAAT                  |                      |              |        |          |         |       |       |      |

Table 1: Continue...
### Table 1: Characterization of Single Nucleotide Polymorphism Marker in the Chinese Giant Salamander Andrias davidianus

| Marker | Sequence 1 | Length 1 | Base | Frequency 1 | Length 2 | Frequency 2 | Length 3 | Frequency 3 |
|--------|------------|----------|------|-------------|----------|-------------|----------|-------------|
| RAD_003 | TCATTGTCCACACTGACCACAAAT | 424 | A/C | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | T/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.083 | 2.00/1.83 | 0.1667 | 0.1554 | 0.659 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | C/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.083 | 2.00/1.18 | 0.1667 | 0.1554 | 0.659 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | C/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_011 | GCTCGAATCCCTACAGAGCC | 310 | A/G | 0.083 | 2.00/1.38 | 0.3333 | 0.2837 | 0.364 |
| RAD_194 | AATTGCCTTCAGGTATTAGAGCC | 370 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5091 | 0.000 |
| RAD_194 | AATTGCCTTCAGGTATTAGAGCC | 370 | C/T | 0.050 | 2.00/1.11 | 0.1000 | 0.0966 | 0.815 |
| scaffold2196 | ACTATTGGGAGTTTAGATGGTGTACG | 270 | C/G | 0.167 | 2.00/1.38 | 0.3333 | 0.2837 | 0.364 |
| scaffold1637 | CTACGAATTTAAATCAGCGGTGTC | 304 | T/G | 0.050 | 2.00/1.11 | 0.1000 | 0.0966 | 0.815 |
| scaffold1637 | CTACGAATTTAAATCAGCGGTGTC | 304 | C/T | 0.050 | 2.00/1.11 | 0.1000 | 0.0966 | 0.815 |
| scaffold1637 | CTACGAATTTAAATCAGCGGTGTC | 304 | C/T | 0.050 | 2.00/1.11 | 0.1000 | 0.0966 | 0.815 |
| scaffold1894 | TCTCTCTCAACTGAGTCTCAGAGA | 310 | A/T | 0.050 | 2.00/1.11 | 0.1000 | 0.0966 | 0.815 |
| RAD_029 | GAAAGGAACTCCAGAAGACAGT | 362 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_029 | GAAAGGAACTCCAGAAGACAGT | 362 | A/G | 0.450 | 2.00/1.98 | 0.9000 | 0.5034 | 0.000 |
| RAD_029 | GAAAGGAACTCCAGAAGACAGT | 362 | C/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_029 | GAAGGAACCTCCAGGAATCAGT | 362 | 224 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_029 | GAAGGAACCTCCAGGAATCAGT | 362 | 266 | C/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_029 | GAAGGAACCTCCAGGAATCAGT | 362 | 310 | A/G | 0.417 | 2.00/0.95 | 0.8333 | 0.5044 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 181 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 230 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 251 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 298 | C/T | 0.467 | 2.00/1.99 | 0.9333 | 0.5062 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 150 | A/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 201 | A/C | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 236 | T/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 239 | C/G | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 253 | A/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 262 | A/C | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_132 | GCCCTAATCTGCCACAACACC | 348 | 263 | A/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |
| RAD_320 | GCATGCCAGCTTGCAATG | 338 | 115 | A/C | 0.450 | 2.00/1.98 | 0.9000 | 0.5034 | 0.000 |
| RAD_320 | GCATGCCAGCTTGCAATG | 338 | 151 | A/G | 0.433 | 2.00/1.97 | 0.8667 | 0.4994 | 0.000 |
| RAD_320 | GCATGCCAGCTTGCAATG | 338 | 160 | C/T | 0.433 | 2.00/1.97 | 0.8667 | 0.4994 | 0.000 |
| RAD_320 | GCATGCCAGCTTGCAATG | 338 | 175 | C/T | 0.500 | 2.00/2.00 | 1.0000 | 0.5085 | 0.000 |

Table 1: Continue...
| Marker   | Nucleotide Sequence 1 | Base Change | Base Change Proportion | Heterozygosity | Allele Frequency 1 | Allele Frequency 2 |
|----------|----------------------|-------------|------------------------|----------------|--------------------|--------------------|
| GCA      | CTG                 | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| TGC      | GCATTCCAGAAATGGGTATGG | T/G         | 0.433                  | 2.00/1.97      | 0.8667             | 0.4994             |
| CCG      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| TGG      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| CCG      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| CCG      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
| GGA      | GGAACCAGAATTCGAGAACCTGA | C/T         | 0.400                  | 2.00/1.92      | 0.8000             | 0.4881             |
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