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PROOF
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Development and Validation of 31 SNP Markers of Topmouth Culter, *Culter alburnus* by Whole-Genome Resequencing

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Abstract

Culter alburnus is one of the most important commercial freshwater fishes and widely distributed in China. In this study, a total of 31 novel single nucleotide polymorphism (SNP) markers were developed based on whole-genome resequencing. The analysis of the 31 SNP genotypes in 60 fish showed that the observed heterozygosity and expected heterozygosity ranged from 0.283 to 0.650 and 0.280 to 0.504, respectively. The minor allele frequency ranged from 0.167 to 0.458, and the polymorphism information content ranged from 0.239 to 0.375. Among these SNPs, only one SNP were found to deviate from Hardy-Weinberg equilibrium significantly (P<0.01). These SNP markers could be a valuable tool for population conservation in this species.

Introduction

The topmouth culter (*Culter alburnus*) belongs to the order Cypriniformes, family Xenocyprididae and inhabits the major rivers, lakes, and reservoirs in China (*Li et al.,* 2018). It is the most important commercial freshwater fishes, among the species of the genus *Culter*, *C. alburnus* has the longest body size. Because *C. alburnus* has the advantage of rapid growth, low oxygen tolerance, and disease resistance, hybridization breeding with *Megalobrama amblycephala* became important (National Reg no: GS-02-001-2020). However, wild *C. alburnus* populations are declining and their germplasm resources have been threatened in recent years because of the overfishing and habitat changes caused by environmental pollution and water-related construction (Qi et al., 2013). This has necessitated population genetic investigations on *C. alburnus* to protect its wild resources.

Previous studies have performed valuable genetic evaluations of this species using mitochondrial markers, amplified fragment length polymorphism (AFLP) markers, and simple sequence repeat (SSR) markers (Wang et al., 2007; Chen et al., 2009; Liu et al., 2013; Sun et al., 2021). Wild *C. alburnus* in China has low genetic diversity, and the significant genetic differentiation, in addition, the genetic diversity of wild populations is lower than that of cultured populations (Qi et al., 2013; Qi et al., 2015). The historical effective population size of *C. alburnus* has been found to correlate with the uplift of the Tibetan Plateau, as indicated by demographic history reconstruction through whole-genome

resequencing (Jiang et al., 2023). Single nucleotide polymorphisms (SNPs) are characterized by a wide genomic distribution, high polymorphism levels, and good reproducibility, and have been used in molecular ecology, evolution, and conservation genetics (Vignal et al. 2002; Shan et al., 2022). SNP markers have significant advantages, such as highly abundance, wide distribution, site-specificity, and low error-detection rate, over SSR markers (Zhou et al., 2022). In this study new SNP loci of *C. alburnus* investigated for utilization on genetic evaluation of this specie.

Materials and methods

In 2023, cultured *C. alburnus* samples were randomly selected from the Fishery Research Institute of the Anhui Academy of Agricultural Sciences, Hefei, Anhui, China. The parents of these cultured populations were wild populations originating from the Yangtze and Huaihe Rivers, which are two different water systems in China. Genomic DNA was extracted from the muscle tissue samples collected from 150 individuals using the TlANamp Genomic DNA Kit (DP304, Tiangen, Beijing, China), according to the manufacturer's instructions. DNA quality and concentration were determined using NanoDrop 2100 (Thermo Scientific, DE, USA) and 1.0% agarose-gel electrophoresis. Ninety DNA samples were randomly selected from the 150 samples and sequenced using an Illumina HiSeq 4000 platform. SNP detection was performed by gatk4 (https://gatk. broadinstitute.org/hc/en-us/articles/ 360035890471-Hard-filtering-germline-short-variants), and the SNP filter expression parameters were set as follows: QD<2.0, MQ<40.0, SOR>5.0, MQRankSum<−12.5, FS>60.0, QUAL<30 (DePristo *et al*., 2011). After quality control and strict filtration, 1060.57 Gb of clean data and 9561,979 putative SNPs were obtained. A total of 31 SNPs with high heterozygosity were selected to analyze the polymorphisms in the remaining 60 samples using PCR. A 20 µL PCR reaction volume contained 10 µL of 2×Master Mix, 0.5 µL each of the forward and reverse primers (10 mmol/L), 1 µL of DNA template (20 ng), and 8 µL of dd H_2O. The PCR parameters were as follows: 95°C for 5 min; followed by 32 cycles of 94°C for 30 s, annealing temperature for 40 s, and 72°C for 50 s; and final extension at 72°C for 10 min.

The amplification products were sequenced using an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA), and SNP genotypes were tested and analyzed using DNAMAN V6.0. The observed number of alleles (*Na*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), minor allele frequency (*MAF*), and deviations from the Hardy–Weinberg equilibrium (P_{HWE}) were calculated using the chi-square test with POPGENE 3.2. Polymorphism information content (*PIC*) was estimated from allele frequencies using Cervus 3.0 (Kalinowski *et al*., 2007).

Results and Discussion

A total of 31 SNP markers were polymorphic and bi-allelic and were amplified successfully in the 60 individuals of *C. alburnus* (Table 1). The *Ho*, *He, MAF* and *PIC* of the SNPs were 0.283–0.650, 0.280–0.504, 0.167–0.458, and 0.239–0.375, respectively. The mean *Ho*, *He* and *PIC* were 0.394, 0.362, and 0.292, respectively. These results indicate that cultured *C. alburnus* has a lower genetic diversity. In a previous study, the wild population of *C. alburnus* from Dianshan Lake (Shanghai, China) showed high genetic diversity measured using 11 microsatellite DNA markers; with mean *Ho* and *He* of 0.6288 and 0.7946, respectively (Chen et al., 2009). In addition, among the present SNPs, six loci deviated significantly from Hardy–Weinberg equilibrium (HWE) after Bonferroni adjustments (P<0.05). Several factors, including random mating deviations, mutations, selection, genetic drift, and migration, can disrupt HWE (Zhang et al., 2024). In the current study involving cultured *C. alburnus*, deviations from HWE may be attributed to a lack of heterozygosity stemming from historical founder effects, artificial selection, and the impact of inbreeding (Chen et al., 2017). A study revealed that French rainbow trout (*Oncorhynchus mykiss*) exhibit a relatively high level of inbreeding, which is likely attributable in part to a founder effect and to "sweepstakes reproductive success." (D'Ambrosio et al., 2019). These results suggested that the number of breeding parents should be increased and genetic exchanges among the populations should be promoted to improve the genetic diversity of cultured *C. alburnus* during the subsequent breeding processes. The SNPs identified in this study will be valuable tools for population conservation and management of *C. alburnus.*

Ethical Statement

The experimental protocol was established, according to the ethical guidelines of the Basel Declaration and was approved by the Experimental Animal Welfare and Ethical of Anhui Academy of Agricultural Sciences (NO. AAAS 2024-06).

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Author Contribution

Huan Wang performed the experiment, designed, and analyzed the data, and wrote the original manuscript. Huaxing Zhou and Yuting Hu collected the materials, measured parameters and analyzed the data. Jun Ling and He Jiang collected the materials and

Table 1. Summary information of the 31 SNP markers developed for topmouth culter *Culter alburnus*

Locus	Primer sequences (5'-3')	Mutation type	Amplification length (bp)	*Ho*	*He*	*MAF*	P_{HWE}	*PIC*
JAOTNS010003905	F:AGATCTCGCACAGGTCGATT R:AGGTCAGCGCAGATGTTTCT	A/G	124	0.367	0.323	0.200	0.282	0.269
JAOTNS010003744	F:AACAGCACAGGGATTTCTGC R:ATGACATCCCAGCATCAGTG	A/G	138	0.333	0.484	0.400	0.015*	0.365
JAOTNS010003713	F:ACCTCTGCATCTGCCACTG R:CAGGAGCAGTCTCCATGTCA	T/C	146	0.533	0.495	0.433	0.548	0.371
JAOTNS010003696	F:TTCCACCACTTCATTCACCA R:TGAGTGCTCGACGTAAAACG	T/A	157	0.417	0.352	0.225	0.146	0.288
JAOTNS010003632	F:TTTTGCTGGGACCTGTATCC R:CATATCCTTATGGATGCGCC	T/C	181	0.500	0.424	0.300	0.157	0.332
JAOTNS010003585	F:TCAATCTACAGGCAGGGGTC R:CGCAAAGTCTTGTATTGCCA	T/C	251	0.383	0.313	0.192	0.073	0.262
JAOTNS010003549	F:CTCTACCGGTGAGATGGCTC R:ACAGGATCACTGCCCTTTCA	A/T	182	0.333	0.280	0.167	0.132	0.239
JAOTNS010003508	F:AACCACTGCAGCCTGACTCT R:TCAGTTTGTGCGTGAGCAAT	A/G	177	0.350	0.291	0.175	0.110	0.247
JAOTNS010003484	F:GGACCACCGTAGTGTTCGTT R:TGCTCCACTGTGATTTCCAA	A/T	124	0.300	0.323	0.200	0.579	0.269
JAOTNS010003033	F:ATGTCGACGCTCACACAGAC R:GTGTTGAAAACTGAGGGCGT	A/G	234	0.417	0.333	0.208	0.047*	0.275
JAOTNS010002888	F:GAGAGGATGACAGAGTCGCC R:AACGGGAGTTCAGAGTGGTG	T/C	243	0.333	0.280	0.167	0.132	0.239
JAOTNS010002678	F:ACCTCCTGAACCTCCATGTG R:TGGTACAAGCAAGCGAACAC	A/G	233	0.467	0.361	0.233	0.021*	0.294
JAOTNS010002589	F:ATTCGGACATGCCCTAAGTG R:AGCCTCTCAGGCACCATCTA	T/C	172	0.367	0.504	0.383	0.034*	0.375
JAOTNS010002489	F:GGAGGAACAAACAAACAATGA R:GACCAACAGTGAAGGAACGC	A/G	200	0.383	0.352	0.225	0.479	0.288
JAOTNS010002366	F:TCAGAGGTCGTAGGTGCTGA R:AGCCTGCTCTTCCGTGTCTA	A/G	310	0.383	0.333	0.208	0.230	0.275
JAOTNS010002188	F:AGTGTCTTGGCACCAAAAGG R:GAACCAAAAAGCTCCCTTCC	A/G	218	0.383	0.313	0.192	0.073	0.262
JAOTNS010002055	F:TTTCAAACGTTTCCACTCCC R:GTTTTGAGCGCTGTTGAGTG	A/G	221	0.383	0.313	0.192	0.073	0.262
JAOTNS010001952	F:TTTCAAACGTTTCCACTCCC R:GTTTTGAGCGCTGTTGAGTG	A/G	219	0.400	0.323	0.200	0.059	0.269
JAOTNS010001890	F:GCCTGAAAGTCATAGGTGGC R:GATCTCGCCTTTCACTGGAG	T/C	283	0.400	0.323	0.200	0.059	0.269
JAOTNS010001555	F:AGCTGACAGGAGGGAACAGA R:TACTTGGGTCTGTAACCGGG	G/T	170	0.283	0.493	0.425	0.001*	0.369
JAOTNS010001422	F:GTGGAAAACATCGTGCATTG R:TCCACAATGAAAGCTGAGGA	T/C	157	0.383	0.370	0.242	0.770	0.299
JAOTNS010001200	F:CAGACAGGCTGCATCCAATA R:TCACGTGACCTAAGGCATCA	A/G	310	0.417	0.370	0.242	0.317	0.299
JAOTNS010001167	F:ACATCCATTACAAAACCGGC R:TTTAGATCAGGTTCAGCGGG	A/G	202	0.333	0.342	0.217	0.837	0.282
JAOTNS010000844	F:CTCACTGAGCCATCGGATTT R:CGCCTTACTACCCACCTCTG	A/G	180	0.650	0.501	0.458	0.020*	0.373
JAOTNS010000619	F:TACAAATGACTGCGGGTCAA R:AGCCACATGGATCACCTTTC	A/G	183	0.383	0.333	0.208	0.230	0.275
JAOTNS010000510	F:GTGATGGACACTGGGGTTTT R:CATCCGGAGAGAGACCGTAT	A/G	154	0.333	0.323	0.200	0.795	0.269
JAOTNS010000455	F:ATGATCATGTGGGTGGCTCT R:TTCAGACCGTTGTGCATCTG	A/G	202	0.367	0.323	0.200	0.282	0.269
JAOTNS010000388	F:CTGCGCTACATGCACATTCT R:ATGGAACCCAGCCTAAATCC	A/T	137	0.383	0.370	0.242	0.770	0.299
JAOTNS010000204	F:GAGGTGATTTCTGCATGGGT R:ACGGCTTGGCAGTACAATCT	G/T	172	0.450	0.386	0.258	0.196	0.310
JAOTNS010000034	F:CTCACGAATGTGAACACGGT R:CGACACCCTTGCCTCATTAT	A/T	194	0.417	0.333	0.208	0.250	0.275
JAOTNS010000005	F:GAAGCCCTGACTTTGATGGA R:TGCTAAAATGGCCAGAGGAC	G/T	193	0.383	0.352	0.225	0.479	0.288
Mean±SD	/	/	195.710±48.614	0.394±0.071	0.362±0.067	0.246±0.083	/	0.292±0.040

Note: *Ho* represents the observed heterozygosity, *He* represents the expected heterozygosity, *MAF* represents minor allele frequency, P_{HWE} represents the results for Hardy–Weinberg equilibrium, **P*<0.05, PIC represents polymorphism information content.

corrected English grammar. Guoqing Duan designed, edited and corrected the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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