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Isolation and characterization of 32 microsatellite loci for topmouth culter (*Culter alburnus* Basilewsky)

S.-L. Liu[1,2], Z.-M. Gu[1], Y.-Y. Jia[1], J.-L. Zhao[2], W.-P. Jiang[1], Q. Li[1] and F. Li[1]

[1]Zhejiang Institute of Freshwater Fisheries, HuZhou, China
[2]Key Laboratory of Freshwater Fisheries Germplasm Resource, Shangai Ocean University, Ministry of Agriculture, Shanghai, China

Corresponding author: Z.-M. Gu
E-mail: guzhimin2006@163.com

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ABSTRACT. The topmouth culter (*Culter alburnus*) is an economically important freshwater fish, which is widely distributed throughout large rivers, reservoirs, and lake areas of China. We report here the isolation and characterization of 32 new polymorphic microsatellite loci isolated from genomic DNA in this species enriched by $(CA)_{12}$ and $(GA)_{12}$ probes. The variability of these microsatellites was tested on 30 individuals cultured. The average allele number was 6.6 per locus, ranging from 3 to 12. The observed heterozygosity was from 0.4667 to 0.9000, and the expected heterozygosity was from 0.6163 to 0.9085. After using Bonferroni's correction for multiple tests, there was no evidence of linkage disequilibrium between pairs of loci, but deviations from Hardy-Weinberg equilibrium were found in 3 loci. These microsatellites can be used to study QTL of economic importance, population genetic diversity and the construction of genetic maps for *C. alburnus* in the future.

Key words: *Culter alburnus*; Microsatellite; Genetic diversity

INTRODUCTION

Topmouth culter (*Culter alburnus* Basilewsky) is an economically important fish and is widely distributed in large rivers, reservoirs, and lakes in China (Luo and Chen, 1998). Because of market demand for this fish on the rise, culture production has expanded significantly over the past decades (Wang et al., 2007). The development of molecular genetic markers would seem particularly valuable for these studies, such as the genetic difference between cultured population and wild population, preservation of genetic variability, and prevention of inbreeding depression.

Microsatellites are codominant, highly polymorphic, and ideal for genetic diversity, population structure and genetic mapping studies (Ma et al., 2012; Liu et al., 2013). A few microsatellites are available for *C. alburnus* (Chen et al., 2009; Li et al., 2010; Qi et al., 2013), but unfortunately, the number of SSRs available for this species is limited. In this study, we developed and characterized 32 novel polymorphic microsatellites isolated from the *C. alburnus* genome, which can be useful to describe the levels of genetic diversity and population structure within and between populations in this species, thus providing a powerful tool for identifying conservation priorities, developing management strategies, and facilitating selective breeding.

MATERIAL AND METHODS

Briefly, approximately 300 ng genomic DNA were digested with *Mse*I restriction enzyme (NEB) at 37°C for 2 h. These fragments were ligated to *Mse*I adapters and then amplified by PCR using *Mse*I-N primers (5'-GATGAGTCCTGAGTAAN-3') following the program of 94°C for 4 min, 28 cycles of 94°C for 30 s, 53°C for 1 min and 72°C for 1 min, and 72°C for 5 min. The PCR products were then hybridized with biotinylated probe $(CA)_{12}$ and $(GA)_{12}$ in 300 µL hybridization solution (4X SSC, 0.1% sodium dodecyl sulfate, 0.5 µM probe) at 55°C for 30 min. Subsequent probe-bound DNA fragments were enriched for CA and GA repeats using streptavidin-coated magnetic beads (Promega, Madison, WI, USA) at room temperature for 30 min, followed by two washing steps. Recovered DNA fragments were amplified with *Mse*I-N primer as described above. The PCR products, after being purified with the Gel Extraction kit (Tiangen, Beijing, China), were ligated to pGEM-T vector (Promega) and transformed into *Escherichia coli* DH5α competent cells (Tiangen, Beijing, China). Positive clones were picked out and tested by PCR using *Mse*I-N primers.

Of 150 clones, 128 positive clones with DNA fragments above 400 bp were chosen, cultured with shaking for 6 h (37°C, 300 rpm), and sequenced. After sequence analysis, 105 clones were found to contain microsatellites (with 5 or more repetitions). In total, 52 PCR primer pairs were successfully designed using Primer Premier 5.0 (Premier Biosoft International, CA, USA).

The primer pairs were tested on 30 cultured individuals of *C. alburnus* collected from Hu-Zhou, Zhejiang Province, China. Amplification reactions (10 µL) contained 1X PCR buffer with 1.5 mM $MgCl_2$, 0.1 µM of each primer, 10 µM dNTP, 20 ng DNA, and 0.25 U Taq polymerase (Tiangen). PCR amplification was carried out under the following conditions: pre-denaturation for 3 min at 94°C, then 30 cycles of denaturation at 94°C for 30 s, 58°C for 30 s and extension at 72°C for 30 s, and final extension at 72°C for 5 min. The PCR products were visualized on 8% polyacrylamide gels, followed by silver staining and gel fixation. Sizes of amplified microsatellites were determined by reference to a standard base pair ladder, pUC18 (Tiangen).

The Genepop 4.0 software (Rousset, 2008) was used to calculate the number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy-Weinberg equilibrium. MICRO-CHECKER (Van Oosterhout et al., 2004) was used to infer the most probable technical cause of departure from Hardy-Weinberg equilibrium (HWE), and the sig-

nificance was adjusted using the sequential Bonferroni correction (Rice, 1989).

RESULTS AND DISCUSSION

In total, 32 of 52 microsatellite loci were polymorphic (Table 1), while the remaining 20 loci were monomorphic or resulted in poor or no amplification in *C. alburnus*. In the stock,

Table 1. Characteristics of the 32 microsatellite loci isolated from *Culter alburnus*.

Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	N_A	H_O	H_E	P (HWE)	GenBank accession No.
Cal001	F: TTTCCTCTAACTCCACATT R: ATCACGACTGAGACCTATC	$(AC)_{16}$	182-216	9	0.7667	0.8316	0.0893	KC134212
Cal003	F: AGAGACATTGCTGCATCAT R: TCGCCGTAAGTAGTAGGT	$(CA)_{27}$	163-211	7	0.8000	0.7407	0.3430	KC134214
Cal004	F: TCTTTGTTTCCGCCCTATTT R: CAGTGGCTGTTCTGATGCTC	$(GT)_{10}(GA)_{18}$	228-258	5	0.7333	0.7605	0.1129	KC134215
Cal006	F: ACACCGCCCACTTTTACACC R: TTCCTCCAAACCTGTTCCCA	$(AC)_{16}$	167-197	5	0.7667	0.7356	0.1592	KC134217
Cal009	F: CTATCAGCCTCGGTTTGGGG R: ACGGCACAGGTGCAGGAAGA	$(TG)_{26}$	182-212	5	0.6333	0.6163	0.0875	KC134220
Cal010	F: ATTAGCAGATACAACAACGG R: TAACAGAACAGCCCAATAGA	$(TG)_{29}$	218-258	6	0.8333	0.8316	0.2735	KC134221
Cal011	F: AAACCACAGCAAAGTTCATA R: GAAGGACAGGATGTGACTCT	$(TG)_{17}$	271-295	4	0.6000	0.6763	0.0883	KC134222
Cal013	F: TGGCTCATTACACCTCCT R: TTACTTGTCTGATCCGCATA	$(GT)_{26}(GA)_{10}$	146-182	11	0.9000	0.9085	0.5700	KC134224
Cal014	F: GAGAAAGTGCTGGTCAATCA R: TGTGGTTCAGTTCTTCAGGA	$(CA)_{21}$	155-187	5	0.8000	0.7288	0.4479	KC134225
Cal016	F: TTGTAGAAGAAGTCCGAGTG R: TTAGCATTAGTCAGCGTGAA	$(TG)_{42}$	284-324	9	0.8333	0.8531	0.2164	KC134227
Cal017	F: ATTCATTGAGCCGAACTGTC R: CAACCGCAACATTACAAAGA	$(TG)_{26}$	208-240	10	0.7667	0.8904	0.0509	KC134228
Cal018	F: CCACAAGGACAAGGATTATG R: GCCATGAACACAACTTACC	$(CA)_{28}$	144-180	9	0.8333	0.8825	0.1364	KC134229
Cal020	F: CTCTTCCATCCAGATAGCAA R: GCCACACCTGTTGAGATC	$(TG)_{30}$	284-344	6	0.7667	0.8040	0.1578	KC134231
Cal022	F: CCGTTCGTTTGCTTTCAG R: TGGTGGTGATGGTGATGA	$(AC)_{27}ATA(CG)_5$	305-331	5	0.6667	0.7638	0.1957	KC134233
Cal024	F: TGAAAGACCCGACAAAGAAG R: GAGAACCAAACCAGACAACA	$(AC)_{16}$	260-292	6	0.7333	0.8107	0.1702	KC134235
Cal025	F: CTTGCTTCATACTTGCTCTG R: GCGACATTGCTGACTCTA	$(GT)_{33}$	268-308	12	0.8333	0.8553	0.0698	KC134236
Cal026	F: AAACCGCTTTATCCTTACTC R: GCAGTGTATGTCAGTTGGAG	$(CA)_9CG(CA)_5$	146-170	8	0.8000	0.8610	0.0723	KC134237
Cal027	F: GAGGAACGATACAGAGAATGA R: GGCAGACTCAATGTGGTAG	$(GT)_{18}$	181-211	7	0.8000	0.8537	0.1454	KC134238
Cal028	F: GGTCCTGCTGGGTAAAGA R: CAGATGCGGTCAGAGATG	$(TG)_{18}$	136-160	6	0.7333	0.7757	0.1215	KC134239
Cal029	F: GCGTCTGTATTTCCTGTCGG R: TTCCAAAAGCATCGTAAGCC	$(GT)_{16}$	163-191	7	0.8000	0.8249	0.1162	KC134240
Cal030	F: GCATTCTGTGGTATGTATGTG R: AGAGCTGTTGTTGATGATGT	$(AC)_{15}$	193-219	8	0.7000	0.8111	0.0554	KC134241
Cal043	F: TGCCAAGTGTTCGTTACATA R: TGTGGATGAAGTGTCCTACT	$(AC)_{13}$	155-180	9	0.8667	0.8712	0.3067	KF111423
Cal045	F: TCCTCAGCAGTTCTCAGT R: GCAATTACCAAAGACACAGA	$(TG)_{28}$	283-325	8	0.8333	0.8503	0.3301	KF111425
Cal046	F: TCTGTGACAAGAGACTGAAC R: AGCCTCTGACTTGGACTG	$(GT)_{16}$	203-239	4	0.7667	0.7023	0.4708	KF111426
Cal047	F: CTCACAGAGTCAGATTAGGA R: AACACTGTAACCATAGAAGC	$(CA)_{17}CG(CA)_{17}$	288-330	7	0.4667	0.7835	0.0000*	KF111427
Cal048	F: TCATACAACTACCAGCAACA R: TTCCTTCGTGGCATTCAG	$(AC)_6G(CA)_{10}$	158-186	5	0.7667	0.7689	0.1698	KF111428
Cal049	F: GTTACCTTGGTGTCCTTGAG R: CATTATGACGCACATCTGAG	$(AG)_{18}$	221-247	6	0.7667	0.8435	0.1779	KF111429
Cal050	F: AGAACAGTACAGAGAGCATT R: GGATTGATAGTGAAGTAGAGC	$(AG)_{15}$	162-184	6	0.8000	0.7435	0.1283	KF111430
Cal051	F: TTAGGTGAATCCTCAGTTGT R: TTCTGTCAGTGTCCTCTTAC	$(CT)_{12}$	160-178	3	0.5333	0.6605	0.0610	KF111431
Cal052	F: GAATCTGCCGTTCTCACTAA R: TACCTGTCCACCTCAATCAA	$(GA)_{14}$	131-153	6	0.6333	0.8288	0.0008*	KF111432
Cal053	F: TCATCAACTCTCACACTCTC R: CCATATCCAGCACTCTAACA	$(CT)_{20}$	237-261	4	0.7333	0.7452	0.2901	KF111433
Cal054	F: CTCTGAAAGAAAGACCTCCT R: GCTACTGAGTTGTCATCCTA	$(CT)_{16}$	338-360	4	0.4667	0.7249	0.0005*	KF111434

Locus name, repeat motif, allele number (N_A), allele size range, observed heterozygosity (H_O), expected heterozygosity (H_E) and P values of deviations from Hard-Weinberg equilibrium (HWE) (statistical significance at $P < 0.05$) are listed in the Table.

the number of alleles per locus ranged from 3 to 12 with an average of 6.6, and H_O ranged from 0.4667 to 0.9000, whereas H_E ranged from 0.6163 to 0.9085. Evidence of null alleles was only statistically significant for loci Cal047, Cal052, and Cal054. We found no evidence of large allele dropouts and stuttering. The loci Cal047, Cal052, and Cal054 showed deviation from Hardy-Weinberg equilibrium ($P < 0.05$), which may be related to appearance of null alleles and relatively small number of samples. These data did not show significant linkage disequilibrium between loci in the study population. These microsatellites can be used to study QTL of economic importance, population genetic diversity, patterns of kinship and reproductive success of farmed stocks, with the goal of better protecting wild stocks, improving the productivity of farmed stocks, and the construction of genetic maps for *C. alburnus* in the future.

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