ONLINE RESOURCES

Ten polymorphic microsatellite loci developed from *Triplophysa anterodorsalis*

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Introduction

Triplophysa anterodorsalis is a genus Triplophysa (Rendahl) fish which belongs to the family Balitoridae, subfamily Nemacheilinae. There are 112 nominal species in the genus *Triplophysa* all over the world, and ~60% of species are found in China (Chen and Yang 2005; Froese R. and Pauly D. 2008 FishBase: www.fishbase.org, ver. (06/2008)). *T. anterodorsalis* is an endemic fish, distributed in upper reaches of the Yangtze river, Jinsha river and its tributaries (Ding 1994). But, since 2002, four cascade hydropower stations were under construction in the lower reaches of Jinsha river (Chen *et al.* 2005). Fragmentation caused by these dams might disrupt the connectivity of hydro-ecosystem and the gene flow between local fish populations (Fu *et al.* 2003; Jiang *et al.* 2007; Huang *et al.* 2011).

Due to human activities, such as overfishing, dam construction, water contamination and habitat alteration, wild fisheries of this species have declined dramatically, and the distribution ranges have been shrinking rapidly in the last decades (Gao *et al.* 2011). In the long run, a good understanding of the genetic diversity, population structure and differentiation of *T. anterodorsalis* is required in order to establish adequate management plans for the conservation of this species. While the need for restoration of *T. anterodorsalis*, has been recognized, a eight-year management project based on population genetics has been initiated to define the efficiency of these conservation measures since 2011 (Yu and Chen 2011).

In the present study, we isolated and characterized 10 microsatellites from *T. anterodorsalis*. These markers will be a useful tool for studies of population demographic history, genetic structure and conservation purposes in this species.

Materials and methods

A partial genomic library for the repeat motif $(GT)_{13}$ was constructed using the FIASCO protocol (Zane *et al.* 2002) with a few modifications (Zhu *et al.* 2005). Genomic DNA was extracted from fin clips of a wild *T. anterodorsalis* captured in the lower reach of Jinsha river basin (26°57′46.98″ N, 102°52′09.36″ E, Qiaojia, Yunnan, China), using a proteinase K/phenol–chloroform method and RNAse treated (Sambrook and Russell 2001). DNA samples were pooled and digested with *Mse*I restriction enzyme (Promega, Madison, USA). Size-selected fragments (200–800 bp) were ligated to *Mse*I adaptors (OligoA: 5-TACTCAGGACTCAT-3′ and oligoB: 5′-GACGATGAGTCCTGAG-3′). Then the ligated fragments were hybridized with 5′-biotinylated probe $(GT)_{13}$ at 62°C for 30 min and then captured by streptavidin magnetic particles (Roche, Branford, USA). After removing those nonspecific binding and unbound DNA by several non-stringent and stringent washes, DNA fragments containing microsatellites were amplified by PCR, products were ligated to pMD-18T vector (Takara, Dalian, China) and transformed into competent cell DH5-α *Escherichia coli*. Clones, 94 were found with SSR from the positive individuals sequenced by the ABI 3730 XL sequencer and analysed by the software FINCHTV ver. 1.4 (Geospiza, Seattle, USA). 67 primer pairs flanking the target region were designed by the software Primer Premier 5.0 (Ren *et al.* 2004).

Microsatellite loci were tested using 32 wild individual samples DNA of *T. anterodorsalis*. The PCR reaction contained 0.5 U *Taq* polymerase (Takara, Dalian, China), 10 μm of each primer, 30 ng template DNA, 2 mM dNTPs, 1.5 mM $MgCl_2$and 1 μL 10× PCR buffer (Takara, Dalian, China) in a total volume of 10 μL. The thermal profile consisted of predenaturation at 95°C for 5 min, 35 cycles with 50 s

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Table 1. Characteristics of 10 microsatellite markers from *Triplophysa anterodorsalis.*

Locus	Accession no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	A	Size range (bp)	H_O	H_E	P (HWE)
QQ2-9	KC506779	$(TG)_{25}$	F: TATGGCAGCACTGAGGGAA R: GCAAACATGAATGAAAATAAAGC	64.0	13	178–214	0.871	0.900	0.926
QQ2-13	KC506780	$(AC)_{19}$	F: AGCGGTGAAGGAAATAACG R: CGAAAAGTGAGGTGAAAGG	62.0	9	156–178	0.767	0.876	0.010
QQ2-22	KC506781	$(AG)_7$	F: GGGCAGCCATGTTACAGC R: GCAGGGATTGTTGGTGGG	52.0	3	127–139	0.645	0.654	0.013
QQ2-31	KC506782	$(AC)_{17}$	F: ACAGGCATTTCTTGATAA R: TGTCCCCATAAAACATAG	52.0	4	146–160	0.667	0.745	0.120
QQ2-57	KC506783	$(TG)_{29}$	F: GTCCTGAGTAACGACAAAAACA R: TGCCCAACCTATCCAACAC	50.0	5	120–142	0.75	0.71	0.044
QQ2-59	KC506784	$(TG)_8$	F: ACCCGCTGAGTGACTGAC R: CACAATCCCACATACATGC	62.0	3	155–163	0.625	0.643	0.363
QQ4-54	KC506785	$(AC)_8$	F: GCACAAGAACCAGCCA R: CTACATAGTTGTGGAAAGA	46.0	3	230–240	0.742	0.605	0.239
QQ4-58	KC506786	$(AC)_{12}$	F: AGTGAAAGTGAATGTTCTGGAT R: TCCTTCGATTTGACCCAC	46.0	4	222–246	0.656	0.544	0.488
QQ4-75	KC506787	$(AC)_9$	F: GGCAAGAGGAAGGCGAC R: GAGGTAGGCACAAGGAACACT	46.0	2	162–166	0.875	0.5	0.008
QQ4-88	KC506788	$(CTAT)_8$	F: TGGAACTCACGAGAAGACAT R: CAAAAGCACCATCAGCAA	46.0	3	153–161	0.594	0.675	0.670

T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity; A, observed number of alleles;
*significant deviation from HWE after Bonferroni correction (adjusted $P = 0.005$).

at 94°C, 50 s at 48–55°C (table 1) and 50 s at 72°C , followed by a final extension of 7 min at 72°C. The PCR protocol was performed in an ABI 9700 Thermocyler. Amplification products were loaded onto 10% nondenaturing polyacrilamide gels, run at 10 W for about 3 h in 0.5× TBE buffer and stained with GelRed (Biotium, Hayward, USA). Gel pictures were taken with the Ultraviolet Gel Document System (Syngene). Number and size of alleles were analysed using Quality One 4.6.2 (Bio-Rad, Hercules, USA). Observed and expected heterozygosity were performed using PopGene 1.31 (Yeh *et al.* 1999). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) at each locus were calculated using GenePop 4.0 (Rousset 2008). Null alleles were tested using the software Micro-Checker ver. 2.2.3 (Oosterhout *et al.* 2004).

Results and discussion

One hundred and twenty positive clones were sequenced with M13 primers in one direction using ABI 3730 XL (Applied Biosystems, Foster City, USA). Ninety-four sequences contained microsatellites, and 92 possessed sufficiently long flanking sequences appropriate for primer design. Sixty-seven pairs of primers were designed, and finally, 10 polymorphic loci were found. Conditions and characteristics of the 10 loci are given in table 1. Sequences of these loci have been deposited in GenBank under accession nos. KC506779–KC506788.

The allele number of these polymorphic markers ranged from 2 to 13. Observed and expected heterozygosity in the population ranged from 0.594 to 0.875 and from 0.500 to 0.900, respectively. No significant LD was detected among the loci. No loci significantly ($P < 0.005$) deviated from HWE after Bonferroni correction (Rice 1989). The detailed characterization of these molecular markers is presented in table 1. In conclusion, the present study has identified 10 new markers for *T. anterodorsalis* in a limited survey. These loci should provide sufficient levels of genetic diversity to allow parentage analysis for assessing the genetic diversity in fish being used for releasing programme intended to supplement the dwindling natural stock and delineation of the fine-scale population structure.

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References

Chen D. Q., Chang J. B. and Gu H. B. 2005 Impacts of Jinsha river first stage project on ecology and environment of nature reserve and its countermeasures. *J. Yangtze. River. Sci. Res. Inst.* **22**, 21–24 (in Chinese).

Chen X. Y. and Yang J. X. 2005 Triplophysa rosa sp. nov.: a new blind loach from China. *J. Fish. Biol.* **66**, 599–608.

Ding R. (ed). 1994 *The fishes of Sichuan*, China, pp. 641. Sichuan Publishing House of Science and Technology, Chengdu, Sichuan, China (in Chinese).

Fu C. Z., Wu J. H., Chen J. K., Wu Q. H. and Lei G. C. 2003 Freshwater fish biodiversity in the Yangtze River basin of China: patterns, threats and conservation. *Biodiver. Conserv.* **12**, 1649–1685.

Gao S. B., Tang H. Y., Qiao Y., Yang Z. and Chen J. S. 2011 Status of fishery resources in the main stream of the lower reaches of Jinsha River. *J. Hydroecol.* **34**, 44–49 (in Chinese).

Huang F., Xia Z. Q., Zhang N. and Lu Z. H. 2011 Does hydrologic regime affect fish diversity? -A case study of the Yangtze Basin (China). *Environ. Biol. Fish.* **92**, 569–584.

Jiang H., Xie S. G., Zhao W. Q. and Chang J. B. 2007 Changes of fish assemblages after construction of Eetan Reservoir in Yalong River. *Acta Hydrobio. Sin.* **31**, 532–539 (in Chinese).

Oosterhout C. V., Hutchinson W. F., Wills D. P. M. and Shipley P. 2004 Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Ren L., Zhu B. Q., Zhang Y. B., Wang H. Y., Li C. Y., Su Y. H. and Ba C. F. 2004 The Research of applying Primer Premier 5.0 to design PCR primer. *J. Jinzhou. Medi. Col.* **25**, 43–46 (in Chinese).

Rice W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rousset F. 2008 GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol. Ecol. Resour.* **8**, 103–106.

Sambrook J. and Russell D. W. 2001 *Molecular Cloning*, 3rd edition, pp. A1.22. Cold Spring Harbor Laboratory Press, New York, USA.

Yeh F. C., Yang R. C. and Boyle T. 1999 POPGENE version l.31, Microsoft window-based freeware for population genetic analysis. University of Alberta and Centre for International Forestry Research, Edmonton, Cannada.

Yu J. and Chen Y. B. 2011 Construction and adaptability management of fish breeding and releasing station at Xiangjiaba Hydropower Station. *J. Hydroecol.* **32**, 135–139 (in Chinese).

Zane L., Bargelloni L. and Patarnello T. 2002 Strategies for microsatellite isolation: a review. *Mol. Ecol.* **11**, 1–16.

Zhu B., Liao X., Shao Z., Rosenthal H. and Chang J. 2005 Isolation and characterization of microsatellites in Chinese sturgeon, *Acipenser sinensis*. *Mol. Ecol. Notes* **5**, 888–892.

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