GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Development and characterization of microsatellite markers via cross-species amplification of *Paramisgurnus dabryanus*

G. Chen, S. Huang, J. Gao, X. Bai, W. Wang and X. Cao

College of Fisheries, Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei, China

Corresponding author: X. Cao
E-mail: caoxiaojuan@mail.hzau.edu.cn

Genet. Mol. Res. 14 (2): 5694-5698 (2015)
Received January 25, 2013
Accepted April 29, 2015
Published May 29, 2015
DOI http://dx.doi.org/10.4238/2015.May.29.1

ABSTRACT. The large-scale loach, *Paramisgurnus dabryanus*, is a small freshwater fish of major economic importance in many Asian countries, particularly China and South Korea. Fifteen polymorphic microsatellite (simple sequence repeat) markers were obtained through cross-species amplification between this loach and a related species, *Misgurnus anguillicaudatus* (GenBank accession numbers: KC117456 to KC117470). The number of alleles per locus ranged from 5 to 12 among 40 individuals, and the average observed and expected heterozygosities were 0.344 and 0.828, respectively. Three loci showed significant deviations from Hardy-Weinberg equilibrium. These polymorphic loci could provide a valuable tool for investigations of the population genetics, phylogeography, and conservation genetics of *P. dabryanus*.

Key words: *Paramisgurnus dabryanus*; Microsatellite markers; Cross-amplification; Polymorphism

 ©FUNPEC-RP www.funpecrp.com.br

INTRODUCTION

The large-scale loach, *Paramisgurnus dabryanus*, is a loach species that is distributed widely across East Asia, from Russia to China. Because of its high economic and medicinal value, this loach is widely cultured in China and South Korea (You et al., 2009). Recently, the wild population of *P. dabryanus* has substantially decreased due to overfishing and water pollution. Research of this loach has mostly focused on its breeding physiology (You et al., 2008), growth (Wang et al., 2009), development (Zhang et al., 2001), and toxicology (Li et al., 2003). The genetic diversity, population structure, and molecular marker-assisted breeding of this species are currently poorly understood, primarily due to a lack of variable genetic markers.

Microsatellites have become one of the most popular molecular markers that are used in a wide variety of genetic investigations (Russell et al., 1997; Stevanato et al., 2013). There are several methods for microsatellite isolation (Zane et al., 2002). In contrast, to isolate microsatellites from target species *de novo*, cross-species amplification is a cost-effective method resulting in the rapid development of microsatellites from closely related taxa (Schlotterer et al., 1991; Zheng et al., 1995, Gu et al., 2012). To date, only six microsatellite sequences of *P. dabryanus* have been reported in a single study (You et al., 2012). Therefore, screening for more polymorphic microsatellites in this loach is very important and necessary for future genetic investigations. In this study, we obtained 15 polymorphic microsatellites for *P. dabryanus* from a related species, *Misgurnus anguillicaudatus*, through cross-species amplification.

MATERIAL AND METHODS

In order to characterize isolated microsatellites, 40 *P. dabryanus* individuals were collected from Hubei (23 individuals) and Henan (17 individuals) provinces, China, in 2012. Fin samples were dissected from these loaches and stored in 100% ethanol immediately after removal. Genomic DNA was extracted from fins following the method described in Lassner et al. (1989), which was further modified by Torres et al. (1993).

M. anguillicaudatus, which is a species related to *P. dabryanus*, was used in this study to obtain microsatellites by cross-species amplification. Sixty-three pairs of microsatellite primers from *M. anguillicaudatus* (Table 1, Morishima et al., 2008; DDBJ database) were randomly selected and synthesized for polymerase chain reaction (PCR) amplification. PCR was performed in a 10-μL reaction mixture containing 8-40 ng DNA (a mixed DNA sample of six *P. dabryanus* individuals), 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl), 120 μM dNTPs, 0.15 μM of each primer, and 1 U Taq DNA polymerase (all reagents were purchased from Dingguo Bio., Beijing, China). Amplification was achieved in a T 100^{111} thermal cycler and the conditions were as follows: 94°C for 5 min, 94°C for 40 s, annealing temperature (53.1°-62.2°C; efforts were made to optimize the annealing temperature) for 45 s, 72°C for 50 s for 35 cycles, with a final step of 10 min at 72°C. PCR products were checked by 1.5% agarose gel electrophoresis and visualized under a UV transilluminator after ethidium bromide staining. Based on the results of agarose gel electrophoresis, PCR products with clear bands and reasonable sizes were selected and then sequenced by the Shanghai ShengGong Biological Engineering Technology & Services Co., Ltd., Shanghai, China to ensure that the products were genuine microsatellites. After filtering out the 63 microsatellite loci with poor

 ©FUNPEC-RP www.funpecrp.com.br

cross-amplification, combined with the sequencing results, the remaining loci (genuine microsatellites) were tested in 40 *P. dabryanus* individuals for polymorphism analyses. The PCR products were separated on 6% non-denaturing polyacrylamide gel. Gels were then stained with 1 g/L silver nitrate in water and visualized under ultraviolet light. The size of alleles was estimated using a 501 bp DNA marker (PUC18DNA/msp1) combined with image analysis, as described by Nelson et al. (1998). The number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity, and departure from Hardy-Weinberg equilibrium (HWE) were estimated in POPGENE version 1.32 (Yeh et al., 1999).

Table 1. Names of 63 microsatellites loci of Misgurnus anguillicaudatus and their DDBJ accession numbers.

Locus	DDBJ Accession No.	Locus	DDBJ Accession No.	Locus	DDBJ Accession No.	Locus	DDBJ Accession No.	Locus	DDBJ Accession No.
Mac2	AB060172	Mac48	AB081626	Mac167	AB303453	Mac361	AB303496	Mac497	AB303578
Mac11	AB060181	Mac49	AB060186	Mac190	AB303455	Mac364	AB303498	Mac519	AB303584
Mac21	AB081619	Mac50	AB060187	Mac229	AB303459	Mac375	AB303500	Mac523	AB303585
Mac23	AB081621	Mac51	AB081627	Mac235	AB303461	Mac387	AB303506	Mac532	AB303586
Mac24	AB060178	Mac60	AB081631	Mac242	AB303463	Mac404	AB303512	Mac541	AB303588
Mac25	AB060179	Mac62	AB081632	Mac262	AB303468	Mac408	AB303514	Mac559	AB303592
Mac31	AB081622	Mac63	AB081633	Mac264	AB303470	Mac425	AB303516	Ma574	AB303594
Mac36	AB060180	Mac103	AB303436	Mac276	AB303473	Mac429	AB303518	Mac576	AB303595
Mac37	AB060181	Mac105	AB303437	Mac277	AB303474	Mac449	AB303519	Mac605	AB303605
Mac39	AB081623	Mac123	AB303441	Mac291	AB303477	Mac456	AB303522	Mac612	AB303607
Mac43	AB081624	Mac133	AB303444	Mac293	AB303478	Mac462	AB303524	Mac627	AB303610
Mac45	AB060185	Mac140	AB303445	Mac320	AB303483	Mac466	AB303525		
Mac47	AB081625	Mac165	AB303451	Mac331	AB303487	Mac477	AB303575		

RESULTS AND DISCUSSION

Of the 63 microsatellite loci from *M. anguillicaudatus*, 39 showed no amplification products in *P. dabryanus*, while 6 presented very weak and multiple bands, which were not scorable. Hence, 18 pairs of primers resulted in suitable and scorable PCR products in *P. dabryanus*. After sequencing these PCR products, 15 loci were verified to be microsatellites. The 15 microsatellite sequences were submitted to GenBank (accession numbers KC117456 to KC117470). Characteristics of the 15 microsatellite loci in *P. dabryanus* are listed in Table 2. All of the 15 loci were highly polymorphic with an average of 9.07 alleles per locus (ranging from 5 to 12). The average observed and expected heterozygosities were 0.344 and 0.828, respectively. Three loci were found to deviate from HWE after Bonferroni correction ($P < 0.05$).

The present study successfully demonstrated cross-species amplification of microsatellite primers in Cobitidae (15/63; 23.8%), confirming previous studies in Phasianidae (Gu et al., 2012), Muridae (Sun at al., 2009), and Serranidae (Dong et al., 2008), which all showed that cross-species amplification was a very effective method for microsatellites development. These results might suggest that many microsatellites and their flanking regions are relatively conserved in Cobitidae, which would facilitate the construction of a syntenic map in this family. Adequate levels of variation at the 15 microsatellite loci suggest that these markers would be potentially useful for studies on parentage and kinship analysis, population genetics, and phylogeography in *P. dabryanus*.

 ©FUNPEC-RP www.funpecrp.com.br

Table 2. Characteristics of fifteen polymorphic microsatellite loci in 40 individuals of *Paramisgurnus dabryanus*.

Locus	GenBank Accession No.	Primer sequences (5'-3')	Size range (bp)	Repeat motif	N_A	H_E	H_O	Ta (°C)	PHW
Mac133-SSR	KC117457	F: CTGCATGCTCTTGCATAGGA	192-242	(TG)5	11	0.878	0.435	55.8	0.120
		R: CATTCATATTCCACCCTCTCG							
Mac190-SSR	KC117459	F: CACTGCAACAAGCATTGCATA	221-256	(GA)8	8	0.855	0.368	53.9	0.135
		R: TTTTACCCCCAAATGGATCA							
Mac229-SSR	KC117469	F: CTCCAGCATTCCCACAATTC	170-235	(CT)5(CTGT)5	8	0.768	0.256	53.9	0.166
		R: GGACCCTGGGTGTGACAATA							
Mac361-SSR	KC117470	F: GCGTCACAAAGTTTCTTCAG	258-299	(TC)12(AG)8	12	0.894	0.025	53.9	0.100
		R: TGTAAAGAGAGGCTGTGAGT							
Mac404-SSR	KC117463	F: CTTTGCTCAGCCATGATCAG	168-212	(GT)7(GA)6	9	0.859	0.550	55.8	0.772
		R: AGCGTAAGACATCAGCATTC							
Mac425-SSR	KC117460	F: CTACGGAAGTGCTGCAGACA	85-144	(TC)3	10	0.819	0.461	61.2	0.140
		R: CATCCTAGGCTCAGGCCAAA							
Mac477-SSR	KC117462	F: GCTGAGACTCTTTATGTCTCAC	84-125	(GT)5	12	0.867	0.125	60.0	0.132
		R: GCTATCAAGGGAACTGAATGG							
Mac576-SSR	KC117468	F: AACTATTGCAGGGTTACCAC	161-209	(GT)5	11	0.907	0.297	58.1	0.322
		R: GTATGGATGCTTTCTCGACA							
Mac264-SSR	KC117461	F: GACAAGCTTCCTCTCCAAGT	76-186	(CT)26CA(CT)7	11	0.834	0.600	58.1	0.021*
		R: TGCASAGAGACGGTGAGTGA							
Mac429-SSR	KC117467	F: TATGTGAGGAAGATAAGCAG	121-176	(GT)8	5	0.880	0.050	55.8	0.018*
		R: GAATCCAGATCAAAGCAATC							
Mac449-SSR	KC117458	F: CCCTCCACTTCAACCCTACA	192-293	(TG)5	5	0.548	0.325	58.1	0.281
		R: GACCCTGCTGTCATCTCACA							
Mac456-SSR	KC117466	F: TCTCCTCTGACACCATCAGG	194-259	(AC)11	11	0.892	0.100	62.0	0.110
		R: GTACGGACACGAGTCTGGAA							
Mac532-SSR	KC117465	F: CAGTGAGCGAGTTCAGTAGG	310-418	(TG)16	8	0.719	0.250	62.0	0.350
		R: TCGTGTTGGTCTTCTGGTTC							
Mac574-SSR	KC117456	F: AGTTCATGCCTCCAAAG	170-245	(TG)7	7	0.850	0.700	55.8	1.000
		R: GTTTTCAGGCAGACCAA							
Mac627-SSR	KC117464	F: CGATGCATTGAAAATGTCCT	207-269	(AC)12	8	0.845	0.625	55.8	0.032*
		R: CTGCTCTTTTCACAGTACCT			9.07	0.828	0.344		
Total									

Ta, annealing temperature; N_A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; PHW, probability of Hardy-Weinberg equilibrium; *meant $P < 0.05$

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#31201719) to Xiaojuan Cao.

REFERENCES

Dong Y, Han J and Cai H (2008). Cross-species amplification and characterization of polymorphic microsatellite loci in Hong Kong grouper, *Epinephelus Akaara*. *J. Beijing Normal Univ. (Nat. Sci.)* 44: 511-514.

Gu L, Liu Y, Wang N and Zhang Z (2012). A panel of polymorphic microsatellites in the Blue Eared Pheasant (*Crossoptilon auritum*) developed by cross-species amplification. *Chin. Birds* 3: 103-107.

Lassner MW, Peterson P and Yoder JI (1989). Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. *Plant Mol. Biol. Rep.* 7: 116-128.

Li XY, Liu YD, Song LR, Lei LM et al. (2003). The toxicity of microcystins to embryo and larvae of loach (*Paramisgurnus dabryanus* Sauvage). *Acta Hydrobiol. Sin.* 3: 318-319.

Morishima K, Nakayama I and Arai K (2008). Genetic linkage map of the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). *Genetica* 132: 227-241.

Nelson RJ, Beacham TD and Small MP (1998). Microsatellite analysis of the population structure of a vancouver island sockeye salmon (*Oncorhynchus nerka*) stock complex using nondenaturing gel electrophoresis. *Mol. Mar. Biol. Biotechnol.* 7: 312-319.

Russell JR, Fuller JD, Macaulay M, Hatz BG, et al. (1997). Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theor. Appl. Genet.* 93: 714-720.

Schlotterer C, Amos B and Tautz D (1991). Conservation of polymorphic simple sequence in cetacean species. *Nature* 354: 63-65.

Stevanato P, Trebbi D, Biancardi E, Cacco G, et al. (2013). Evaluation of genetic diversity and root traits of sea beet accessions of the Adriatic Sea coast. *Euphytica* 189: 135-146.

Sun B, Bao Y, Zhang L, Zhao Q et al. (2009). Cross-species amplification of rat and mouse microsatellite DNA Loci in the *Niviventer confucianus*. *Chin. J. Zool.* 44: 145-150.

Torres AM, Weeden NF and Martin A (1993). Linkage among isozyme, RFLPs and RAPD markers in *Vicia faba*. *Theor. Appl. Genet.* 85: 937-945.

Wang K, Ling QF, Li Q, Cheng F, et al. HJ (2009). Primary study on the age and growth of *Misgurnus anguillicaudatus* and *Paramisgurnus dabryanus* in the area of Suzhou. *J. Shanghai Ocean Univ.* 18: 163-167.

Yeh FC, Yang R and Boyle T (1999). Popgene version 1.32. Microsoft windows-based freeware for population genetic analysis. University of Alberta, Edmonton, Alberta.

You C, Tong J and Yu X (2012). Microsatellite DNA analysis on genetic diversity of seven populations of *Paramisgurnus dabryanus*. *J. Hydroecol.* 33: 84-91.

You CH, Yu XM, Tan DQ and Tong JG (2008). Gynogenesis and sex determination in large-scale loach *Paramisgurnus dabryanus* (Sauvage). *Aquacult. Int.* 16: 203-214.

You CH, Yu XM and Tong JG (2009). Detection of hybridization between two loach species (*Paramisgurnus dabryanus* and *Misgurnus anguillicaudatus*) in wild population. *Environ. Biol. Fish.* 86: 65-71.

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

Zhang HY (2001). The embryonic development and fingerling culture of loach, *Paramisgurnus dabryanus* Sauvage. *Acta Hydrobiol. Sin.* 31: 55-62.

Zheng W, Stacey NE, Coffin J and Strobeck C (1995). Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus*. *Mol. Ecol.* 4: 791-792.

 ©FUNPEC-RP www.funpecrp.com.br