Global Journal of Molecular Sciences 6 (1): 22-25, 2011
ISSN 1990-9241

Successful Cross-Amplification of Few Microsatellite Loci Isolated from *Tor tambroides* for Indian Snow Trout, *Schizothorax richardsonii* (Gray, 1832) (Family: Cyprinidae)

[1]*Ashoktaru Barat,* [1]*Suresh Chandra,* [2]*Birender Kumar Singh,*
[1]*Rakesh Matura and* [1]*Prabin Chandra Mahanta*

[1]Molecular Genetics Laboratory, Directorate of Coldwater Fisheries Research (ICAR),
Bhimtal-263136, Nainital, Uttarakhand, India
[2]Department of Zoology, Kumaun University, Nainital, Uttarakhand, India

Abstract: Indian snow trout (*Schizothorax richardsonii*) is an important coldwater fish. A number of 8 microsatellite loci from a mahseer (*Tor tambroides*) were used to study their utility for cross species amplification in *S. richardsonii*. Five of them were successfully optimized through PCR. High levels of heterozygosity (Ho = 0.42-0.95) with allelic numbers on average 3.96 were obtained for these five loci. The present data confirmed the transferability of some heterologous microsatellite loci from one species to another. These five sets of markers would be useful to investigate a fine scale population genetic analysis in *S. richardsonii*.

Key words: *Schizothorax richardsonii* · Indian Snow Trout · Microsatellite Loci · PCR · Cross Species Amplification

INTRODUCTION

Indian snow trout (*Schizothorax richardsonii*) is widely distributed in Himalayan and sub Himalayan streams, rivers along Jammu and Kashmir, Himachal Pradesh, Assam, Sikkim, Bhutan, Nepal, Pakistan and Afghanistan [1]. This species has been considered as a valuable source of fish protein for the Hill community. In recent years, the natural population of the species in the wild resources has been dwindled considerably due to several anthropogenic activities, such as impoundment effect due to dam construction, indiscriminate killing, introduction of exotic fish etc [2]. The species follows allometric growth [3]. Although the species *S. richardsonii* has not yet been included in IUCN list either as an endangered or a threatened one, it's high time that efforts are made to increase the population size. Therefore, breeding and culture of this species was tried at the Directorate of Coldwater Fisheries Research (DCFR) farm, Champawat, Uttarakhand, by collecting the brooders from wild sources. Further refinement of these and to improve growth rate, a genetic breeding program depending on genetic variability studies using molecular markers is needed. A thorough literature survey revealed that there were few partial cytochrome b gene sequences (AF532075-AF532088) and two microsatellite sequences (FN568062 and FN568062) available for *S. richardsonii* in GenBank. However, there is lack of information related to genetic diversity, stock structure analysis using microsatellite markers in this species. Polymerase Chain Reaction (PCR) based microsatellite analysis offered the finest resolution now a day for studying genetic variation in several fish species [4, 5]. Microsatellites are simple DNA sequences that are repeated several times at various points in the organism's DNA. Such repeats are highly variable and can be used as a polymorphic marker. The occurrence of high degree of polymorphism and co-dominant inheritance has made them one of the most popular genetic markers for studies on genetic diversity, population genetic structure on fishes [6-9]. Microsatellites are tandem repeats of 1-6bp and developed from anonymous genomic sequences [5, 10]. But development of species-specific primers for PCR amplification of microsatellite loci is expensive and time consuming. It has been observed that primers developed to amplify markers in one species may amplify in related

Corresponding Author: Ashoktaru Barat, Principal Scientist, Molecular Genetics Laboratory, Directorate of Coldwater Fisheries Research (ICAR), Bhimtal-263136, Nainital, Uttarakhand, India. Tel: +91-9410341899.

species as well [11, 12]. Thus, in the present paper it has been tried to study the level of transferability and validity of some microsatellite markers developed earlier by Nguyen *et al*. [13] from *Tor tambroides* under family Cyprinidae for further use in population structure analysis of *S. richardsonii*.

MATERIALS AND METHODS

Samples Collection: The fish Samples of were collected by cast net from five different rivers of Kumaon and Gharwal regions of Uttarakhand: Gola (Ranibag, n=8; 29°27'30"N, 79°28'45"E), Uttarvahini (Garampani, n=11; 29°27'30"N, 79°28'45"E), Chirapani (Champawat, n=8; 29°19'59"N, 80°6'0"E), Alaknanda, (Barangana, n=8; 30°26'03"N, 79°21'94"E) and Kosi (Katli, n=9; 29°35'50"N, 79°39'52" E). The sampling sites were selected to cover genetic variation on a wide geographical distribution range of the species. The Muscle samples were collected through dorsal part and preserved in 95% ethanol. All specimens were fixed in 10% formalin in the field as a voucher.

DNA Extraction and Pcr Amplification: Total genomic DNA was isolated from muscle tissue by using the standard phenol-chloroform extraction protocol described by Sambrook and Russel, [14]. Amplification of each DNA samples was performed in a 10µl reaction mixture containing 10x Taq assay PCR buffer A (100mM Tris, pH 9.0, 500mM Kcl, 15mM $MgCl_2$, 0.1% Gelatin) (Genei, India), 200µM of each dNTPs (Genei, India), 5pmol of each primer (Ocimum Biosolutions, India), 0.5U Taq DNA Polymerase (Genei, India) and 25ng of DNA. One negative control was performed for each set of amplifications. Amplifications were performed on ABI 9700 Thermal Cycler (ABI, USA) with the following parameters: 4 min of initial denaturation at 94°C followed by 35 cycles of 45s at 94°C, 45s at locus specific annealing temperatures (Table 1) and 60s at 72°C, ending with a final step of extension at 72°C for 7mins. After PCR, 5µl of formamide dye was added to each reaction. The samples were denatured at 95°C for 5min. and immediately placed on ice. Finally PCR products were separated through 6% denaturing polyacrylamide gel in 1xTBE buffer. Φ174 Hinf I digest (Fermentas, USA) was used as a size marker for the microsatellite alleles. Detection of alleles was carried out by silver staining [15]. The gel image was documented and analyzed in UV-Gel Documentation Unit (Alpha Imager 3400, Alpha Innotech Corporation, USA).

Statistical Analysis: Eight primers were selected (Tt1 B01, Tt1 C06, Tt1C10, Tt1 F02, Tt2 B02, Tt2 D01, Tt2 F04 and Tt2 F07) on the basis of the allelic richness reported by Nguyen *et al*. [12] for the present study on *S. richardsonii*. Out of eight primers five (Tt1 F02, Tt2 F04, Tt2 F07, Tt1 B01 and Tt1C10) could successfully amplified target fragments of the expected size. All five loci exhibited polymorphism in the individuals tested. We used Genetic Data Analysis Software GDA v 1.1 [16] to obtain number of alleles (A), observed heterozygosity (Ho), expected heterozygosity (He), to test for linkage disequilibrium and deviation from Hardy-Weinberg Equilibrium (HWE). The total number of alleles ranged from 3-4 with an average of 3.96 alleles per locus. However, number of alleles originally reported by Nguyen *et al*. [13] was in the range of 1-3. It was observed that all the eight primers mentioned above were annealed at 57°C [13], which were different in the present study Table 1. Observed and expected heterozygosity ranged from 0.42-0.95 and 0.70-0.74, respectively. The probability test did not detect any significant deviation in allele frequencies from that expected under ($P < 0.001$) Hardy-Weinberg equilibrium. A test for genetic differentiation was performed to test the hypothesis that the sample sets had genetic heterogeneity. The genetic heterogeneity was tested based on the genotype rather than on allele frequencies. The combined probability over all loci and the sample sets was found to be significant ($P > 0.0001$), indicating that sample sets differ significantly in their genotype frequencies.

RESULTS AND DISCUSSION

Primer sequences and the specific annealing temperature (Ta °C) in the resources species and *S. richardsonii* are given in Table 1. The optimum annealing temperatures to get the scorable bands in *S. richardsonii* differed from that reported for the resources species. Five out of the eight primer pairs tested yielded successful amplification in *S. richardsonii*. All primer pairs amplified only a single locus. It is evident from Table 1 that amplification success was higher when primers were from the resource species within the subfamily Cyprininae than Results suggested that certain sequences flanking tandem repeats are conserved within the subfamily Cyprininae and, to some extent, also between the subfamilies of Cyprinidae. The results of this studies demonstrate that the microsatellite marker sets published by Nguyen *et al*. [12] for *Tor tambroides* (Fam: Cyprinidae) produces quantifiable microsatellite

Table 1: Results of PCR amplifications using the microsatellite primer set developed by Nguyen *et al*, [13] on genomic DNA samples from Indian Snow Trout A, number of alleles observed; H_o, observed heterozygosity; H_e, expected heterozygosity; P, value of the exact test

Locus Name	Primer sequence (5'-3')	Acc. No.	Ann. temp	Repeat Sequence	Allele Size	A	H_o	H_e	P
Tt1 F02	F-CATGGACCAAATTACAAGGATTT	DQ778026	53°C	$(TG)_2TA(TG)_4TC(TG)_2$	240bp	4	0.706	0.734	0.077
	R-AACCTGTGAGGGATGTCCAG								
Tt2 F04	F-ATGCCAGCTACAGGTCCAAT	DQ778032	52°C	$(AC)_{11}$	150bp	4	0.865	0.702	0.271
	R-CGTGTGTATGATGCCACCTC								
Tt2F07	F-GAGACGACTCTAGTCGCTGACA	DQ778033	56°C	$(AG)_9$	153bp	4	0.950	0.747	0.092
	R-GTGTGGCCAGTGTAGCTGAA								
Tt1B01	F-GAGGGGCATTTTGTTCTTGA	DQ778019	56°C	$(AC)_8AT(AC)_3$~$(TC)_3$	245bp	3	0.425	0.714	0.066
	R-GCTTCCCCTCATAAGCCTTC								
Tt1C10	F-GCTGAAGCAGGTGAATCTGA	DQ778022	55°C	$(TG)_{13}$	188bp	4	0.670	0.742	0.170
	R-TGATGCCTGTCAAACCTGTG								

fragments for the Indian Snow Trout, *S. richardsonii* (Fam: Cyprinidae) after standardizing the annealing temperature and optimizing other PCR profiles for each locus. Our results also suggest that PCR primers designed for microsatellite loci from *Tom tambroides* may be useful for genetic analysis for the present snow trout though the average number of alleles per locus is low which may be due to small sample size. The small sample size also affected the power of exact tests for conformations to Hardy-Weinberg expectations (HWE).

CONCLUSION

The development of microsatellite markers is a time consuming and highly expensive also. Hence, it was tried to use some available markers from public domain. The present study had shown the utility of five polymorphic microsatellite loci through cross-species amplification and exhibited considerable promise to evaluate stock structure, genetic variation, conservation genetics and molecular breeding of *S. richardsonii*. T`he information generated here using these novel microsatellite loci would be able to solve some issues, 1) potential transferability of some heterologous primers from one species to another, 2) population genetic studies of *S. richardsonii* may be initiated and 3) it may also reduce the time and cost for development of microsatellite markers.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Department of Biotechnology, Govt. of India and Indian Council of Agricultural Research, New Delhi for financial assistance in carrying out the present studies.

REFERENCES

1. Talwar, P.K. and G.A. Jhingran, 1991. Inland fisheries of India and adjacent countries. Vol I and II, Oxford and IBH Publishing Company Pvt. Limited, New Delhi.
2. Sehgal, K.L., 1999. Coldwater fish and fisheries in the Himalayas: rivers and streams. Fish and Fisheries at higher altitudes: Asia. FAO Fisheries technical paper. (Ed) T. Petr, 385: 41-63, FAO, Rome.
3. Goel, C., A. Barat, V. Pande, S. Ali and R. Kumar, 2011. Length-weight relationship of snow trout (Schizothorax richardsonii) based on linear and nonlinear models from hill stream of Uttarakhand, India. World J. Fish and Marine Sci., 3(6): 485-488.
4. Chistiakov, D.A., B. Hellemans and F.A.M. Volckaert, 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture, 255: 1-29.
5. Liu Z.J. and J.F. Cordes, 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture, 238: 1-37.
6. Wright, J.M. and P. Bentzen, 1994. Microsatellite: Genetic markers for the future. Rev. Fish Biol. Fisher., 4: 384-388.
7. Sonstebo, J.H., R. Borgstrom and M. Heun, 2007. Genetic structure of brown trout (*Salmo trutta* L.) from the Hardangervidda mountain plateau (Norway) analyzed by microsatellite DNA: a basis for conservation guidelines. Conserv. Genet., 8: 33-44.
8. Eldridge, W.H. and K. Killebrew, 2008. Genetic diversity over multiple generations of supplementation: an example from Chinnook salmon using microsatellite and demographic data. Conserv. Genet., 9: 13-28.

9. Sugaya, T., M. Sato, E. Yokoyama, Y. Nemoto, T. Fujita, H. Okouchi, K. Hamasaki and S. Kitada, 2008. Population genetic structure and variability of Pacific herring Clupea pallasii in the stocking area along the Pacific coast of northern Japan. Fisheries Sci., 74: 579-588.
10. Zhan, A., Y. Wang, B. Brown and H. Wang, 2009. Isolation and characterization of novel microsatellite markers for Yellow Perch (*Perca flavescens*). International J. Molecular Sci., 10: 18-27.
11. Morris, D.B., K.R. Richard and J.M. Wright, 1996. Microsatellite from Rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study in Salmonids. Can. J. Fish Aquat. Sci., 53: 120-126.
12. Scribner, K.T., J.R. Gust and R.L. Fields, 1996. Isolation and characterization of novel salmon microsatellite loci: Cross-species amplification and population genetic applications. Can. J. Fish Aquat. Sci., 53: 833-841.
13. Nguyen T., M. Baranski, M. Rourke and H. McPartlan, 2007. Characterization of microsatellite DNA markers for a mahseer species, *Tor tambroides* (Cyprinidae) and cross-species amplification in four congeners. Mol. Ecol. Resour, 7: 109-112.
14. Sambrook, J. and D.W. Russel, 2006. The Condensed Protocol from Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp: 1-800.
15. Riesner, D.G., R.Z. Steger, R.A. Owens, M. Wagenhofer, W. Hillen, S. Vollbach and K. Henko, 1989. Temperature-gradient gel electrophoresis of nucleic acids: analysis of conformational transition, sequence variation and protein Nucleic acid interactions. Electrophoresis, 10: 377-389.
16. Lewis, P.O. and D. Zaikin, 2001. Genetic Data Analysis Software, Version 1. Available at http:// hydrodictyon.eeb.uconn.edu/people/plewis/softwa re.php.