Short Communication

Discrimination of Seven Species of Bagrid Catfishes Under The Genus *Mystus* Using RAPD Fingerprinting

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Received: May 20, 2016; revised: December 5, 2016; accepted: December 16, 2016

Abstract: A comparative random amplification of polymorphic DNA (RAPD) analysis is carried out for the first time on seven species of *Mystus* (Bagrid catfishes) distributed in two river basins, in the northeastern India, to evaluate the degree of genetic similarity among them. This study used thirteen arbitrary oligodecamer RAPD markers amplifying 639 reproducible and scorable bands of size 285 to 2150 bp which are assigned to 204 loci. Similarity index values obtained for each pairwise comparison among the seven species ranged between 0.208 to 0.454. The dendogram grouped all the seven species into two major clusters based on their genetic differences by reflecting the morphological differences among them. Cluster A includes *M. rufescens, M. ngasep, M. bleekeri, M. cavasius* and *M. falcarius* whereas *M. tengara* and *M. dibrugarensis* are fall under cluster B .The dendogram also clearly showed the genetic similarity of *M. bleekeri* populations of the upper and lower Brahmaputra basin by clustering together with a similarity index value of 0.454. Thus, the results have inferred that RAPD markers are useful in distinguishing the species of *Mystus* and establishing their interrelationship based on their genetic composition.

Key words: Interrelationship, *Mystus*, RAPD, Species-segregation

Introduction

Fishes of the genus *Mystus* Scopoli (with nearly 40 species) are small to medium-sized bagrid catfishes found predominantly in freshwater habitats in the West, South and Southeast Asia (Ng & Rohan Pethiyagoda, 2013). The genus is characteristic in having a thin needle-like first infraorbital, twisted and thickened metapterygoid loosely attached to the quadrate by means of ligament or a small extent of cartilage (Mo, 1991). The actual diversity of the genus is not fully known, as evidenced by the recent publications on species-resurrections from synonymy, redescriptions and new-species descriptions (Darshan *et al.,* 2011, 2013). Although these fishes are very common in all the freshwater habitats of the low-lying plains of India, especially in the northeast India, molecular characterization of this group of fishes is poorly studied, most probably because of deficient tissue collection and *Mystus* species are endemic to a specific basin (eg. *Mystus rufescens* and *M. falcarius* occur only in Chindwin River system; while *M. tengara, M. blekeri and M. cavasius* are found only in Ganga-Brahmaputra system) or even restricted to a particular region of the specific basin (eg. *M. dibrugarensis* is restricted to Dibrugar, Assam and its nearby areas in Brahmaputra basin only) and are not easily accessible to the researchers.

RAPD analysis is a technique for rapidly detecting genomic polymorphisms, utilizing a single short oligonucleotide primer in a polymerase chain reaction (PCR) and the technique also have the advantage that no prior knowledge of the genome is necessary for successful application (Welsh and McClelland, 1990). The technique is reasonably low cost and protocol is fairly simple in comparing to other DNA-based techniques, such as amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) (Karp *et al.,* 1997). RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms and has provided with important applications in fish (Bartish *et al*., 2000). A comprehensive literature survey revealed that there are a few reports on the genetic data of this group of fishes. Grag *et al*. (2009) reported on the genetic diversity of the two populations of *Mystus vittatus* collected from two separate water bodies in Madhya Pradesh state of India. Saini *et al*. (2011) used RAPD technique to discriminate six species of Bagrid fishes of two genera, *Mystus* and *Sperata*.

RAPD technique has been applied to the study of phylogenetic relationships in tilapiine and cichlid species (Bardakci and Skibinski, 1994) and among wild species of Indian major carps (Phale *et al*., 2009). In the present study, we have collected seven species of *Mystus* from the Chindwin and Ganga-Brahmaputra basins and analyzed the genetic similarity to discriminate the species of *Mystus,* by using RAPD-PCR technique.

Materials and methods

Samples of *Mystus rufescens, M. ngasep* and *M. falcarius* were collected from the Chindwin drainage of Manipur while *Mystus bleekeri* (includes two populations:Dibru River population of upper-Brahmaputra River and Guwahati population of Lower-Brahmaputra River)*, M. tengara, M. cavasius* and *M. dibrugarensis* were collected from the Brahmaputra drainage (Table 1).

Table 1. Fish Collection data of seven species of *Mystus* of northeast India.

Species	No. of samples	Drainage	Collection site	Latitude/Longitude
M. falcarius	15	Chindwin basin	Lokchao R. Manipur	24°15′N, 94°19′E
M. rufescens	24	Chindwin basin	Lokchao R. Manipur	24°15′N, 94°19′E
Mystus ngasep	36	Chindwin basin	Nambul R, Manipur	24°48′N, 93°55′E
M. dibrugarensis	33	Brahmaputra basin	Dibru R. , Assam	27°34′N, 95°19′E
M. bleekeri	05	Lower Brahmaputra	Guwahati, Assam	26°11′N, 91°45′E
M. bleekeri	05	Upper Brahmaputra	Dibru R. , Assam	27°34′N, 95°19′E
M. cavasius	32	Brahmaputra	Guwahati, Assam	26°11′N, 91°45′E
M. tengara	22	Brahmaputra	Guwahati, Assam	26°11′N, 91°45′E

About 100 mg of upper lobe of caudal fin was collected from the freshly caught fish specimens, kept in 80% ethanol and stored at -20° C until use. The voucher specimens were preserved in 10% formalin and deposited in Manipur University Museum of Fishes (MUMF) and Rajiv Gandhi University Museum of Fishes (RGUMF). Fishes were identified following the standard works of Vishwanath *et al.* (2007) and Darshan *et al.* (2010).

Genomic DNA was extracted from the ethanol fixed fin tissue, following the phenol-chloroform extraction protocol (Sambrook and Russel, 2001) and resuspended in TE buffer (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.0). Concentration of DNA was measured with a UV 1 spectrophotometer (Thermo Scientific, England) by measuring optical densities at 260 nm and 280 nm to calculate the quantity and purity.

RAPD-PCR reactions were carried out in sterile PCR tubes in a reaction volume of 25 ml containing: template DNA (50 ng), 2.5 ml of 10x buffer, 1.5 ml of $MgCl_2$ (25 mM), 2 mM dNTPs mix (100 mM), 5 pmoles of Random primer (Operon series, USA), 0 .5 U *Taq* DNA polymerase and autoclaved water, by using an Eppendorf mastercycler gradient PCR. The reaction conditions were: denaturation at 94 °C for 4 minutess, followed by 35 cycles of 94 °C for 1 minutes and 36 °C for 1 minute, elongation at 72 °C for 2 minutes, with a final elongation at 72 °C for 7 minutes. A negative control was included for each set of amplifications. Amplified product was separated on 1.2% agarose gel (SRL, India) stained with ethidium bromide, by using submarine gel electrophoresis in 1X TBE buffer (Tris-borate EDTA; 89.0 mM Tris, 2.0 mM EDTA, 89.0 mMboric acid), pH 8.0, for 2 hours at constant voltage of 70 V. Gel pictures were taken by UV-Gel Documentation Unit (Alpha Imager 3400, Alpha Innotech Corporation, USA). Size of the bands was estimated by 1 kb DNA ladder (Fermentas Life Sciences, Germany) which run in every gel.

In the primary analysis, 60 random primers of 10 mer from Operon Technologies (arbitrary primers from OPA, OPY and OPX series) were tested on 2 samples each from each taxon. Based on the screening data, 13 primers which can generates very strong amplification with at least 85-90% reproducible polymorphic DNA bands in all the species under study were selected for the analysis (Table 2).

Table 2. Primer codes and sequences of the Operon Technologies random primers used in the present study.

Sl. No.	Primer codes	Sequence (5' to 3')
1	OPA 1	CAGGCCCTTC
2	OPA2	TGCCGAGCTG
3	OPA3	AGTCAGCCAC
4	OPA4	AATCGGGCTG
5	OPA5	AGGGGTCTTG
6	OPA10	GTGATCGCAG
7	OPA13	CAGCACCCAC
8	OPA18	AGGTGACCGT
9	OPX12	TCGCCAGCCA
10	OPX17	GACACGGAGC
11	OPY2	CATCGCCGCA
12	OPY4	GGCTGCAATG
13	OPY5	GGCTGCGACA

Only five representatives of each taxon group under study were randomly selected due to limitation of lane in the gel. All the amplicons produced by a primer were run at the same time in a single gel. DNA banding patterns generated by RAPD were scored 1 for presence and 0 for absence for each amplified band. A pool scoring matrix for all the taxon was generated depending on the band pattern (0, 1 matrix), produced by the thirteen primers.

Software Alpha View SA, (version 3.2.3) was used for the estimation of molecular weight of the RAPD markers in base pairs by comparison with the known molecular weight (bp) of the ladder (1 Kb) in the gel. Genetic similarity and dendrogram were analyzed using the NTSYS-PC (version 2.20e) software.

Results and discussion

A comparative RAPD analysis was carried out on the seven species of *Mystus* distributed in the northeast India to observe the degree of genetic similarity and interrelationship among them (Fig. 1). The thirteen RAPD markers selected for this study amplified 639 bands ranging from 285 to 2150 bp, which were assigned to 204 loci with a mean of 15 loci per primer. Among these 204 RAPD loci, 11 were found to be monomorphic and 20 were polymorphic as well as regarded as potential species specific bands.

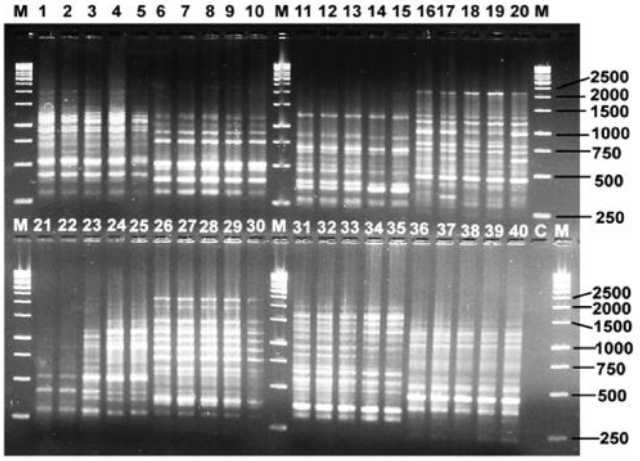

Fig. 1. RAPD profiles of 7 species of *Mystus* generated by using OPA 13 marker. (1-5: *M. ngasep*, 6-10: *M. bleekeri* from the Lower Brahmaputra River, 11-15: *M. tengara*, 16-20: *M.cavasius*, 21-25: *M. rufescens*, 26-30: *M. bleekeri* from the upper Brahmaputra River, 31-35: *M. dibrugarensis*, 36-40: *M. falcarius*, C: control & M: Lader.

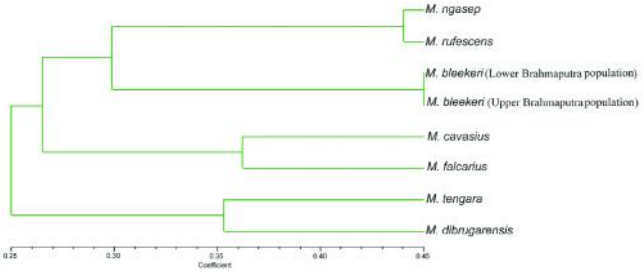

Fig. 2. Dendogram obtained by the Jaccard similarity index and method of UPGMA for seven species of *Mystus* distributed in northeast India.

Table 3. Jaccard's similarity index of 7 species of *Mystus* obtained from 13 RAPD markers. (MN: *Mystus ngasep*, MBLB: *M. bleekeri* from Lower Brahmaputra River, MT: *M. tengara*, MC: *M. cavasius*, MR: *M. rufescens*, MBUB: *M. bleekeri* from Upper Brahmaputra River, MD: *M. dibrugarensis*, MF: *M. falcarius*).

Row\cols	MN	MBLB	MT	MC	MR	MBUB	MD	MF
MN	1.000							
MBLB	0.350	1.000						
MT	0.267	0.227	1.000					
MC	0.270	0.318	0.238	1.000				
MR	0.444	0.288	0.258	0.271	1.000			
MBUB	0.273	0.454	0.262	0.285	0.275	1.000		
MD	0.238	0.216	0.353	0.304	0.259	0.221	1.000	
MF	0.252	0.240	0.217	0.363	0.252	0.208	0.248	1.000

The similarity index values obtained for each pairwise comparison among the seven species of *Mystus* ranged between 0.208 to 0.454 (Table 3). Maximum similarity was observed between the two populations of *M. bleekeri* with a value of 0.454 followed by *M. rufescens* and *Mystus ngasep* and the minimum was shared between *M. falcarius* and *M. M. bleekeri* (Upper Brahmaputra population).

Dendogram (Fig. 2) obtained has grouped all the seven species into two major clusters A and B, reflecting their morphological differences. Cluster A comprises *M. rufescens, M. ngasep, M. bleekeri* (both the populations: the Dibru River population of Upper and the Guwahati population of lower Brahmaputra River), *M. cavasius* and *M. falcarius* which are morphologically distinct in having a long base adipose-fin (without interdorsal) and cranial fontanel reached the base of the occipital process. Species in cluster B (*M. tengara* and *M. dibrugarensis*) have a short cranial fontanel which does not reach the base of the occipital process and a short adipose-fin base (with long interdorsal). Cluster A is again sub-divided into clusters A_1 and A_2. The fishes under the cluster A_1 (*M. ngasep, M. rufescens,* and *M. bleekeri*) are also morphologically distinct in having a prominent body stripe while those fishes under A_2 were lacking body stripes.

The dendogram has also clearly revealed that *M. rufescens* is genetically closer to *M. ngasep* with a higher similarity index value of 0.444, even though both are resolved into well separated clusters representing two separate distinct species. Results of the present study also showed that the two populations of *M. bleekeri* are genetically more similar, most probably due to regular gene flow between the two populations as there is no assumable geographic barrier between the upper and lower Brahmaputra drainage. Thus, the two populations of *M. bleekeri* are clustered together. Present results inferred that RAPD markers have proved to be useful in discriminating the species of *Mystus* and establishing their interrelationship based on their genetic composition. For better understanding of the interrelationship among this group of fishes, more work needs to be done using Type-I or dominant markers.

Acknowledgements

The authors are grateful to the University Grants Commission (UGC), New Delhi, for financial assistance through the Centre with Potential for Excellence in Biodiversity (CPEB) scheme and Directorate of Cold water Fisheries research (DCFR), Bhimtal for providing all research facilities.

References

Bagra, K., Kadu, K., Nebeshwar-Sharma, K., Laskar, B. A., Sarkar, U. K., Das, D. N. 2009. Ichthyological survey and review of the checklist of fish fauna of Arunachal Pradesh, India. Check List. 5(2): 330-350.

Bardakci, F. and Skibinski, D.O.F. 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity. 73:117-123.

Bartish, I.V., Gorkava, L.P., Rumpunen, K., Nybom, H. 2000. Phylogenetic relationship and differentiation among and within populations of Chaenomeles Lindl. (Rosaceae) estimated with RAPD's and isozymes. Theor Appl Genet. 101: 554-563.

Darshan, A., Anganthoibi, N. and Vishwanath, W. 2010. Redescription of the striped catfish *Mystus carcio* (Hamilton) (Siluriformes: Bagridae). Zootaxa. 2475: 48-54.

Darshan, A., Vishwanath, W., Mahanta, P. C. and Barat, A. (2011). *Mystus ngasep*, a new catfish species (Teleostei: Bagridae) from the headwaters of Chindwin drainage in Manipur, India. Journal of Threatened Taxa. 3: 2177-2183.

Darshan, A., Mahanta, P. C., Barat A. and Kumar, P. (2013). Redescription of the striped catfish *Mystus tengara* (Hamilton, 1822) (Siluriformes: Bagridae). India. Journal of Threatened Taxa. 5: 3536-3541.

Ferraris, C. J. Jr. 2007. Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes) and catalogue of Siluriform primary types. Zootaxa. 1418: 1-628.

Garg, R.K., Silawat, N., Sairkar, P., Vijay, N., Mehrotra, N.N. 2009. RAPD analysis for genetic diversity of two populations of *Mystus vittatus* (Bloch) of Madhya Pradesh, India. African Journal of Biotechnology. 8(17): 4032-4038.

Karp, A., Kresovich, S., Bhat, K.V., Ayand, W.G. and Hodgkin, T. 1997. Molecular Tools In Plant Genetic Resources Conservation: A Guide To The Technologies, IPGRI Technical Bulletin No. 2, International Plant Genetic Resources Institute, Rome, Italy.

Kottelat, M. and Whitten, T. 1996. Freshwater Biodiversity in Asia with special reference to Fish: World Bank Technical Paper No. 343. The World Bank, Washington, DC. Pp: 59.

Mo, T. P. 1991. Anatomy, relationships and systematics of the Bagridae (Teleostei: Siluroidei) with a hypothesis of siluroid phylogeny. Theses Zoologicae. 17: 1-216.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G.A. B. and Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403: 853-858.

Ng, H. H. and Pethiyagoda, R. 2013. *Mystus zeylanicus*, a new species of bagrid catfish from Sri Lanka (Teleostei: Bagridae). Ichthyological Exploration of Freshwaters. 24(2): 161-170.

Phale, S.R., Chauhan, S., Bhute, Y. V., Baile, V.V. 2009. Detection of genetic variation in the wild population of Indian majour carps using random amplified polymorphic DNA fingerprinting. Journal of Fish Aquatic Science. 4(1):63-70.

Rohlf, J. 1998. Numerical Taxonomy and Multivariate Analysis System Version 2.20e, Exeter Software. Application Biostatistics. New York.

Saini, A., Dua, A., Mohindra, V., Lakra, W. S. 2010. Molecular discrimination of six species of Bagrid catfishes from Indus river system using randomly amplified polymorphic DNA markers. Molecular Biology Report. 38(5): 2961-2965

Sambrook, J. and Russell, D.W. 2001. Molecular cloning - A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Vishwanath, W., Lakra, W.S. and Sarkar, U.K. 2007. Fishes of North east India. National Bureau of Fish Genetic Resources, Lucknow, UP, India. Pp: 264.

Welsh, J. and McClelland, M. 1990. Fingerprinting genome-using PCR with arbitrary primers. Nucleic Acids Research. 18: 7213-7218.