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Characterization of polymorphic microsatellite markers and genetic diversity in wild bronze featherback, *Notopterus notopterus* (Pallas, 1769)

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Abstract Six polymorphic microsatellite DNA loci were identified in the primitive fish, bronze featherback, *Notopterus notopterus* for the first time and demonstrated significant population genetic structure. Out of the six primers, one primer (NN90) was specific to *N. notopterus* (microsatellite sequence within the RAG1 gene) and five primers were product of successful cross-species amplification. Sixty-four primers available from 3 fish species of order *Osteoglossiformes* and families *Notopteridae* and *Osteoglossidae* were tested to amplify homologous microsatellite loci in *N. notopterus*. Fifteen primer pairs exhibited successful cross-priming PCR product. However, polymorphism was detected only at five loci. To assess the significance of these six loci (including NN90) in population genetic study, 215 samples of *N. notopterus* from five rivers, *viz* Satluj, Gomti, Yamuna, Brahmaputra and Mahanadi were analyzed. The five sample sets displayed different diversity levels and observed heterozygosity ranged from 0.6036 to 0.7373. Significant genotype heterogeneity ($P < 0.0001$) and high F_{ST} (0.2205) over all loci indicated that the samples are not drawn from the same genepool. The identified microsatellite loci are promising for use in fine-scale population structure analysis of *N. notopterus.*

Keywords *Notopterus notopterus* · Microsatellite markers · Polymorphism · Genetic diversity

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Introduction

The significance of microsatellite markers derives from their random abundance in the genome, single locus nature, simplicity of assay, high levels of allelic diversity, Mendelian inheritance, co-dominance and selective neutrality. The microsatellites DNA markers have become proven tools for direct assessment of genetic variation and population level evolution [1–3]. Although microsatellite primers cannot generally be used universally, a certain level of microsatellite amplification between closely related species (cross-species microsatellite amplification) is possible [4]. The success rate of cross-species microsatellite amplification is directly related to the evolutionary divergence between the species from which the microsatellite loci have been isolated (the source species) and the species to which the heterologous loci are being applied [5]. The cross species microsatellite amplification is sufficiently successful that a diverse range of evolutionary genetic studies in various species groups have been conducted based solely on cross-amplified microsatellites [6]. Scribner and Pearce [4] reviewed cross-species amplification of microsatellite markers in various taxonomic groups. Among piscine species, cross-species amplification of the microsatellite primers was successfully demonstrated in Cichlidae [7], Salmonidae [8–10] and Cyprinidae [11–13], Notopteridae [14], Percidae [15] and in Acipenseridae [16]. Use of heterologous sequences can circumvent the extensive preliminary work and cost involved to develop microsatellite enriched genomic libraries for individual species [7].

Notopterus notopterus commonly known as bronze featherback, is a teleostean fish which represents prehistorical lineage, as it belongs to primitive order Osteoglossiformes [17–19] and family Notopteridae. Therefore,

genetic diversity, phylogeographic and phylogenetic exploration through application of molecular markers in the fishes of this order are of considerable interest. The featherback, *N. notopterus,* is widely distributed in South-east Asia, Bangladesh, Cambodia, India, Laos, Burma, Nepal, Pakistan, and virtually all river basins of peninsular Thailand and Malaysia; Sumatra and Java. In India, this fish is widely distributed in Indus, Ganges–Brahmaputra, Mahanadi, Krishna, Cauvery, and other river basins in peninsular India [20].The fish has been categorized as an important commercial fish by FAO [21] for food as well as for ornamental trade. Populations of bronze featherback in wild are declining and therefore categorized as one of the threatened species of the country [22]. This species is listed on the IUCN Red List as Least Concern (LC) due to lack of data [23]. Rainboth [24] has described the biology and other information on this fish. However, the genetic studies in the species have been limited to a small part of vast distribution [25, 26] and genetic relatedness with another Notopterid fish, *Chitala chitala* [27].

The objective of the present study was to test if primers developed for other fish species of order *Osteoglossiformes* are sufficiently conserved to amplify as homologous microsatellite loci in *N. notopterus*. The study also presents the results on genetic variability and divergence in five populations of *N. notopterus* from rivers of four different basins in India to evaluate the potential of identified microsatellite loci in population structure of the commercially and evolutionary important species.

Materials and methods

Sample collection and isolation of genomic DNA

The fish specimens were obtained through commercial riverine catches from five rivers, *viz* Satluj (Hari ke patan, n = 81), Gomti (Sultanpur, n = 66), Yamuna (Yamunanagar, n = 26) Brahmaputra (Assam, n = 18) and Mahanadi (Cuttack, n = 24) during September 2000 to January 2008. These sampling sites were selected to cover genetic variation on a wide geographical distribution of the species. The river Satluj belongs to the Indus basin and river Brahmaputra which originates from South-western Tibet and flows southwest through the valley of Assam, river Gomti and Yamuna are tributary of Ganga River System. The Mahanadi is a major river in East Central India. This river flows through the states of Chhattisgarh and Orissa, into the Bay of Bengal. The blood samples were collected through caudal puncture and fixed in 95 % ethanol in 1:5 (blood:ethanol) ratio and dorsal white muscles (50 mg) were stored in 95 % ethanol at 4 °C until use.

PCR amplification and electrophoresis

The genomic DNA was extracted from blood and muscles using proteinase K, phenol: chloroform protocol [28]. PCR amplification was carried out in a 25 µl reaction mixture that included 1X PCR buffer (10 mM Tris–HCl pH 9.0; 50 mM KCl; 0.01 % gelatin), 0.2 mM of each dNTP, 2.0 mM of $MgCl_2$, 5 pmol of each primer, 1.5 U Taq DNA polymerase and 25–50 ng of template DNA. PCR (MJ PTC-200 thermal cycler, MJ Research, Inc., MA, USA) cycles were as follows: (1) one cycle of denaturation at 94 °C for 5 min; (2) 25 cycles of denaturation at 94 °C for 30 s, relevant annealing temperature for 30 s, elongation at 72 °C for 1 min (3) a final elongation of one cycle at 72 °C for 4 min and stored at 4 °C. PCR products were resolved through vertical non-denaturing polyacrylamide (19:1 acrylamide:bisacrylamide) gels electrophoresis (10 × 10.5 cm, Amersham Biosciences, Hong Kong). Electrophoresis was performed with 1× TBE buffer for 5 h at 10 V cm^{-1} at 4 °C. For cross-species amplification experiments, 10 % PAGE gels were used. For loci exhibiting successful amplification, gel concentration was optimized according to allele size for better resolution. The amplified microsatellite loci were visualized through silver staining (silver staining kit, Amersham Biosciences). Alleles were designated according to PCR product size, calculated relative to a molecular marker (pBR322 DNA/*Msp*I digests) with software BIOVIS Gel 1D.

For genotyping total of 215 individuals were amplified for six locus in 12.5 µl reaction volumes containing 1× PCR buffer (10 mM Tris–HCl, pH 9.0 50 mM KCl; 0.01 %gelatin), 2.0 mM of $MgCl_2$, 0.2 mM of dNTP, 5 pmol of forward (labelled) and reverse primer each and 1.5 U of Taq Polymerase and template DNA with the same PCR reaction conditions used above. Genotyping was performed on an ABI337 automated DNA Sequencer and the individuals analyzed using Gene Mapper V. 3.2. Data was analysed using the software GENETIX 4.02 [29] to obtain expected (He) and observed (Ho) heterozygosity values.

Screening of primers and genetic diversity analysis

Microsatellite primers from *Chitala chitala*, *Scleropages formosus* and *Arapaima gigas* (Table 1) were tested for amplification of homologous loci. The three species are termed as resource species in the study. This cross-species amplification experiment was carried out with 14 specimens of *N. notopterus* from following locations, Satluj (Hari ke patan, n = 5), Gomti (Sultanpur, n = 5) and Brahmaputra (Assam, n = 4). The optimum annealing temperature, to achieve a scorable band pattern, was determined through experimental standardization for each

Table 1 Primers of microsatellite loci tested for cross-species amplification in *Notopterus notopterus*

Species	Number of primer pairs tested	Microsatellite Locus	GenBank accession number	Reference	Successful primer pair amplified in *N. notopterus*
Chitala chitala	31	*Cch1, Cch2, Cch4, Cch6, Cch9, Cch10, Cch13, Cch15, Cch18, Cch20*	DQ525389-98	Punia et. al 2006	6
		Cch3, Cch5, Cch7, Cch25	DQ525333-36		
		Cch26,Cch27,Cch28, Cch29,Cch30, Cch31, Cch32,Cch33,Cch8, Cch11,Cch12,Cch14 Cch16, Cch17,Cch19, Cch21	DQ786637-52		
		NCG 3-12a	DQ496226		
Scleropages formosus	18	*D11, D13, D14, D16, D27, D31, D32, D33, D35, D37, D38, D42, D72, D85, D88, D92, D94, D95*	AF219953-219971	Yue et.al 2000	–
Arapaima gigas	14	*AGCTm-1,-3,-4,-5,-7,-8,AGCAM-2, -4,-13,-15,-16,- 18, -20, -26*	AY176172-85	Farias et. al 2003	–
Notopterus notopterus	1	*NN90*	AF508062	Venkatesh et. al 1999	1

primer pair. The primers yielding scoreable amplified products were again evaluated with larger sample size (215) individuals from five rivers to evaluate their suitability in quantification of genetic divergence in *N. notopterus*. Individual fish genotypes for each locus were determined and allele frequencies and heterozygosity (observed and expected) values were calculated using Genetix 4.02 software [29]. Tests for conformity to Hardy–Weinberg expectations (probability test) and genetic differentiation were performed through Genepop 3.3d [30].

Results

Identification of polymorphic Microsatellite loci in *N. notopterus*

Out of a total of 64 primer pairs tested, 15 primers from another *Notopterid* fish, *C. chitala* provided successful amplification (23.4 %) of homologous loci in *N. notopterus*. The primers of *Scleropages formosus* and *Arapaima gigas* did not amplify any locus. Out of the 15 successful primers, 9 primers were consistent monomorphic, while 6 loci exhibited polymorphism. Though, all these six primer pairs consistently amplified single locus, but the locus *Cch8* was not considered for genotyping analysis due to higher molecular weight. One species-specific primer of *N. notopterus* available (microsatellite sequence within the *RAG1* gene, referred to here as the *NN90* locus [31]) was also assessed and found to be polymorphic. Comparison of repeat motif with that found in resource species (*C. chitala*) indicate that microsatellite loci differ with simple change of repeat motif units, except for two loci *Cch2* and *Cch20*. In *Cch 2* locus, compound repeats were found in both species but with a GTT separating the two subunits (GA) and (TG) in *C. chitala*. In locus *Cch20,* the repeat motif in *N. notopterus* was tetranucleotide (GAGT) and completely different from *C. chitala* which has dinucleotide repeat (GA). With the inclusion of *NN90* locus, total 6 loci were available for genotyping and assessment of samples of *N. notopterus* from 5 riverine localities.

Genetic Diversity in *N. notopterus* population from five rivers

A total of 215 *N. notopterus* samples from five rivers were analyzed with six microsatellite loci to test their potential in genetic variation analysis. A total of 198 alleles were identified. The total number of alleles per locus and their size (bp) ranges are given in Table 2. The maximum of 15 alleles were detected at the locus *Cch2*. The mean number of alleles per locus were 6.0000 (Satluj), 6.8333 (Gomti), 5.3333 (Yamuna), 6.3333 (Brahmaputra) and 8.5000 (Mahanadi). The expected heterozygosity (He) values ranged from 0.5260 (Satluj) to 0.7586 (Mahanadi), whereas observed heterozygosity (Ho) varied from 0.6036 (Satluj) to 0.7373 (Brahmaputra). In total out of 30 tests (population x loci) performed, 13 tests indicated significant deviation from Hardy–Weinberg expectations ($p < 0.05$) after validation with P_{score} test and application of sequential Bonferroni corrections of probability level. There was evidence of possibility of null alleles at some of the loci (Table 2). No significant linkage disequilibrium was detected between comparisons of these loci indicating all six loci segregate independently to each other.

Table 2 Characteristics of microsatellites including; F (forward primer), R (reverse primer), Ta (annealing temperature), N_a (allele observed), H_e (expected heterozygosity), H_O (observed heterozygosity), P (agreement to HW expectations)

Locus	Primer sequence	Core Sequence	Ta (°C)	River	Na	Size range (bp)	He	Ho	HW (P)	Genetic (P) homogeneity
Cch2	F: ACCCAAGCCATGTTAAGTGGTC	$(GA)_7$ $(TG)_9$	60	Satluj	11	195–235	0.8240	0.8025	0.1069	0.0000*
	R: GGCGAGTCCACGATTTCAAG			Gomti	13	171–217	0.8460	0.6724	0.0000*,[a]	
				Yamuna	12	155–221	0.8416	0.9600	0.2556	
				Brahmaputra	10	165–207	0.8472	0.8333	0.0396	
				Mahanadi	15	173–229	0.8762	0.6522	0.0000*,[a]	
Cch18	F: TGTAGGAGCCGGAGGTGGGAGAA	$(CA)_{18}$	60	Satluj	8	118–140	0.5414	0.5802	0.3588	0.0000*
	R: CTGCAGCAGTAGGCCTGTGAG			Gomti	5	118–140	0.5673	0.4727	0.0666	
				Yamuna	5	118–140	0.6467	0.6250	0.3581	
				Brahmaputra	9	118–148	0.7022	0.8333	0.6136	
				Mahanadi	12	118–148	0.8255	0.8750	0.9198	
Cch10	F: TCGTTATTTCTGACATTCAAGTGC	$(GT)_{23}$	60	Satluj	7	86–112	0.7640	0.5769	0.0000*,[a]	0.0000*
	R: TACAAGCTCCATGCACAATTA			Gomti	5	92–112	0.5538	0.1556	0.0000*,[a]	
				Yamuna	4	104–110	0.4653	0.4167	0.1350	
				Brahmaputra	5	102–112	0.6484	0.5000	0.0582	
				Mahanadi	4	100–110	0.6213	0.0000	0.0000[a]	
Cch20	F: GGAGGGATGCTGTGCACTATAAAG	$(GAGT)_4$	58	Satluj	3	150–158	0.5120	1.0000	0.0000*	0.0000*
				Gomti	4	152–158	0.5569	1.0000	0.0000*	
	R: CCGTTGGGTCTGTCTGTATAT			Yamuna	4	154–160	0.6775	1.0000	0.0000*	
				Brahmaputra	4	154–164	0.7006	1.0000	0.0000*	
				Mahanadi	5	150–158	0.6696	1.0000	0.0000*	
Cch39	F: TCAAGCATGCGTGTTAGTCTATGG	$(GT)_{17}$	58	Satluj	3	158–164	0.0603	0.0370	0.0090	0.0000*
				Gomti	5	156–164	0.5898	0.5758	0.0000*	
	R: TGGGGAGCGGGGGTTGTT			Yamuna	2	160–164	0.3336	0.4231	0.5472	
				Brahmaputra	4	154–164	0.3719	0.4444	1.0000	
				Mahanadi	8	154–174	0.7736	0.8000	0.0000*	
NN90	F: GCACCTGCAGTAAACCACA	$(AC)_{20}$	60	Satluj	4	160–172	0.4541	0.6250	0.0013	0.0000*
	R: CTCCCAATGCTCTCCCAATA			Gomti	9	158–180	0.6786	0.7600	0.2699	
				Yamuna	5	158–172	0.3951	0.4783	1.0000	
				Brahmaputra	6	166–180	0.6953	0.8125	0.3420	
				Mahanadi	7	162–176	0.7851	0.3636	0.0000*,[a]	

* $P < 0.05$

[a] Possible Presence of Null allele

Table 3 Pairwise F_{ST} between five natural populations of *Notoperus notopterus* studied through Six polymorphic microsatellite loci (p values for F_{ST} were significant; $p < 0.05$)

	Satluj	Gomti	Yamuna	Brahmaputra	Mahanadi
Satluj		0.1320	0.0943	0.3003	0.2425
Gomti			0.0294[#]	0.2676	0.3107
Yamuna				0.2945	0.1946
Brahmaputra					0.1481
Mahanadi					

[#] $P > 0.05$ shows insignificant p values for Pairwise F_{ST}

Table 4 Comparison of F_{ST} and R_{ST} values based on six polymorphic microsatellites in *Notopterus notopterus*

Population pair	F_{ST}	R_{ST}
Satluj and Gomti	0.1320	0.3096
Satluj and Yamuna	0.0943	0.1881
Satluj and Brahmaputra	0.3003	0.6470
Satluj and Mahanadi	0.2425	0.0308
Gomti and Yamuna	0.0294	0.0348
Gomti and Brahmaputra	0.2448	0.5377
Gomti and Mahanadi	0.1574	0.1385
Yamuna and Brahmaputra	0.2945	0.4082
Yamuna and Mahanadi	0.1946	0.0436
Brahmaputra and Mahanadi	0.1481	0.4201

Significant genetic heterogeneity ($P < 0.05$) was evident at all the loci. Pairwise comparison of F_{ST} values from Gomti and Mahanadi exhibited significantly higher $_{ST}$ value (0.3107, $P < 0.0001$) however Gomti and Yamuna exhibited insignificant $_{ST}$ value (0.0294, $P = 0.10$). The high F_{ST} value of the Brahmaputra and Mahanadi sample compared with other localities (Table 3) was supported by statistical significance. Comparative account of F_{ST} and R_{ST} values between all possible pairs of samples are given in Table 4. R_{ST} was found to be higher than F_{ST} at 7 out of the 10 possible populations pairs (Table 4). Overall loci populations Fst value was 0.2205.

Discussion

The study demonstrates successful cross-species amplification of microsatellite loci in *N. notopterus* and seven polymorphic loci were found, of which six loci were used to assess genetic variation in samples from five riverine locations. The optimum annealing temperature to get scorable band in *N. notopterus* varied from that reported in the literature. Therefore, optimization of PCR conditions is a matter of necessity for the primers identified through cross-species amplification. Most of the successful primer pairs showed comparable allele sizes compared with the species from which they were developed, as reported in other studies also [13, 32, 33]. However, *Cch20* showed quite different amplification products among the species examined with comparatively larger allele sizes in the *Chitala chitala* (with the size range of 200–202 bp) than that amplified in *N. notopteus* (with the known size range of about 160–180 bp). The likelihood that primer pairs developed for one species amplify in another species is higher, if the two species are closely related [4] such as within genus, subfamilies or families. The reports of success are limited between families and rare between orders. In *N. notopterus*, only the primers from *C. chitala* of same family *Notopteridae*, successfully amplified homologous loci. Similarly, the primer *NN90* of *N. notopterus* origin amplified and was useful to study genetic diversity of *C. chitala* [33]. Conversely, in *C. chitala,* 5 out of 19 primers were reported to be common with *Sclerophagus formosus,* a fish from different family *Osteoglossidae* under same order *Osteoglossiformes* [14], however, no such conserved loci were found in *N. notopterus*. Here, it is to mention that even 2 primer of *Sclerophagus formosus* were found conserved across the orders, as reported in a siluriform fish, *Horabagrus brachysoma* [34]. The present study points out the usefulness of cross-species amplification of microsatellites in family *Notopteridae,* to establish markers for population genetics studies. The findings in this paper have provided a useful set of markers for phylogeography and population genetics studies of the *N. notopterus*. Microsatellite loci are typically characterized by high mutation rates.

The homologous loci in two species can differ with respect to repeat motifs. Normally the numbers of repeat motif units are altered due to deletion and addition. Therefore, the mutations does not alter the relationship of microsatellite loci sequences for homologous loci and microsatellite loci are suggested to follow stepwise mutation model (SMM) and result in changes is allele size [35]. However, there are also possibilities of infinite allele model of mutation. This was reported in the present study where repeat motif is different at Cch20.

Genetic diversity is generally the result of long-term evolution and it represents the evolutionary potential of a species. To survive and adapt to an unstable environment, a species has to evolve and accumulate genetic variation [36, 37] Tables 2 and 3 in this study clearly demonstrate the presence of fair levels of polymorphism in *N. notopterus*, with the total number of alleles across the six loci being high, equaling 198.

Some loci deviated significantly from the Hardy–Weinberg expectations. The determination of inbreeding coefficient (F_{IS}) through partitioning of genetic variability

as suggested by Wright [38] and Weir [39] has been widely used to determine if the population has excess or deficit of heterozygote. Out of 9 tests that deviated significantly for Hardy–Weinberg expectations, 8 were associated with deficit of heterozygote (+ve F_{IS}) and 5 indicated excess of heterozygote (−ve F_{IS}). Deficit of heterozygotes could be due to possible null alleles at some of the loci in some population or indicate the violation of assumption of Hardy–Weinberg expectations such as lack of random mating or directional selection in the population.

Several evolutionary forces, such as mutations, random genetic drift, gene flow and natural selection, influence the variation patterns of genomes and populations [40]. Selection and mutations have locus-specific effects while genetic drift and gene flow act at genome-wide scale under a standard neutral model [41]. Random genetic drift due to isolation and mutations may be the important factor contributing to genetic differentiation among populations in *N. notopterus*. The Fst over all loci and populations indicated significant genetic differentiation and implied that 22.05 % of the total variation could be attributed to genetic divergence between samples from different riverine localities. The F_{ST} value is a useful measurement of genetic differentiation among populations. In this study, the pairwise F_{ST} values were between 0.0294 and 0.3107. Our results show that the Mahanadi has an extremely significant differentiation with the Gomti and a significant differentiation with the Satluj, Yamuna and Brahmaputra. Brahmaputra also shows significant differentiation with all the populations. *N. notopterus* have poor swimming abilities and inhabits sluggish water in wetlands, floodplains which may or may not be fed with rivers [24]. This fish lay eggs in small clumps on submerged vegetation [20] and thus are not easily dispersed, further indicating its poor dispersal capability, neither through movement of adult fish or the passive transportation of eggs and consequently leads to restricted gene flow between populations and are expected to exhibit high genetic differentiation [42]. The rivers Yamuna and Gomti are part of Ganga river system, low differentiation value possibly could be due to ancestral proximity or accomplished through some gene flow happening over the generations through continuity of the river system. Under stepping stone model of migration, the gene exchange between neighbours can nullify the genetic differentiation [43].

The microsatellite dataset revealed higher R_{ST} when compared to F_{ST} in 7 out of 10 possible population pairs. This indicated that fragmentation has more bias towards allelic size as R_{ST} is computed based on allele size rather than allelic identity. On a historical scale, F_{ST} reflects more recent events, since it compares variance in allele frequencies between different populations and does not take into account past mutations. In the present study, all the pairwise comparisons involving river Brahmaputra have high R_{ST} than F_{ST}, however, same is not true for river Mahanadi samples. This possibly indicates that accumulation of mutations is responsible for genetic divergence in Brahmaputra population, while river Mahanadi differentiation is the consequence of the random genetic drift.

The present investigation identifies six polymorphic microsatellite markers for *N. notopterus* using heterologous primers useful to discriminate the population structure of *N. notopterus*. The genetic variation detected from these markers also reveals that *N. notopterus* in different rivers studied, has distinct population substructure and is not a part of single gene pool.

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References

1. O'Connell M, Wright JM (1997) Microsatellite DNA in fishes. Rev Fish Biol Fish 7:331–363
2. Neff BD, Gross MR (2001) Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. Evolution 55:1717–1733
3. Chistiakov DA, Hellemans B, Volckaert FAM (2006) Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255:1–29
4. Scribner KT, Pearce JM (2000) Microsatellites evolutionary and methodological background and empirical applications at individual, population, and phylogenetic levels. In: Baker A (ed) Molecular methods in ecology. Blackwell Science Ltd., London, pp 235–271
5. Primmer CR, Moller AP, Ellegren H (1996) A wide-range survey of cross-species microsatellite amplification in birds. Mol Ecol 5:365–378
6. Palo JU, Mäkinen HS, Helle E, Stenman O and Väinö lä R (2001) Microsatellite variation in ringed seals (*Phocahispida*): genetic structure and history of the Baltic Sea population. Heredity 86:609–617
7. Zardoya R, Vollmer DM, Craddock C, Streelman JT, Karl S, Meyer A (1996) Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid (Pisces: *Perciformes*). Proc R Soc Lond B 263:1589–1598
8. Scribner KT, Gust JR, Fields RL (1996) Isolation and characterization of novel salmon microsatellite loci: cross species amplification and population genetics applications. Can J Fish Aqua Sci 53:833–841
9. Smith CT, Koop BF, Nelson RJ (1998) Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol Ecol 7:1613–1621
10. Cairnney M, Taggart BJ, Hoyheim B (2000) Characterization of microsatellite and minisatellites loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol Ecol 9:2175–2178

11. Zheng W, Stacey NE, Coffin J, Strobeck C (1995) Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus*. Mol Ecol 4:791–792
12. Mohindra V, Anshumala, Punia P, Narain L, Kapoor D, Lal KK (2005) Microsatellite loci to determine population structure of *Labeo dero* (Cyprinidae). Aquat Living Resour 18:83–85
13. Chaturvedi A, Mohindra V, Singh RK, Lal KK, Punia P, Bhaskar R, Mandal A, Narain L, Lakra WS (2011) Population genetic structure and phylogeography of cyprinid fish, *Labeo dero* (Hamilton, 1822) inferred from allozyme and microsatellite DNA marker analysis. Mol Biol Rep 38:3513–3529
14. Mandal A, Lal KK, Mohindra V, Singh RK, Punia P, Chauhan UK, Lakra WS (2009) Evaluation of genetic variation in the clown Knifefish, *Chitala chitala,* using allozymes, RAPD, and microsatellites. Biochem Genet 47:216–234
15. Wirth T, Saint-Laurent R, Bernatchez L (1999) Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross-species amplification within the family Percidae. Mol Ecol 8:1960–1963
16. McQuown EC, Sloss BL, Sheehan RJ, Rodzen J, Tranah GJ, May B (2000) Microsatellite analysis of genetic variation in sturgeon: new primer sequences for *Scaphirhynchus* and *Acipenser*. Trans Am Fish Soc 129:1380–1388
17. Taverne L (1975) A propos de Tselfatia Arambourg, C., 1943 (Cénomanien du Maroc), de Protobrama (Cénomanien du Liban) et du rapport éventuel des Tselfatioïdes fossiles (Crétacé) et des Téléostéens du super-ordre des *Ostéoglossomorphes*. Ann Soc R Zool Belg 105(1–2):53–72
18. Nelson JS (1994) Fishes of the World. Wiley, New York
19. Otero O, Gayet M (2001) Palaeoichthyofaunas from the Lower Oligocene and Miocene of the Arabian plate, palaeoecological and palaeobiogeographical implications. Palaeogeogr Palaeoclimatol Palaeoecol 165:141–169
20. Talwar KP, Jhingran AG (1991) Inland fishes of India and adjacent countries. Oxford & IBH, New Delhi
21. Casavas I, Doulman DJ, Petr TO, Padro J, Debas L (1996) Cambodia-rehabilitation and development needs of the fishery sector. FAO, Rome
22. CAMP (1998) Conservation Assessment and Management Plan for freshwater fishes of India. Zoo outreach organization and NBFGR, Lucknow, p 156
23. Ng HH, (2010) *Notopterus notopterus*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. http://www.iucnredlist.org. Downloaded on 07 September 2012
24. Rainboth WJ (1996) Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome, p 265
25. Takagi Akira P, Ishikawa Satoshi, Thuok Nao, Sitha Hort, Masanori Nakatani, Mutsumi Nishida, Hisashi Kurokura (2006) Tandem repeat sequence segments in control region of bronze featherback *Notopterus notopterus* mitochondrial DNA. Fish Sci 72(6):1319
26. Takagi AP, Ishikawa S, Nao T, Song SL, Hort S, Thammavong K, Saphakdy B, Phomsouvanhm A, Nishida M, Kurokura H (2010) Genetic differentiation and distribution routes of the bronze featherback *Notopterus notopterus* (Osteoglossiformes: Notopteridae) in Indochina. Biol J Linnean Soc 101:575–582
27. Lal KK, Mandal A, Singh RK, Punia P, Kapoor D, Chauhan UK, Singh SP, Mohindra V (2006) Genetic divergence in two feather back fishes, *Chitala chitala* and *Notopterus notopterus*. J Appl Ichthyol 22(5):369–373
28. Ruzzante DE, Taggart C, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53:634–645
29. Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1997) GENETIX version 4.02, Genetics logiciel sous windows pour Ia ge' ne' Tique des populations, available at: http://www.univmontp2.fr/genetix/genetix.htm
30. Raymond M, Russet F (1998) GENEPOP Version 3.3d Population Genetics Software for Exact Test and Ecumenicism, available at: http://www.cefe.cnrs-mop.fr
31. Venkatesh B, Ning Y, Brenner S (1999) Late changes in spliceosomal introns define clades in vertebrate evolution. Proc Natl Acad Sci USA 96(18):10267–10271
32. Van Hooft WF, Hanotte O, Wenink PW, Groen AF, Sugimoto Y, Prins HH, Teale A (1999) Applicability of bovine microsatellite markers for population genetic studies on African buffalo (*Syncerus caffer*). Anim Genet 30:214–220
33. Mandal A, Mohindra V, Singh RK, Punia P, Lal KK (2011) Mitochondrial DNA variation in Indian populations of endangered Feather-Back Fish, *Chitala chitala*. Mol Biol Rep 39(2):1765–1775
34. Gopalakrishnan A, Abdul Muneer PM, Musammilu KK, Lal KK, Kapoor D, Mohindra V (2006) Primers from order *Osteoglossiform* and *Siluriform* detect polymorphic microsatellite loci in suncatfish, Horabagrus brachysoma (Teleostei: Bagridae). J Appl Ichthyol 22(5):456–458
35. Zhu Y, Strassmann JE, Queller DC (2000) Insertions, substitutions, and the origin of microsatellites. Genet Res 76:227–236
36. Lande R (1996) The meaning of quantitative genetic variation in evolution and conservation. In: Szaro RC, Johnston DW (eds) Biodiversity in managed landscapes. Oxford University Press, New York, pp 27–40
37. Oostermeijer JGB, Luijten SH, den Nijs JCM (2003) Integrating demographic and genetic approaches in plant conservation. Biol Conserv 113:389–398
38. Wright S (1965) The interpretation of population structure by Fstatistics with special regard to systems of mating. Evolution 19:395–420
39. Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
40. Gaut B, Le Thierry d'Ennequin M, Peek AS, Sawkins MC (2000) Maize as a model for the evolution of plant nuclear genomes. Proc Natl Acad Sci USA 97:7008–7015
41. Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet 4:981–994
42. Ward RD, Grewe PM (1994) Appraisal of molecular genetics techniques in fisheries. Rev Fish Biol Fish 4:300–325
43. Hartl DL, Clark AG (1997) Principles of population genetics. Sinauer Associates Inc Publishers, Sunderland