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Genetic variation and phylogenetic relationship between two species of yellow catfish, *Horabagrus brachysoma* and *H. nigricollaris* (Teleostei: Horabagridae) based on RAPD and microsatellite markers

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Abstract The two species of yellow catfish, *Horabagrus brachysoma* and *H. nigricollaris* are categorized as 'endangered' and 'critically endangered' respectively in their wild habitat. Proper knowledge of genetic structure and variability of these endangered species are highly essential for the management, conservation and improvement of fish stocks. Therefore, genetic variation and phylogenetic relationships between these species of yellow catfish sampled from Chalakkudy River in the hot spot of biodiversity-Western Ghats region, Kerala, India were analyzed by using Random amplified polymorphic DNA (RAPD) and microsatellite markers. 85 RAPD and five microsatellites loci were detected to analyze the genetic variation and phylogenetic relationships among these species. Out of 85 RAPD loci produced only 52.94% were polymorphic whereas in microsatellite, all 5 loci were polymorphic (100%). Species-specific RAPD bands were found in both species studied. In microsatellite, the number of alleles across the five loci ranged from 1 to 8. The observed heterozygosities in *H. brachysoma* and *H. nigricollaris* were 0.463 and 0.443, respectively. Here, both RAPD and microsatellite methods reported a low degree of gene diversity and lack of genetic heterogeneity in both species of *Horabagrus* which strongly emphasize the need of fishery management, conservation and rehabilitation of these species.

Keywords Horabagrus · Phylogeny · RAPD · Genetic variation · Microsatellites · Polymorphism

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Introduction

The *Horabagrus* genus contains only two species, *H. brachysoma* (Gunther, 1864) (Fig. 1a) and *H. nigricollaris* [1] (Fig. 1b), which are endemic and cultivable yellow catfish belonging to the family Horabagridae found in southern part of the biodiversity hotspot—Western Ghats, India. The genus *Horabagrus* was originally placed in the Bagridae family [2] but work carried out by de Pinna [3] suggested that *Horabagrus* was more closely related to Schilbeidae family. But later he pointed out some features of *Horabagrus* were distinct from both Schilbeidae and Bagridae and he placed *Horabagrus* in its own family. Recently, Hardman [4] recommended recognition of this group as family Horabagridae based on cytochrome *b* evidence with which Sullivan et al. [5] supported the study by phylogenetic analysis of *rag1* and *rag2* nuclear genes.

Horabagrus brachysoma (Fig. 1a) is a large freshwater catfish distributed in selected west flowing rivers originating from the Western Ghats of Kerala and Karnataka viz., Chaliyar, Chalakkudy, Periyar, Meenachil, Pampa and Nethravathi Rivers [6, 7]. It is confined to the lower stretches of the river (approximately within 30 km distance) and is not migratory. *H. nigricollaris* (Fig. 1b) is only found in hill streams of the Chalakudy River at Vettilappara (N 10°17′, E 76°32′) area, Kerala, India [8].

Fig. 1 Two species of *Horabagrus*. **a**. *H. brachysoma*, **b**. *H. nigricollaris*

Both *H. brachysoma* and *H. nigricollaris* are quite similar in appearance. *H. brachysoma* has a large round black mark on shoulder surrounded by a light yellow ring [6] whereas *H. nigricollaris* has the black stripe extends over the neck, forming a "black collar" (hence the scientific name) [1]. Still there is some discrepancy in their identification. The attractive golden yellow color of these species makes them valuable for aquarium in India and abroad. *H. nigricollaris* is sleeker in body shape than *H. brachysoma*. *H. brachysoma* reaches about 45 cm while *H. nigricollaris* reaches about 27 cm [9]. Over-exploitation (especially for trade), destructive fishing methods which leads to habitat alteration, pollution and related anthropogenic interventions on their natural habitats have considerably reduced the wild population of these species during the last few years [10, 11]. The workshop on Conservation Assessment Management Plan to evaluate the status of freshwater species of India, held at NBFGR in 1997 categorized *H. brachysoma* as 'endangerd' and *H. nigricollaris* as "critically endangered" based on International Union for Conservation of Nature and natural resources (IUCN) criteria [12]. Since both species are endangered the information on stock structure is highly essential. Moreover, these species are being used as food and ornamental fish culture from Peninsular India. Recently, we have reported the stock structure and genetic variation in *H. brachysoma* based on these markers [7, 13] and allozymes [10]. Here, the genetic discrimination and phylogenetic relationships between two species are requisite as both species show much morphological similarities and they are rare in wild. Shivanandan [14] has depicted a comparison based on morphological characters of these species. However, differentiation of species only through morphological features is inefficient and even inaccurate. Thus, efficient use of genetic resources in fish breeding programs requires knowledge about genetic diversity. Worldwide demands on these fish necessitate to work on conservation of germplasm and their further genetic improvement, planning and implementation of proper conservation and management strategies.

The random amplified polymorphic DNA (RAPD) [15, 16] analysis is particularly attractive for analysis of genetic diversity and phylogeny [17]. RAPD has been used for phylogenetic studies for species and subspecies identification of fish [18–20]. Also, stock identification of several species has been carried out using RAPD technique [21–23]. Recently, Saini et al. [24] has been reported the RAPD markers for discrimination of 6 bagrid catfish species from Indus River, India. Also, few other authors have been carried out RAPD analyzes in catfishes [22, 25–27]. Microsatellites are well studied co-dominant DNA maker [28]. Due to their high variability, these genetic markers have been widely used in phylogenetic analysis [29, 30], genetic mapping [31], and population structure studies [7, 32–34]. Several investigators have made use of microsatellite markers in various catfishes [25, 35, 36]. In the present work, we analyzed the genetic diversity and phylogenetic relationships between two species of yellow catfish, *H. brachysoma* and *H. nigricollaris* collected from Chalakkudy River, Kerala using RAPD and microsatellites

markers. The observation of present study reveals that *H. nigricollaris* is more severely affected in its genetic heterogeneity when compared with *H. brachysoma*. Moreover, we report the genetic relatedness between two species and their distribution of genetic polymorphism across Chalakkudy River.

Materials and methods

Sampling

Specimens of *H. brachysoma* and *H. nigricollaris* were obtained through commercial catches from the River Chalakkudy. *H. brachysoma* were collected form the lower stretches of the River at Kanakkankadavu site (10°08′N; 76°07′E) while *H. nigricollaris* were collected at upper hilly streams of the River at Vettilappara (N 10°17′, E 76°32′) site. A total of 60 specimens were used from the *H. brachysoma* whereas only 21 specimens of *H. nigricollaris* could collect for this study.

RAPD analysis

Blood samples were used for the DNA extraction following the procedures of Taggart et al. [37]. After quantification, the DNA was diluted to final concentration of 25 ng/µl. Thirty decamer primers (20 from OPA series and 10 from OPAH series, Operon technologies, Almeda) were used for RAPD analysis. An initial screening resulted in selection of 8 primers that produced clear, high intensity and reproducible amplification products and these 8 primers used for the population genetic analysis (Table 1). Amplification reactions had a final volume of 25 µl and contained 1× reaction buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 pmol of primer, 0.2 mM dNTPs, 2 U *Taq* DNA polymerase (Genei, Bangalore, India) and 25 ng of template DNA. RAPD–PCR reactions were run in a PTC 200 thermal cycler (M.J. research, Inc., Watertown, Massachusetts, USA) and subjected to initial denaturation step at 95°C for 3 min followed by 40 cycles of 94°C for 3 min, 40°C for 1.30 min and 72°C for 2 min and a final elongation at 72°C for 10 min. To check for DNA contamination, a negative control was set up by omitting the DNA from the reaction mixture. The resulting products were electrophoretically separated through 1.5% agarose gels stained with ethidium bromide (5 µg/ml) in TBE buffer (90 mM Tris–borate and 2 mM EDTA, pH 8.0) and the gels were documented using Imagemaster 1D gel documentation system (Amersham Biosciences, USA). Gel images were used for the analysis of the amplified products and the numbers of bands were counted. A binary matrix was produced whereby the presence (dominant) and absence (recessive) of each DNA fragment for each sample was recorded 1 or 0, respectively. Faint or poorly amplified fragments were excluded from the analysis. The DNA marker applied along with RAPD samples helped to determine the molecular sizes of the DNA fractions of the fish samples. The data of RAPD were analyzed by POPGENE Version 1.31 [38].

Microsatellite analysis

Five Siluriform specific microsatellite primers (microsatellite flanking regions) were downloaded from NCBI site and used to study the cross-species amplification of microsatellites in *H. brachysoma* and *H. nigricollaris* (Table 2). These five microsatellite primers were from the species *Pangasius hypophthalmus* (three) [36]; *Clarias macrocephalus* (one) [39] and *Clarias gariepinus* (one)

Table 1 Comparative assessment of genetic variations in *H. brachysoma* (*Hb*) and *H. nigricollaris* (*Hn*) with RAPD markers

Primer Code	Sequences (5′–3′)	Total no. of bands			No. of polymorphic bands	Percentage of polymorphic bands (p%)	Observed No. of alleles (Na)		Effective No. of alleles (Ne)		Average Heterozygosity (H)	
		Overall	*Hb*	*Hn*			*Hb*	*Hn*	*Hb*	*Hn*	*Hb*	*Hn*
OPA-07	GAAACGGGTG	9	7	5	5	55.55	1.125	1.125	1.116	1.103	0.060	0.057
OPA-09	GGGTAACGCC	10	9	7	4	40.00	1.100	1.100	1.099	1.099	0.050	0.050
OPA-11	CAATCGCCGT	11	9	7	7	63.63	1.167	1.000	1.165	1.000	0.083	0.000
OPA-20	GTTGCGATCC	8	7	4	6	75.00	1.250	1.250	1.250	1.245	0.125	0.125
OPAH-01	TCCGCAACCA	13	12	11	7	53.85	1.154	1.000	1.150	1.000	0.076	0.000
OPAH-02	CACTTCCGCT	9	9	8	3	33.33	1.111	1.000	1.103	1.000	0.053	0.000
OPAH-04	CTCCCCAGAC	16	13	13	9	56.25	1.125	1.000	1.125	1.000	0.063	0.000
OPAH-08	TTCCCGTGCC	9	7	8	4	44.44	1.000	1.000	1.000	1.000	1.000	0.000
Total	–	85	73	63	45	52.94	–	–	–	–	–	–
Mean	–		–		–		1.129	1.047	1.126	1.045	0.064	0.023
St. Dev	–		–		–		0.337	0.213	0.331	0.204	0.167	0.104

Table 2 Comparative assessment of genetic variations in the five microsatellite loci in *H. brachysoma* (Hb) and *H. nigricollaris* (Hn)

Loci	Observed number of alleles (Na)			Effective number of alleles (Ne)			Observed Heterozygosity (Hob)			Expected Heterozygosity (Hex)			Inbreeding Co-efficient (F_{IS})		
	Overall	*Hb*	*Hn*	Overall	*Hb*	*Hn*	Overall	*Hb*	*Hn*	Overall	*Hb*	*Hn*	Overall	*Hb*	*Hn*
Phy01	8	5	4	4.625	2.036	2.459	0.6	0.655	0.524	0.792	0.519	0.498	0.235	−0.287	0.263
Phy05	2	2	1	1.317	1.578	1	0.28	0.483	0	0.243	0.373	0	−0.163	−0.318	–
Phy07	2	1	2	1.497	1	2	0.42	0	0.729	0.335	0	0.412	−0.266	—	−0.314
Cma03	3	3	2	2.391	2.046	1.63	0.42	0.345	0.424	0.588	0.52	0.452	0.278	0.327	0.155
Cga06	3	2	2	2.166	1.942	1.8	0.76	0.828	0.667	0.544	0.494	0.455	−0.412	−0.706	−0.194
Total	18	13	11	–	–	–	–	–	–	–	–	–	–	–	–
Mean	3.6	2.6	2.2	2.399	1.721	1.579	0.496	0.462	0.443	0.5	0.481	0.458	–	–	–
St. Dev	2.51	1.14	1.095	1.322	0.446	0.909	0.186	0.316	0.361	0.217	0.221	0.265	–	–	–

[40]. Amplifications were performed in 25 µl reaction mixture containing 1× reaction buffer (10 mM Tris, 50 mM KCl, 0.01% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 pmol of each primer, 0.2 mM dNTPs, 2 U *Taq* DNA polymerase (Genei, Bangalore, India) and 25 ng of template DNA. The PCR reactions were performed in a PTC 200 gradient thermal cycler (M.J. Research, Inc., Watertown, Massachusetts, USA). All thermal cycling reactions were configured with heated lids. The amplification cycle consisted of a 5 min denaturation at 94°C followed by 25 cycles of 94°C denaturing for 30 s, usually 50–60°C annealing for 30 s (annealing temperature depending upon the T_m value of primer) and 72°C extension for 2 min. Cycling was concluded with a 2 min extension at 72°C. The amplified products were checked in 10% polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining (Amersham Pharmacia Kit). Molecular weights of the bands were calculated with reference to the molecular weight marker (*pBR*322 DNA/*Msp*I digest) with the software Image Master ID Elite. In the analysis of microsatellites, various parameters include number of alleles, allelic frequencies, percentage of polymorphic loci, observed and expected heterozygosity, linkage disequilibrium, conformity of allele frequencies to that expected under Hardy- Weinberg equilibrium and estimates of population differentiation including F-statistics were estimated using POPGENE version 1.31 [38]. The expected frequency of null alleles was calculated according to Van Oosterhout et al. [41] using MICRO-CHECKER (available from http://www.microchecker.hull.ac.uk).

Results

Genetic variability in both *horabagrus* species

A total of 85 different RAPD loci were detected consistently with 8 decamer primers in both species of *Horabagrus*. Among these 85 loci, 73 numbers of loci were produced in *H. brachysoma,* whereas only 63 were observed in *H. nigricollaris*. All the 8 amplified primers were polymorphic and out of the 85 total RAPD fragments amplified, 45 (52.94%) were found polymorphic and the remaining 40 (47.06%) were found shared by all the individuals of both species (Table 1). The number and size of the fragments were ranged from 8 (OPA07 and OPA-20) to 16 (OPAH-04) and 150–2000 bp respectively. The average number of bands in overall primers was 10.62. For overall primers, the observed (Na) and effective (Ne) number of alleles in *H. brachysoma* were 1.129 and 1.126 respectively whereas in *H. nigricollaris,* Na and Ne were 1.047 and 1.045 respectively (Table 1). Average heterozygosity (H) (Nei, 1978) for overall primers was 0.064 in *H. brachysoma* and 0.023 in *H. nigricollaris*. Comparison of RAPD gel pattern of both species of *Horabagrus* is shown in Fig. 2a–c.

Cross-species amplification of five microsatellite primers obtained from other catfishes such as *Pangasius hypophthalmus, Clarias macrocephalus* and *Clarias gariepinus* were amplified with a total of five loci, one in each primer. Among these five loci, 4 loci were polymorphic (80%) in each *Horabagrus* species even though the overall polymorphism was 100%. In *H. brachysoma*, the locus *Phy07* is monomorphic while in *H. nigricollaris* the monomorphic locus was *Phy05*. There were 18 alleles obtained in five loci. But only 13 and 11 alleles were amplified in *H. brachysoma* and *H. nigricollaris* respectively. The locus *Phy01* showed maximum number of 8 alleles and the loci *Cma03* and *Cga06* had three alleles each. The mean observed number of alleles was 3.6 and the mean effective number of alleles was 2.399. The observed heterozygosities (H_{ob}) in all four polymorphic loci in *H. brachysoma* were 0.462 whereas in *H. nigricollaris* was 0.443 and in both species it was 0.496 (Table 2). The five amplified microsatellite loci in *H. brachysoma* have been sequenced [7]. The zymogram of loci *Phy01*, *Phy05* and *Cma03* are given Fig. 2d–f. There was no significant association indicative of linkage

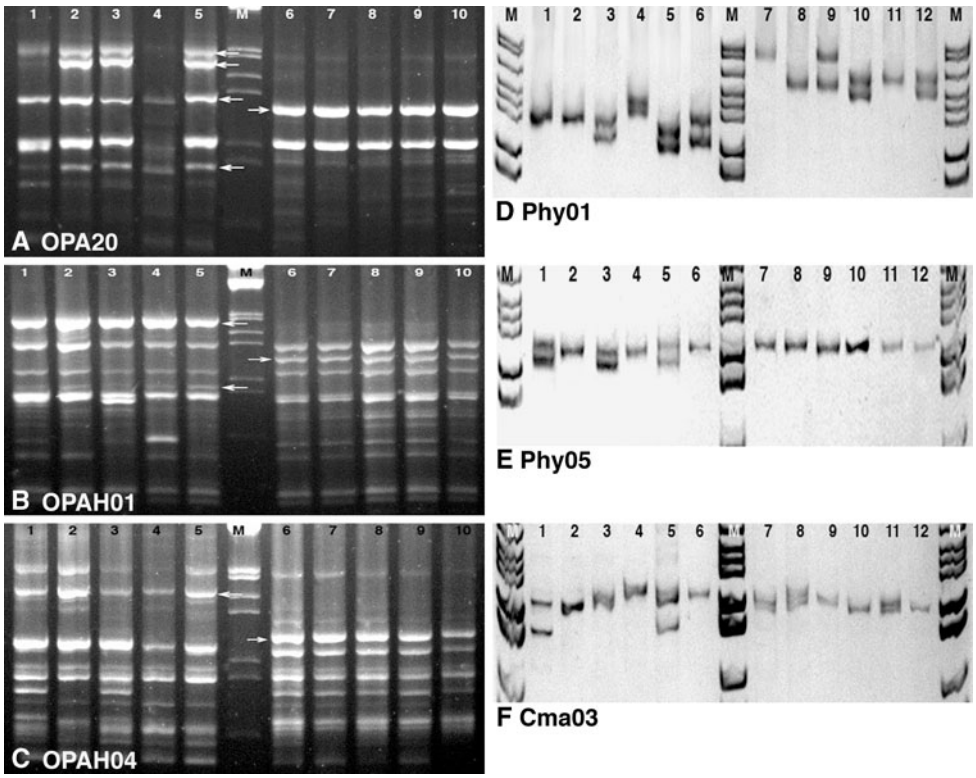

Fig. 2 Comparison of RAPD pattern of *Horabagrus brachysoma* and *H. nigricollaris* with primers OPA20 (**a**), OPAH01 (**b**), and OPAH04 (**c**). *Lanes 1–5 H. brachysoma; 6–10 H. nigricollaris. Lane M* Molecular weight marker-λDNA with *Eco*R1 and *Hind*III double digest. The *arrow* shows the polymorphic RAPD loci in both species. **d–f**: Microsatellite pattern of locus *Phy01* (**d**), *Phy05* (**e**) and *Cma03* (**f**). *Lanes 1–6 H. brachysoma; 7–12 H. nigricollaris. Lane M* molecular weight marker (*pBR*322 with *Msp*I cut)

disequilibrium ($P > 0.05$) between any pair of microsatellite loci; therefore assumed that allelic variation at microsatellite loci could be considered independent. The probability test revealed that the observed allele frequencies in three polymorphic loci (*Phy01*, *Phy05* and *Cga06*) out of 4 in *H. brachysoma* and 2 loci (*Phy07* and *Cga06*) in *H. nigricollaris* showed significant deviation ($P < 0.05$) from Hardy–Weinberg equilibrium. In *H. nigricollaris*, two loci were showed positive value of F_{IS} and this positive value of F_{IS} indicated the deficiency of heterozygotes.

Species-specific markers

Several RAPD fragments showed fixed frequencies in a particular species and these can be used as species-specific markers. Among the 85 RAPD fragments obtained with 8 primers 34 were identified as species-specific markers in both species of *Horabagrus*. Among these, 22 were specific to *H. brachysoma* and the remaining 12 were specific to *H. nigricollaris*. All the eight primers could produce the species-specific markers. The primers OPA07, OPA11 and OPAH04 gave the maximum numbers (six) of unique bands in both species of *Horabagrus* while the primer OPAH02 only produced one species-specific RAPD band (in *H. brachysoma*). The details of species-specific RAPD bands detected in both species are given in Table 3.

In microsatellite, only 12 species-specific bands were observed in both species of *Horabagrus*. Among these, 7 alleles observed in *H. brachysoma* and 5 observed in *H. nigricollaris*. The locus *Phy01* produced 7 species-specific markers while the locus *Cga06* produced 2 specific markers and all other loci produced single marker each. The list of species-specific microsatellite markers produced in both species of *Horabagrus* is shown in Table 4.

Frequency of null alleles in microsatellites

The expected frequency of null alleles was calculated using MICRO-CHECKER and all the genotypes of the loci showing deviation from Hardy–Weinberg equilibrium were tested for null alleles. The estimated null allele frequency was not significant ($P > 0.05$) in all the five tested loci using different algorithms, indicating the absence of null alleles and false homozygotes. In addition, there was no instance of non-amplified samples in repeated trials with any of the primer pairs. Therefore, for population genetic analysis, information from all the five loci was considered.

Phylogenetic relationship between species

The phylogenetic relationships [42] between two species of *Horabagrus* by RAPD and microsatellite were developed. It was observed that *H. brachysoma* and *H. nigricollaris* were closely related having the high genetic identity and less genetic distance. The genetic distance between two species was by the value 0.534 in RAPD and 0.439 in

Table 3 Species- specific RAPD markers with size in bp in each species

S. No.	RAPD Primers	Size (bp)	Species
1	OPA07	1000	*H. brachysoma*
2		950	*H. nigricollaris*
3		700	*H. brachysoma*
4		600	*H. nigricollaris*
5		200	*H. brachysoma*
6		150	*H. brachysoma*
7	OPA09	1200	*H. brachysoma*
8		900	*H. nigricollaris*
9		800	*H. brachysoma*
10		750	*H. brachysoma*
11	OPA11	700	*H. brachysoma*
12		500	*H. nigricollaris*
13		300	*H. brachysoma*
14		250	*H. nigricollaris*
15		200	*H. brachysoma*
16		150	*H. brachysoma*
17	OPA20	1950	*H. brachysoma*
18		1750	*H. brachysoma*
19		1300	*H. brachysoma*
20		1200	*H. nigricollaris*
21		800	*H. brachysoma*
22	OPAH01	1750	*H. brachysoma*
23		1150	*H. nigricollaris*
24		850	*H. brachysoma*
25	OPAH02	1650	*H. brachysoma*
26	OPAH04	2000	*H. brachysoma*
27		1900	*H. nigricollaris*
28		1600	*H. brachysoma*
29		1500	*H. brachysoma*
30		1300	*H. nigricollaris*
31		1000	*H. nigricollaris*
32	OPAH08	1800	*H. brachysoma*
33		300	*H. nigricollaris*
34		200	*H. nigricollaris*

Table 4 Species-specific markers in microsatellite and their frequencies in each *Horabagrus* species

Locus	App. size	*H. brachysoma*	*H. nigricollaris*
Phy01	235	–	0.191
Phy01	215	–	0.333
Phy01	205	–	0.119
Phy01	190	0.086	–
Phy01	180	0.627	–
Phy01	176	0.155	–
Phy01	162	0.086	–
Phy05	155	0.241	–
Phy07	205	–	0.500
Cma03	151	0.172	–
Cga06	125	0.414	–
Cga06	115	–	0.333

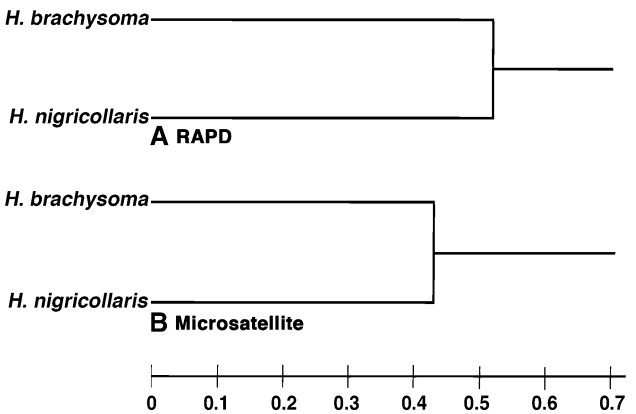

Fig. 3 Comparison of UPGMA dendrograms of RAPD (**a**) and microsatellite (**b**) markers in *H. brachysoma* and *H. nigricollaris*. *Scale bar* denotes the genetic distance between two species

microsatellites whereas genetic identity was 0.586 in RAPD and 0.645 microsatellites. The UPGMA dendrogram [43] of these two markers between the species are given in Fig. 3.

Discussion

Genetic variation and phylogenetic relationships between two species of *Horabagrus* by RAPD and microsatellite were developed and its comparative assessment between the two species was discussed in the present study. The study reveals that both *Horabagrus* species are genetically distinct even though they show much genetic and morphological similarity. Relatively less polymorphism was noticed in *H. nigricollaris* when compared with *H. brachysoma. H. nigricollaris* is more severely affected due to its less genetic heterogeneity. Inbreeding due to less population size could be one of the reasons for less polymorphism [40]. In RAPD, less polymorphism was noticed in both *Horabagrus* species. Generally RAPD shows higher polymorphic bands as seen in the channel catfish, *Ictalurus punctatus* and *I. furcatus* (61.05%) [26].

Deficiency in heterozygocity in microsatellite loci were observed in both species of *Horabagrus*. But, Usmani et al. [44] reported the significant excess of heterozygocity in other bagrid catfish, *Mystus nemurus* (H_{ob} = 0.499 and H_{ex} = 0.482). The deficiency of heterozygocity in microsatellites in yellow catfish could be due to inbreeding, a situation caused by over-exploitation leading to a decline of the species in the wild. Over-exploitation leading to drastic decline of the yellow catfish has been recorded in

Chalakkudy River of Kerala since 1997 as per latest IUCN norms [12]. Non-random mating and less effective population size could be major reasons for heterozygote deficiency [41] especially in *H. nigricolalris* in the present study. Deficiency in heterozygocity in microsatellite loci also observed in *H. brachysoma* samples which indicates the lack of genetic diversity due to inbreeding can be resulted in poor growth and disease susceptibility. The estimated null allele frequency was not significant ($P > 0.05$) in all five tested loci using different algorithms, indicating the absence of null alleles and false homozygotes in both *Horabagrus* species samples. In addition, there was no instance of non-amplified samples in repeated trials with any of the primer pairs. Therefore, for comparison of genetic analysis, information from all the five loci was considered.

We report 34 species-specific RAPD and 12 species-specific microsatellite bands in both species of *Horabagrus* (Tables 3, 4). The species-specific bands could be quantitative trait loci (QTL), which could exhibit some desirable characters such as temperature or salt tolerance, or resistant to some diseases which can be used for marker assisted selection. Several authors detected and analyzed the QTLs based on RAPD markers in different fish species [19, 45]. Also there are reports that QTLs used for marker assisted selection [46]. Some promising RAPD markers could be sequenced and so converted to a SCAR marker to study desirable traits as has been done in other fish species [47, 48]. Microsatellites become the preferred marker for animal gene mapping and ease of typing via PCR [49–51]. Several authors detected QTLs from specific microsatellite markers [45, 52]. The species-specific microsatellite markers can be used for selective breeding and would be very useful for tracking parentage in selection experiments [53]. Our future aim will be the construction of linkage map and identification of QTLs in these endangered fish species for better aquaculture programs.

The low degree of gene diversity and lack of genetic heterogeneity in the samples of both species of *Horabagrus* were observed, which strongly emphasize the need of fishery management, conservation and rehabilitation of this species. Indiscriminate collection for international trade as ornamental species is considered to be one of the major reasons for the massive reduction in the numbers of *Horabagrus* species [8]. The extensive habitat alterations (due to damming and de-forestation) and destructive fishing practices are serious concerns in Chalakkudy River [7]. Strong management strategies such as ban of destructive fishing practices and development of captive breeding technology, cryopreservation of germplasm and small scale aquaculture program should be implemented for the conservation and management of these endangered species.

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