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SHORT RESEARCH NOTE

Molecular identification of five Indian sciaenids (pisces: perciformes, sciaenidae) using RAPD markers

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Abstract The randomly amplified polymorphic DNA (RAPD) markers were used to detect interspecific genetic variability and genetic relatedness among five Indian sciaenids namely *Otolithes cuvieri*, *Johnieops sina*, *Johnieops macrorhynus*, *Johnieops vogleri* and *Protonibea diacanthus* for the first time. Eight RAPD primers (OPA01, OPA06, OPA07, OPA18, OPP12, OPP14, OPP16 and OPP11) generated 40 species specific diagnostic bands. The highest genetic divergence was detected between *J. macrorhynus* and *P. diacanthus* (0.586) where as the lowest one was observed between *J. sina* and *J. vogleri* (0.274).

Keywords Sciaenids · RAPD · Molecular Identification

The family sciaenidae is widely distributed throughout the world with approximately 70 genera and 300 species including about 30 species from Indian waters (Mohanraj et al., 2003). The Indian sciaenids including the commercially important species selected for the present study (*Otolithes cuvieri*, *Johnieops sina*, *Johnieops macrorhynus*, *Johnieops vogleri* and *Protonibea diacanthus*) contribute approximately 4.6% to the total Indian marine fish production.

Conventionally, sciaenid fishes have been identified based on morphological, meristic and anatomical characters (Taniguchi, 1970; Mohan, 1981). However, there are ambiguities due to morphological closeness (Menezes et al., 1993) and the synonym used in the Fishbase also point to the ambiguous identification with respect to some sciaenid species of genus Johnieops and Johnius (Froese & Pauly, 2006). Therefore, application of molecular tools can provide valuable information for species identification and complement the traditional taxonomic data and validation of systematic position of sciaenid fishes. RAPD-PCR technique has been used for species identification in a wide range of organisms including fishes in recent years (Liu & Chordes, 2004). The RAPD technique allows detection of DNA polymorphisms by random amplification of multiple regions of the genome by polymerase chain reaction (PCR) using single arbitrary primers designed independently of target DNA sequence. In the present study, RAPD markers were used for the first time to resolve taxonomic ambiguities and document interspecific genetic variability among five species of sciaenids, *O. cuvieri*, *J. sina*, *J. macrorhynus*, *J. vogleri* and *P. diacanthus.*

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The sciaenid samples were collected from New Ferry Wharf (Latitude 18°96′ N and Longitude 72°85′ E), Mumbai, on the North Western coast of India during February–March, 2006. Approximately 100 mg of white muscle tissue from 10 individuals of each species were preserved in 95% ethanol until used. DNA was isolated following Ruzzante et al. (1996) with minor modifications. A total of 50 arbitray primers (OPA, OPB and OPP series, Operon Ltd., USA) with random sequence were screened. The PCR amplifications were carried out in the thermal cycler (MJ Research, PTC-200) in a reaction of 25 µl, containing 50 ng genomic DNA, 1 × PCR buffer (10 mM Tris–HCL, pH 9.0, 50 mM KCl, 0.01% gelatin), 1.5 Mm $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of primer and 1.5 units of Taq DNA polymerase. The amplification conditions were 94°C for 5 min followed by 40 cycles at 94°C for 1 min, 40°C for 1 min and 72°C for 2 min with a final extension of 72°C for 2 min. After amplification, 8 µl of PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide and TAE buffer (40 mM Tris, 20 mM Acetic acid, 1 mM EDTA) to visualize the band patterns generated by each primer. The molecular weight of each band was estimated using a standard molecular marker (Eco RI/digested lambda DNA) with Image master 1D Elite ver. 3.01 (GE Amersham Biosciences, USA). All fragments were designated by primer name followed by its size (bp). Each individual was scored for the presence or absence of a particular amplified band. The RAPD profiles for the five species were classified as described by Callejas & Ochando (2002). The RAPD genotype data were analysed using software TFPGA (Ver. 1.3, Miller, 1997) and the allele frequencies were estimated based on Lynch & Milligan's (1994) with Taylor expansion. Matrix of unbiased genetic distance (Nei, 1978) and bootstrap (1000 replicates) values were calculated.

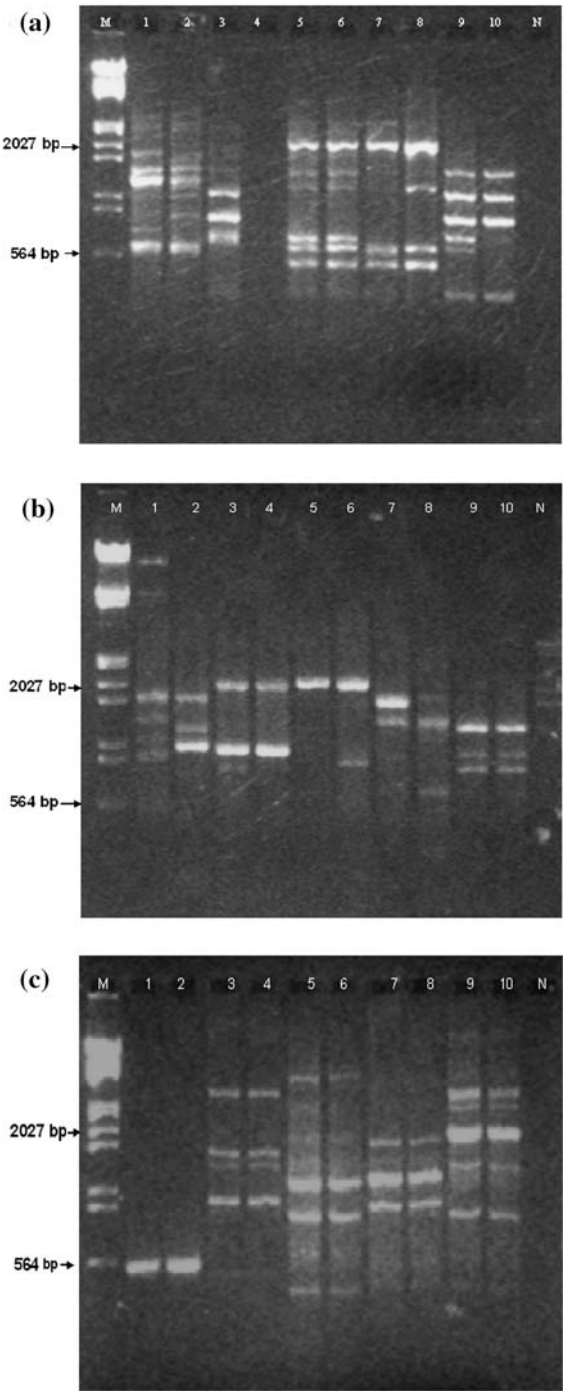

Fig. 1 (**a**) RAPD bands amplified by primers OPA-01 in Sciaenids, M- Standard Molecular weight marker; λ DNA Eco RI/*Hin*d III, 1–2 *Otolithes cuvieri*, 3–4 *Johnieops sina*, 5–6 *Johnieops macrorhynus*, 7–8 *Protonibea diacanthus*, 9–10 *Johnieops vogleri* and N- Negative (**b**) RAPD bands amplified by primers OPA-07 in Sciaenids, M- Standard Molecular weight marker; λ DNA Eco RI/*Hin*d III, 1–2 *Otolithes cuvieri*, 3–4 *Johnieops sina*, 5–6 *Johnieops macrorhynus*, 7–8 *Protonibea diacanthus*, 9–10 *Johnieops vogleri* and N- Negative (**c**) RAPD bands amplified by primers OPP-14 in Sciaenids, M- Standard Molecular weight marker; λ DNA Eco RI/*Hin*d III, 1–2 *Otolithes cuvieri*, 3–4 *Johnieops sina*, 5–6 *Johnieops macrorhynus*, 7–8 *Protonibea diacanthus*, 9–10 *Johnieops vogleri* and N-Negative

Table 1 Genotype frequencies at 85 RAPD loci in five sciaenids species

Locus	*Otolithes cuvieri*	*Johnieops sina*	*Johnieops macrorhynus*	*Johnieops vogleri*	*Protonibea diacanthus*
OPA01-2053	–	–	–	–	+(80)*
OPA01-1836	+(40)	–	+(80)	–	+
OPA01-1390	+(60)	–	+	+(80)	–
OPA01-1198	+	+(60)	–	–	+
OPA01-1014	–	–	–	–	+(80)*
OPA01-941	–	+**	–	–	–
OPA01-649	-	+(80)	+	+	–
OPA01-465	+(50)	–	–	–	+(60)
OPA01-379	–	–	+(80)	+	–
OPA01-292	–	–	–	–	+**
OPA01-171	–	–	+(80)*	–	–
OPA06-3757	+**	–	–	–	–
OPA06-2475	+(80)*	–	–	–	–
OPA06-1854	–	+	+(80)	+	–
OPA06-1057	+**	–	–	–	–
OPA06-423	+(40)	+	+	+(80)	–
OPA06-394	–	–	–	+**	–
OPA06-314	+(30)	–	–	–	+
OPA06-215	–	–	+**	–	–
OPA06-189	–	–	–	–	+**
OPA07-2004	–	–	–	+**	–
OPA07-1771	+(80)	+	+(60)	+(60)	–
OPA07-1302	–	+(80)	+(80)	+(80)	–
OPA07-1082	+(80)	+	+	+	–
OPA07-828	+(80)	+(80)	–	–	–
OPA07-675	–	+**	–	–	–
OPA07-372	–	–	–	–	+**
OPA07-320	+**	–	–	–	–
OPA07-302	–	–	–	+**	–
OPA07-272	–	–	+**	–	–
OPA18-1550	+**	–	–	–	–
OPA18-1075	–	+(80)*	–	–	–
OPA18-991	+(80)	+	+	+	–
OPA18-679	–	–	+**	–	–
OPA18-532	–	+**	–	–	–
OPA18-345	–	+(80)	+(80)	+(60)	–
OPA18-227	–	+(80)	+(70)	+(80)	–
OPA18-212	+**	–	–	–	–
OPA18-180	–	–	–	–	+**
OPA18-109	–	+(80)	+(80)	+(80)	–
OPA18-79	–	–	–	+(60)*	–
OPA18-40	–	+**	–	–	–
OPP12-2497	–	+	+	+(80)	–
OPP12-1618	–	+	+	+	–
OPP12-1169	–	+	+	+	–
OPP12-954	+(80)*	–	–	–	–
OPP12-728	–	+(80)	+(80)	+(80)	–
OPP12-318	+	+(80)	+(80)	+(80)	–
OPP12-212	–	+(80)	+(80)	+	–
OPP12-202	+**	–	–	–	–
OPP12-175	–	–	–	+**	–
OPP12-156	–	+**	–	–	–
OPA14-4697	–	–	+**	–	–
OPA14-3770	–	+(80)	+	+(80)	–
OPA14-3163	–	+**	–	–	–
OPA14-2130	+(80)	+	+	+	+
OPA14-1636	–	+(70)	+(70)	+(80)	–

Table 1 continued

Locus	*Otolithes cuvieri*	*Johnieops sina*	*Johnieops macrorhynus*	*Johnieops vogleri*	*Protonibea diacanthus*
OPA14-1373	–	+	+(80)	+(80)	–
OPA14-1186	–	–	+**	–	–
OPA14-787	–	+	+	+(60)	–
OPA14-670	+(80)	+(80)	+(60)	–	–
OPA14-352	+(80)*	–	–	–	–
OPA14-244	–	–	+**	–	–
OPP16-2816	–	+	–	+(80)	+(80)
OPP16-2313	–	+(80)	–	+	–
OPP16-1899	+	–	–	–	+(80)
OPP16-1379	+	–	–	–	+(80)
OPP16-966	–	+(80)	+	–	–
OPP16-774	–	–	+**	–	–
OPP16-445	–	+**	–	–	–
OPP16-286	–	–	–	+**	–
OPP16-221	–	–	–	–	+**
OPP16-211	–	–	–	+**	–
OPP16-190	+**	–	–	–	–
OPP11-2055	+(80)	+(80)	+(80)	+(80)	–
OPP11-1967	–	+	+	+	–
OPP11-1728	–	–	–	+**	–
OPP11-1221	–	–	–	–	+**
OPP11-1015	–	+**	–	–	–
OPP11-873	–	–	+**	–	–
OPP11-760	+**	–	–	–	–
OPP11-701	+**	–	–	–	–
OPP11-501	–	+**	–	–	–
OPP11-460	–	–	–	–	+**

+Presence of band in all individuals

–Absence of band in all individuals

+( )Percentage of individuals exhibiting a band is given in parentheses

** Species Specific Marker

* Species Exclusive Marker

Out of 50 arbitrary primers used, 8 primers viz. OPA01, OPA06, OPA07, OPA18, OPP12, OPP14, OPP16 and OPP11 were selected on the basis of reproducibility and resolution of banding patterns in all the five species. These 8 primers amplified a total of 85 loci in the size range from 40 to 4697°bp. The number of stable and clear RAPD bands generated per primer varied between 9 and 12. The RAPD profile for five species is depicted in the Fig. 1a–c. *Genotype frequencies observed in 10 individuals analyzed for each of the five sciaenid species are given in Table 1. The species specific diagnostic markers (present in all the individuals of a particular species) were evident at 40 loci in the five sciaenid species (Table 1). Species exclusive markers (present in some individuals of a particular species) in medium or low frequencies were also detected in all the five species (Table 1).

Estimates of Nei's (1978) unbiased genetic distance (D) demonstrated sufficient genetic divergence to discriminate the samples of different species (Table 2). The genetic distance for the species of genus *Johnieops* ranged from 0.274 between *J. sina* and *J. vogleri* to 0.384 between *J. vogleri* and *J. macrorhynus*. Between the genera, the genetic distance ranged from 0.392 between *Otolithes* and *Protonibea* to 0.586 between *Protonibea* and *Johnieops*.

The RAPD markers have been successfully used in genetic diversity studies in the past for several species (Williums et al., 1990; Callejas & Ochando, 2002; Govindaraju & Jayasankar, 2004; Das et al., 2005). Our study generated RAPD profiles with species specific bands in five sciaenid species through eight random

Table 2 Nei's, 1978 genetic distance average within species, between species (above diagonal) and net between species (below diagonal) in the five sciaenids

Sl. No.	Species	1	2	3	4	5	Within group
1	*O. cuvieri*	*	0.535	0.515	0.392	0.549	0.097
2	*J. sina*	0.458	*	0.338	0.538	0.274	0.058
3	*J. macrorhynus*	0.422	0.265	*	0.586	0.384	0.089
4	*P. diacanthus*	0.326	0.492	0.525	*	0.565	0.034
5.	*J. vogleri*	0.464	0.208	0.302	0.511	*	0.074

primers for the first time. The average genetic distance between all five species was considerably higher than the average genetic distance within the species. This suggested the existence of a separate gene pool for these species. Within the genus *Johnieops*, *J. sina* and *J. vogleri* were genetically closer to each other than *J. macrorhynus*. The species diagnostic profiles confirmed that *J. sina* and *J. vogleri* should be considered as separate species. At intergeneric level, the genera *Otolithes* and *Protonibea* were genetically closer to each other (D = 0.392) than the genus *Johnieops* (D = 0.538–0.586). It is interesting to note that the genetic distance (0.2971) observed between the genera *Protonibea* and *Otolithes* was more or less similar to the range observed at interspecific level within the genus *Johnieops* (0.274–0.384). Menezes et al. (1993) have reported genetic distance in the range of 0.334–0.612 between four genera of sciaenids *Otolithes*, *Kathala*, *Johnieops* (*Johnius*) and *Pennahia* based on allozyme variation.

The identified arbitrary primers have provided consistent band patterns in the five sciaenid species and distinct species diagnostic profiles demonstrated that RAPD fingerprinting is a promising tool in sciaenid taxonomy. The primers identified in the study will be useful in DNA based identification of other sciaenid species. In addition, the technique and the data will also facilitate the species identification in juvenile forms, fishery products and to the management of the exploited populations.

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