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Detection of Genetic Variation and Genetic Diversity in Two Indian Mudskipper Species (*Boleophthalmus boddarti*, *B. dussumieri*) using RAPD Marker

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Abstract

Due to the environmental changes and habitat destruction the mudskipper fish population is decreasing in recent years. To predict the fish population structure, frequent manual survey and molecular methods are widely used. Molecular markers such as RAPD, microsatellite, allozyme, D-loop haplotype are frequently adopted to assess the population structure of an organism. In this study ten-arbitrary primers were screened to estimate the genetic relationships and diversity of two mudskipper species (*Boleophthalmus boddarti* and *B. dussumieri*) in Vellar estuary, Tamilnadu, India. By this RAPD marker study, the genetic diversity (H) in *B. boddarti* was more (0.0116 ± 0.0066) than in *B. dussumieri* (0.0056 ± 0.0024) in Vellar estuary (India). The genetic distance between *B. boddarti* and *B. dussumieri* was 1.7943. By observing the species specific bands and the phylogenetic analysis it is revealed that these two species clearly deviated into separate clusters emphasizing the distinct species status.

Keywords: *Boleophthalmus boddarti, B. dussumieri,* genetic diversity, Mudskipper, RAPD

Introduction

Mudskippers (Gobiidae: Oxudercinae) live in intertidal habitat of the mudflats and in mangrove ecosystem (Murdy, 1989) and these fishes are uniquely adapted to a completely amphibious lifestyle (Graham, 1997). Being amphibious, they are uniquely adapted to intertidal habitats, unlike most fish in such habitats which survive the retreat of the tide by hiding under wet seaweed or in tidal pools (Graham, 1997). Anatomical and behavioural adaptations that allow them to move effectively on land as well as in the water (Harris, 1960). As their name implies, these fish use their fins to move around in a series of skips. They can also flip their muscular body to catapult themselves up to 2 feet (60 cm) into the air (Piper, 2007). They are found in mangrove ecosystems and mudflats of East Africa and Madagascar east through the Sundarbans of Benga, Southeast Asia to Northern Australia, southeast China and southern Japan, up to Samoa and Tonga Islands (Murdy, 1989). They grow to a length of about 9.5 cm and are carnivorous opportunist feeder (Murdy, 1989). They feed on small prey such as small crabs and other arthropods (Murdy, 1989). The two mudskipper species such as, *Boleophthalmus boddarti* and *B. dussumieri* are the residential fishes inhabiting the mudflats of the Vellar estuary and the waterways of Pichavaram mangrove forests, Tamil Nadu, India. The year 2004 tsunami made rapid changes in the morphology of the mudflats by shifting the dominant soil type-clay rich soil (clay 60%) into sandy soil (sand 70%) (Ravi, 2005). Most strikingly, the population of the mudskippers was totally eliminated in some areas and in other areas the population become highly reduced (3 to 12 individuals/sq.m. to 1 to 3 individuals/sq.m. (Ravi, 2005). So it is very essential the periodic manual survey or molecular study to assess the population structure of these declining species in their native habitat.

Random amplified polymorphic DNA (RAPD) analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al*., 1990). The method is simple and rapid technique for determining genetic diversity, variation and no prior knowledge of the genome under study is required (Hadrys *et al.,* 1992). RAPD analysis also has been used to evaluate genetic diversity for species and subspecies identification in guppy (Dinesh *et al*., 1993), tilapia (Bardakci and Skibinski, 1994; Dinesh *et al*., 1996), brown trout and Atlantic salmon (Elo *et al*., 1997), largemouth bass (Williams *et al*., 1998), Indian major carps (Barman *et al*., 2003) and damsel fishes (Parveen *et al*., 2011). But in Indian mudskippers it has not been used so far and there is no such study was explained the population structure of these species. In this study, the genetic diversity was analysed and accessed the

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genetic variation in *B. boddarti* and *B. dussumieri* using RAPD marker.

Materials and methods

Sample collection and DNA isolation

Tissue samples (fin clips) of *B. boddarti* and *B. dussumieri* (n = 20/species) were collected from Vellar estuary mangroves (Lat 11°29′ N; Lon 79°46′ E) and stored at 95% ethanol. Genomic DNA was extracted from the stored tissues by proteinase- K, Sodium Chloride based protocol (Taggart *et al*., 1992). DNA samples from individuals of each species were diluted to 25 ng/µl with deionized distilled water and used for PCR amplification. Ten commercially available decamer random primers (*An1- An10*) from Chromous Biotech Pvt Ltd (Bangalore, India) were used for this study.

Polymerase Chain Reaction

The amplification reaction was carried out in a 25-µl reaction volume containing 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 2.5 mM $MgCl_2$, 0.001% gelatin, 100 µM each of dATP, dCTP, dGTP, and dTTP, 0.2 µM of each primer, 1 U of *Taq* DNA polymerase (Bangalore Genei, India) and 25 ng of weight genomic DNA. RAPD-PCR was performed in a thermocycler (Lark, India) for 40 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 35°C for 30 seconds, and extension at 72°C for 60 seconds. The final extension was carried out at the same temperature for 5 minutes. The resulting products were electrophoretically analyzed through 1.5% agarose gels, stained with ethidium bromide, and visualized using a UV transilluminator (Maniatis *et al*., 1982). Subsequently the gel was photographed using a gel documentation system (Lark, India).

RAPD Data Analysis

Sizes of RAPD bands were determined by comparison with a 500-bp ladder and genetic similarity/distance between the two fish species was estimated using PopGene Software (Version 1.31, Yeh *et al*., 1999) Nei and Li's (1979) genetic similarity (GS) among the two species was computed and converted by PopGene into genetic distance (GD) according to Hillis and Mortiz's (1990) formula, GD = 1-GS. The GS reflects the proportion of the bands shared between individuals and values range from (1) when present to (0) when absent.

Phylogenetic relationship based on genetic distance values generated from RAPD data among two species and a dendrogram was also plotted by unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and Nei (1978) NEIGHBOUR procedure of PYLIP (Version 3.5c, Felsenstien, 1993) using PopGene software (Version 1.31, Yeh *et al*., 1999).

Results

Genetic variation

The number of amplified bands observed varied, depending on the primers, species and individuals. All the RAPD profiles obtained in this analysis are shown in Fig. 1 a, b. In general, the number of resolved amplified fragments varied from 3 to 7, with the size range varying from 30 to 2500 bp. On average, each random primer amplified 5.50 bands in *B. boddarti*, and 4.90 in *B. dussumieri* (Tab. 1). The total scorable bands were 104, under which the total numbers of species-specific bands were 74 (Tab. 1).

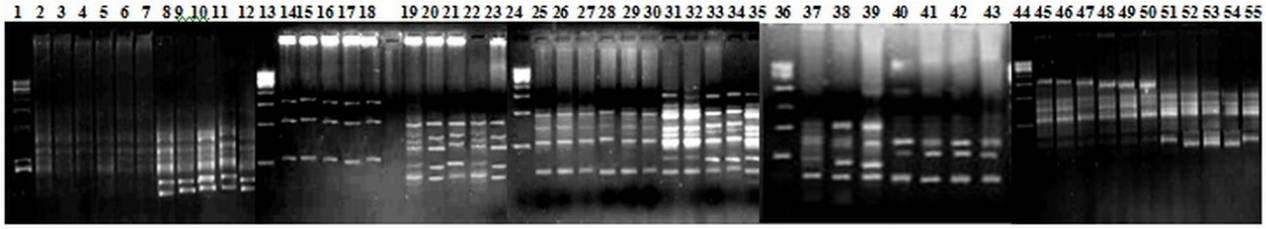

Fig. 1 (a). RAPD profiles generated with arbitrary primers in two mudskipper species with primer (*An1-An5*) Lanes 1, 13, 24, 36 and 44: 500-bp DNA ladder. Lanes 2-7; 14-18; 25-30; 37-39; 45-50: *B. boddarti*. Lanes 8-12; 19-23; 31-35; 40-43; 51-55: *B. dussumieri*

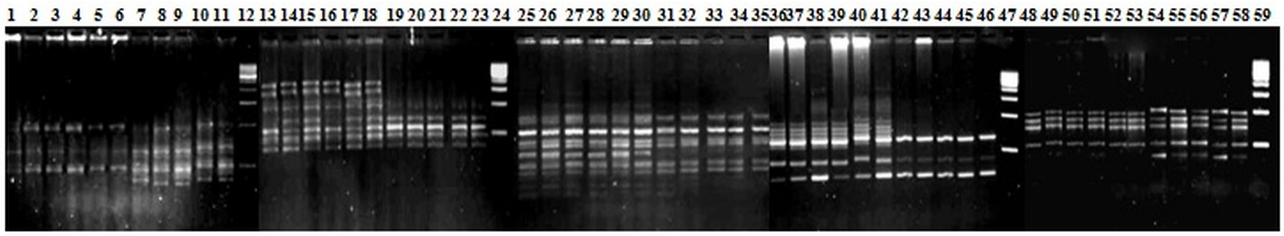

Fig. 1 (b). RAPD profiles generated with arbitrary primers in two mudskipper species with primer (*An6-An10*). Lanes 12, 24, 47 & 59: 500-bp DNA ladder. Lanes 1-6; 13-18; 25-30; 36-41; 48-53: *B. boddarti*. Lanes 7-11; 19-23; 31-35; 42-46; 54-58: *B. dussumieri*

Tab. 1. Number and size of the bands generated by ten RAPD primers and molecular weight of the diagnostic fragments in two mudskipper species

RAPD primer	No. of fragments		Size range and diagnostic fragments (bp)			
			B. boddarti		*B. dussumieri*	
	B. boddarti	*B. dussumieri*	Size range	Dia. frag.	Size range	Dia. frag.
An1	6	4	1875-30	1875	1239-557	1239
				1290		1044
				585		841
				30		557
An2	6	3	1516-73	1516	2103-575	2103
				1159		1539
				357		575
				73		
An3	7	4	2113-70	2113	1440-70	1440
				1391		1034
				1002		638
				866		
				598		
				87		
An4	6	7	1113-308	1113	1254-109	1254
				849		971
				678		700
				515		539
				414		414
				308		224
						109
An5	5	4	887-66	887	1170-514	1170
				736		867
				576		760
				251		514
				66		
An6	6	3	1056-329	1056	1022-463	1022
				739		664
				645		463
				505		
				375		
				329		
An7	5	7	1509-757	964	2500-757	2500
						1982
						926
An8	6	7	1246-365	-	1246-365	603
An9	3	6	1256-746	1256	1418-720	1418
				968		1293
				746		1199
						1120
						921
						720
An10	5	4	1077-345	1077	1058-547	1058
				923		942
				345		

Although there was some variation observed between individuals within the species, most of the bands were not variable among different individuals of a given species. Intra-species genetic variation was also detected in these two species (Tab. 2), but the level of polymorphism was low. The RAPD profile of each species was unique in terms of

Tab. 2. Overall observed number of alleles (Na), Effective number of alleles (Ne), Nei's (1973) gene diversity (H), Shannon's Information index (I)

Species	Na	Ne	H	I
B. boddarti	1.0337 ± 0.1815	1.0196 ± 0.1199	0.0116 ± 0.0066	0.0175 ± 0.0079
B. dussumieri	1.0112 ± 0.1060	1.0110 ± 0.1037	0.0056 ± 0.0024	0.0077 ± 0.0029
Overall	1.8652 ± 0.3435	1.8377 ± 0.3590	0.4219 ± 0.0765	0.5874 ± 0.0422

numbers and positions of bands. The inter-specific genetic similarity and distance are presented in Tab. 2. The genetic identity and distance between *B. boddarti* and *B. dussumieri* was 0.1662, 1.7943 respectively (Tab. 3).

Tab. 3. Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) of two mudskipper species

	B. boddarti	*B. dussumieri*
B. boddarti	******	0.1662
B. dussumieri	1.7943	******

Genetic diversity

The overall observed and expected polymorphic loci in *B. boddarti* and *B. dussumieri* is given in Tab. 3. The genetic diversity (H) in *B. boddarti* was more (0.0116 ± 0.0066) than in *B. dussumieri* (0.0056 ± 0.0024). The UPGMA- neighbour joining tree (Fig. 2) grouped the two species into separate clusters emphasizing the distinct species status of *B. boddarti* and *B. dussumieri*.

Discussion

This is the first report on the use of RAPD markers for studying genetic variation genetic diversity in this mudskipper species. The low levels of within-species genetic variation exhibited in *B. dussumieri* due to their less population for long period of time with in a limited area. This is an indication of possible high rate of inbreeding in *B. dussumieri* within less effective population size. Relatively higher levels of intraspecific genetic variation exhibited in *B. boddarti* (Tab. 2) may be due to comparatively higher effective population size. The RAPD profiles generated here for each species can be used for systematic inference. The phylogenetic tree obtained from the RAPD data emphasizing the distinct species status of *B. boddarti*, and *B. dussumieri*. (Fig. 2).

The genetic similarity between *B. boddarti* and *B. dussumieri* in this study was 0.1662. Whereas, the interspecies genetic similarity was high (0.336) in Indian major

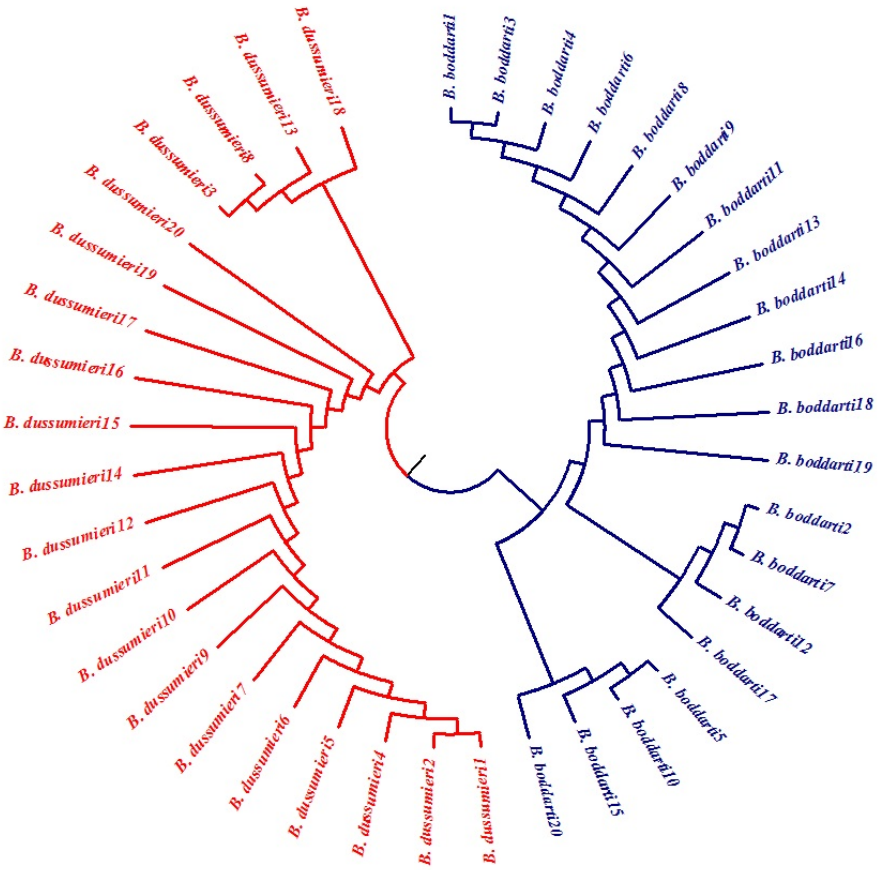

Fig. 2. Dendrogram Based Nei's (1978) Genetic distance: Method = UPGMA -Modified from NEIGHBOR procedure of PHYLIP Version 3.5

carp and Tilapia by RAPD analysis (Barman *et al*., 2003; Dinesh *et al*., 1996; Eknath and Doyle, 1990). Similar to this present study, RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms (Bardakci and Skibinski, 1994; Greef and Triest, 1999). The genetic distance is more between genus than between species and the hypothesis is also proved in this study by this marker. Same type of work also proved in earlier studies in Indian major carps (Barman *et al*., 2003).

Despite some limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species, particularly in mudskippers for which very little genetic information is available now. This study represents a first step towards the generation of DNA markers for purposes, such as species diagnosis, detection of molecular markers linked to economic traits, assessment of genetic diversity and studies on molecular systematics. Further studies with other molecular markers such as microsatellite, allozyme, D-loop haplotype, *CO1* and *Cyt b* gene sequences are essential to clarify and confirm the genetic relationships among all Indian mudskipper species.

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