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Short Communication

RAPD fingerprinting of the ornamental fish *Badis badis* (Hamilton 1822) and *Dario dario* (Kullander and Britz 2002) (Perciformes, Badidae) from West Bengal, India

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Abstract

We used random amplification of polymorphic DNA (RAPD) to generate species-specific diagnostic fragment patterns for the molecular identification of the ornamental aquarium fish species *Badis badis* and *Dario dario*. Seven arbitrary oligodecamer primers produced a total of 116 bands of which 98.23% were polymorphic. The size of the amplified products was in the range 340 bp to 2170 bp. Intraspecies genetic similarity was 0.879 ± 0.023 for *B. badis* and 0.840 ± 0.014 for *D. dario* while interspecies genetic similarity was 0.602 ± 0.017, with cluster analysis displaying separate taxonomic and evolutionary status for these fish. The results show that RAPD was useful for the molecular identification of aquarium fish species, with morphological traits also being important.

Key words: Badis badis, *Dario dario*, diagnostic markers, RAPD molecular identification, West Bengal.

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India is endowed with a vast fish biological resource representing more than 10% of world fish diversity (Das and Pandey, 1998). However, most indigenous aquarium fish have not been genetically characterized and documented and are being indiscriminately caught from their natural environments leading to their depletion and probable extinction. Taxonomic methods of fish identification and characterization are primarily based on morphological characters which may sometimes be insufficient to identify a species, particularly in the early stages of development when only a few morphological characters are observable (Ayoma *et al.*, 2000). The ornamental freshwater fish *Badis badis* (Hamilton, 1822) and *Dario dario* (Kullander and Britz 2002) (Actinopterygii, Perciformes, Badidae) can be distinguished morphologically, with *B. badis* being distributed throughout Asia in Bangladesh, Bhutan, India and Nepal, while *D. dario* is found in the North Eastern region of India in Assam, Bihar and West Bengal. These species have undergone a series of revisions in taxonomic status at genus and species level. Hamilton (1822) placed these fish in the genus *Labrus* and named them *Labrus badis* and *Labrus dario* respectively. Much later, Talwar and Jhingran (1991) considered both *B. badis* and *D. dario* to be *B. badis*, while Tomey (1999) considered *D. dario* to be a subspecies of *B. badis* (*B. badis bengalensis*) but, more recently, Kullander and Britz (2002) revised the family Badidae and placed *Badis badis bengalensis* in the new genus *Dario*.

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The objective of the study described in this paper was to use random amplified polymorphic DNA (RAPD) markers to develop genetic baseline data for these fish and also provide genetic data to clarify the taxonomic status of *B. badis* and *D. dario* which have undergone recent changes in nomenclature and systematic position.

Specimens of *B. badis* and *D. dario* were collected from sites in the Nadia district (22°56'11.36" N, 88°29'36.35" E), North 24 Parganas district (22°45'25.79" N, 88°25'4.59" E) and Cooch Bihar region (26°18'18.48" N, 89°26'51.71" E) of the Indian state of West Bengal. A total of 20 specimens were collected for each species and preserved in 90% ethanol. For each fish, Genomic DNA was extracted from muscle following the protocol of Sambrook and Russel (2001) with minor modifications. The polymerase chain reaction (PCR) was carried out in a 25 μL reaction mixture consisting of 100 ng DNA, 100 pM of decamer primer, 10 mM dNTP mixture (Dynazyme), 10X PCR buffer (Dynazyme), 1.5 mM $MgCl_2$ and 0.05 units of Taq polymerase (Dynazyme). Reactions were carried out using a GeneAmp PCR system 2400 (Applied Biosystems) programmed for one step of 4 min at 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 34 °C and 2 min at 72 °C, followed by a final extension step of 10 min at 72 °C. All primers used were 10 base oligomers obtained from Operon Technologies (Alameda, USA). Twenty oligodecamer primers from Kit

A and Kit B were initially screened for amplification of genomic DNA by PCR and seven oligodecamer primers were selected depending on repeatability and reproducibility of amplified fragment patterns. The RAPD products were analyzed by electrophoresis in 1.6% (w/v) agarose gels run for 2 h using 0.5 X TBE buffer (0.89 M Tris-borate, pH 8.3, 0.02 M EDTA, for 10X). Gels were stained with 0.5 μg mL^{-1} ethidium bromide and photographed under UV light using a BioRad gel documentation system. The DNA fragment sizes were estimated by comparison with a standard 100 bp ladder (Sigma) using Bio1-D software. The RAPD fragments were scored for either the presence (1) or absence (0) of a homologous amplification product. Genetic similarity was calculated and the unweighted pair group method with averages (UPGMA) was used to construct a dendrogram (Nei, 1978) of the scored data using *PopGene-v 1.31*(Yeh, 1999).

Seven primers (OPA-03, OPA-08, OPB-08, OPB-12, OPB-14, OPB-17, and OPB-18) produced 116 fragments with 98.23% polymorphism (Figure 1), of which 47 fragments were generated from *B. badis* and 69 fragments from *D. dario*. Numbers and sizes of scorable fragments varied from 1 to 12, with a size range of 340 bp to 2170 bp (Table 1). The two species could be unambiguously identified using 26 RAPD markers, of which 9 occurred in *B. badis* and 17 in *D. dario*. In *B. badis* primer OPA-8 produced one diagnostic band, OPA-3 two bands and OPB-14 and OPB-18 three bands each, while in *D. dario* primers OPB-17 and OPB-18 produced one diagnostic band each, OPA-8 three bands, OPB-14 four bands and OPB-8 five bands (Table 1). The highest number of fragments for *B. badis* were the 9 produced by primer OPB-08, while for *D. dario* the highest number was the 12 produced by primer OPA-03. Genetic similarity ranged from 0.836 to 0.906 for *B. badis* and 0.819 to 0.857 for *D. dario*, while the average intraspecies genetic similarity was 0.879 ± 0.023 for *B. badis* and 0.840 ± 0.014 for *D. dario*. Interspecies genetic similarity between the two species was 0.602 ± 0.017 and the UPGMA dendrogram (Figure 2) grouped the two species into separate clusters emphasizing the distinct species status of *B. badis* and *D. dario*.

Callejas and Ochando (2001) used 10 primers and observed 48 species-specific RAPD diagnostic bands which were able to unambiguously differentiate eight *Barbus* species from the Iberian Peninsula, with three markers in *B. guiraonis,* four in *B. bocagei*, *B. microcephalus* and *B. scalateri*, five in *B. graellsii*, seven in *B. comiza,* eight in *B. hassi and* thirteen in *B. meridionalis*. Dinesh *et al.*, (1993) used 9-mer primers to generate DNA fingerprints for freshwater fish belonging to the families Anabantidae, Belontidae, Chararidae, Cyprinidae and Salmonidae. Ruzainah *et al.* (2003) used five primers and RAPD analysis to diagnostically identify the eel-loaches *Pangio filinaris* and *Pangio piperata*, the genetic similarity between these two species (0.609 ± 0.22) being comparable to the average genetic similarity (0.602 ± 0.17) estimated by

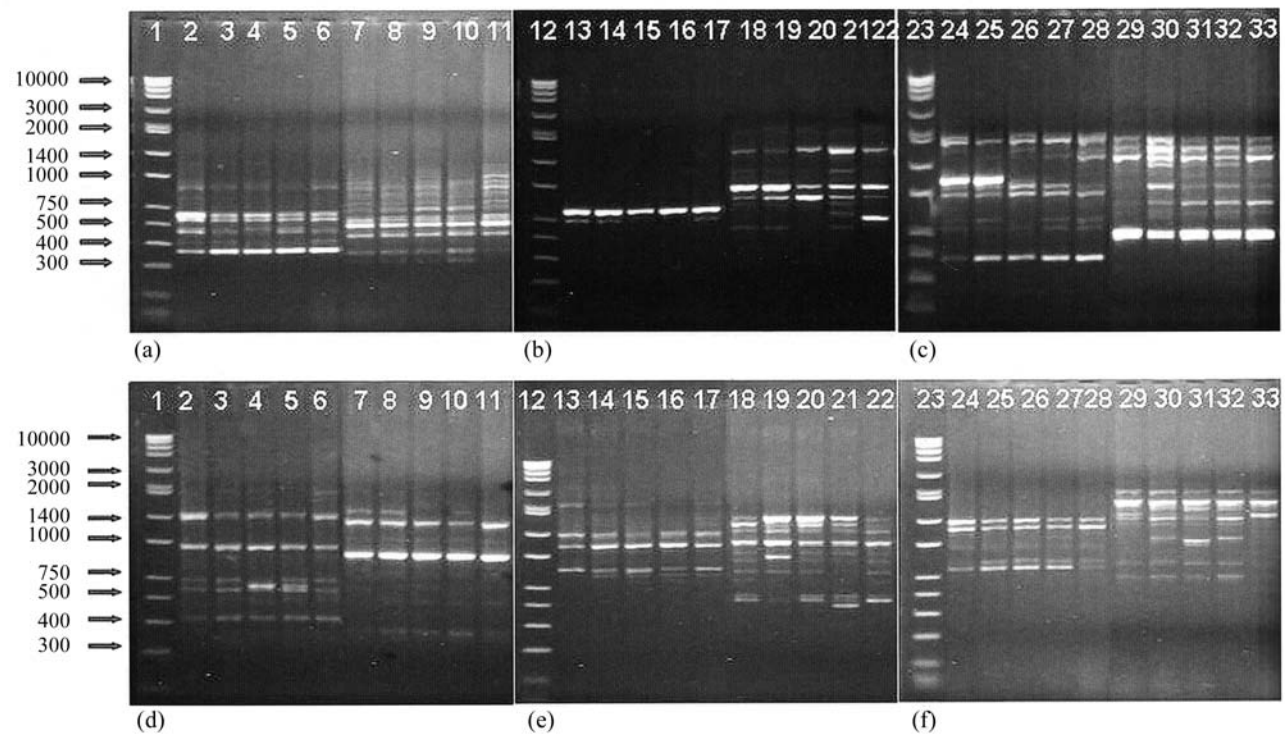

Figure 1 - The RAPD fragment patterns of *Badis badis* (lanes 2-6, 13-17, 24-28) and *Dario dario* (lanes 7-11, 18-22, 29-33) using primer (a) OPA-03, (b) OPA-08 (c) OPB-08 (d) OPB-12 (e) OPB-14 (f) OPB-18. Lane 1, 12, 23 represent wide range DNA molecular weight ladder.

Table 1 - Number and size of fragments amplified by different RAPD primers used and molecular weight of diagnostic markers for *Badis badis* and *Dario dario*.

Primer	Sequence (5' to 3')	G+C content (%)	Number of fragments		Size range and diagnostic fragments (bp)			
			Badis badis	*Dario dario*	*Badis badis*		*Dario dario*	
					Size range	Diag. frag.	Size range	Diag. frag.
OPA-3	AGTCAGCCAC	60	4-6	8-12	930-370	530 370	990-340	950 780 460
OPA-8	GTGACGTAGG	60	1-2	3-9	820-720	820	2070-580	1470 970 920
OPB-8	GTCCACACGG	70	4-9	6-10	1690-420	-	1500-670	1490 1450 930 860 670
OPB-12	CCTTGACGCA	60	4-6	3-4	1500-410	-	1420-870	-
OPB-14	TCCGCTCTGG	70	4-7	6-10	2130-860	1390 1150 920	1500-480	1500 1480 1440 1230
OPB-17	AGGGAACGAG	60	3-6	3-4	1500-680	-	1460-640	930
OPB-18	CCACAGCAGT	60	3-4	6-8	1400-850	1400 1240 820	2000-750	1500

Diag. frag.: diagnostic fragments

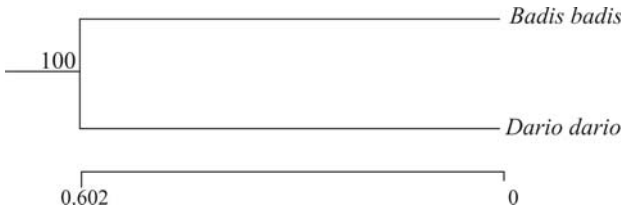

Figure 2 - UPGMA dendrogram showing genetic identity based distinct separation of *Badis badis* and *Dario dario*.

us for *B. badis* and *D. dario*. Brahmane *et al.*, (2006) used RAPD to delineate populations of commercially important anadromous *Tenualosa ilisha* populations from rivers draining into the Bay of Bengal and Arabian Sea.

Our RAPD study confirms the taxonomic position of two species of commercially important ornamental fish found in India. We developed a protocol for the application of RAPD-PCR and produced baseline molecular data for *B. badis* and *D. dario*. Though RAPD produced several diagnostic markers for identification of *B. badis* and *D. dario* further development of a sequence characterized amplified region would be more useful. Due to their economic value in terms of the aquarium fish trade, identification of unique markers for species identification is essential in aquaculture programs, decreasing harvesting pressure on the naturally occurring species and leading to accurate pairing in breeding programs for commercial activity and for genetic monitoring of cultured populations for acceptable levels of genetic diversity. This RAPD methodology can also be applied to other commercially important ornamental fish species found in India, to create molecular documentation of fish species and genetic assessment of indigenous fish resources.

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