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Molecular characterization of eight Indian Snakehead species (Pisces: Perciformes Channidae) using RAPD markers

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Abstract Murrels (Perciformes; Channidei; Channidae) are unique group of freshwater air breathing fishes having a confined distribution to African and Asian continents. The phylogenetic relationship among eight Channid species *viz. Channa aurantimaculata, Channa bleheri, Channa diplogramma, Channa gachua, Channa marulius, Channa punctatus, Channa stewartii* and *Channa striatus* were investigated using RAPD markers. Eight random oligodecamers viz. OPAC03, OPAC05, OPAC07, OPAC09, OPAC19, OPA10, OPA11 and OPA16 were used to generate the RAPD profile. Estimates of Nei's (Genetics, 89:583–590, 1978) unbiased genetic distance (D) demonstrated sufficient genetic divergence to discriminate the samples of different species and the values ranged from 0.3292 to 0.800 The present RAPD analyses strongly substantiate the view of earlier morphological and osteological studies of Channid species, the closer association among species in "*gachua*" and "*marulius"* groups.

Keywords Pisces · Indian Snakeheads · Molecular characterization · RAPD

Introduction

Murrels are primary freshwater fishes inhabiting African and Asian continents [1]. They are commonly called Snakeheads and are characterized by peculiar morphological features, such as elongated cylindrical body, long and entirely soft-rayed dorsal and anal fins, a large mouth with well-developed teeth on both upper and lower jaws, and an accessory air-breathing apparatus known as the suprabranchial organ [2, 3]. They have flattened heads; possess large scales on their heads and eyes being located in the dorsoventral position on the anterior part of the head.

The family Channidae consists of two genera viz., *Channa* and *Parachanna*. The Asian genus *Channa*, which presently contains 26 valid species, is widely distributed in Iran and southern Asia (the Indian subcontinent including Sri Lanka, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia, and Philippines) and the Far East (China, Taiwan, Korea, and southern Russia) [4–8]. The African genus *Parachanna* contains three valid species, which are restricted to central West Africa [9, 10]. So far Snakeheads have been identified conventionally based on morphological and anatomical characters [11]. However, there are ambiguities due to morphological closeness and changing colour patterns from juvenile to adult stage. Inspite of their economic and scientific importance to date there is very limited information available on the extent of molecular genetic structure in these species [1, 12]. Hence the present study attempted to investigate the genetic identity of eight Channid species distributed in Indian waters using RAPD markers.

Molecular techniques have become major tools for systematic ichthyologists and may also be useful to fishery biologists for ratification of taxonomic problems at species and population levels [13–15]. Random amplified polymorphic DNA (RAPD) technique allows detection of DNA polymorphisms by randomly amplifying multiple regions of the genome by polymerase chain reaction (PCR) using single arbitrary primers designed independent of target

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DNA sequence [16, 17]. Randomly amplified polymorphic DNA marker has widely been used to reveal the genetic identity of fish species [18, 19].

Materials and methods

Live Snakehead samples of *Channa striatus* were collected from river Periyar (10°.10′ N, 76°.13′ E) Kerala, *Channa punctatus* were collected from river Tamirabarani (80°.44′ N 77°.44′ E), *Channa marulius* from Bhavanisagar Dam (11°.58′ N 77°.58′ E) Tamilnadu, *Channa diplogramma* from river Pampa (9°.27′ N 76°.78′ E) Kerala and *Channa aurantimaculata, Channa bleheri* and *Channa stewartii* from river Brahmaputra (24°.8 N′ 89°.42′ E) Assam. Approximately 100 mg of fin tissue from five individuals of each species was preserved in 95% ethanol. DNA was isolated from preserved samples following Ruzzante et al [20] with minor modifications. A total of 40 arbitrary primers (OPAC and OPA series Operon Technologies Ltd. USA) with random sequence were screened. Eight primers which gave reproducible results viz. OPAC03, OPAC05, OPAC07, OPAC09, OPAC19, OPA10, OPA11 and OPA16 were selected. The PCR amplifications were carried out in the Veriti 96 well Thermal Cycler Applied Biosystems in a reaction volume of 25 µl containing 50 ng genomic DNA, 10× PCR buffer (10 mM Tris–HCl pH 9.0 50 mM KCl 0.01% gelatin), 2.5 mM of each dNTP, 5 pmol of primer and 0.7 units of *Taq* DNA polymerase. The amplification conditions were 94°C for 5 min followed by 29 cycles at 94°C for 1 min 40°C for 1 min and 72°C for 2 min with a final extension at 72°C for 10 min. After amplification 8 µl of PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide and 1× TBE buffer to visualize the band patterns generated by each primer. The molecular weight of each band was estimated using a standard molecular marker (Lambda DNA/*Eco* RI *Hin*dIII Double Digest) with Image master 1D Elite Ver.3.01 (GE Amersham Biosciences USA) [21]. The RAPD genotype was analyzed using Popgene software (Ver. 1.3) [22].

Results and discussion

In the present study out of 40 RAPD arbitrary primers used eight primers viz. OPAC03, OPAC05, OPAC07, OPAC09, OPAC19, OPA10, OPA11 and OPA16 showed reproducible result and with good resolutions in banding patterns whereas the other thirty-two primers produced highly inconsistent amplification products or did not amplify at all and hence they were excluded from further analysis. Amplification using eight primers resulted in fragments ranging in length between 312 and 3,029 bp which were assigned to 93 loci. The number of stable and clear RAPD bands generated per primer varied in the range of 5–12. The number and size of the bands generated strictly depend upon the nucleotide sequence of the primer used and the source of the template DNA resulting in the genome-specific fingerprints of random DNA bands [23]. The RAPD fingerprints generated in the *Channa* species were consistent and showed species specific diagnostic markers in the Channa species studied except in *C. aurantimaculata, C. marulius* and *C. diplogramma* (Table 1; Figs. 1, 2, 3, 4, 5 6). Estimates of Nei's [24] unbiased genetic distance (D) demonstrated the genetic distance to discriminate the different species (Table 2) and the values ranged from 0.3292 to 0.8000.

The morphological and osteological comparisons made by previous authors in eight species of *Channa* examined indicated two phylogenetic groups: *gachua* group and *marulius* group [11]. *Gachua* group comprises *Channa amphibeus, Channa aurantimaculata, Channa barca, Channa bleheri, Channa gachua, Channa punctatus* and *Channa stewartii* whereas *Channa marulius* and *Channa striatus* have found their place in marulius group. UPGMA dendrogram constructed on the basis of genetic distance revealed that the genetic relationship was very close among *Channa aurantimaculata and Channa bleheri* as well as *C. gachua and C. stewartii* whereas *Channa marulius, Channa striatus and Channa diplogramma* were found to be genetically distant (Fig. 7).

Morphologically the members of the *marulius* group are differentiated from *gachua* group in having a prominent V shaped sharp isthmus grouped sensory pores arrangement absence of big cycloid scales on the lower jaw where as the members of the gachua group have U-shaped isthmus

Table 1 Average no of bands amplified in each species no. of polymorphic bands and species specific bands within eight Channid species by OPAC03 OPAC05 OPAC07 OPAC09 OPAC19 OPA10 OPA11 and OPA16 primers

Species	Average no of bands amplified	Number of polymorphic bands	Species specific bands
C. a	32	3	
C. b	44	13	(OPAC07-2,778 bp)
C. d	41	6	
C. g	46	10	(OPA11-646 bp, OPA16-2,617 bp)
C. m	44	3	
C. p	43	4	(OPAC09-921 bp)
C. st	50	8	(OPAC03-1,703 bp)
C. s	63	3	(OPAC09-312 bp, OPAC19-390 bp)

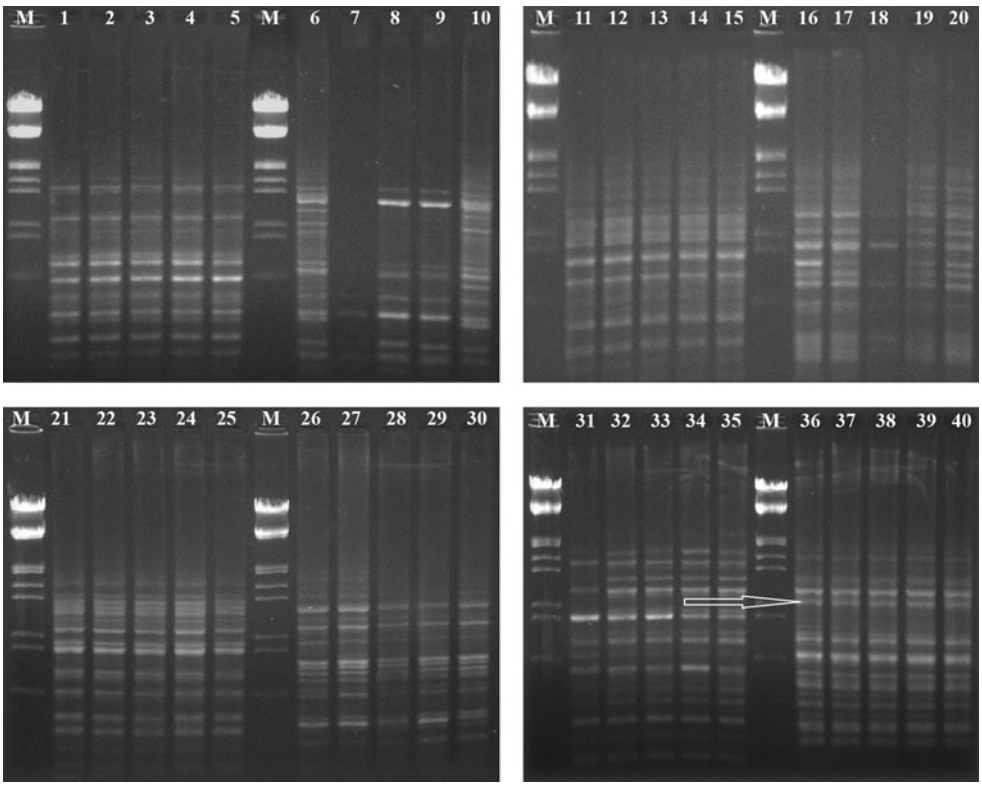

Fig. 1 RAPD bands amplified by primer OPAC-03 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/ *Eco* RI *Hind* III Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

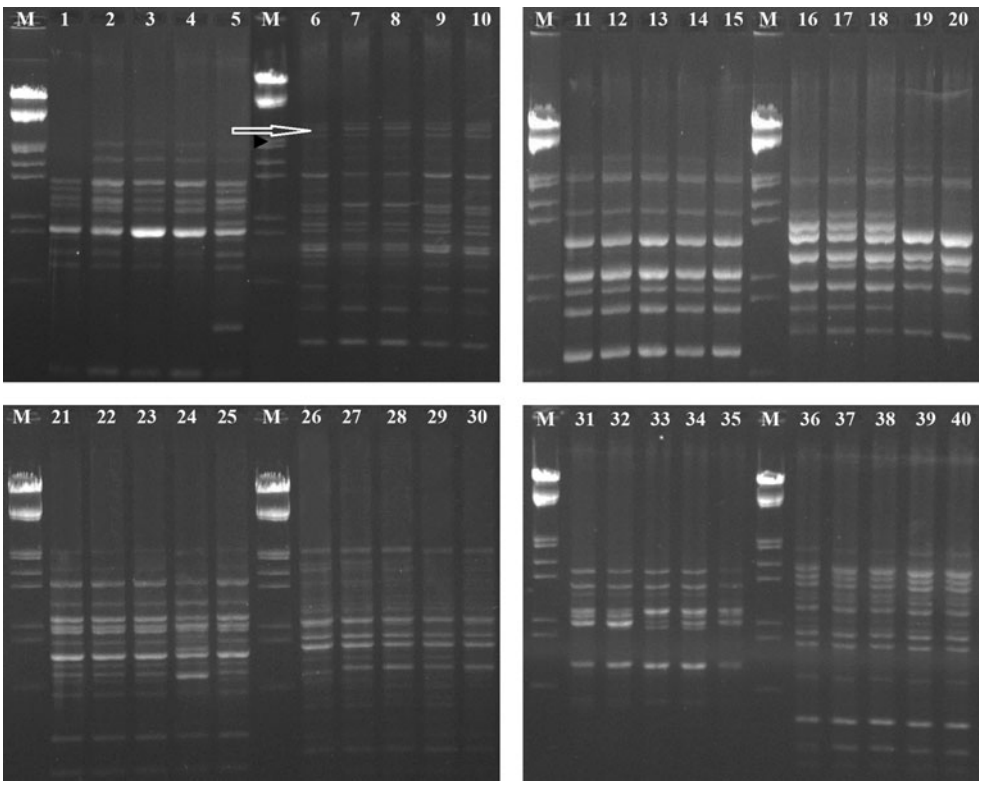

Fig. 2 RAPD bands amplified by primer OPAC-07 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/ *Eco* RI *Hin*dIII Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

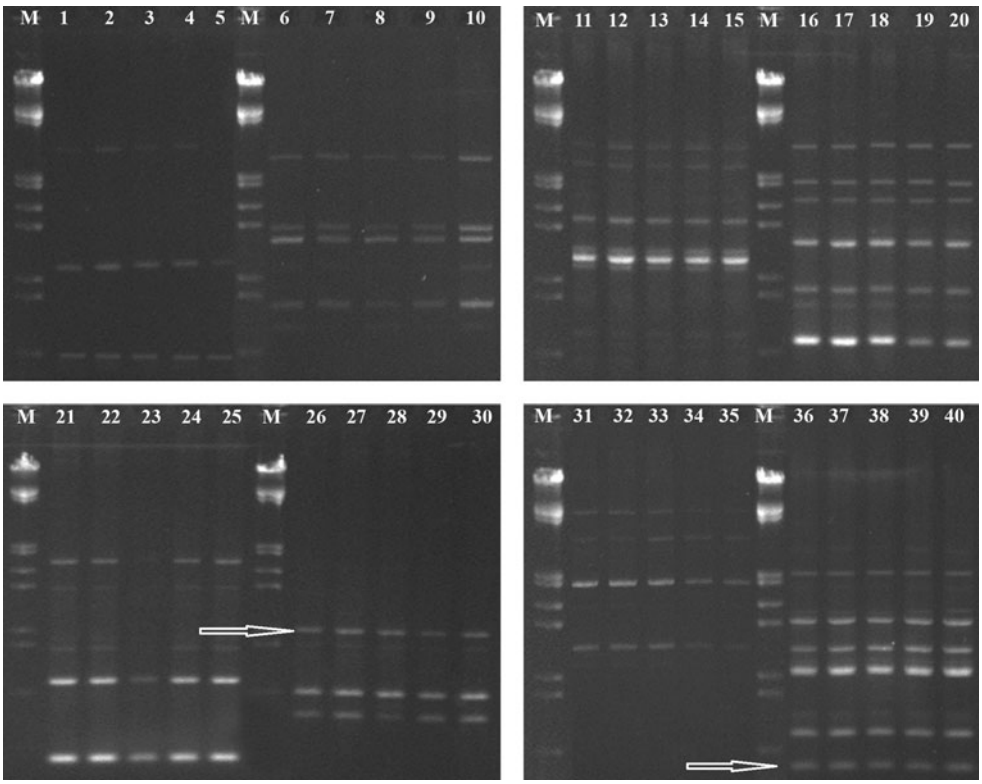

Fig. 3 RAPD bands amplified by primer OPAC-09 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/*Eco* RI *Hin*dIII Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

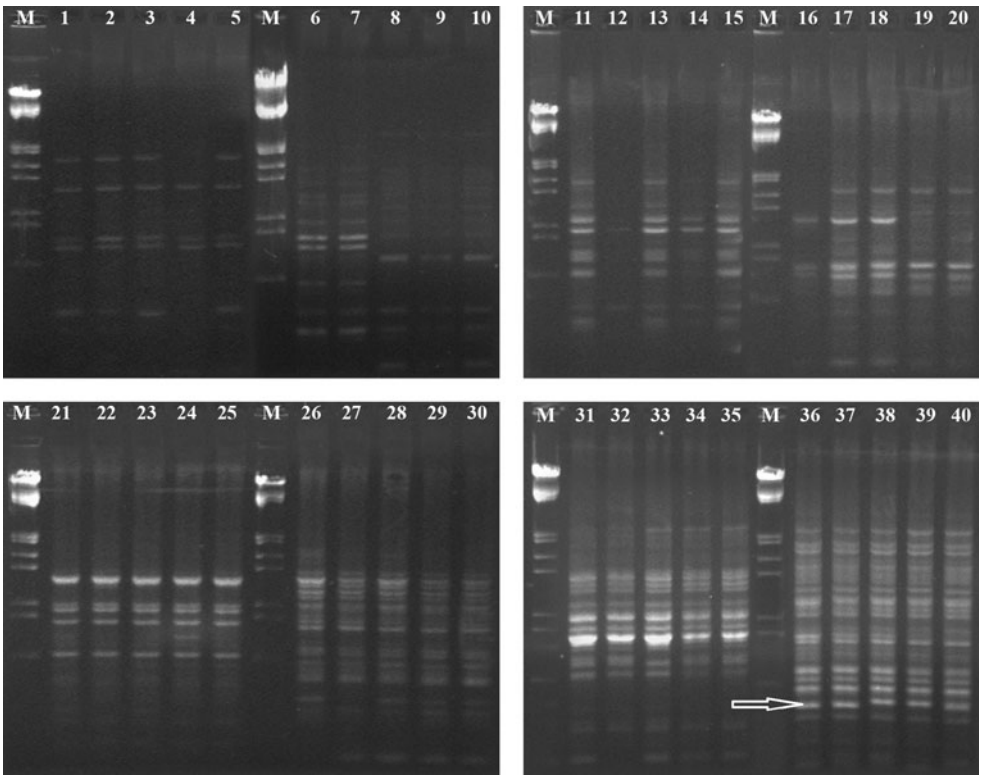

Fig. 4 RAPD bands amplified by primer OPAC-19 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/*Eco* RI *Hin*dIII Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

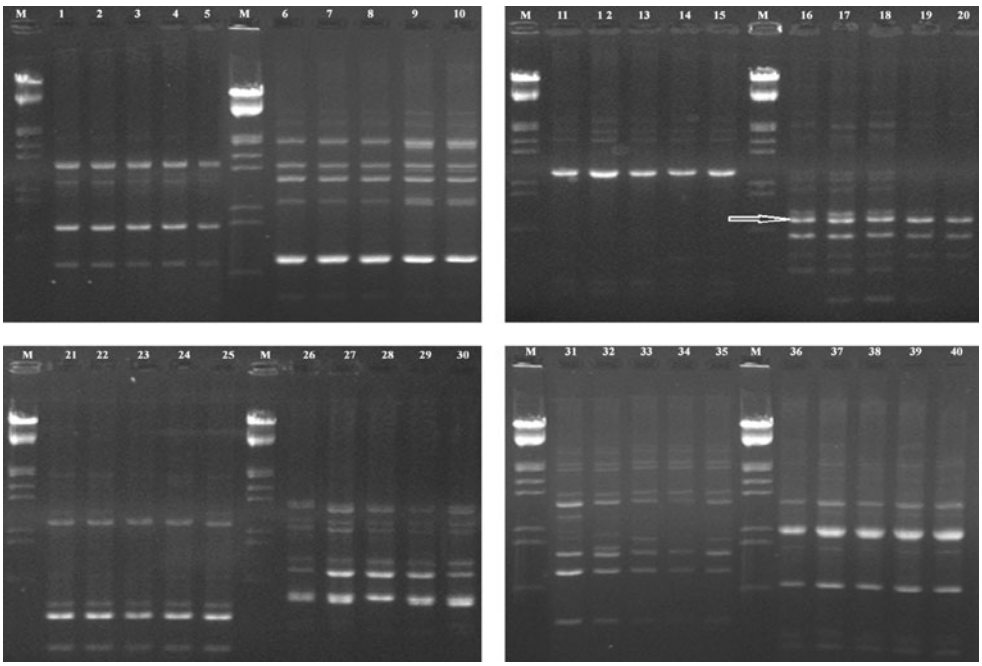

Fig. 5 RAPD bands amplified by primer OPA-11 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/*Eco* RI *Hin*dIII Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

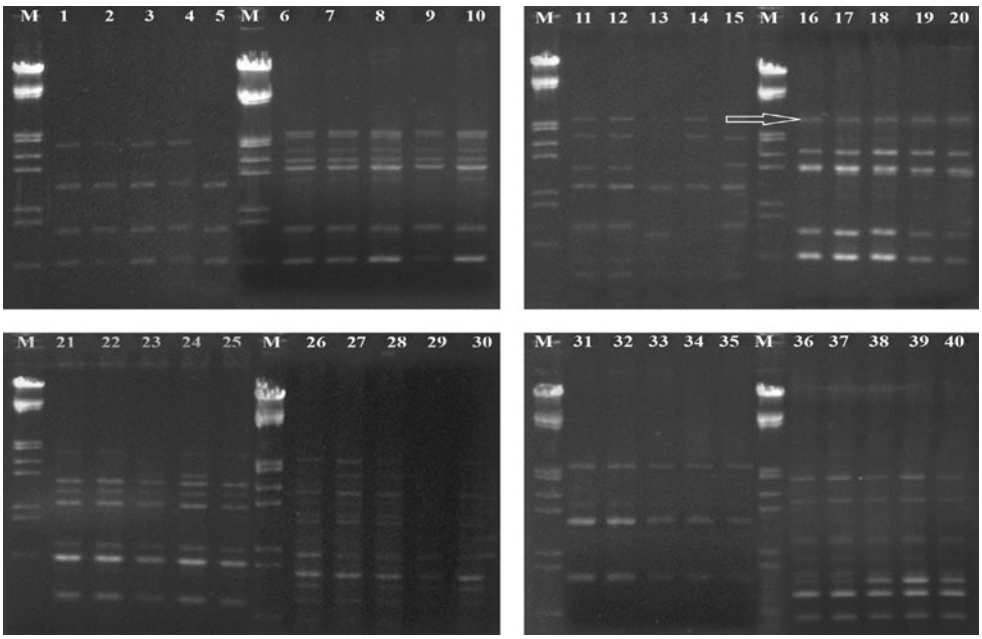

Fig. 6 RAPD bands amplified by primer OPA-16 in eight species of Indian Channids *M* Standard Molecular weight marker; marker (Lambda DNA/*Eco* RI *Hin*dIII Double Digest) *1–5 Channa aurantimaculata*, *6–10 Channa bleheri*, *11–15 Channa diplogramma*, *16–20 Channa gachua*, *21–25 Channa marulius*, *26–30 Channa punctatus*, *31–35 Channa stewartii*, *36–40 Channa striatus*, → highlighting species specific bands

single sensory pore arrangement presence of one or two big cycloid scales on each side of the lower jaw (Fig. 8). *Marulius* group has 3–4 numbers of branchial tooth plates where as *gachua* group has one or no branchial tooth plates in the epibranchial region. The present study gives a genetical support to the morphological observations made by previous authors [11].

In the present study least genetic distance was observed between *Channa aurantimaculata* and *Channa bleheri* (0.3292) and *Channa gachua* and *Channa stewartii* (0.3790). *Channa marulius, Channa striatus and Channa diplogramma* were found to be phylogenetically closer. In conclusion the arbitrary primers have provided consistent band pattern in the eight *Channa* species and hence RAPD represents a useful and reliable tool for species discrimination and for detecting genetic relationship in *Channa* species. The present study can be useful for resolving the taxonomic ambiguities as well as for devising effective

Table 2 Nei's (1972/1978) genetic distance among eight species of genus *Channa* estimated from RAPD profiles

Pop ID	*C. a*	*C. d*	*C. g*	*C. m*	*C. p*	*C. b*	*C. st*	*C. s*
C. a	****							
C. d	0.6054	****						
C. g	0.5058	0.7232	****					
C. m	0.5481	0.8000	0.4278	****				
C. p	0.3666	0.6785	0.5586	0.4797	****			
C. b	0.3292	0.5349	0.4590	0.5986	0.4433	****		
C. st	0.3766	0.6590	0.3790	0.5498	0.4263	0.5310	****	
C. s	0.7224	0.7005	0.6586	0.5777	0.5078	0.7095	0.5389	****

C. a-Channa aurantimaculata, C. b-Channa bleheri, C. d-Channa diplogramma, C. g-Channa gachua, C. m-Channa marulius, C. p-Channa punctatus, C.st-Channa stewartii and *C. s-Channa striatus*

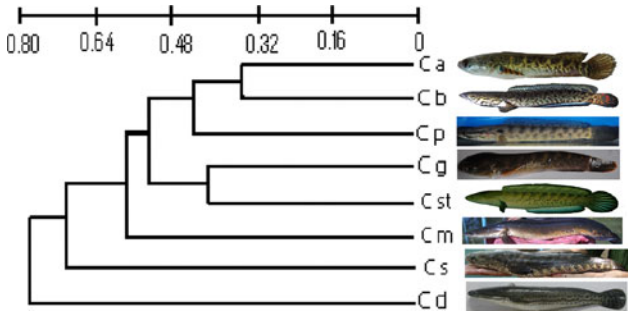

Fig. 7 UPGMA dendrogram of eight Channid species based on RAPD profiles. (*C. a-Channa aurantimaculata C. b-Channa bleheri C. d-Channa diplogramma C. g-Channa gachua C. m-Channa marulius C. p-Channa punctatus C. st-Channa stewartii C. s-Channa striatus*)

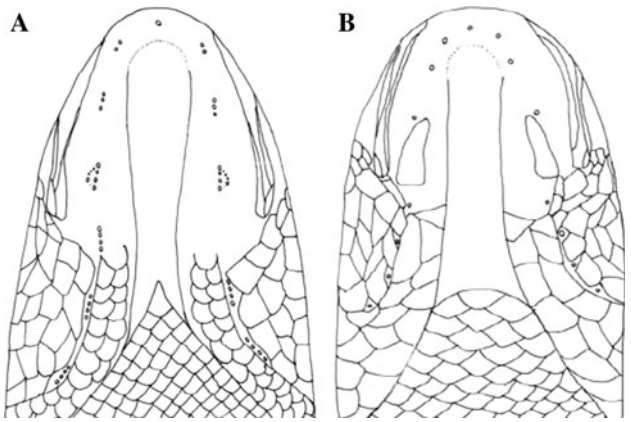

Fig. 8 Ventral view of (**a**) *Channa marulius* and (**b**) *Channa gachua* showing sensory pores and sides of lower jaw (MUMF-Per/0025151.6 mm SL) and (MUMF-Per/0004 112.8 mm SL), respectively

management strategies, future aquaculture plans and conservation measures. As the *Channa* species show variable color patterns during different life stages the technique and the data will also be helpful for the species identification in juvenile forms.

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References

1. Xia Li, Musikasinthorn P, Kumazawa Y (2006) Molecular phylogenetic analyses of Snakeheads Perciformes: Channidae) using mitochondrial DNA sequences. Ichthyol Res 53:148–159
2. Musikasinthorn P (1998) *Channa panaw*, a new channid fish from the Irrawaddy and Sittang River basins, Myanmar. Ichthyol Res 45:355–362
3. Musikasinthorn P (2003) Channoidei (Snakeheads) In: Hutchins M, Thoney A, Loiselle PV, Schlager N (eds) Grzimek's animal life encyclopedia, 2nd edn. vols 4, 5. Fishes I–II. Gale Group, Farmington Hills, pp 437–447
4. Musikasinthorn P (2000) *Channa aurantimaculata*, a new channid fish from Assam (Brahmaputra River basin), India, with designation of a neotype for *C. amphibeus* (McClelland, 1845). Ichthyol Res 47:27–37
5. Berra TM (2001) Freshwater fish distribution. Academic Press, San Diego
6. Musikasinthorn P, Taki Y (2001) *Channa siamensis* (Günther, 1861), a junior synonym of *Channa lucius* (Cuvier *in* Cuvier and Valenciennes, 1831). Ichthyol Res 48:319–324
7. Zhang C-G, Musikasinthorn P, Watanabe K (2002) *Channa nox*, a new channid fish lacking a pelvic fin from Guangxi, China. Ichthyol Res 49:140–146
8. Courtenay WR, Williams JD (2004) Snakeheads (Pisces, Channidae): a biological synopsis and risk assessment. US Geological Survey Circular 1251, p 143
9. Bonou CA, Teugels GG (1985) Révision systématique du genre *Parachanna* Teugels et Daget, 1984 (Pisces: Channidae). Rev Hydrobiol Trop 18:267–280
10. Teugels GG (1992) Channidae. In: Leveque C, Paugy D, Teugels GG (eds) The fresh and brackish water fishes of west Africa, vol 2. ORSTOM et MRAC, Paris, pp 655–658
11. Vishwanath W, Kh Geethakumari (2009) Diagnosis and interrelationships of fishes of the genus *Channa* Scopoli (Teleostei: Channidae) of northern. Ind J Threatened Taxa 1(2):97–105
12. Nagarajan M, Haniffa MA, Gopalakrishnan A, Basheer VS, Muneer Abdul (2006) Gnetic variability of *Channa punctatus*

populations using randomly polymorphic DNA. Aquacult Res 37:1151–1155
13. Çiftci Y, Okumus I (2002) Fish population genetics and applications of molecular markers to fisheries and aquaculture: I- basic principles of fish population genetics. Turk J Fish Aquat Sci 2: 145–155
14. Okumus I, Çiftci Y (2003) Fish population genetics and molecular markers: II- molecular markers and their applications in fisheries and aquaculture. Turk J Fish Aquat Sci 3:51–79
15. Povh AJ, Lopera-Barrero NM, Lupchinski E Jr, Gomes PC, Lopes TS (2008) Genetic monitoring of fish repopulation programs using molecular markers. Cien Inv Agr 35(1):1–10
16. Welsh J, McClelland M (1990) Fingerprinting genome using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218
17. Gil LA (2007) PCR-based methods for fish and fishery products authentication. Trends Food Sci Technol 18(11):558–566
18. Bardakci F (2001) Random amplified polymorphic DNA (RAPD) Markers. Turk J Biol 25:185–196
19. Ali BA, Huang T-H, Qin D-N, Wang X-M (2005) A review of random amplified polymorphic DNA (RAPD) markers in fish research. Rev Fish Biol Fish 14:443–453
20. Ruzzante DE, Taggart CT, Cook C, 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
21. Archana Saini, Anish Dua, Vindhya Mohindra, Lakra WS (2010) Molecular discrimination of six species of Bagrid catfishes from Indus river system using randomly amplified polymorphic DNA markers. Mol Biol Rep. doi:10.1007/s11033-9960-1
22. Yeh FC, Yang RC, Boyle T (1999) POPGENE 32- Version 1.31. Population genetics software. http://www.ualberta.ca/ ~fyeh/fyeh
23. Welsh J, McClelland M (1994) Fingerprinting using arbitrarily primed PCR: applications to genetic mapping, population biology, epidemiology and detection of differentially expressed RNAs. In: Mullis KB, Ferre F, Gibbs RA (eds) The polymerase chain reaction, Brikhauser, Boston, pp 295–303
24. Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590