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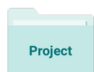

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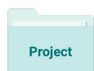

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Genetic relatedness among fish species of Genus *Channa* using mitochondrial DNA genes

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ABSTRACT

The family Channidae is represented by 26 species, out of which 23 species are found in Asia. However, the taxonomy and phylogeny of the Channid fishes found in India are poorly understood. In the present study, eight species of Channa (*Channa striata*, *Channa punctatus*, *Channa marulius*, *Channa gachua*, *Channa stewartii*, *Channa aurantimaculata*, *Channa barca* and *Channa bleheri*) were investigated using partial sequences of 16S rRNA and Cytochrome c Oxidase subunit I (COI) of mitochondrial genes to differentiate among the eight species and study their relationships. The sequence analysis of the genes revealed two distinct groups, which are genetically distant from each other and exhibit identical phylogenetic resolution. The partial sequences of both the genes provided sufficient phylogenetic information to distinguish all the eight species of Channa.

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1. Introduction

The family Channidae includes airbreathing freshwater fishes, popularly known as snakeheads or murrels and consists of two genera *Channa* and *Parachanna*. The Asian genus *Channa*, which presently contains 26 valid species, is distributed in Iran and southern Asia (the Indian subcontinent), South Eastern Asia (Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia and Philippines) and the far East (China, Taiwan, Korea and Southern Russia) (Li et al., 2006; Vishwanath and Geetakumari, 2009). The African genus *Parachanna* contains only three valid species confined to the Central West Africa (Li et al., 2006).

These fishes have a physiological need to breathe atmospheric air, which they do with a suprabranchial organ: a primitive form of a labyrinth organ. These are economically important species having great potential for aquaculture and capture fisheries throughout southern and southeastern Asia. Vishwanath and Geetakumari (2009) recently reported that the North-Eastern India is an important denizen for about nine species of Channa. However, taxonomy and phylogeny of these species have not been studied using molecular techniques.

Nagarajan et al. (2006) identified distinct genetic populations of *Channa punctatus* from Southern India using RAPD markers. As a major phylogenetic study, Li et al. (2006) reconstructed the phylogenetic relationships among 20 species of snakeheads using mitochondrial genes ND2 and adjacent tRNAs. The ND2 segment is fast evolving in vertebrates (Lopez et al., 1997). Compared to ND2 and tRNA regions, mitochondrial 16S rRNA gene and the protein coding cytochrome c oxidase subunit I (COI) genes have been extensively used for fish phylogenetics (Brown, 1985; Bermingham and Lessios, 1993; Santos et al., 2003; Vinson et al., 2004; Ward et al., 2005; Lakra et al., 2009). In this study the partial sequence data of 16S rRNA and COI genes were used to differentiate among the eight species of Channa from the North Eastern Hill (NEH) region of India and to investigate their genetic relationships.

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2. Materials and methods

2.1. Sample collections

Eight species belonging to genus *Channa* (*Channa striata*, *C. punctatus*, *Channa marulius*, *Channa gachua*, *Channa stewartii*, *Channa aurantimaculata*, *Channa barca* and *Channa bleheri*) were collected from upper (Latitude 27° 30′ 07N and Longitude 95° 19′ 48E) and lower part of Assam (Latitude 26° 09′ 52N and Longitude 91° 45′ 35E), North-Eastern part of India. For species identification and nomenclature, taxonomic keys prepared by Vishwanath and Geetakumari (2009) and Jayaram (2010) were followed. The taxonomic identification of the species was confirmed by the taxonomists Dr. B. K. Bhattacharya and Prof. W. Vishwanath (Vishwanath and Geetakumari, 2009). Voucher specimens were maintained in the divisional laboratory of NBFGR, Lucknow. Samples were collected during October, 2008–February, 2009 and approximately 25 mg of fin clips from five individuals of each species were preserved in 95% ethanol until used.

2.2. DNA isolation

DNA was isolated following Ruzzante et al. (1996) with minor modifications. Briefly, samples were homogenized separately in incubation buffer (10 mM Tris–HCl and 10 mM ethylene diamine tetra-acetic acid (EDTA), pH8.0). After digestion with lysis buffer (10 mM Tris–HCl, 10 mM EDTA, pH8.0, 1% SDS and 200 μg/ml Proteinase K) at 37 °C for overnight, the digests were deproteinized by successive phenol/chloroform and iso-amyl alcohol extraction and DNA was recovered by ethanol precipitation, drying and resuspension in TE buffer. The concentration and purity of isolated DNA was estimated at wavelength 260/280 nm using a UV spectrophotometer. The DNA was diluted to get a final concentration of 100 ng μl^{-1}.

2.3. Amplification and sequencing

The mitochondrial 16S rRNA gene was amplified in a 50 μl reaction volume with 1X Taq polymerase buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 2 U of Taq polymerase and 100 ng genomic DNA using the thermal cycler PTC 200 (MJ Research). The primers used for the amplification of the partial 16S rRNA gene were 16SAR (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SBR (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al., 1991). The thermal profile used was 35 repetitions of a three step cycle consisting of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min and extension at 72 °C for 1.5 min including 4 min for initial denaturation at 94 °C and 7 min for the final extension at 72 °C.

COI gene was also amplified in a final concentration of 50 μl volume with a final concentration of 1X Taq polymerase buffer, 2 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 2 U of Taq polymerase and 100 ng of genomic DNA. The primers used for the amplification of the COI gene were FishF1-5′TCAACCAACCACAAAGACATTGGCAC3′ and FishR1-5′TAGACTTCTGGG TGGCCAAAGAATCA3′ (Ward et al., 2005). The thermal regime consisted of an initial step of 2 min at 95 °C followed by 35 cycles of 40 s at 94 °C, 40 s at 54 °C and 1 min 10 s at 72 °C followed in turn by final extension of 10 min at 72 °C.

PCR products were visualized on 1.2% agarose gels and the most intense products were selected for sequencing. Products were labeled using the BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc.) and sequenced using an ABI 3730 capillary sequencer following manufacturer's instructions.

2.4. Sequence analysis

Sequences were aligned using ClustalW (Thompson et al., 1997) and submitted to GenBank under the accession numbers (HM117172–HM117251). The extent of sequence differences between species was calculated by averaging pair-wise comparisons of sequence difference across all individuals. The 16S rRNA sequences of the five individuals of each species were aligned to yield a final alignment varying from 504 bp (*C. marulius*) to 573 bp (*C. barca*). The COI sequences of the five individuals of each species were aligned to yield a final alignment ranging from 561 bp (*C. marulius*) to 583 (*C. stewartii*). Pair-wise evolutionary distance among haplotypes was determined by the Kimura 2-Parameter method (Kimura, 1980) using the software program MEGA 3.1 (Molecular Evolutionary Genetics Analysis) (Kumar et al., 2004). The number of polymorphic sites and nucleotide diversity (Pi), nucleotide composition and number of transition and transversion between species were determined by DnaSp ver 5.10 (Rozas et al., 2009). Gaps were considered as missing data on the phylogenetic reconstructions. Neighbor Joining (NJ) and Maximum Parsimony (MP) trees were constructed using MEGA 3 using *Thunnus orientalis* from NCBI (GenBank Accession No. AB185022) as an out-group. To verify the robustness of the internal nodes of NJ and MP trees, bootstrap analysis was carried out using 1000 pseudoreplications (Felsenstein, 1993).

3. Results

3.1. 16S rRNA sequence analysis

A total of 40 individuals from *C. striata*, *C. punctatus*, *C. marulius*, *C. gachua*, *C. stewartii*, *C. aurantimaculata*, *C. barca* and *C. bleheri*) were used for partial sequence analysis of 16S rRNA and COI genes, which yielded 80 sequences. Simplicity and un-

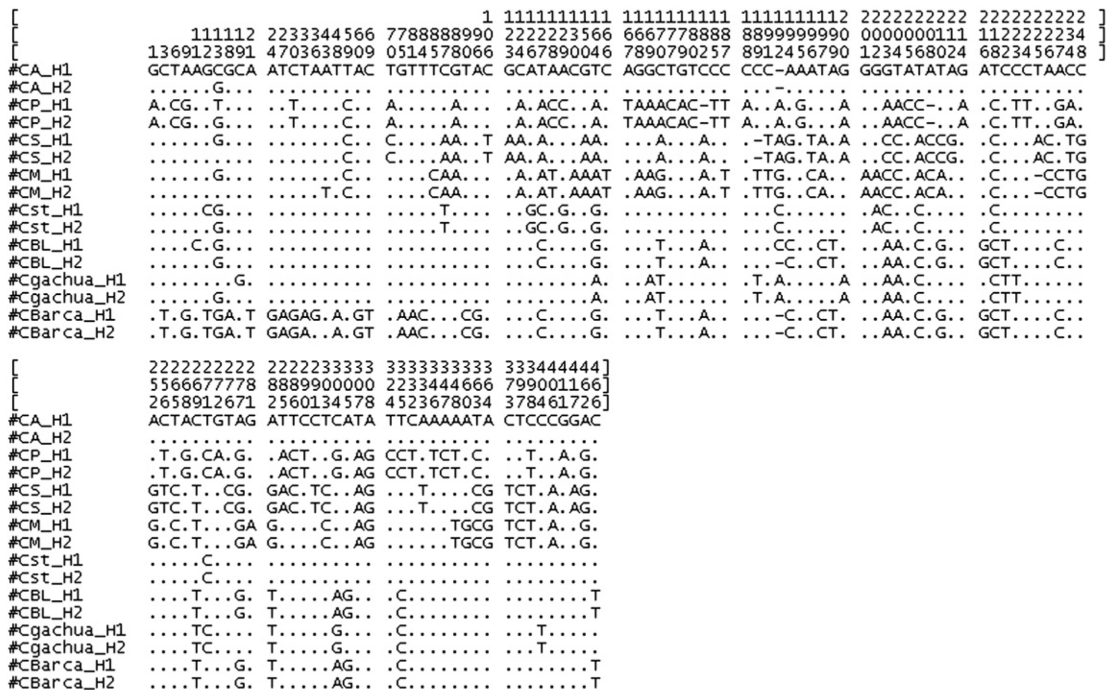


Fig. 1. Alignment of partial DNA sequences of the mitochondrial gene, 16S rRNA of eight Indian channids (only variable sites are reported) (H1: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: *C. punctatus*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: *C. gachua*, Cb: *C. barca*).

ambiguity were observed among the sequences of the both mitochondrial regions as there were no insertions, deletions and stop codons in the sequences.

Sequencing of the 16S rRNA gene produced an average of 550 bp (range 504–573) nucleotide base pairs per taxon. Multiple alignments resulted in a consensus length of 493 sites including base pairs and gaps. Two haplotypes each were observed in all the species for 16S rRNA gene. Of the 493 sites, "373, 119, 115 and 4 were conserved, variable, parsimony informative and singleton" respectively. The polymorphic sites are given in Fig. 1. The analysis revealed nucleotide frequencies as $A = 29.90\%$, $T = 21.60\%$, $G = 22.50\%$ and $C = 26.00\%$. As expected, average transitional pairs ($si = 27$) were more frequent than transversional pairs ($sv = 16$) with an average ratio of 1.66. Pair-wise nucleotide divergence and sequence divergences are given in Table 1. Average intra-specific sequence diversity was 0.091. The highest interspecies sequence diversity (0.1546) was *C. marulius* and *C. barca*, whereas the lowest value (0.0347) was between species *C. bleheri* and *C. barca*. Interspecies nucleotide differences ranged from 10 to 75, whereas intraspecies nucleotide differences ranged from 1 to 2 (Table 1).

Pair-wise genetic distance values (Kimura 2-parameter) based on 16S rRNA using MEGA 3.1 are given in Table 2. The average genetic distance of individuals among channid species was estimated as 0.093 and within species as 0.002.

Table 1
Pair-wise nucleotide differences (below diagonal) and sequence divergence (above diagonal) in 16S rRNA sequences derived from eight Indian channids.

	CAH1	CAH2	CPH1	CPH2	CSH1	CSH2	CMH1	CMH2	CstH1	CstH2	CBLH1	CBLH2	CgH1	CgH2	CbH1	CbH2
CAH1		0.0020	0.1155	0.1155	0.1043	0.1022	0.1043	0.1043	0.0245	0.0224	0.0490	0.0469	0.0367	0.0367	0.0837	0.0816
CAH2	1		0.1155	0.1134	0.1022	0.1043	0.1022	0.1063	0.0224	0.0204	0.0469	0.0449	0.0388	0.0347	0.0816	0.0796
CPH1	56	56		0.0021	0.1340	0.1340	0.1361	0.1381	0.1193	0.1173	0.1276	0.1237	0.1049	0.1049	0.1452	0.1472
CPH2	56	55	1		0.1320	0.1340	0.1340	0.1381	0.1173	0.1152	0.1255	0.1216	0.1049	0.1008	0.1493	0.1431
CSH1	51	50	65	64		0.0020	0.0613	0.0654	0.1102	0.1082	0.1143	0.1104	0.1122	0.1082	0.1472	0.1452
CSH2	50	51	65	65	1		0.0634	0.0634	0.1122	0.1102	0.1163	0.1125	0.1102	0.1102	0.1493	0.1472
CMH1	51	50	66	65	30	31		0.0041	0.1061	0.1041	0.1122	0.1084	0.1122	0.1082	0.1546	0.1505
CMH2	51	52	67	67	32	31	2		0.1102	0.1082	0.1163	0.1125	0.1122	0.1122	0.1526	0.1526
CstH1	12	11	58	57	54	55	52	54		0.0020	0.0509	0.0490	0.0468	0.0427	0.0837	0.0816
CstH2	11	10	57	56	53	54	51	53	1		0.0488	0.0469	0.0448	0.0407	0.0836	0.0816
CBLH1	24	23	62	61	56	57	55	57	25	24		0.0020	0.0468	0.0427	0.0387	0.0367
CBLH2	23	22	60	59	54	55	53	55	24	23	1		0.0428	0.0388	0.0367	0.0347
CgH1	18	19	51	51	55	54	55	55	23	22	23	21		0.0041	0.0796	0.0775
CgH2	18	17	51	49	53	54	53	55	21	20	21	19	2		0.0755	0.0734
CbH1	41	40	71	73	72	73	75	74	41	41	19	18	39	37		0.0020
CbH2	40	39	74	73	71	72	70	72	40	40	18	17	38	36	1	

H1: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: C. *punctata*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: *C. gachua*, Cb: *C. barca.*

Table 2
Pair-wise genetic distances (Kimura 2-parameter) of eight Indian channids based on 16S rRNA sequences.

	CAH1	CAH2	CPH1	CPH2	CSH1	CSH2	CMH1	CMH2	CstH1	CstH2	CBLH1	CBLH2	CgH1	CgH2	CbH1	CbH2
CAH1																
CAH2	0.002															
CPH1	0.127	0.127														
CPH2	0.127	0.125	0.002													
CSH1	0.107	0.105	0.147	0.145												
CSH2	0.105	0.107	0.147	0.147	0.002											
CMH1	0.108	0.105	0.148	0.145	0.063	0.065										
CMH2	0.108	0.111	0.150	0.150	0.067	0.065	0.004									
CstH1	0.025	0.023	0.130	0.127	0.112	0.114	0.108	0.113								
CstH2	0.023	0.021	0.127	0.125	0.109	0.112	0.115	0.110	0.002							
CBLH1	0.050	0.047	0.140	0.137	0.120	0.123	0.118	0.123	0.052	0.049						
CBLH2	0.047	0.045	0.137	0.135	0.118	0.120	0.115	0.121	0.049	0.047	0.002					
CgH1	0.036	0.038	0.112	0.113	0.117	0.115	0.118	0.118	0.045	0.043	0.043	0.041				
CgH2	0.036	0.034	0.110	0.107	0.112	0.115	0.113	0.118	0.040	0.038	0.038	0.036	0.004			
CbH1	0.089	0.086	0.162	0.167	0.164	0.167	0.177	0.174	0.088	0.089	0.041	0.038	0.082	0.070		
CbH2	0.086	0.084	0.174	0.172	0.162	0.164	0.153	0.165	0.086	0.086	0.038	0.036	0.079	0.074	0.002	

H1: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: *C. punctata*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: *C. gachua*, Cb: *C. barca.*

Interspecies distance ranged from 0.021 to 0.177 and the intraspecies distance ranged from 0.002 to 0.004. The highest interspecies genetic distance (0.177) was between *C. marulius* and *C. barca* and the lowest genetic distance (0.021) was between *C. stewartii* and *C. aurantimaculata*.

All five sequences for each species were included in the phylogenetic analysis. The NJ and MP trees revealed identical phylogenetic relationship among the species (Fig. 2). Two major clusters were obtained with the first cluster formed by the

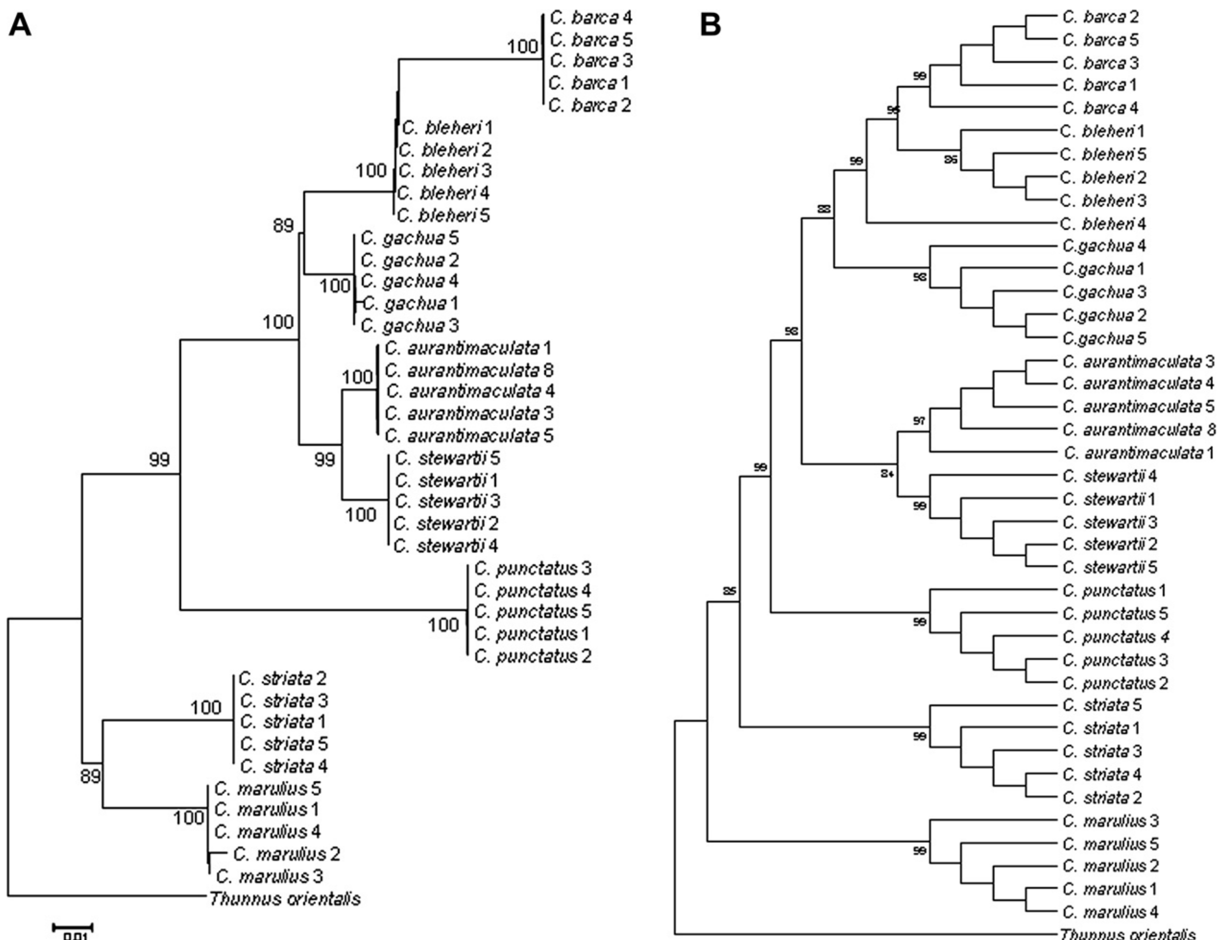


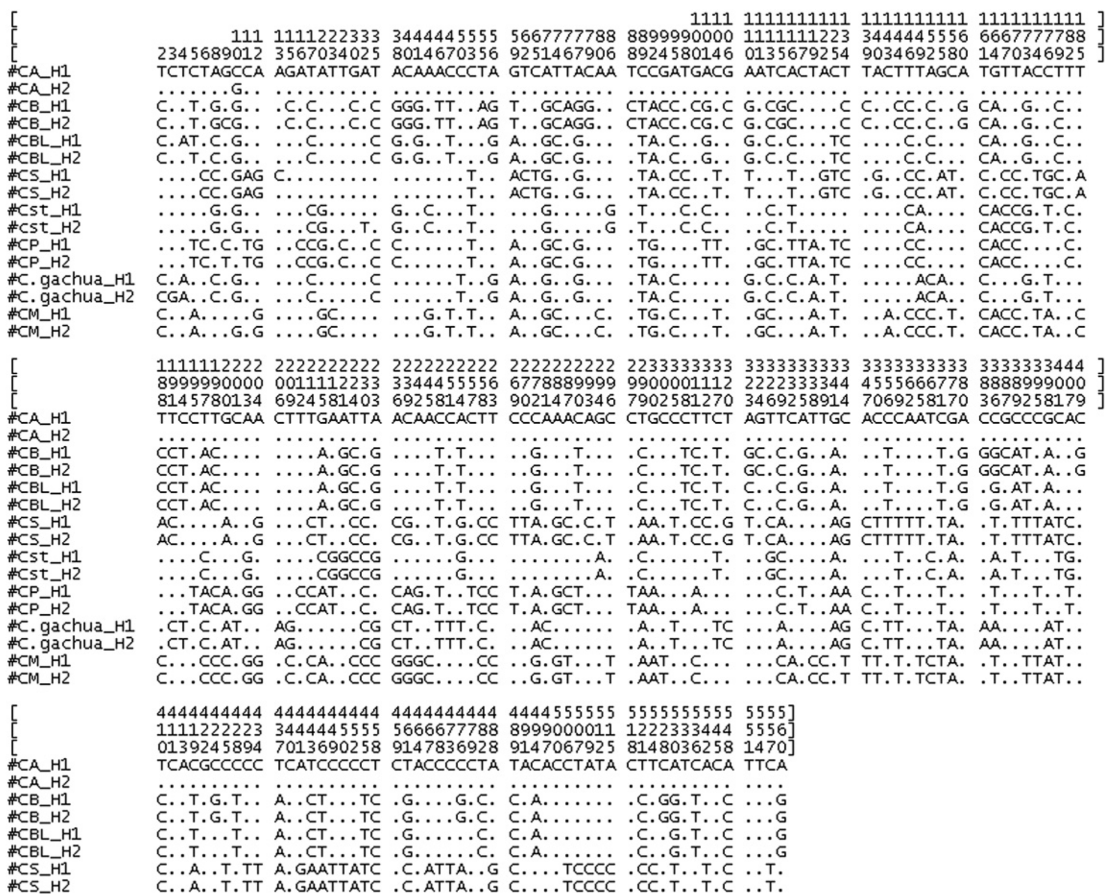
Fig. 2. A. NJ B. MP phylogenetic tree of Indian channids inferred from DNA sequences of mitochondrial gene 16S rRNA.

species *C. barca*, *C. bleheri*, *C. gachua*, *C. stewartii*, *C. aurantimaculata* with *C. punctatus* as a sub cluster. The second cluster was formed by *C. striata* and *C. marulius.* In both the trees, these clusters were supported by high bootstrap values (NJ 89–100%; MP 85–99%).

3.2. COI sequence analysis

Sequencing the COI gene produced an average of 561 nucleotide base pairs per taxon. No insertions, deletions or stop codons were observed in any sequence. Two haplotypes were observed in all the eight species. Of the 561 sites, "347, 214, 211 and 3 were conserved, variable, parsimony informative and singleton" respectively. The polymorphic sites are given in Fig. 3. As expected, all variable changes within species were third codon position transitional substitutions. The analysis revealed nucleotide frequencies as $A = 24.10\%$, $T = 28.60\%$, $G = 16.60\%$ and $C = 30.70\%$. As expected, average transitional pairs ($si = 61$) were more frequent than transversional pairs ($sv = 31$) with an average ratio of 1.96.

Average intergeneric and interspecies sequence divergence values are given in Table 3. Average sequence diversity among the Channid was 0.203, whereas average interspecies sequence diversity was 0.201. The highest interspecies sequence diversity (0.2460) was between *C. barca* and *C. striata and* the lowest value (0.0463) was between *C. bleheri* and *C. barca.* The average interspecies nucleotide differences ranged from 26 to 138 and intraspecies nucleotide difference ranged from 0 to 1 (Table 3). Pair-wise genetic distance values (K2P) based on COI sequences using MEGA 3.1 were given in Table 4. The average genetic distance between species was estimated as 0.203. The average distance within species was 0.002. The interspecies distance with COI ranged from 0.049 to 0.2460 and the intraspecies distance ranged from 0.002 to 0.004. The highest genetic distance (0.330) was between *C. striata* and *C. barca*, whereas the lowest interspecies distance (0.049) was found between *C. bleheri* and *C. barca*. The NJ and MP tree revealed identical phylogenetic relationship among the species (Fig. 4). Two major clusters were obtained with the first cluster formed by *C. barca*, *C. bleheri*, *C. gachua*, *C. stewartii*, and *C. aurantimaculata*. The second clade was formed by *C. striata* and *C. marulius* with *C. punctatus* as a sub cluster. As with 16S rRNA, the clusters were supported by high bootstrap values (NJ 85–100%; MP 90–98%).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#CA_H1         TCTCTAGCCA AGATATTGAT ACAAACCCTA GTCATTACAA TCCGATGACG AATCACTACT TACTTTAGCA TGTTACCTTT
#CA_H2         .......G.. .......... .......... .......... .......... .......... .......... ..........
#CB_H1         C..T.G.G.. .C.C...C.C GGG.TT..AG T..GCAGG.. CTACC.CG.C G.CGC....C C..CC.C..G CA..G..C..
#CB_H2         C..T.GCG.. .C.C...C.C GGG.TT..AG T..GCAGG.. CTACC.CG.C G.CGC....C C..CC.C..G CA..G..C..
#CBL_H1        C.AT.C.G.. ...C.....C G.G..T...G A..GC.G... .TA.C..G.. G.C.C...TC ....C.C... CA..G..C..
#CBL_H2        C..T.C.G.. ...C.....C G.G..T...G A..GC.G... .TA.C..G.. G.C.C...TC ....C.C... CA..G..C..
#CS_H1         ....CC.GAG C......... .......T.. ACTG..G... .TA.CC..T. T...T..GTC .G..CC.AT. C.CC.TGC.A
#CS_H2         ....CC.GAG .......... .......T.. ACTG..G... .TA.CC..T. T...T..GTC .G..CC.AT. C.CC.TGC.A
#Cst_H1        .....G.G.. ...CG..... G..C...T.. ...G.....G .T...C.C.. ..C.T..... ....CA.... CACCG.T.C.
#cst_H2        .....G.G.. ...CG...T. G..C...T.. ...G.....G .T...C.C.. ..C.T..... ....CA.... CACCG.T.C.
#CP_H1         ...TC.C.TG ..CCG.C..C C.......T.. A..GC.G... .TG....TT. .GC.TTA.TC ....CC.... CACC....C.
#CP_H2         ...TC.T.TG ..CCG.C..C C.......T.. A..GC.G... .TG....TT. .GC.TTA.TC ....CC.... CACC....C.
#C.gachua_H1   C.A..C.G.. ...C.....C ......T..G A..G..G... .TA.C..... G.C.C.A.T. .....ACA.. C...G.T...
#C.gachua_H2   CGA..C.G.. ...C.....C ......T..G A..G..G... .TA.C..... G.C.C.A.T. .....ACA.. C...G.T...
#CM_H1         C..A.....G ....GC.... ...G.T.T.. A..GC...C. .TG.C...T. .GC...A.T. ..A.CCC.T. CACC.TA..C
#CM_H2         C..A...G.G ....GC.... ...G.T.T.. A..GC...C. .TG.C...T. .GC...A.T. ..A.CCC.T. CACC.TA..C

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#CA_H1         TTCCTTGCAA CTTTGAATTA ACAACCACTT CCCAAACAGC CTGCCCTTCT AGTTCATTGC ACCCAATCGA CCGCCCGCAC
#CA_H2         .......... .......... .......... .......... .......... .......... .......... ..........
#CB_H1         CCT.AC.... ....A.GC.G ....T.T... ..G...T... .C...TC.T. GC.C.G..A. ..T....T.G GGCAT.A..G
#CB_H2         CCT.AC.... ....A.GC.G ....T.T... ..G...T... .C...TC.T. GC.C.G..A. ..T....T.G GGCAT.A..G
#CBL_H1        CCT.AC.... ....A.GC.G ....T.T... ..G...T... .C...TC.T. C..C.G..A. ..T....T.G .G.AT.A...
#CBL_H2        CCT.AC.... ....A.GC.G ....T.T... ..G...T... .C...TC.T. C..C.G..A. ..T....T.G .G.AT.A...
#CS_H1         AC....A..G ...CT..CC. CG..T.G.CC TTA.GC.C.T .AA.T.CC.G T.CA....AG CTTTTT.TA. .T.TTTATC.
#CS_H2         AC....A..G ...CT..CC. CG..T.G.CC TTA.GC.C.T .AA.T.CC.G T.CA....AG CTTTTT.TA. .T.TTTATC.
#Cst_H1        ....C...G. ....CGGCCG ......G... ........A. .C......T. ..GC....A. ...T..C.A. .A.T...TG.
#Cst_H2        ....C...G. ....CGGCCG ......G... ........A. .C......T. ..GC....A. ...T..C.A. .A.T...TG.
#CP_H1         ...TACA.GG ..CCAT..C. CAG.T..TCC T.A.GCT... TAA...A... ...C.T..AA C..T...T.. .T...T..T.
#CP_H2         ...TACA.GG ..CCAT..C. CAG.T..TCC T.A.GCT... TAA...A... ...C.T..AA C..T...T.. .T...T..T.
#C.gachua_H1   .CT.C.AT.. AG......CG CT..TTT.C. ..AC...... .A..T...TC ...A....AG C.TT...TA. AA....AT..
#C.gachua_H2   .CT.C.AT.. AG......CG CT..TTT.C. ..AC...... .A..T...TC ...A....AG C.TT...TA. AA....AT..
#CM_H1         C...CCC.GG .C.CA..CCC GGGC....CC ..G.GT...T .AAT..C... ...CA.CC.T TT.T.TCTA. .T..TTAT..
#CM_H2         C...CCC.GG .C.CA..CCC GGGC....CC ..G.GT...T .AAT..C... ...CA.CC.T TT.T.TCTA. .T..TTAT..

[              4444444444 4444444444 4444444444 4444555555 5555555555 5555]
[              1111222223 3444445555 5666677788 8999000011 1222333444 5556]
[              0139245894 7013690258 9147836928 9147067925 8148036258 1470]
#CA_H1         TCACGCCCCC TCATCCCCCT CTACCCCCTA TACACCTATA CTTCATCACA TTCA
#CA_H2         .......... .......... .......... .......... .......... ....
#CB_H1         C..T.G.T.. A..CT...TC .G....G.C. C.A....... .C.GG.T..C ...G
#CB_H2         C..T.G.T.. A..CT...TC .G....G.C. C.A....... .C.GG.T..C ...G
#CBL_H1        C..T...T.. A..CT...TC .G......C. C.A....... .C..G.T..C ...G
#CBL_H2        C..T...T.. A..CT...TC .G......C. C.A....... .C..G.T..C ...G
#CS_H1         C..A..T.TT A.GAATTATC .C.ATTA..G C....TCCCC .CC.T..T.C ..T.
#CS_H2         C..A..T.TT A.GAATTATC .C.ATTA..G C....TCCCC .CC.T..T.C ..T.
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Fig. 3. Alignment of partial DNA sequences of the mitochondrial gene, COI of eight Indian channids (only variable sites are reported) (H: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: *C. punctatus*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: *C. gachua*, Cb: *C. barca*).

Table 3
Pair-wise nucleotide differences (below diagonal) and sequence divergence (above diagonal) in COI sequences derived from eight Indian channids.

	CAH1	CAH2	CPH1	CPH2	CSH1	CSH2	CMH1	CMH2	CstH1	CstH2	CBLH1	CBLH2	CgH1	CgH2	CbH1	CbH2
CAH1		0.0018	0.1747	0.1747	0.2014	0.1996	0.1890	0.1907	0.1140	0.1159	0.1337	0.1319	0.1426	0.1444	0.1693	0.1711
CA-H2	1		0.1765	0.1765	0.1996	0.1978	0.1907	0.1889	0.1123	0.1140	0.1319	0.1301	0.1408	0.1426	0.1675	0.1693
CPH1	98	99		0.0017	0.1746	0.1729	0.1657	0.1675	0.1818	0.1836	0.1782	0.1765	0.1889	0.1907	0.2192	0.2174
CPH2	98	99	1		0.1747	0.1729	0.1658	0.1675	0.1818	0.1836	0.1782	0.1764	0.1889	0.1907	0.2192	0.2192
CSH1	113	112	98	98		0.0018	0.1747	0.1729	0.2068	0.2086	0.2032	0.2014	0.1889	0.1907	0.2442	0.2460
CSH2	112	111	97	97	1		0.1729	0.1711	0.2050	0.2068	0.2014	0.1996	0.1872	0.1889	0.2424	0.2442
CMH1	106	107	93	93	98	97		0.0018	0.1907	0.1925	0.1979	0.1961	0.2086	0.2103	0.2389	0.2406
CMH2	107	106	94	94	97	96	1		0.1889	0.1907	0.1969	0.1943	0.2068	0.2086	0.2371	0.2389
CstH1	64	63	102	102	116	115	107	106		0.0018	0.1497	0.1479	0.1426	0.1426	0.1360	0.1854
CstH2	65	64	103	103	117	116	108	107	1		0.1515	0.1497	0.1426	0.1444	0.1854	0.1872
CBLH1	75	74	100	100	114	113	111	110	84	85		0.0018	0.1266	0.1283	0.0481	0.0499
CBLH2	74	73	99	99	113	112	110	109	83	84	1		0.1283	0.1301	0.0463	0.0481
CgH1	80	79	106	106	106	105	117	116	80	80	71	72		0.0018	0.1693	0.1711
CgH2	81	80	107	107	107	106	118	117	80	81	72	73	1		0.0018	0.1729
CbH1	95	94	123	123	137	136	134	133	103	104	27	26	95	1		0.0018
CbH2	96	95	122	123	138	137	135	134	104	105	28	27	96	97	1	

H1: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: *C. punctata*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: *C. gachua*, Cb: *C. barca.*

Table 4
Pair-wise genetic distances (Kimura 2-parameter) of eight Indian channids based on COI sequences.

	CAH1	CAH2	CPH1	CPH2	CSH1	CSH2	CMH1	CMH2	CstH1	CstH2	CBLH1	CBLH2	CgH1	CgH2	CbH1	CbH2
CAH1																
CA-H2	0.002															
CPH1	0.210	0.213														
CPH2	0.210	0.213	0.002													
CSH1	0.248	0.245	0.212	0.212												
CSH2	0.245	0.243	0.209	0.209	0.002											
CMH1	0.231	0.234	0.192	0.192	0.215	0.212										
CMH2	0.234	0.231	0.195	0.195	0.212	0.209	0.002									
CstH1	0.132	0.130	0.226	0.226	0.266	0.263	0.241	0.238								
CstH2	0.135	0.132	0.229	0.229	0.269	0.266	0.244	0.241	0.002							
CBLH1	0.156	0.154	0.221	0.221	0.256	0.254	0.252	0.250	0.180	0.182						
CBLH2	0.154	0.151	0.218	0.218	0.254	0.251	0.250	0.247	0.177	0.180	0.002					
CgH1	0.166	0.164	0.235	0.235	0.228	0.226	0.263	0.260	0.166	0.169	0.146	0.149				
CgH2	0.169	0.166	0.238	0.238	0.231	0.228	0.266	0.263	0.169	0.171	0.149	0.151	0.002			
CbH1	0.207	0.205	0.288	0.288	0.326	0.323	0.323	0.320	0.230	0.233	0.051	0.049	0.207	0.210		
CbH2	0.210	0.207	0.285	0.287	0.330	0.326	0.326	0.323	0.233	0.236	0.053	0.051	0.210	0.213	0.002	

H1: Haplotype 1, H2: Haplotype 2, CA: *Channa aurantimaculata*, CP: *C. punctata*, CS: *C. striata*, CM: *C. marulius*, Cst: *C. stewartii*, CBL: *C. bleheri*, Cg: C. gachua, Cb: *C. barca.*

4. Discussion

Phylogenetic relationships based on morphological characters and molecules are mostly concordant (Bernardi and Crane, 2005; Ward et al., 2005). The eight species of *Channa* from the North-Eastern region of India were found genetically distinct from each other and partitioned into two groups based on the partial sequence information of both 16S rRNA and COI genes

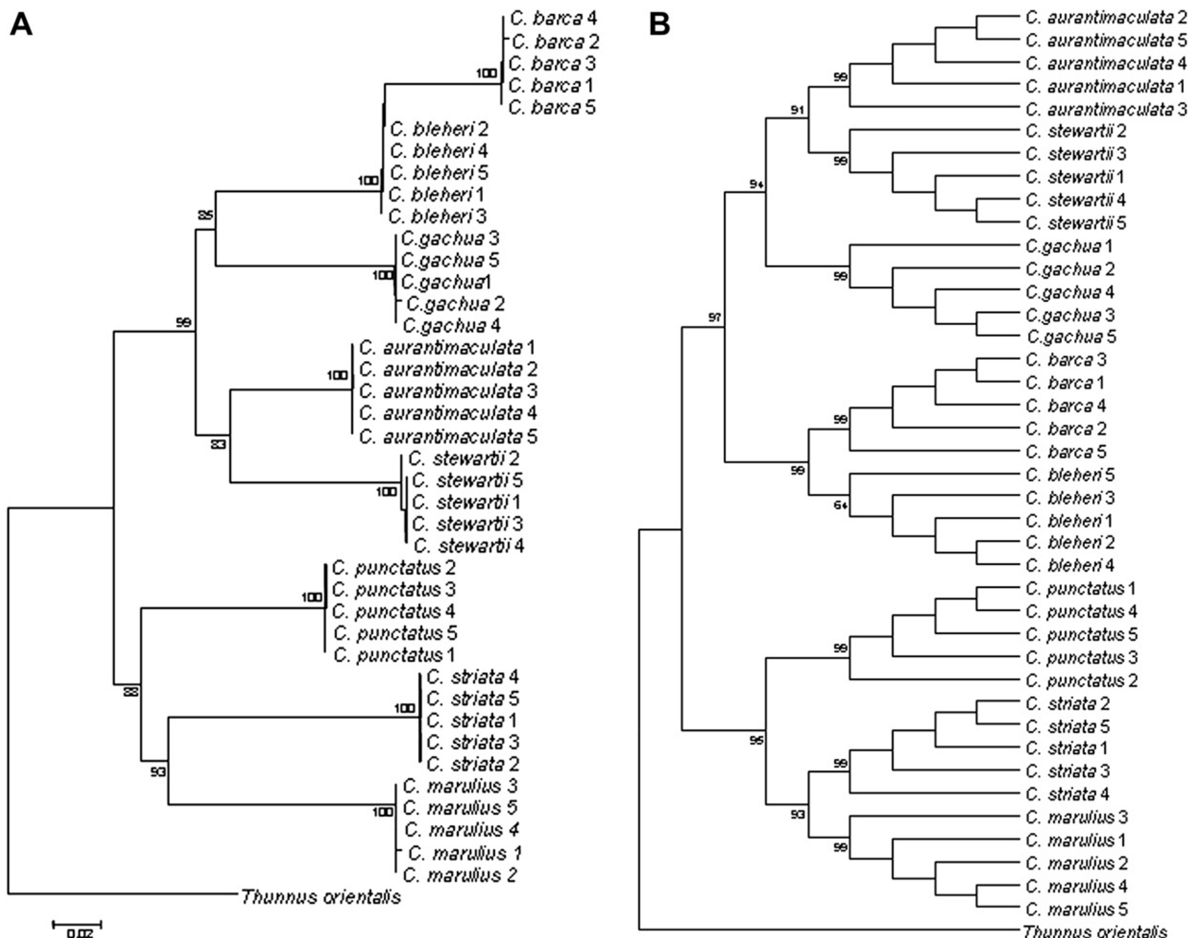

Fig. 4. A. NJ B. MP phylogenetic tree of Indian channids inferred from DNA sequences of mitochondrial gene COI.

without any haplotype sharing or overlapping. The genetic divisions of eight *Channa* species, identified by our data correspond with the taxonomic sub-divisions of Vishwanath and Geetakumari (2009). Estimates of genetic divergence with both 16S rRNA and COI genes were sufficient enough to discriminate individuals of different snakehead species. These values showed conformity with the previous reports in other teleosts for both 16S rRNA (5.88–19.5% in sciaenids; Lakra et al., 2009 and 7.15–21.68% in mullids; Apostolidis et al., 2001) and COI (1.04–35.72% in Australian fishes; Ward et al., 2005).

The observed transitions vs. transversion ratios in Channids are also comparable to those of many teleosts (Ward et al., 2005; Chakraborty et al., 2006a). Transitions outnumbered transversions in the present study in accordance with the previous reports on mtDNA in fish (Vinson et al., 2004). Generally, a much larger excess of transitions related to transversion is typically observed in teleost mtDNA (Ward et al., 2005). The GC content of 16S rRNA (48.50%) and COI (47.30%) region was on relatively high in all the *Channa* species. Ward et al. (2005) reported an overall higher GC content in fishes based on complete mtDNA genome ranging from 38.4 to 43.2% and with COI alone, 42.2–47.1%, which was mostly attributable to 3rd base variation. In our study also, the snakeheads exhibited more nucleotide changes at 3rd position, consistent with most mutations being synonymous.

Relatively high degree of K2P nucleotide divergence with 16S rRNA gene (interspecies; 2.24–11.55%) was observed among the *Channa* species indicating its ability to adequately describe inter-relationships of snakeheads. Vinson et al. (2004) reported high nucleotide divergence (8.3%) among the sciaenids in Northern Brazil using 16S rRNA gene sequences. Chakraborty et al. (2006a,b) also showed similar results in ribbon fishes and silver biddies, indicating the usefulness of this gene sequence for accurate identification of the species. DNA barcoding based on partial sequence information of COI gene has been widely used for species identification of fishes (Ward et al., 2005; Spies et al., 2006; Lakra et al., 2009). Our results revealed that COI gene is an effective marker for DNA barcoding of *Channa* species from India.

In conclusion, our results are congruent with the taxonomic divisions of channids, based on morphological and osteological characters reported by Vishwanath and Geetakumari (2009) and hence both the mitochondrial genes-16S rRNA and COI can be used as a robust molecular markers for species identification in Channids, thus providing a valuable extension to Linnaean system.

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