Mol Biol Rep (2011) 38:841–846
DOI 10.1007/s11033-010-0175-2

Evaluation of cytochrome b mtDNA sequences in genetic diversity studies of *Channa marulius* (Channidae: Perciformes)

Maria Habib · W. S. Lakra · Vindhya Mohindra · Praveen Khare · A. S. Barman · Akanksha Singh · Kuldeep K. Lal · Peyush Punia · Asif A. Khan

Received: 1 August 2009 / Accepted: 12 April 2010 / Published online: 5 May 2010

Abstract *Channa marulius* (Hamilton, 1822) is a commercially important freshwater fish and a potential candidate species for aquaculture. The present study evaluated partial Cytochrome b gene sequence of mtDNA for determining the genetic variation in wild populations of *C. marulius*. Genomic DNA extracted from *C. marulius* samples ($n = 23$) belonging to 3 distant rivers; Mahanadi, Teesta and Yamuna was analyzed. Sequencing of 307 bp Cytochrome b mtDNA fragment revealed the presence of 5 haplotypes with haplotype diversity value of 0.763 and nucleotide diversity value of 0.0128. Single population specific haplotype was observed in Mahanadi and Yamuna samples and 3 haplotypes in Teesta samples. The analysis of data demonstrated the suitability of partial Cytochrome b sequence in determining the genetic diversity in *C. marulius* population.

Keywords *Channa marulius* · Cytochrome b · mtDNA · Polymorphism · Genetic divergence

Introduction

Channa marulius is a commercially important fish of South East Asia valued as an esteemed table fish and also used for ornamental trade. *C. marulius* is a freshwater fish inhabiting reservoirs, lakes, swamps and large marshy water areas and has been reported even at an altitude of 475 m MSL [1]. The distribution of this fish is native to India, Bangladesh, China, Thailand and Cambodia, Sri Lanka and Pakistan. In India, it is reported from the rivers of Indo-Gangetic plains and also in many parts of Peninsular India. *C. marulius* is the fastest growing among murrels and largest of the family Channidae, reaching a length of 120–122 cm [2, 3]. The fish is considered as a potential aquaculture species in addition to an important capture fishery resource. The culture of this giant murrel has not been traditionally popular for its highly voracious feeding habits at tertiary level. *C. marulius* is considered to be a local migrant, and travels for a short distance for feeding purpose or for locating suitable breeding grounds in new water bodies to avoid stress conditions of existing habitat. Due to declining abundance of *C. marulius* in wild, the fish has been assessed at lower risk- near threatened (LRnt) status [4].

In addition to protein and nuclear DNA markers, different mtDNA gene sequences have been used to determine variation at interspecific and intraspecific levels in fishes. The fast rate of mtDNA evolution coupled with maternal inheritance have made mtDNA an extremely useful genetic system for studying gene flow, hybrid zones, population structure and other population related questions. Even conservative protein coding genes like Cytochrome b tend to show intraspecific variation mainly in 3rd position of codon which can be used to identify stocks. Variation in mtDNA Cytochrome b gene has been used for population studies in fishes across taxonomic orders such as, Clupeiformes [5]; Acipenceriformes [6, 7]; Squaliformes [8]; Salmoniformes [9, 10]; Anguilliformes [11]; Cypriniformes [12] Siluriformes [13] and Perciformes [14, 15].

M. Habib · W. S. Lakra (✉) · V. Mohindra · P. Khare · A. S. Barman · A. Singh · K. K. Lal · P. Punia
National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow, UP 226002, India
e-mail: lakraws@hotmail.com

A. A. Khan
Department of Zoology, Faculty of Life Science, Aligarh Muslim University, Aligarh, UP 202002, India

Overall, the research on Murrels (Family: Channidae), using molecular markers for phylogeny as well as population genetics has been limited. Cytochrome b region was successfully amplified in Murrels for the purpose of inferring Phylogenetic relationships [16]. Molecular phylogeny of family Channidae has been determined using NADH dehydrogenase subunit 1 and 2 of mitochondrial DNA [17]. Intra specific genetic variability in *Channa punctatus*, from a part of its native distribution in peninsular India was examined using allozyme and RAPD markers [18, 19]. Information on stock structure is required for conservation management of native populations of *C. marulius* [20]. The information of stock structure is vital for planning management and conservation of natural resources, besides it is useful in genetic improvement programs [21]. Identification of molecular markers that are suitable for determination of genetic variation and divergence is the primary requisite for such studies. The foremost necessity is to identify polymorphic molecular markers for determining genetic variation and divergence. The present study analyses partial sequences of mtDNA Cyto b gene in the samples of *C. marulius* collected from 3 distant rivers namely, Mahanadi, Teesta and Yamuna, to determine genetic variation and evaluate their potential in determining genetic differentiation in natural populations of *C. marulius*.

Materials and methods

Sample collection

Specimens of *C. marulius* were collected through commercial catches from rivers Teesta ($n = 11$) at Teesta Barrage (26°45′ 16.83″N; 88°36′ 02.83″E), Mahanadi ($n = 7$) at Cuttack (21°58′N; 86°07′E) and Yamuna ($n = 5$) at Yamuna Nagar (29°58′ 47.01″N; 76°54′ 47.40″E), belonging to different basins (Fig. 1). River Teesta is tributary of Brahmaputra River which later joins Ganga River System (as Jamuna in Bangladesh). River Yamuna is a tributary of Ganga River System. River Mahanadi is an independent river originating from central plateau in India and draining into Bay of Bengal. The blood was extracted from individual specimen at collection site through caudal puncture and fixed in 95% ethanol in ratio 1:5.

DNA preparation

Total Genomic DNA was extracted from blood samples using Phenol–Chloroform Method [22]. DNA was amplified using Universal Primers for MtDNA Cytochrome b region; L14841 and H15149 [23]. PCR conditions were: 94°C, 5 min: 30 cycles of 94°C for 30 s; 55°C for 1 min; 72°C for 1 min 30 s and final extension for 10 min at 72°C.

DNA sequencing

Double stranded PCR product was purified using elution method from low melting Agarose gel. The purified PCR Product was used in setting up sequencing reaction with same set of primers using Mega Bace ET Terminator Dye kit. The sequencing reaction was performed for 30 cycles of: 95°C for 10 s; 50°C for 20 s; 60°C for 2 min. PCR products were precipitated using ethanol and ammonium acetate and were dissolved in Mega Bace Loading Buffer. The DNA sequencing was carried out on an automated DNA sequencer, MegaBace 500 (GE Healthcare) using manufacturer's recommendations.

Analysis of DNA sequences

DNA sequences were aligned using ClustalW [24] and were analysed for determining parameters of population genetic variation. MEGA 4.1 [25] was used to estimate parameters of Genetic Variation. Sequence Composition and Molecular diversity indices, Genetic Differentiation and F*st* values were calculated using the software Arlequin 3.11 [26] and haplotype diversity was estimated using DnaSP 4.5 [27]. For comparison sequence of Cytochrome b gene of *C. marulius* available in Genbank (Accession no: AY763771) was treated as a separate population from unspecified location in analysis with the sequences from present study.

Results

Sequence composition

Out of total of 349 bp of Cytochrome b mitochondrial gene amplified, 307 bp fragment was analyzed to determine genetic variation. The average frequencies of four nucleotides for all the 23 samples of *C. marulius* are A: 25.49%; T: 28.16%; G: 15.79%; C: 30.55%. Nucleotide sequences of Cytochrome b in *C. marulius* were A + T rich (53.65%) and transition to transversion ratio was 3.1. Cytochrome b sequence data generated 5 haplotypes (Table 1); haplotype H01 and H05 were observed in Mahanadi and Yamuna respectively, whereas H02, H03 and H04 were seen in Teesta only. (Table 2; GenBank Accession no: GQ415663–GQ415667, GQ464027–28 for Mahanadi; GQ415673–GQ 415677, GQ464053–GQ464061 for Teesta; GQ415668–GQ415672 for Yamuna.) Total 23 variable sites were identified (Table 1) and 5 of them were parsimony informative.

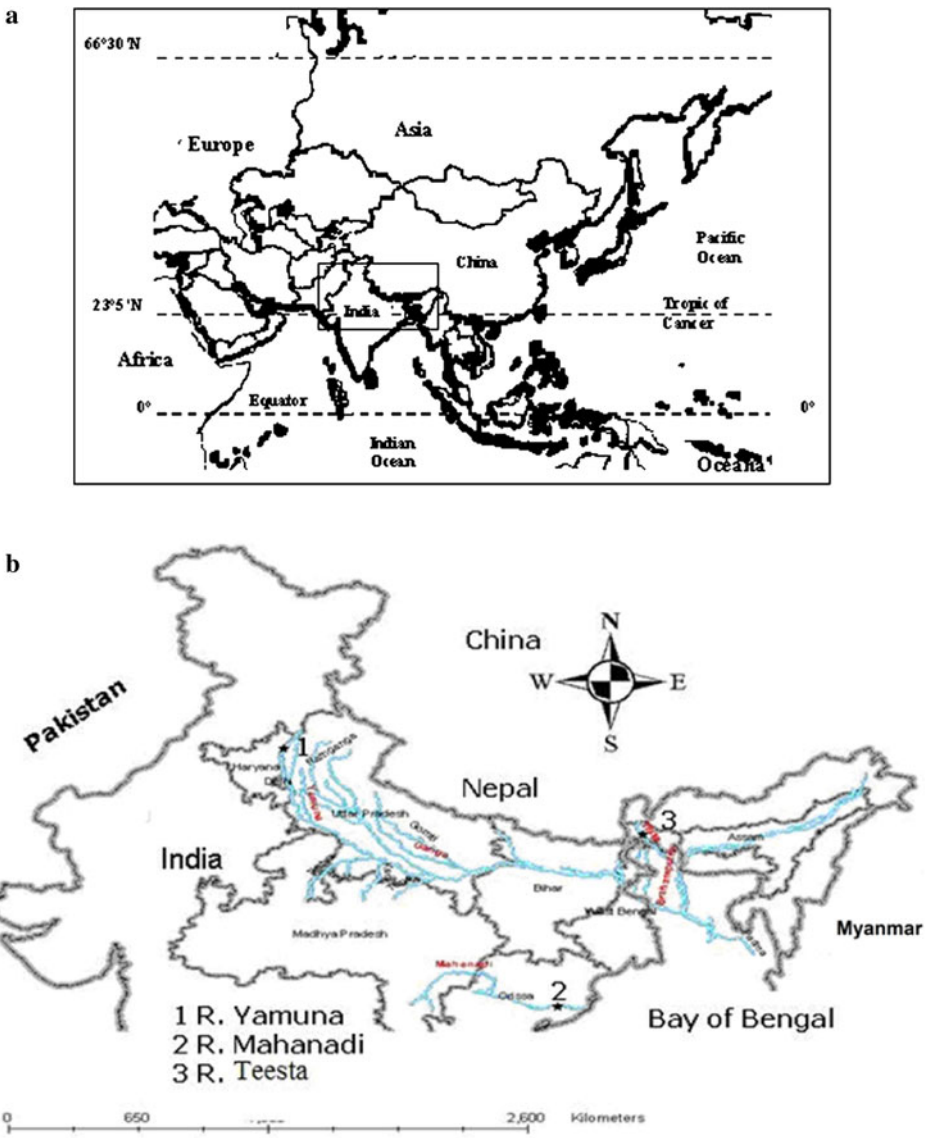

Fig. 1 **a** General map of the region, study area is located within the box. **b** Locations of sampling station (*) across different river basins for population structure study of *C. marulius*

Table 1 Different haplotypes and their consensus sequences detected in 3 populations of *C. marulius* (the *dots* refer to the identical positions to reference consensus sequence)

Position→	9	19	21	60	69	75	87	99	108	111	129	165	171	186	189	216	222	225	237	246	252	273	294	GenBank
Consensus sequence→	T	C	C	A	C	C	A	C	T	T	T	A	T	T	C	T	C	T	G	C	T	C	C	acc no ↓
H01	G	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	C	.	.	.	.	.	GQ415663
H02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	GQ415673
H03	.	.	A	G	T	T	T	T	C	C	C	C	C	C	.	C	T	.	A	T	C	A	T	GQ464061
H04	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	GQ415675
H05	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	GQ415668

Nucleotide and haplotype diversity

Mean no of polymorphic loci was estimated to be: 0.333 ± 0.577 and mean nucleotide diversity (pi) was 0.0128. Haplotype diversity (Hd) was found to be 0.763 and variance of Hd was 0.002 ± 0.046. Haplotype and nucleotide diversity for Mahanadi and Yamuna samples was 0.00 as both these rivers gave single haplotype

Table 2 No of haplotype detected in 3 populations of *C. marulius*

Haplotype	Mahanadi	Teesta	Yamuna
H01	7	0	0
H02	0	2	0
H03	0	8	0
H04	0	1	0
H05	0	0	5

whereas for Teesta samples haplotype diversity (Hd) was 0.473 with variance 0.02614 ± 0.162 and Nucleotide diversity (pi) was 0.01236.

Molecular diversity indices and genetic differentiation

Analysis of Molecular Variance (AMOVA) within 3 populations (Teesta, Yamuna and Mahanadi) revealed that out of total variation, only 10.11% was contributed due to variation within population, however 89.8% was attributed to differentiation among populations and population structuring revealed by high and significant F*st* value of 0.89 (Table 3). Population pair wise F*st* values (Table 4) ranged from 0.80 to 1.00 and Co-efficient of differentiation for all 3 populations was 0.680. Mean *P* distance over all the 3 populations were 0.013 and mean distance (d) within groups ranged from 0.000 to 0.012.

Sequences obtained in this study for *C. marulius* from 3 populations were compared with that of Accession no AY763771 of *C. marulius*. AMOVA analysis with all the 4 populations revealed among population variation of 90.59% and within population variation of 9.41% and a significant F*st* value of 0.905.

Discussion

Understanding of population genetic structure of a species provides critical information for developing conservation and management strategies for natural fish populations as well as fishes having threatened status. Results obtained upon analysis of 307 bp mtDNA Cytochrome b sequences in the present study, revealed high genetic differentiation in *C. marulius* collected from three different rivers. The Cytochrome b fragment amplified in the present study, using universal primer [28], has been reported to be useful in detecting intraspecific variation in several species as this region of mtDNA Cytochrome b is found to be polymorphic across orders. The order of most represented bases; C > T>A > G is the order in Cytochrome b of Percid fishes [29] and the level of G observed in *C. marulius* was in accordance with the consistent level in other Snakeheads [30]. Nucleotide Sequences of Cytochrome b in *C. marulius* were A + T rich (53.65%), which are similar to many other fishes (Johns and Avise 1998). The universal primers [28] used in the present study to amplify the region of 307 bp of Cytochrome b of mitochondrial genome, were found to be polymorphic in *C. marulius*. This region has been found to be polymorphic and has been used successfully for intraspecific genetic diversity analysis in various fish species, like *Salmo trutta* [31]; *Cyprinodon variegates* [32]; *Sardina pilchardus* [33] and *Lates calcarifer* [34].

An interesting finding in the study was that 2 (Mahanadi and Yamuna samples) out of 3 populations exhibited unique single haplotype each. AMOVA revealed low within population variation in *C. marulius* (10.11%) and high among population variation (89.8%). It is reported that a migratory fish species has 85 and 15% of its diversity within and between local populations, respectively and 67.6 and 32.4% for a non migratory fish [35]. The genetic divergence level between populations, observed in this study was even higher than that reported for a non migratory fish. Murrels are local migrants travelling only for a short distance for the purpose of feeding or for locating suitable breeding grounds or in search of new water to avoid stress conditions of existing ecosystem [36]. F*st* value also supported the presence of significant genetic difference between populations of Mahanadi, Teesta and

Table 4 Population pair wise F*st* values (below diagonal) and *P* values for pairwise F*st* (above diagonal) between 4 different populations of *C. marulius* from Indian sub continent

	Mahanadi	Teesta	Yamuna	NCBI
Mahanadi	0.000	0.000*	0.000*	0.000*
Teesta	0.91336	0.000	0.000*	0.000*
Yamuna	1.00000	0.80418	0.000	0.000*
NCBI	1.00000	0.87665	1.00000	0.000

* $P < 0.05$

Table 3 AMOVA analyses of cytochrome b sequences for 3 populations of *C. marulius*

Source of variation	df	Sum of squares	Variance components	% of variation	Fixation index	*P* value
Among populations	2 (3)	31.932 (38.400)	1.18276 Va (1.1930 Va)	89.89 (90.59)	F*st*: 0.89889 (0.9058)	0.00000 ± 0.00000
Within populations	41 (44)	5.455 (5.455)	0.13304 Vb (0.1239 Vb)	10.11 (9.41)		
Total	49 (47)	37.386 (43.85)	1.31580 (1.31699)			

AMOVA values obtained by inclusion of one NCBI sequence (accession no AY763771) are given in *brackets*

Yamuna rivers. Such high intraspecific diversity could be expected as the 3 rivers belong to different river basins and therefore, it is likely that populations investigated, could have evolved in isolation after fragmentation from common ancestors. It is hence evident that these 3 population samples belong to reproductively isolated populations. Inclusion of sequence from Genbank (Accession no: AY763771) also revealed more or less similar results. It confirmed that such a high level of genetic differentiation is likely to be the characteristic of species *C. marulius* possibly influenced by the restricted dispersion of the species. It was suggested that samples originating from a single parent can result in a single population specific haplotype as mtDNA is maternally inherited [37]. Keeping in view that murrels exhibit parental care where their young ones move along with the maternal parent in shoals and so remain localized for long time. Hence, smaller sized specimens have more possibility to be the progeny of single or limited number of parents. Therefore, care was taken to obtain samples from large sized specimen (more than 1.5 kg) in the present study. At the same time, it is accepted that more mtDNA variation, existing in Mahanadi and Yamuna rivers, in low frequency as observed in Teesta samples, however, could have been under estimated due to limited sample size available.

Results obtained demonstrated that partial mtDNA Cytochrome b fragment (307 bp) is observed to be a potential marker for studying variation both within as well as among populations in *C. marulius*. Our results clearly demonstrated the differences occurring within the same population as in case of Teesta (3 haplotypes) as well as in between the populations like Mahanadi and Yamuna. Such studies have provided useful information in case of many other fish species. The success of conservation programs and effective management policies depend on the levels of genetic divergence within and between species and developing strategies to maintain the natural genetic diversity [38]. In a number of reports, mtDNA has proved useful for resolving population groupings in cases where morphological analyses were either inadequate or controversial [39] and therefore assisted in determining priorities for conservation. In case of *Sardina pilchardus* [33], mtDNA Cytochrome b sequence analyses, indicated that no strong hydrographical or environmental factors acted as sub structuring force in the Adriatic population of the fish and so from a fishery management perspective, the conclusions gave precious demographic information to define the pattern of exploitation of this fish species. The Cytochrome b fragment will be a promising marker to determine distribution and pattern of genetic variation across wide native distribution of *C. marulius*. Information on genetic stocks developed in the process will be useful to plan stock specific strategies for conservation and management of wild populations of *C. marulius*. Nevertheless, the utility of mtDNA Cytochrome b fragment in determining genetic divergence of wild population of *C. marulius* is proved beyond ambiguity.

Acknowledgments The authors thank Sh. A.K. Pathak (Scientist and OIC ARIS Cell, NBFGR) for providing the locality map. Excellent technical cooperation from Sh. Akhilesh Mishra, Sh. Rajesh Kumar and Sh. R.S Sah is duly acknowledged.

References

1. Munro ISR (1955) The marine and freshwater fishes of Ceylon. Dept. Ext. Affairs, Canberra, 348 pp, 56 pls
2. Bardach JE, Ryther JH, McLarney W (1972) Aquaculture: the farming and husbandry of freshwater and marine organisms. Wiley, New York, 867p
3. Talwar PK, Jhingran AG (1992) Inland fishes of India. Rec Ind J 3:19–24
4. CAMP (1998) Report of the workshop conservation assessment and management plan (CAMP) for freshwater fishes of India. Organized by Zoo Outreach Activity and National Bureau of Fish Genetic Resources, Lucknow
5. Lecomte F, Grant WS, Dodson JJ, Rodriguez-Sanchez R, Bowen BW (2004) Living with uncertainty: genetic imprints of climate shifts in east Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Mol Ecol 13:2169–2182
6. Fontana F, Conterio F, Gandolfi G, Tagliavini J, Rosenthal H, Bronzi P, McKenzie DJ (2007) Mitochondrial DNA sequences of 6 sturgeon species and phylogenetic relationships within Acipenseridae. J Appl Ichthyol 15(4–5):17–22
7. Pages M, Desse-Berset N, Tongard C, Brosse L, Hänni C, Berrebi P (2009) Historical presence of the sturgeon *Acipenser sturio* in the Rhone Basin determined by the analysis of ancient DNA cytochrome b sequences. Conserv Genet 10:217–224
8. Murray WB, Wang JY, Yang SC, Stevens JD, Fisk A, Svavarsson J (2008) Mitochondrial cytochrome b variation in sleeper sharks (Squaliformes: Somniosidae). Mar Biol 153:1015–1022
9. Oleinik AG, Skurikhina LA, Brykov VA (2007) Divergence of Salvelinus sp from north eastern Asia based on mitochondrial DNA. Ecol Freshw 16:87–98
10. Bouza C, Vilas R, Castro J (2008) Mitochondrial haplotype variability of brown trout populations from north-western Iberian Peninsula, a secondary contact area between lineages. Conserv Genet 9:917–920
11. Daemen E, Cross T, Ollevier F, Volckaert FAM (2001) Analysis of genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. Mar Biol 139:755–764
12. Fayazi J, Moradi M, Rahimi G, Ashtyani R, Galledari H (2006) Genetic differentiation and phylogenetic relationships among *Barbus xanthopterus* (Cyprinidae) populations in south west of Iran using mitochondrial DNA markers. Pak J Biol Sci 9(12):2249–2254
13. So N, VanHoudt JKJ, Volckaert FAM (2006) Genetic diversity and population history of the migratory catfishes *Pangasianodon hypophthalmus* and *Pangasius bocourti* in the Cambodian Mekong River. Fish Sci 72:469–476
14. Hsu KC, Shih NT, Ni HI, Tsao Shao K (2007) Genetic variation in *Trichiurus lepturus* (Perciformes: Trichiuridae) in water of Taiwan: several species or cohort distribution? Raffles Bull Zool 14:215–220
15. Brown J, Stapien CA (2008) Ancient divisions, recent expansions: phylogeography and population genetics of the round Gobi *Appolonia melanostoma*. Mol Ecol 17:2598–2615

16. Abol-Munafi AB, Ambok MA, Ismail P, MinhTam B (2007) Molecular data from the cytochrome b for the phylogeny of Channidae (Channa sp) in Malaysia. Biotechnology 6(1):22–27
17. Li X, Musikasinthorn P, Kumazawa Y (2006) Molecular phylogenetic analysis of snakeheads (Perciformes: Channidae) using mitochondrial DNA sequences. Ichthyol Res 53:148–159
18. Haniffa MA, Nagarajan M, Gopalakrishnan A, Basheer VS, Muneer A (2006) Genetic variability of *Channa punctatus* populations using randomly amplified polymorphic DNA. Aquac Res 37:1151–1155
19. Hanniffa MA, Nagarajan M, Gopalakrishnan A, Musammilu KK (2007) Allozyme variation in threatened freshwater fish spotted Murrel (*Channa punctatus*) in south Indian river system. Biochem Genet 45(3/4):363–373
20. Ponniah AG, Sarkar UK (2000) Fish biodiversity of north east India. NBFGR-NATP Publ 2:24–25
21. Abdul Muneer PM, Gopalakrishnan A, Musammilu KK, Mohindra V, Lal KK, Basheer VS, Lakra WS (2009) Genetic variation and population structure of endemic yellow catfish, *Horabagrus brachysoma* (Bagridae) among three populations of Western Ghat region using RAPD and microsatellite markers. Mol Biol Rep 36:1779–1791
22. Ruzzante DE, Taggart CT, Cook D (1996) Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Can J Fish Aquat Sci 53:2695–2705
23. Kocher TD, Thomas WK, Meyer A, Edwards SV, Pabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–6200
24. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research advance access. Nucleic Acids Res 22:4673–4680
25. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
26. Excoffier LG, Schneider LS (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinformatics Online 1:47–50
27. Rozas J, Sánchez-Delbarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497
28. Kocher TD, Conroy JA, McKaye KR, Stauffer JR (1993) Similar morphologies of cichlid fish in Lakes Tanganyika and evolution, edited by M. H. A. Keenleyside. Chapman and Hall, Malawi are due to convergence. Mol Phylogenet Evol 2:158–165
29. Song CB, Near TJ, Page LM (1998) Phylogenetic relationships among Percid fishes as inferred from mitochondrial cytochrome b DNA sequence data. Mol Phylogenet Evol 10:343–353
30. Abol-Munafi AB, Ambok MA, Ismail P, MinhTam B (2007) Molecular data from the cytochrome b for the phylogeny of Channidae (Channa sp) in Malaysia. Biotechnology 6(1):22–27
31. Apostolidis AP, Triantaphyllidis C, Kouvatsi A, Economidis PS (1997) Mitochondrial DNA sequence variation and phylogeography among *Salmo trutta* L (Greek brown trout) populations. Mol Ecol 6(6):531–542
32. Finne KL (2001) Phylogeographic structure of the Atlantic pupfish, *Cyprinodon variegatus* (Cyprinodontidae), along the eastern coast of North America. Unpublished M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia
33. Tinti F, di Nunno C, Guarniero I, Talenti M, Tommasini S, Fabbri E, Piccinetti C (2002) Mitochondrial DNA sequence variation suggests the lack of genetic heterogeneity in the adriatic and ionian stocks of *Sardina pilchardus*. Mar Biotechnol 4:163–172
34. Marshall CRE (2005) Evolutionary genetics of barramundi (*Lates calcarifer*) in the Australian region. Unpublished Ph.D Thesis, School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia
35. Vrijenhoek RC (1998) Conservation genetics of freshwater fish. J Fish Biol 53(Suppl A):394–412
36. Chondar SL (1999) Biology of fin fishes and shellfishes. SCSC Publishers, Howrah, India
37. Avise JC (1994) Molecular markers, natural history, and evolution. Chapman and Hall, New York
38. Lakra WS, Goswami M, Gopalakrishnan A (2009) Molecular identification and phylogenetic relationships of seven Indian Sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome c oxidase subunit I mitochondrial genes. Mol Biol Rep 36:831–839
39. Bowen BW, Meylan AB, Perran R (1992) Global population structure and natural history of Green Turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881