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Low genetic diversity and absence of population differentiation of hilsa (*Tenualosa ilisha*) revealed by mitochondrial DNA cytochrome *b* region in Ganga and Hooghly rivers

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We investigated the mtDNA cytochrome *b* based genetic structure of anadromous clupeid hilsa, *Tenualosa ilisha,* from the rivers Ganga and Hooghly. Six different haplotypes were observed, in sample size of 240, with a single dominant haplotype present in both rivers. Analysis of molecular variance (AMOVA) of Ganga and Hooghly populations does not suggest existence of population structuring in hilsa. AMOVA conducted on the whole population from Ganga and Hooghly suggested existence of a single population, migrating to Ganga and Hooghly rivers through the estuaries for spawning and breeding.

Key words: Hilsa, *Tenualosa ilisha*, Ganga, Hooghly, Bay of Bengal, West Bengal, India, mtDNA cytochrome *b*.

INTRODUCTION

The tropical shad, hilsa, *Tenualosa ilisha* (Hamilton, 1822), is a commercially important fish in West Bengal, India. Hilsa is abundantly available in Ganga and Hooghly estuary and constitutes a major seasonal fishery (De, 2001). The largest hilsa catch comes from Ganges delta and upper Bay of Bengal region with Bangladesh taking the largest share (>100000 ton/year), followed by India (25000 ton/year) and Burma (5000 ton/year), which toge-

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ther constitutes a fishery of more than 200000 tonnes in Bay of Bengal region (FAO, 1997). Large numbers of people are dependent on the hilsa fishery throughout the Indian subcontinent (especially in West Bengal, India) in the form of subsistence fishery, artisanal and commercial fishery. Many researchers have investigated differences in hilsa races. Day (1873) mentioned two classes of hilsa, Mojumdar (1939) indicated three types of hilsa, Pillay (1952, 1954) concluded that there were three stocks of hilsa distinguishable by relative size of body. Pillay et al. (1963) concluded based on non meristic characters that Chilka lake on East coast of India and Saurashtra coast on the West have their own stocks of hilsa with very little intermingling among them, Ganga with two populations and Hooghly hilsa different from hilsa stocks of Ganga, Padma, Chilka, Godavari and Saurashtra coast. Ghosh et al. (1968) distinguished three forms of hilsa based on the height of body namely; slender, broad and broader distributed in the entire stretch from Allahabad on Ganga to Lalgola on Padma in downstream.

Tagging experiments by Pillay et al. (1963) concluded that among populations of Hooghly, Padma and Ganga, there is little or no movement between rivers and little intermingling of three populations. Thus, aim of the study was to analyze population structure of commercially important tropical anadromous fish *T. ilisha* from Ganga and Hooghly using mitochondrial DNA cytochrome *b* gene sequence polymorphism.

MATERIALS AND METHODS

T. ilisha sampling g was conducted at five different locations on Ganga and Hooghly rivers during the 2006 monsoon (Figure 1). Samples were collected along the Hooghly freshwater stretch at Nawabganj, Feeder canal at Farakka on Bhagirathi-Hooghly, and upstream Ganga at Allahabad, downstream Ganga at Beniagram and Lalgola. The two sample sites at Feeder canal and Beniagram, though close distance wise represent two different flows of the Ganga river, one entering river Hooghly to feed the Kolkata port and the other down the Farakka barrage entering Bangladesh after flowing in India for a few kilometers representing two different migratory paths of hilsa (Table 1). Liver tissues were preserved in 95% ethanol and genomic DNA was extracted following Sambrook and Russel (2001). The mitochondrial DNA (mtDNA) cytochrome *b* region of *T. ilisha* was polymerase chain reaction (PCR) amplified using a set of forward and reverse primers (Kocher et al., 1989), L14841 (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'), H15149 (5'-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'). The PCR mixture contained 1.5 mM $MgCl_2$, 0.2 µM of each dNTP, 100 ng of template DNA, 2.0 unit of Taq polymerase (Finnzyme) and 5 µl of PCR buffer (1x is 10 mM Tris-HCl, pH 8.8 at 25°C, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100). Reaction volume was made 20 µl by adding sterile double distilled water. PCR was programmed for one cycle at 94°C for 4 min, 35 cycles at 94°C for 1 min, annealing at 50°C for 1 min, and extension reaction was carried out at 72°C for 2 min. A final extension was performed after the 35^{th} cycle at 72°C for 10 min.

PCR amplified product was visualized in 1.6% agarose gel. The DNA sequencing of hilsa mitochondrial DNA cytochrome *b* gene fragment was performed using forward primer L14841. DNA sequencing was performed on the ABI-3100 DNA sequencing machine. The mt cytochrome *b* DNA sequences were confirmed by BLAST searching with GenBank and aligning using CLUSTALW2 with *Tenualosa macrura* mtDNA cytochrome *b* sequence (Thompson et al., 1997). Haplotype diversity (h), nucleotide diversity within populations (π), inter-population nucleotide divergences (d_{xy}), intra-population divergences (d_x or d_y) and mean net inter-population nucleotide divergences (d_A) were quantified by Arlequin 3.01 software (Excoffier et al., 2006). Population genetic structure analysis was conducted between the two rivers and considering both rivers as single unit. The gene flow (N_{em}) values between the two rivers was analyzed for haploid data using formula (N_{em}) = (1 - F_{st}/2F_{st} for haploid). Unweighted pair group method with arithmetic mean (UPGMA) was used to construct the topology using MEGA3 (Kumar et al., 2004) software (Figure 2). *T. macrura* was used as out-group (accession no. AY390588).

RESULTS AND DISCUSSION

The cytochrome *b* gene primers amplified a PCR fragment of 360 bp. Out of total 360 bp, 300 bp region of the sequence was used to analyze the population structure of hilsa. In total, five polymorphic sites were detected which constituted 1.66% of 300 bp region of cytochrome *b* gene. All variations occurred at third codon position and were silent and did not result in amino acid substitution. The polymorphic sites gave a total of six observed haplotypes in the sample size of 240. All haplotype sequences were deposited in GenBank under accession numbers FJ179450-FJ179455. Haplotype A (97.1%) was most commonly observed in Ganga and Hooghly popula-tions. The second most common haplotype was B (1.3%), and other four haplotypes (C, D, E and F) occurred in only one individual in four different populations (0.04%). Haplotype A was observed at all sites: Beniagram, Lalgola, Allahabad on river Ganga and at Nawabganj on river Hooghly and at Feeder canal. Haplotype B was observed in Beniagram and Nawabganj. Haplotypes C and D were observed in river Ganga at Benia-gram and haplotype E and F were observed in river Hooghly at Feeder canal (Table 2). Genetic variation in river Ganga and Hooghly measured as haplotype diversity was 0.0641 ± 0.0306 and 0.0508 ± 0.0282, respectively. Total haplotype diversity for Ganga and Hooghly was 0.0575 ± 0.0209.

Nucleotide diversity in Ganga and Hooghly was observed to be 0.0002 ± 0.0005 and 0.0002 ± 0.0004, respectively and the total nucleotide diversity for both populations was 0.0002 ± 0.0005. Haplotype diversity and nucleotide diversity for both population was low. Total number of nucleotide transitions in mtDNA cyt *b* gene region was five with three transitions in each population. The nucleotide composition for Ganga population was C (27%), T (29.33%), A (25%), G (18.67%), and for Hooghly population it was C (27%), T (29.33%), A (24.99%) and G (18.68%). The overall nucleotide composition was C (27%), T (29.33%), A (24.99%) and G (18.67%).

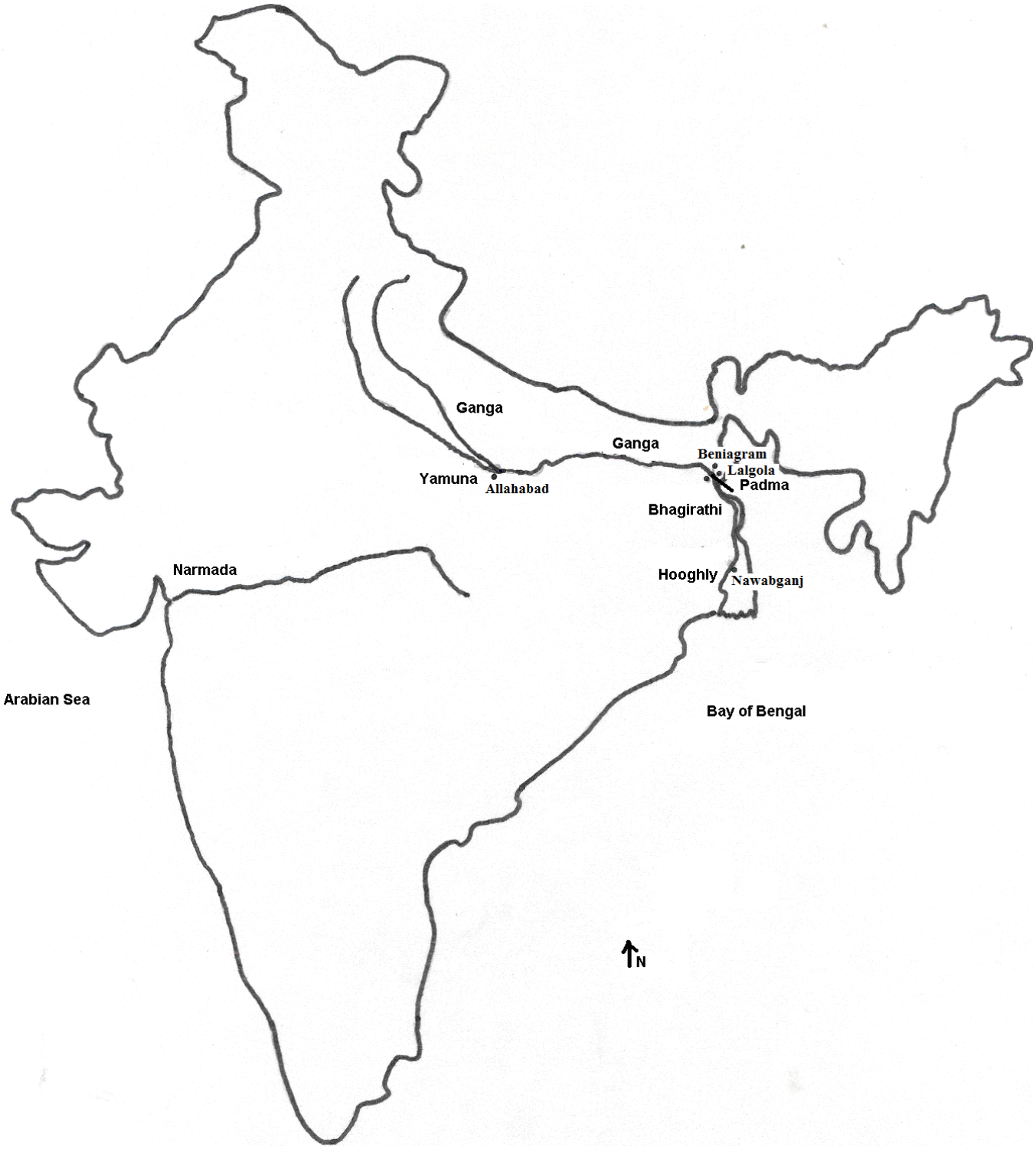

Figure 1. Map of India representing sampling sites (not to scale).

Inter population nucleotide divergences (d_{xy}) was low, average 0.0664, intra population divergences (d_x or d_y) were 0.0650 and 0.0684 for Ganga and Hooghly populations, respectively with average 0.0667. Mean net inter population nucleotide divergences (d_A) was negative (-0.0002) indicating no net divergences among populations (Table 3). F_{st} values within Ganga were -0.0039 and within Hooghly were -0.0043 (Table 4).

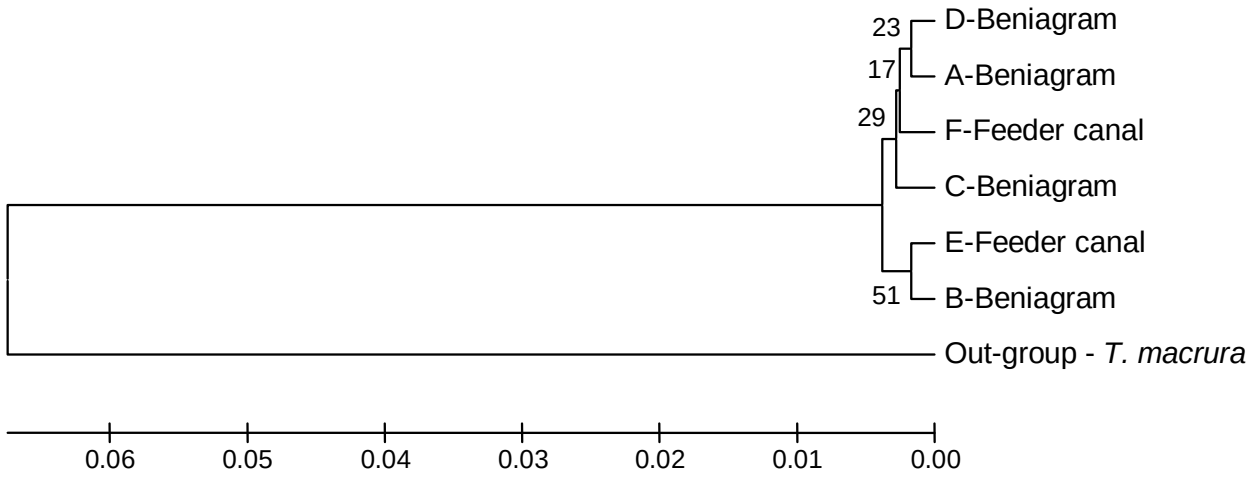

Figure 2. The UPGMA tree (Kimura-2-parameter) constructed using MEGA3.

Table 1. Sampling locations of *Tenualosa ilisha*.

River	Sampling site	Geographical Co-ordinate	Number of samples (*N*)
Ganga	Beniagram (West Bengal)	24°47’58.60’’N, 87°55’22.32’’E	80
Ganga	Lalgola (West Bengal)	24°26’45.83’’N, 88°12’04.26’’E	33
Ganga	Allahabad (Uttar Pradesh)	25°24'52.75"N, 81°53'29.25"E	10
Hooghly	Nawabganj (West Bengal)	22°47’33.30’’N, 88°21’06.71’’E	31
Bhagirathi-Hooghly	Feeder Canal (West Bengal)	24°48’00.86’’N, 87°55’04.21’’E	86
Total sample size			*N* = 240

Table 2. The haplotypes and haplotype frequencies of cytochrome *b* gene fragment of *Tenualosa ilisha* from rivers Ganga, Hooghly.

Haplotype (GenBank acc. no.)	Relative frequencies of haplotype	Sample size (*N*) each haplotype	Nucleotide Variable site				
			0	1	1	2	2
			8	2	4	3	6
			1	9	4	4	1
A (FJ179450)	0.971 ± 0.007	233	A	T	A	A	G
B (FJ179451)	0.013 ± 0.011	3	A	T	A	G	G
C (FJ179452)	0.004 ± 0.004	1	A	T	A	G	A
D (FJ179453)	0.004 ± 0.004	1	A	C	A	G	A
E (FJ179454)	0.004 ± 0.004	1	G	C	A	G	A
F (FJ179455)	0.004 ± 0.004	1	A	T	G	A	G

Table 3. Estimates of interpopulation d_{xy} (upper triangular), intrapopulation d_x or d_y (on diagonal and italics), and net nucleotide divergence d_A (below triangle) of cytochrome *b* fragment among Ganga and Hooghly samples of *Tenualosa ilisha*.

Population	Ganga	Hooghly
Ganga	0.0650	0.0664
Hooghly	-0.0002	0.0684

Interpopulation F_{st} between Ganga and Hooghly was -0.0041 (P = 0.8035 ± 0.0132) (Table 5). The gene flow (N_{em}) values between Ganga and Hooghly was infinite (Table 6) suggesting free flow of gene pool and mixing of hilsa between Ganga and Hooghly. Analysis of molecular variance (AMOVA) of Ganga and Hooghly populations (Table 7) yielded among group F_{ct} = 0.005 (P > 0.05), among population within groups F_{sc} = - 0.0166 (P > 0.05) and within population F_{st} = -0.0115 (P > 0.05). This river

Table 4. Population specific F_{st} values for Ganga and Hooghly samples of *Tenualosa ilisha*.

Population	F_{st}
Ganga	-0.0039
Hooghly	-0.0044

Table 5. Pairwise F_{st} values from cytochrome *b* gene fragment among populations of *Tenualosa ilisha* from Ganga, Hooghly below diagonal and P value above diagonal.

Population pairwise F_{st} value	Ganga	Hooghly
Ganga	0.000	0.8095 ± 0.0115
Hooghly	-0.0041	0.000

Table 6. N_{em} values from cytochrome *b* gene fragment among populations of *Tenualosa ilisha* from Ganga, Hooghly.

N_{em} values (N_{em} = (1-F_{st}/2F_{st} for haploid)	Ganga	Hooghly
Ganga	-	
Hooghly	Infinity	-

Table 7. Analysis of molecular variance of cytochrome *b* gene fragment of *Tenualosa ilisha* from 1) Ganga, at Lalgola, Beniagram and Allahabad, 2) Hooghly, at Nawabganj and Feeder canal.

Parameter	D.f	Variance	%total	Φ statistics	*P*- value
Among area	1	0.00017	0.50	0.0050	0.596 ± 0.004
Among populations within areas	3	- 0.0005	-1.65	-0.0166	0.920 ± 0.002
Within populations	235	0.0335	101.15	-0.0115	0.934 ± 0.002

Table 8. Analysis of molecular variance of cytochrome *b* gene fragment of *Tenualosa ilisha* from Ganga and Hooghly.

Parameter	D.f	Variance	%Total	Φ statistics	*P*- value
Among populations	1	- 0.0001	-0.41	-0.0041	0.803 ± 0.013
Within populations	239	0.0333	100.41		

based grouping of hilsa samples does not suggest existence of population structuring in hilsa. AMOVA conducted on the whole population from Ganga and Hooghly (Table 8) as a single group revealed no differentiation between Ganga and Hooghly populations (F_{st} = - 0.0041, P > 0.05). Among Ganga and Hooghly rivres, population variation was not detected as indicted by their F_{st} values (Ganga F_{st} = -0.0039, Hooghly F_{st} = -0.0043). The UPGMA tree constructed using Kimura-2-parameter generated two clusters. Cluster I had haplotypes A, C, D and F and the cluster II had haplotypes B and E. The bootstrap support for the clustering was low suggesting non differentiation of population of hilsa in Ganga and Hooghly.

The haplotype A dominated in Ganga and Hooghly observed throughout the migratory route of hilsa in Ganga at Lalgola, Beniagram and also upstream Farakka barrage at the Ganga-Yamuna confluence at Allahabad. In Hooghly, it was observed at Nawabganj and Feeder canal, along the hilsa migratory route in Hooghly. The haplotype B, although available in both rivers constituted a small population in the middle zone of Hooghly estuary at Nawabganj and at Beniagram on Ganga. Haplotypes C, D and E, F was exclusively observed in Ganga and

Hooghly, respectively and represent private or rare haplotypes. During the spawning migration and breeding season, hilsa congregate in large numbers at Beniagram on Ganga river system and at Feeder canal on Hooghly-Bhagirathi river system due to the physical obstruction at Farakka Barrage; hence, private haplotypes though very few numerically, were observed. Dominance of a single haplotype in the estuaries and low values of total haplotypic diversity (0.0575 ± 0.0209), overall average nucleotide diversity (0.002 ± 0.0005), low inter population nucleotide divergence (d_{xy}), intra population nucleotide divergence (d_x or d_y) in the range of 0.0664 to 0.0667 and negative mean nucleotide divergence values among Ganga and Hooghly populations strongly suggest that hilsa populations in Ganga and Hooghly constitute a single reproductive unit.

The existence of separate races was first suggested by Jenkins (1940). Pillay (1952, 1954, 1957) and Pillay et al. (1963) studied hilsa stock in details. Preliminary biometric studies indicated presence of three stocks of hilsa, distinguishable by the relative height of body. Rahman and Naevdal (2000) using allozymes concluded that the Cox's Bazaar (marine) differed significantly from Barguna and Chandpur regions, while Barguna and Chandpur (inland populations) did not differ significantly from each other. Within the Bay of Bengal, hilsa from Southern India and Myanmar could not be genetically separated from fish collected in Bangladesh (Milton and Chenery, 2001). Lall et al. (2004) concluded homogeneity of hilsa population from Ganga and Hooghly with high gene flow between hilsa populations ascending different rivers for spawning and breeding purpose. Salini et al. (2004), with allozymes and morphometric analysis concluded that Bangladesh, India, Myanmar hilsa were not significantly different from each other and suggested that Bay of Bengal comprised a single population.

The populations of hilsa from rivers Ganga, Yamuna, Hooghly and Narmada were analyzed using random amplification of polymorphic DNA (RAPD) (Brahmane et al., 2006) which concluded that populations from Allahabad, Beniagram and Lalgola from Yamuna and Ganga formed one cluster and the other cluster formed was of populations from Feeder canal, Nawabganj on rivers Bhagirathi and Hooghly and Bhadbhud from river Narmada. Mazumder et al. (2009), using PCR-RFLP of hilsa mitochondrial DNA D-loop region concluded high level of haplotypic and genetic diversity within and significant differentiation among hilsa populations, and also between three ecotypes, river, estuary and marine. Many anadromous and marine fishery population structures resemble hilsa population structure deduced in the present study. Chub mackerel (*Scomber japonicus*) in the Mediterranean Sea shows high gene flow and low degree of differentiation because of high fecundity and voracious feeding behavior (Zardoya et al., 2004).

In tropical estuarine, *Ethmalosa fimbriata* mtDNA cytochrome *b* gene sequence analysis revealed significant population structure due to isolation by distance (Durand et al., 2005). Net nucleotide genetic divergence among localities was nil in cod *Gadus morhua* from Greenland and Iceland (Arnason et al., 2000). The population structure of *Panulirus japonicus* inferred using mtDNA cytochrome oxidase I gene resulted in no significant population subdivision between Ohara, Hamajima and Goto in Japan (Inoue et al., 2007). In Yellowfin (*Thunnus albacares*) and Skipjack (*Katsuwonus pelamis*) tuna mtDNA control region and cytochrome *b* gene sequences, low levels of genetic differentiation was observed between Atlantic and Pacific samples of yellowfin tuna but in skipjack tuna no genetic differentiation was observed due to large population sizes (Ely et al., 2005). In *Engraulis japonica* from Yellow Sea and East China Sea, cytochrome *b* gene fragment studies showed no significant genetic variation in its populations, the biological factors of anchovy such as repeat spawning, long spawning period, and wide spawning ranges contribute to the homogeneity of the anchovy populations in the Yellow Sea and East China Sea (Yu et al., 2005). Many studies have employed morphological, meristic and molecular markers to decipher the population structure *T. ilisha* from Bay of Bengal especially on Ganga and Hooghly river populations and suggested contrasting population structure of hilsa.

We found no evidence of more than one genetic stock of hilsa in Ganga and Hooghly rivers which is supported by otolith core chemistry studies (Milton and Chenery, 2001), allozyme (Lall et al., 2004), morphological and allozyme (Salini et al., 2004). The degree of genetic isolation within the sampling rivers was not detected with F_{st} = - 0.0041. Based on Ward et al. (1994), population structure of hilsa is of marine type. Bay of Bengal is characterized by influx of large quantities of freshwater during monsoon, high turbidity, high to low salinities from marine to freshwater zones in both estuaries, seasonal water circulation, no geographic barriers in the form of islands, and large scale movement of hilsa in Ganga and Hooghly estuaries, high fecundity, continuous migration throughout the monsoon, spawning all along the migratory route, does not allow hilsa populations to get isolated (De, 1986; De and Saigal, 1989; De et al., 1994). Gene flow among basic genetic units is extensive so hilsa behaves as a single panmictic population, and there is no reproductive isolation that can result in genetic divergence. This mitochondrial DNA cytochrome *b* study of hilsa suggests existence of a single population, migrating to Ganga and Hooghly rivers through the estuaries for spawning and breeding purpose. Therefore, management decisions of hilsa stocks throughout its distribution range in India should consider it as a single panmictic population.

Further studies using microsatellite polymorphism and single nucleotide polymorphism is suggested to understand

hilsa population at a larger regional level constituting Myanmar, Bangladesh and India.

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REFERENCES

Arnason E, Petersen PH, Kristinsson K, Sigurgislason H. and Palsson S (2000). Mitochondrial cytochrome b DNA sequence variation of Atlantic cod from Iceland and Greenland. J. Fish Biol.56:409-430.

Brahmane MP, Das MK, Sinha MR, Sugunan VV, Mukherjee A, Singh SN, Prakash S, Maurye P, Hajra A (2006). Use of RAPD fingerprinting for delineating populations of hilsa shad *Tenualosa ilisha* (Hamilton 1822). Genet. Mol. Res. 31:643-652.

Day F (1873). Report on the freshwater fish and fisheries of India and Burma- Calcutta, pp. 22-23 and 35-36.

De DK (1986). Studies on the food and feeding habit of Hilsa, *Hilsa ilisha* (Hamilton) of the Hooghly estuarine system and some aspects of its biology. Ph. D. Thesis, Calcutta University p. 285.

De DK (2001). An overview of the biology of the hilsa, *Tenualosa ilisha* (Hamilton) and its fishery in the Ganga-Hooghly drainage system. International Terubok Conference, Sarawak.

De DK, Saigal BN (1989). Spawning of hilsa, *Tenualosa ilisha* (Ham) in the Hooghly estuary. J. Inland Fish. Soc. India. 21:46-48.

Durand JD, Tine M, Panfili J, Thiaw OT, Lae R (2005). Impact of glaciation and geographic distance on the genetic structure of a tropical estuarine fish, *Ethmalosa fimbriata* (Clupeidae, S. Bowdich, 1825). Mol. Phylogenet. Evol. 36(2):277-287.

Ely B, Vinas J, Bremer JRA, Black D, Lucas L, Covello K, Labrie AV, Thelen E (2005). Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) *and* the skipjack tuna (*Katsuwonus pelamis*). BMC Evol. Biol. 5:1-9.

Excoffier L, Laval G, Schneider S (2006). Arlequin ver 3.01. An integrated software package for population genetics data analysis. Computational and Molecular Population Genetic Lab, Institute of Zoology, University of Berne, Switzerland.

FAO (1997). FAO yearbook of fishery statistics 1997, FAO Fish. Ser. 84: FAO, Rome Italy.

Ghosh AN, Bhattacharya RK, Rao KV (1968). On the identification of the sub-population of *Hilsa ilisha* (Ham) in the Gangetic system with a note on their distribution. Proc. Natl. Inst. Sci. India B 34:44-57.

Inoue N, Watanbe H, Kojima S, Sekiguchi H, (2007). Population structure of Japanese spiny lobster Panulirus japonicus inferred by nucleotide sequence analysis of mitochondrial COI gene. Fish. Sci. 73:550-556.

Jenkins JT (1940). Hilsa investigations in Bengal. Curr. Sci. 7:241-429.

Kocher DT, Thomas WK Meyer A, Edwards SV, Pääbo S, Villablanca FX and Wilson AC (1989). Dynamics of mitochondrial DNA evolution in animals:Amplification and sequencing with conserved primers. Proc. Nat. Acad. Sci. USA. 86:6196- 6200.

Kumar S, Tamura K, Nei M (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings Bioinform. 5:150-163.

Mazumder KK, Alam MS (2009). High levels of genetic variability and differences in hilsa shad, *Tenualosa ilisha* (Clupeidae, Clupeiformes) populations revealed by PCR-RFLP analysis of the mitochondrial DNA D-loop region. Genet. Mol. Biol. 32:190-196.

Milton DA, Chenery SR (2001). Can otolith chemistry detect the population structure of the shad hilsa *Tenualosa ilisha*? Comparison with the results of genetic and morphological studies. Mar. Ecol. Prog. Ser. 222:239-251.

Mojumdar CH (1939). Foreshore fishing in the eastern parts of Bay of Bengal. Sci. Cult. 5:219.

Pillar TVR (1954). Morphological and serological characters of the hilsa, *Hilsa ilisha* (Hamilton) with special reference to racial investigations. J. Asiat. Soc. 20:69-74.

Pillay SR, Rao KV (1963). Observations on the biology and fishery of hilsa, *Hilsa ilisha* (Hamilton), of river Godavari. Proc. Indo-Pac. Fish. 10:37-61.

Pillay TVR (1952). A preliminary biometric study of certain populations of hilsa, *Hilsa ilisha* (Hamilton). Proc. Indo-Pac. Fish. Comm. 4:181-194.

Pillay TVR (1957). A morphometric study of the populations of Hilsa, *Hilsa ilisha* (Hamilton) of the river Hooghly and of the Chilka Lake. *Ibid* 4:344-386.

Pillay TVR, Pillay SR, Ghosh KK (1963). A comparative study of the populations of hilsa, *Hilsa ilisha* (Hamilton) in Indian waters. Proc. Indo-Pac. Fish. 10, 62-104.

Rahman M, Naevdal G (2000). Population genetic studies of hilsa shad, *Tenualosa ilisha* (Hamilton), in Bangladesh waters: evidence for the existence of separate gene pools. Fish. Manag. Ecol. 7:401-411.

Salini JP, Milton DA, Rahman MJ, Hussain MG (2004). Allozyme and morphological variation throughout the geographic range of the tropical shad, hilsa *Tenualosa ilisha*. Fish. Res. 66:53-69.

Sambrook J, Russell, DW (2001). Molecular cloning- A Laboratory Manual. 2nd Ed, Vol. 1, Cold Spring Harbor Laboratory, NY.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The Clustal X window interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24:4876-4882.

Ward RD, Woodward M, Skibinski DOF (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. 44:213-232.

Yu Z, Kong X, Guo T, Jiang Y, Zhuang Z, Jin X (2005). Mitochondrial DNA sequence variation of Japanese anchovy *Engraulis japonicus* from the Yellow Sea and East China Sea. Fish. Sci. 71:299-307.

Zardoya R, Casthilo C, Grande L, Favre-Krey S, Caetano S, Marcato S, Krey G, Patarnello T (2004). Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. Mol. Ecol. 13:1785-1798.