



Assessment of Genetic Variability among Individuals of Freshwater Mud Eel, *Monopterus albus* in a Population of Bangladesh

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Abstract: This study was carried out for the assessment of genetic variability of the species *Monopterus albus* in Bangladesh. Genetic variability of eleven individuals of *Monopterus albus* from “Tangour Haour”, Sunamganj, Bangladesh, was studied based on RAPD analysis. Three arbitrary primers were screened and 24 polymorphic loci were found in 11 individuals of the experimental population. The percentage of average values of polymorphic loci, polymorphism information content and Shannon index were found 32.8, 0.6420 and 0.213 respectively. Highest genetic similarity was found 0.363 (36.3%) between samples (individuals) of 10 and 11 followed in the sample 6 and 7 (0.333, 33.3%). It is indicating a substantial level of genetic variability in the studied samples of the vulnerable species. Inferred from Squared Euclidean genetic distances, highest linkage distance (17.0) was found in sample (S) 4 and (S) 1 individual pair and the lowest linkage distance (4.0) was observed in sample (S) 6 and 7 individual pair. A phylogenetic dendrogram was also constructed by UPGMA method based on Squared Euclidean genetic distances.

Key words: *Monopterus albus*, genetic variability, PCR, RAPD, Bangladesh

I. Introduction

The freshwater mud eel, *Monopterus albus* [1] is common in freshwater, which is a fish species with good taste and high market value. In Bangladesh, the species is available in mud holes, shallow beels, paddy fields, etc. [2]. Eels, including freshwater mud eel have been regarded as one of the best nutritious food for human due to its abundant protein, calcium, unsaturated fat, vitamin A and E, EPA and DHA, etc. Like other eels, freshwater mud eel provides various values for the ecosystem and human being. The unique adaptation that allows this freshwater mud eel is to be successful in their environment [3]. This fish can play a unique role for socio-economic welfare of the area and thus social fishery could be developed in the area with short of culture practice [4]. Raising this species is easy to do and achieves a more profit than some other small size fish culture activities [5], [6]. This fish can easily be cultured in a small tank, aquarium and other vessels. This fish has quite a large economic demand, the populations of the freshwater eel are declining at an alarming rate from the natural water bodies due to water obstruction, habitat destruction, pollution, over exploitation and destructive fishing [7]. The freshwater mud eel is recorded as rare species from floodplains and beels due to disease outbreak [8]. This species is listed in IUCN risk categories [9] or vulnerable or threatened [10]. Very few works have been done on genetic aspects of this freshwater mud eel in Bangladesh as well as in other countries. Genetic diversity among three *Monopterus* species were compared in China [11] whereas in Bangladesh the preliminary level of genetic study of the freshwater mud eel was conducted by RAPD [12]. So, it is very much needed to study genetic characterization of this fish to save in nature from extinction. In this experiment the goal of this research is to assess the genetic variability among individuals of freshwater mud eel (*Monopterus albus*) in an ecologically sensitive area (Tangour Haor) using RAPD technique.

II. Materials and Methods

DNA extraction

Fish samples were collected from “Tangour Haour” and brought to the “Animal Biotechnology Laboratory (ABL)” of the department of Genetic Engineering and Biotechnology (GEB) at Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh, kept them into the glass aquariums. The study was conducted from March to September, 2012. Each fish was dissected and different tissue samples such as liver, kidney, etc.

was isolated from each individual. Isolated tissues were washed by using distilled water and 70% alcohol and preserved separately in 100% alcohol at -20°C. DNA extraction was carried out by using commercially available kit, Bioserve, CAT. NO. 2025 from 11 fish samples. DNA quality was checked by 0.8% agarose gel and image was taken by digital camera (Panasonic™ DMC-fs20, 10 mega pixels). The extracted DNA samples were stored at -20°C.

PCR amplification

Three RAPD primers such as OPB-05 (5' TGCGCCCTTC 3'), OPB-08 (5' GTCCACACGG 3') [12] and OPF-14 (5' TGCTGCAGGT 3') [13] were selected. PCR reactions were performed on each DNA sample in a 10µl reaction mixture containing 1µl of 25mM MgCl₂, 1µl of 10X reaction buffer, 1µl of primer, 0.5µl of each dNTP, 0.2µl of Taq DNA polymerase, 1µl of Template DNA and 5.30µl deionized distilled water. PCR reaction was conducted for pre heating 94°C for 3 minutes, denaturation at 94°C for 1 minutes; annealing temperature for this PCR was 35°C for 1 minute and 2 minutes for elongation at 72°C. A final step of 7 min at 72°C was added to allow complete extension of the amplified fragments. The PCR was run for 40 cycles. The amplified PCR products from each sample were checked by 1.4% agarose gel and the photograph was taken by a 10 mega pixels digital camera (Panasonic™ DMC-FS20).

Data analysis

RAPD data of this experiment was interpreted by using different software and equations. This is as under:

1. AlphaEaseFC 4.0 for identification molecular weight of bands.
2. Statistica for draw dendrogram.
3. Genetic distance analysis from pair wise similarity by following equation

$$D = 1 - \frac{N_{xy}}{N_x + N_y - N_{xy}}$$

Where, D = The genetic distance between sample x and y, N_{xy} = number of band shared by sample x and y, N_x = the number of bands in sample x, N_y = the number of bands in sample y

4. Shannon-Wiener Index among mud eel individuals

$$H_s = - \sum_{i=1}^S (P_i) (\ln P_i)$$

Where, H_s = Symbol for the diversity in a sample of S species or kinds, S = The number of species in the sample, P_i = Relative abundance of ith species or kinds measures, (= S_i/N), N = Total number of individuals of all kinds, S_i = number of individuals of ith species, ln = log to base 2, I = Shannon information Index

5. Nei's genetic similarity was analyzed using pair wise comparison by the following formula [14]:

$$F = \frac{2N_{xy}}{N_x + N_y}$$

Where, F = Nei's genetic similarity, N_{xy} = Number of shared Band between X and Y, N_x = Number of band in X, N_y = Number of band in Y

6. Polymorphism information content (PIC).

$$\text{Polymorphism information content} = 1 - \sum_{j=1}^n P_{ij}^2$$

Where, P_{ij} is the frequency of the jth allele for the ith marker Summed over 'n' alleles.

III. Results

Data scoring

Both side of the gel 1 kb ladder was used as molecular weight marker ranges from 250 bp to 10,000 bp (Generuler™). The size of amplification products were measured in base pairing Alpha EaseFc 4.0 software. Each amplified RAPD bands profile was defined by the presence or absence of bands at particular positions on the gel. Fragments were scored as (1) if present or (0) if absent, separately for each individual for each primer. In this experiment 11 individuals of *M. cuchia* were analyzed. Individual 4, 5 and 7 with three bands between 420 bp and 1180 bp, individuals 1, 8, 10, 11 with two bands and rest of individuals with only one band were found by using primer OPB-05. Furthermore, by using primer OPB-08, individual 3 and 4 with five bands between 273 bp and 1964 bp, individual 1 and 2 with three bands, individual 5, 9, 10, 11 with two bands and rest of individuals with one band were observed. The primer OPF-14 showed five bands in individual 8

between 500 bp and 1500 bp but only two bands were observed in the individuals of 3, 4, 5, 7, 9, 10 while individuals 2, 6 and 11 contain only one band and individual 1 had three bands.

Bands analysis

A total of 73 bands were detected among 11 *Monopterus albus* individual genotypes out of which 24 were polymorphic and 49 were monomorphic bands (Table 1). All markers were shown more or less polymorphism. The highest number of bands (29) amplified by the primer OPB-08 and the lowest number of bands (21) amplified by the primer OPB-05 (Table 1). Marker OPB-05 showed highest polymorphism (47.61%) and marker OPF-14 showed lowest polymorphism (26.08%) among tested individuals. The highest number of bands (2.63) per variety was amplified from the primer OPB-08 and the lowest number of bands (1.90) per sample was amplified by the primer OPB-05 (Table 1). The primer OPF-14 amplified 2.09 numbers of bands per individual sample. Primer OPB-05 also shows highest Polymorphism Information Content (PIC) (0.7108) while an average PIC was 0.6420.

Table 1: RAPD based analysis for eleven individuals of fresh water mud eel

Primer	Size of DNA Bands (bp)	Total Number of DNA Bands	Number of Polymorphic loci	Number of Monomorphic loci	Percentage of Polymorphic loci (%)	Number of Bands per sample	polymorphism information content (PIC)
OPB-05	380-1180	21	10	11	47.61	1.90	0.7108
OPB-08	273-1964	29	8	21	27.58	2.63	0.5372
OPF-14	514-1277	23	6	17	26.08	2.09	0.6781
Total		73	24	49	32.87		0.6420
Average		24.3	8.0	16.3			

Inter-individual pair wise similarity indices

Inter individual pair wise similarity was also studied for this experiment (Table 2). The highest inter individual pair wise similarity found between sample 1 and 2, 3 and 4, 4 and 7, 4 and 8, 4 and 9, 6 and 7, 8 and 9, 10 and 11 whereas rest of the individuals showed lower similarity.

Table 2: Inter-individual pair wise similarity

Sample	Sam 1	Sam 2	Sam 3	Sam 4	Sam 5	Sam 6	Sam 7	Sam 8	Sam 9	Sam 10	Sam 11
Sam1	-----	0	2	0	0	0	0	0	0	0	0
Sam 2		-----	1	1	0	0	1	0	0	0	0
Sam 3			-----	2	1	0	0	1	1	0	0
Sam 4				-----	1	0	0	2	2	0	0
Sam 5					-----	1	0	0	0	1	1
Sam 6						-----	2	1	1	0	0
Sam 7							-----	1	0	0	0
Sam 8								-----	2	0	0
Sam 9									-----	1	0
Sam 10										-----	2
Sam 11											-----

Genetic distance

In this experiment genetic distance was calculated by using the data from pair wise similarity index (Table 2). Among 11 individuals of *M. albus* lowest genetic distance was found between sample 10 and sample 11 (0.778) and relatively higher distance was recorded in other individuals (Table 3), even though highest genetic distance (1) was found in some individuals.

Table 3: Genetic distance among individuals of freshwater mud eel

Sample	Sam1	Sam2	Sam3	Sam4	Sam5	Sam6	Sam7	Sam8	Sam9	Sam10	Sam11
Sam1	-----	1.000	0.800	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sam2		-----	0.923	0.928	1.000	1.000	0.875	1.000	1.000	1.000	1.000
Sam3			-----	0.883	0.928	1.000	1.000	0.942	0.933	1.000	1.000
Sam4				-----	0.944	1.000	1.000	0.833	0.883	1.000	1.000
Sam5					-----	0.909	1.000	1.000	1.000	0.900	0.909
Sam6						-----	0.667	0.917	0.889	1.000	1.000
Sam7							-----	0.917	1.000	1.000	1.000
Sam8								-----	0.847	1.000	1.000
Sam9									-----	0.900	1.000
Sam10										-----	0.778
Sam11											-----

Shannon-Wiener Index of freshwater mud eel

Shannon-Wiener Index (HI) was also observed among the individuals of fresh water mud eel and the value was found 0.213. Evenness of this index was found 0.2080 and 0.1861 among samples (Individuals) 11, 8 & 5, and 2, 7 & 10 respectively (Table 4). Sample 1, 3, 4, 6 and 9 was recorded uneven in its individual genetic makeup.

Table 4: Shannon-Wiener Index among individuals of freshwater mud eel

Sample	Number of sample (Si)	Relative abundance $P_i = S_i/N$	$LN(P_i)$	$P_i * LN(P_i)$	Index HI
Sample 1	7	0.098	-2.32278	-0.2276332	0.2276332
Sample 2	5	0.07	-2.65926	-0.1861482	0.1861482
Sample 3	9	0.126	-2.07147	-0.2610056	0.2610056
Sample 4	10	0.14	-1.96611	-0.2752558	0.2752558
Sample 5	6	0.084	-2.47694	-0.2080628	0.2080628
Sample 6	4	0.056	-2.8824	-0.1614146	0.1614146
Sample 7	5	0.07	-2.65926	-0.1861482	0.1861482
Sample 8	8	0.112	-2.18926	-0.2451967	0.2451967
Sample 9	6	0.084	-2.47694	-0.2080628	0.2080628
			Sum	-2.3531391	2.35313907
	TOTAL=71(N)				Average=0.213

Nei's genetic similarity analysis

Nei genetic similarity (F) was observed among 11 individuals of *M. cuchia* (Table 5). Highest genetic similarity was found 0.363 (36.3%) between individuals/samples 10 and 11 followed in the sample 6 and 7 (0.333; 33.3%). Relatively lower similarity was found among other samples.

Table 5: Nei's genetic similarity among individuals of freshwater mud eel

Band	Sam 1	Sam 2	Sam 3	Sam 4	Sam 5	Sam 6	Sam 7	Sam 8	Sam 9	Sam 10	Sam 11
Sam 1	-----	0	0.25	0	0	0	0	0	0	0	0
Sam 2		----	0.142	0.133	0	0	0.222	0	0	0	0
Sam 3			-----	0.210	0.133	0	0	0.111	0.133	0	0
Sam 4				-----	0.125	0	0	0.210	0.25	0	0
Sam 5					-----	0.2	0	0	0	0.181	0.166
Sam 6						-----	0.333	0.153	0.2	0	0
Sam 7							-----	0.153	0	0	0
Sam 8								-----	0.266	0	0
Sam 9									-----	0.181	0
Sam 10										-----	0.363
Sam 11											-----

Linkage distances

The values of pair-wise comparisons of linkage distances analyzed by using computer software "Statistica" between samples (individuals) were computed from combined data for the three primers, ranged from 7.0 to 17.0 (Table 6). The highest linkage distance (17.0) was found in Sample 4 (S4) and Sample 1 (S1) individual pairs. The lowest linkage distance (4.0) was observed in Sample 6 (S6) and 7 (S7) individual pairs. Comparatively higher linkage distance was observed between all other samples/individuals pair.

Table 6: Squared Euclidean distances (new.sta)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
S1	0	12	12	17	13	11	11	16	13	12	12
S2	12	0	12	13	11	9	7	14	11	10	10
S3	12	12	0	15	13	13	13	14	13	14	12
S4	17	13	15	0	14	14	14	15	14	13	15
S5	13	11	13	14	0	8	10	15	12	9	9
S6	11	9	13	14	8	0	4	11	8	9	9
S7	11	7	13	14	10	4	0	11	10	9	9
S8	16	14	14	15	15	11	11	0	11	14	14
S9	13	11	13	14	12	8	10	11	0	9	11
S10	12	10	14	13	9	9	9	14	9	0	6
S11	12	10	12	15	9	9	9	14	11	6	0

Cluster analysis based on RAPD markers

A cluster analysis using UPGMA based on linkage distance was done to resolve the phylogenetic relationships among 11 individuals of *M. cuchia* genotypes considered for the present study (Figure 1). The UPGMA

clustering system generated five genetic clusters at linkage distance 12. Sample 1 form cluster one alone whereas sample 3 grouped in cluster two and sample 2, 6, 7, 5, 10, 11, 9 form cluster three. Sample 8 and 4 formed as cluster four and five respectively (Figure 1). Cluster three is the biggest group as it contained seven samples (individuals). However, cluster three divided into two sub cluster where one with samples 2, 6, 7, 5, 10, 11 and other with sample 9.

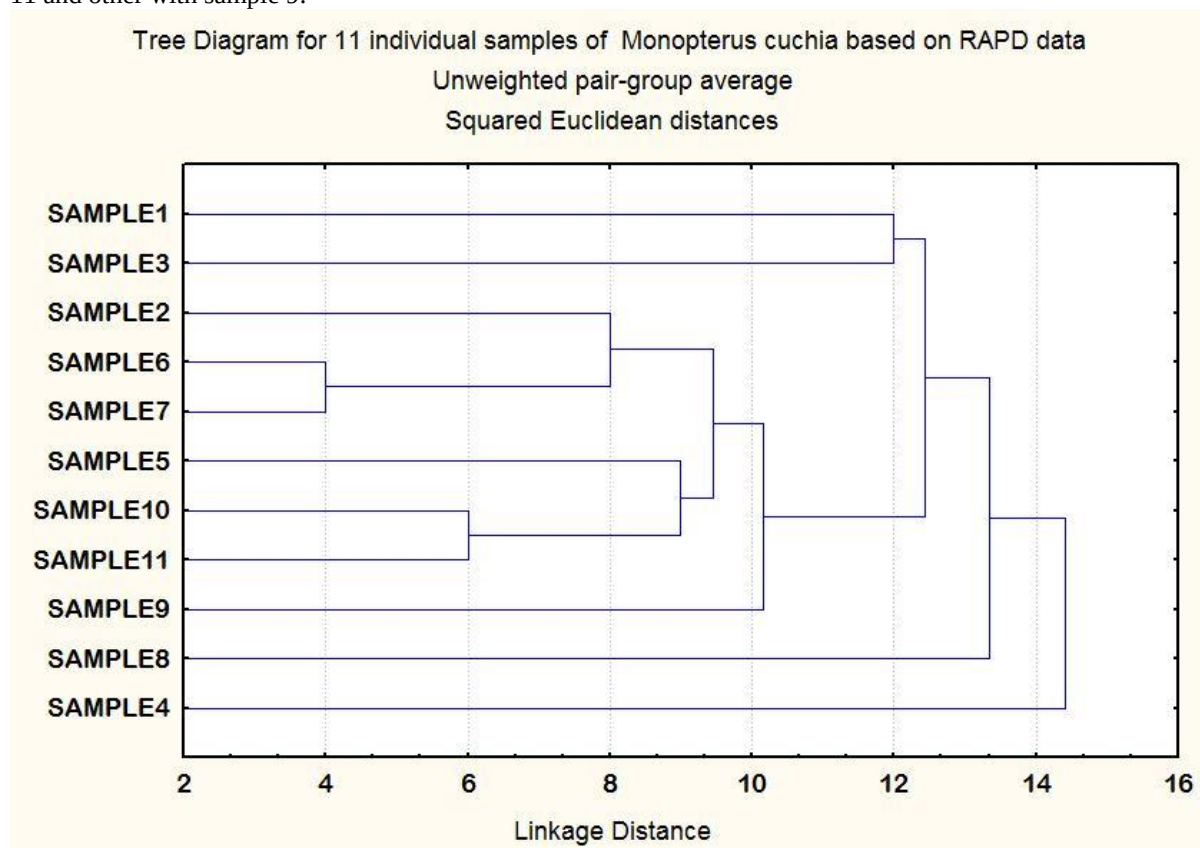


Figure 1: Dendrogram of genetic relationships among 11 individuals of freshwater mud eel

IV. Discussion

The freshwater mud eel, *M. cuchia* has been considered to be a vulnerable fish species in Bangladesh. Genetic variation is a key tool for assessing biological potential of an organism. A population with high level of genetic variation may be better capable of dealing with changes in its surroundings such as fluctuations in water temperature, epidemics, etc. However, to ensure adaptation, expansion, and reestablishment in natural populations, maintaining genetic variation in endangered species is essential. Thus, this study was to observe genetic variability in fresh water mud eel, *Monopterus cuchia*, in Tanguar Haor populatiOn at Sunamgonj district. Three random primers were applied to measure the genetic diversity among 11 individuals of *M. chuchia*. Each 10 bp primer in individual sample amplified 1.90 to 2.63 fragments in length 273-1964 bp, with a high specificity. Experimented data indicated that intra-specific polymorphism in primer OPB-05 was (47.61%), OPB-08 (27.58%), OPF-14 (26.08%) with an average of 32.87 % (Table 1). These data was found different from Alam *et al* [12] with OPB-05 (66.67%) and OPB-08 (83.33%).

Pair wise band sharing based intra-species similarity (F) was found higher between sample 10 and 11 (0.363), with an average 0.361 (36%) (Table 2). Intra-species Shannon diversity (HI) index was found (2.35) with an average of 0.6420 which is indicated the samples were moderately even. On the contrary, a low level of polymorphism was found in five populations of rice field eel, *Monopterus albus* (ranged from 29.51 to 66.39%) in China mainland though two other populations possessed a high proportion of polymorphic loci (82.79%) [15]. But high polymorphic loci were found by Alam *et al* [12] in a population in Bangladesh. Ruzainah *et al* [16] was also found higher SI value with RAPD fingerprinting of two eel loaches, 79.9% SI value for *Pangio piperata* and 88.5% for *Pangio filinaris* then the present study. Linkage distances based on Squared Eulidean distance was found highest between sample 4 and sample 1 (17). Lower distance (4.0) was found between sample 6 and 7, which indicate that sample 4 and sample 1 were distantly related. On the other hand, sample 6 and sample 7 were closely related. All data indicated that moderately related.

The final target of this study was to investigate intra-species genetic variation in Tanguar Haor population of freshwater mud eel. Considering different parameters like percentage of polymorphic loci, Intra-individual Genetic Similarity (F) Shannon's gene diversity (H) and Shannon's Information Index (I) were found moderate degree of genetic variability in the collected samples from Tanguar Haor population at Sunamgonj district that reflects the fact that the species has experienced moderate genetic deterioration. However, the vulnerable state of this species may cause much genetic deterioration in the future if proper steps for conservation and stock enhancement are not taken.

V. Conclusion

In this experiment, the genetic variability of the endangered mud eel, *Monopterus albus* of Tanguar Haor at Sunamgonj district in Bangladesh was analyzed first time by using RAPD technique. This type of experiments is needed to observe with large number of population while only 11 individuals were checked. However, it would be used as a base line study for the future investigation on this species using RAPD technique in Bangladesh.

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