International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:10, No:6, 2016

Genetic Diversity Based Population Study of Freshwater Mud Eel (*Monopterus cuchia*) in Bangladesh

M. F. Miah, K. M. A. Zinnah, M. J. Raihan, H. Ali, M. N. Naser

***Abstract*—**As genetic diversity is most important for existing, breeding and production of any fish; this study was undertaken for investigating genetic diversity of freshwater mud eel, *Monopterus cuchia* at population level where three ecological populations such as flooded area of Sylhet (P1), open water of Moulvibazar (P2) and open water of Sunamganj (P3) districts of Bangladesh were considered. Four arbitrary RAPD primers (OPB-12, C0-4, B-03 and OPB-08) were screened and RAPD banding patterns were analyzed among the populations considering 15 individuals of each population. In total 174, 138 and 149 bands were detected in the populations of P1, P2 and P3 respectively; however, each primer revealed less number of bands in each population. 100% polymorphic loci were recorded in P2 and P3 whereas only one monomorphic locus was observed in P1, recorded 97.5% polymorphism. Different genetic parameters such as inter-individual pairwise similarity, genetic distance, Nei genetic similarity, linkage distances, cluster analysis and allelic information, etc. were considered for measuring genetic diversity. The average inter-individual pairwise similarity was recorded 2.98, 1.47 and 1.35 in P1, P2 and P3 respectively. Considering genetic distance analysis, the highest distance 1 was recorded in P2 and P3 and the lowest genetic distance 0.444 was found in P2. The average Nei genetic similarity was observed 0.19, 0.16 and 0.13 in P1, P2 and P3, respectively; however, the average linkage distance was recorded 24.92, 17.14 and 15.28 in P1, P3 and P2 respectively. Based on linkage distance, genetic clusters were generated in three populations where 6 clades and 7 clusters were found in P1, 3 clades and 5 clusters were observed in P2 and 4 clades and 7 clusters were detected in P3. In addition, allelic information was observed where the frequency of p and q alleles were observed 0.093 and 0.907 in P1, 0.076 and 0.924 in P2, 0.074 and 0.926 in P3 respectively. The average gene diversity was observed highest in P2 (0.132) followed by P3 (0.131) and P1 (0.121) respectively.

***Keywords*—**Genetic diversity, *Monopterus cuchia*, population, RAPD, Bangladesh.

I. Introduction

THE freshwater mud eel, *Monopterus cuchia* is one of the popular fishes of delicacy in the south east Asia. It has excellent health benefits considering its nutritional and medicinal importance [1]. This fish is common in different freshwater habitats of Bangladesh [2], [3], belonging to the order Synbranchiformes and family Synbrachidae [4], [5]. This fish has specialized pharyngeal pouch that helps in respiration both in air and water [6]-[8] and this modification helps to inhabit the fish in mud tunnels as well as in buckets without water for long time [9]. It is an evasive nocturnal and carnivore animal [10]. This fish is sexually dimorphic, mature fishes are summer spawner. The male bears two sperm ducts and female with single oviduct containing around 150-1500 spherical eggs. [11]-[14].

In Bangladesh, this fish is only popularly eaten by the tribal people and a few of other castes; however, it is a high valued export item of Bangladesh to sell it to nearly 15 countries in the world [3] in live condition. Although, 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 of Bangladesh due to habitat loss, change in habitats and over exploitation [15]-[17]. Unsuccessful artificial breeding is another crisis for production of this fish through aquaculture while induced breeding and fry rearing technique of this fish in Bangladesh has not yet been developed [18]. However, it has been observed that this fish can be commercially cultured to meet the market demand which could play great role in the national economy of Bangladesh [19], [20]. This freshwater mud eel could thus contribute to the socio-economic welfare of the area [20]. Very few works have been done on genetic aspects of this freshwater mud eel in Bangladesh as well as in other countries [21]-[26] while genetic diversity is a key tool for breeding programme. In this research, RAPD assay was considered to observe genetic status of this fish at population level in Bangladesh.

II. Materials and Methods

A. Sample Collection

Fish samples were collected from three ecological habitats; flooded area of Sylhet (P1), open water of Moulvibazar (P2) and open water of Sunamganj (P3) districts of Bangladesh. Fifteen individuals from each habitat were considered and collected fish samples were brought to the Fish Breeding House of the Department of Genetic Engineering and Biotechnology (GEB) at Shahajalal University of Science and Technology (SUST), Sylhet, Bangladesh, and kept the fish in aquariums by using proper environment until tissue isolation. Fish samples were identified through different morphometric characteristics [2], [27], [28].

M. F. Miah is with Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh (Corresponding Author, Phone: +8801712865321; fax: +880-821-715257; e-mail: faruque-btc@sust.edu).

K. M. A Zinnah, M. J. Raihan, H. Ali are with Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh (e-mail: raihangeb@gmail.com, hafij16@gmail.com, kazizinnah@gmail.com).

M. N. Naser is with Department of Zoology, University of Dhaka, Dhaka 1000, Bangladesh (e-mail: mnnaser@du.ac.bd).

B. Tissue Isolation

Each collected fish was dissected and different tissue samples such as liver, kidney, etc. were isolated and kept in separate petridishes with water. Isolated tissues were washed with distilled water and 70% alcohol and preserved in small Eppendorfs separately in 100% alcohol at -20 ºC until DNA extraction.

C. DNA Extraction

Commercially available kit, Bioserve, (CAT.NO.2025) was used to extract DNA from fish tissues. A total of 45 fish samples were used for DNA extraction; 15 from each habitat. DNA quality was checked by gel electrophoresis using 0.8% agarose where the gel was run at 70 volt for 40 minute with ethidium bromide staining. 1kb plus DNA ladder (Generuler^{TM}, USA) was used for comparison of DNA quality. Extracted DNA samples were stored at -20 ºC.

D. PCR Amplification

Four RAPD (Randomly Amplified Polymorphic DNA) primers; OPB-12 (CCTTGACGCA), OPB-08 (GTCCACACGG) [21], B-03 (CATCCCCCTG) and C-04 (CCGCATCTAC) [29]; were selected to study genetic diversity among three populations. PCR reaction was done for each sample with 25 µl containing 12.5 µl of Master Mix (GoTaq® G2 Hot Start Green), 2 µl of primer, 2 µl of template DNA and 8.5 µl of nuclease-free water. PCR reaction was conducted for pre heating at 94 ˚C for 3 minutes, denaturation at 94 ºC for 1 minute where the annealing temperature was considered 32 ˚C for the primers OPB-12 and C-04, and 34˚C for the primers B-03 and OPB-08 for 1 minute and 2 minutes for extension at 72 ˚C. A final step of 7 min for 72 ºC was added to allow complete extension of the amplified DNA fragments. The PCR was run for 40 cycles. The amplified PCR product of each sample was checked for banding pattern of DNA by electrophoresis on 2% agarose gel dying with ethidium bromide. 1 kb plus DNA ladder (Generuler^{TM}, USA) was used for measuring the length of DNA. The agarose gel of the amplified PCR products was placed on the gel documentation and the photograph was taken by a 10 mega pixels digital camera (Panasonic"DMC-FS20).

E. Analysis of Banding Data

RAPD data of this experiment was interpreted by using different software and equations where the AlphaEaseFC 4.0 was used for measurement of molecular weight of the bands. Genetic distance was measured by D = 1- Nxy / Nx+Ny- Nxy, where, D is the genetic distance between sample x and y, Nxy is the number of band shared by sample x and y, Nx is the number of bands in sample x and Ny is the number of bands in sample y. Nei's genetic similarity among individuals were measured by F= 2Nxy/ Nx+Ny, where, F is the Nei's genetic similarity, Nxy is the number of shared bands between X and Y, Nx is the number of bands in X and Ny is the number of bands in Y. Linkage distance was calculated considering Squared Euclidean distances by using the software new.sta and intra-individual relationship through dandogram was made by software "Statistica". Allele frequency and average gene diversity were measured according to the Hardly-Weinberg equation which is $(p+q)^2= p^2+2pq+q^2$.

III. Results

Genetic diversity of freshwater mud eel *Monopterus cuchia* was analyzed at populations level based on RAPD assay between three populations, collected from three ecosystems of Bangladesh where 15 individuals were considered from each population.

A. DNA Profiling and Data Scoring

DNA banding pattern was compared based on 1 kb plus DNA ladder (Generuler^{TM}) where the molecular weight of this marker ranges from 75 bp to 20,000 bp. Each amplified band was defined by the presence or absence of bands at particular positions on the gel and fragments were scored as 1 if present or 0 if absent, separately for each individual for each primer. A total of 174 bands were detected among individual genotypes of population one (P1) whereas 138 bands and 149 bands were found in population two (P2) and population three (P3) respectively. Unfortunately, less number of bands from each population was revealed by all the primers (Table I). Number of highest and lowest bands was found 59 and 29 in P1, 43 and 23 in P2 as well as 50 and 29 in P3 respectively while in all cases highest number of bands were recorded by the primer OPB-12 (Table I). In all experimental populations, 100% polymorphic loci were recorded except the P1 (97.5%) where the one monomorphic locus was observed. Total number of polymorphic loci was 84 in P1, 70 in P2 and 75 in P3 respectively (Table I). Furthermore, highest and lowest polymorphic loci were revealed by the primer OPB-12 and OPB-08. The highest number of bands (3.13, 2.87 and 3.33) per sample was amplified from the primer OPB-12 followed by primers OPB-08, C-04 and B-03 respectively (Table I).

B. Genetic Diversity at Population Level

It was used to quantify the differences between three populations in relation to the frequency of a particular trait. Genetic diversity of three experimental populations were observed considering by the study of different parameters such as inter individual pair wise similarity, genetic distance, Nei genetic similarity, linkage distances, cluster analysis and allelic information, etc. Though almost same values in number was observed in three populations, however, the average highest inter individual pair wise similarity was found 2.98 in P1, 1.47 in P2 and 1.35 in P3 respectively (Table II). The highest value was seen 9 in P1 and lowest value 0 in P2 and P3 respectively. Considering genetic distance analysis, it was observed that highest genetic distance 1 was recorded in P2 and P3 and lowest genetic distance 0.444 was found in P2. Though, highest number of values (46) was found in P1, but the highest genetic distance was recorded 0.92 and 0.91 in P3 and P2 respectively (Table II). Nei genetic similarity was recorded high in P1 considering 50 in numbers but highest value was found 0.714 in P2 and 0.667 in P3; and average highest to lowest similarity was observed 0.19, 0.16 and 0.13

in P1, P2 and P3 respectively (Table II). Furthermore, average linkage distance was recorded 24.92, 17.14 and 15.28 in P1, P3 and P2 respectively.

C. Cluster Based Study

Cluster analysis using UPGMA (Unweighted Pair Group Method of Arithmetic Means) based on linkage distance was done to investigate the relationships among three populations of *M. cuchia* genotypes. The UPGMA clustering system generated some genetic clusters with different linkage distance where 6 clades and 7 clusters were found in P1 (Fig. 1), 3 clades and 5 clusters was observed in P2 (Fig. 2) and 4 clades and 7 clusters were detected in P3 (Fig. 3).

TABLE I
RAPD BASED ANALYSIS OF FRESHWATER MUD EEL

Primers	Size of DNA (bp)			No. of Total Bands			No. of Polymorphic Loci			No. of Monomorphic Loci			% Polymorphic loci			No. of Bands/sample		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
OPB-12	126-1322	314-2126	213-2519	59	43	50	31	29	31	00	00	00	100	100	100	3.13	2.87	3.33
C-04	217-872	397-1226	344-1220	47	23	29	21	13	14	00	00	00	100	100	100	3.13	1.53	1.93
B-03	268-1773	138-479	125-385	29	35	29	23	16	12	00	00	00	100	100	100	1.93	2.33	1.93
OPB-08	80-456	80-415	85-462	39	37	41	09	12	18	01	00	00	90	100	100	2.6	2.47	2.73
Total				174	138	149	84	70	75	1	0	0						
Average				43.5	34.5	37.3	21	17.5	18.8	1	0	0	97.5	100	100			

TABLE II
INTER POPULATION GENETIC ANALYSIS

Populations	IIPWS				Genetic Distance				Nei Genetic similarity				Linkage Distance			
	DVN	H	L	A	DVN	H	L	A	DVN	H	L	A	DVN	H	L	A
P1	7	9	1	2.98	46	0.971	0.689	0.89	50	0.473	0.055	0.19	18	35	16	24.92
P2	6	5	0	1.47	33	1	0.444	0.91	32	0.714	0	0.16	16	24	4	15.28
P3	6	8	0	1.35	26	1	0.5	0.92	28	0.667	0	0.13	14	22	8	17.14

IIPWS= Inter–individual pair wise similarity, H=Highest, L=Lowest, A=Average, DVN= Different values in number

TABLE III
ALLELIC FREQUENCY AND GENETIC DIVERSITY

Primers	P1			P2			P3		
	Allele frequency		Average Gene diversity	Allele frequency		Average gene diversity	Allele frequency		Average gene diversity
	p	q		p	q		p	q	
OPB-12	0.066	0.934	0.121	0.051	0.949	0.095	0.056	0.944	0.103
C-04	0.079	0.921	0.141	0.063	0.937	0.11	0.073	0.927	0.13
B-03	0.039	0.961	0.077	0.077	0.923	0.137	0.085	0.915	0.151
OPB-08	0.188	0.812	0.144	0.114	0.886	0.184	0.081	0.919	0.141
Average	0.093	0.907	0.121	0.076	0.924	0.132	0.074	0.926	0.131

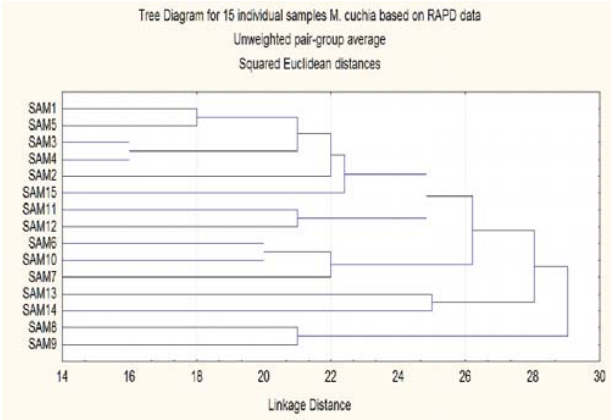

Fig. 1 Genetic relationships among individuals of P1

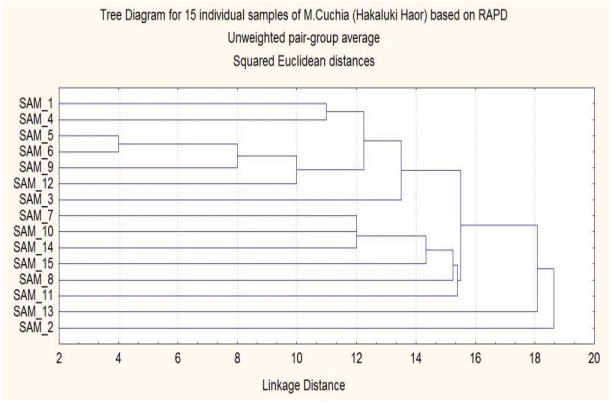

Fig. 2 Genetic relationships among individuals of P2

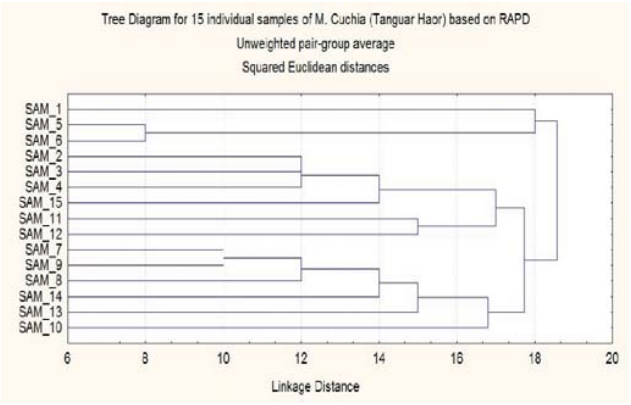

Fig. 3 Genetic relationships among individuals of P3

D.Allelic Information

Considering 4 RAPD primers, the average frequency of the p allele was observed 0.093 in P1, 0.076 in P2 and 0.074 in P3 whereas the average frequency of the q allele was recorded 0.907 in P1, 0.924 in P2 and 0.926 in P3 respectively (Table III). The average gene diversity was observed highest in P2 (0.132) followed by P2 (0.131) and P1 (0.121) respectively (Table III).

IV. Discussion

As the *Monopterus cuchia* is a threatened species of Bangladesh [17], therefore, this study was undertaken for investigating genetic diversity of freshwater mud eel, *M. cuchia* at population level to ensure adaptation, expansion and reestablishment of natural population where three ecological populations such as flooded area of Sylhet (P1), open water of Moulvibazar (P2) and open water of Sunamganj (P3) districts in Bangladesh were considered. Four arbitrary RAPD primers (OPB-12, C04, B-03 and OPB-08) were screened and RAPD banding patterns was analyzed among the populations. A total of 174, 138 and 149 bands were detected among the population of P1, P2 and P3 respectively. 100% polymorphic loci were recorded in P2 and P3 where only one monomorphic locus was observed in P1, recorded 97.5% of polymorphism. Similar result was found by [25] while less polymorphism was recorded in another research in different other population in Bangladesh [21]. Genetic diversity at population level of rice field eel (*Monopterus albus*) was studied by RAPD analysis where 122 polymorphic loci were detected by [30]. Yin et al. [31] assessed the genetic differentiation and variation of the wild and raised swamp eels *Monopterus albus* using RAPD technique and the results showed the percent polymorphic loci was 44.79% and 36.5% while [32] found the percent polymorphic loci of wild samples was 60.6–71% and cultured samples was 54–56.3% by ISSR analysis and these results were differed from the present study. In the rice field eel (*Monopterus albus*), 30 microsatellites were analyzed and sequenced by AFLP where 13 loci exhibited polymorphism and these loci should provide sufficient level of genetic variation [33], while lots of polymorphic loci were recorded in the present study of *M. cuchia* by RAPD assay. The genetic diversity of Asian swamp eel *Monopterus albus* were analyzed with 16 polymorphic novel microsatellites with 11 loci in two natural populations whereas the observed heterozygosity was 0.65 [34] which was also lowest from the present study in *M. cuchia* by RAPD analysis. The average inter-individual pair wise similarity was recorded 2.98, 1.47 and 1.35 in P1, P2 and P3 respectively while only 3 groups of individuals were observed with the ranged of 0 to 2 by [25]. The highest genetic distance 1 was recorded in P2 and P3 and the lowest genetic distance 0.444 was found in P2 while in this case highest genetic distance (average 0.89, 0.91 and 0.92) was recorded from all the populations when compared with the findings of [29] but lowest from the Miah et al. [25] which was an average 0.972315. The average Nei genetic similarity was observed 0.19, 0.16 and 0.13 in P1, P2 and P3 respectively and different values of Nei's genetic similarities were found lower than [25]. The average linkage distance was recorded 24.92, 17.14 and 15.28 in P1, P3 and P2 in orderly while linkage distances were recorded between 7 and 17 by [25]. A genetic relationship was found among the populations where 6 clades and 7 clusters were found in P1, 3 clades and 5 clusters was observed in P2 and 4 clades as well as 7 clusters were detected in P3, but, only 3 clades and 4 clusters were found in the few individuals of a population in Bangladesh [25]. In addition, genotypes and alleles frequency was observed where the frequency of the p and q alleles were observed 0.093 and 0.907 in P1, 0.076 and 0.924 in P2, 0.074 and 0.926 in P3 respectively. The average gene diversity was observed highest in P2 (0.132) followed by P3 (0.131) and P1 (0.121) respectively. In this study, overall, higher genetic diversity has been recorded in this experimental fish which is indicating the good genetic status of this fish in Bangladesh. Wei et al. [30], Li et al. [32] and Lei et al [34] indicated that most of the total genetic variances of different population existed within populations and some variations existed among populations and the present results agreed with among populations. Miah et. al. [25] found higher genetic variability than the present study from a *M. cuchia* population of Bangladesh.

V. Conclusion

In this experiment, the genetic variability of the endangered mud eel, *Monopterus cuchia* was analyzed by using RAPD assay considering three populations from three districs of Bangladesh while genetic diversity was found higher in population 2 and 3 than population 1. The experiments are needed to observe genetic diversity for breeding and conservation programme of this fish in Bangladesh with large number of fish considering RAPD, RFLP, SSR etc. However, this finding would be helpful for the future investigation of this species in Bangladesh for propagation and improvement.

Acknowledgment

We are acknowledged to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for funding through a research project entitled "Development of Artificial Breeding (Induced Breeding and

Selective Breeding) and Production Techniques of Freshwater Mud Eel, *Monopterus cuchia* in Bangladesh".

REFERENCES

[1] L. H. Herbst, S. F. Costa, L. M. Weiss, L. K. Johnson, J. Bartell, R. Davis, M. Walsh, and M. Levi, Granulomatous skin lesions in Moray eel caused by a novel *Mycobacterium* species related to *Mycobacterium triplex*. Infect. Immun., 2001, Vol. 69: pp. 4639-4646.

[2] A. A. K. Rahman. "Freshwater Fishes of Bangladesh," Zoological Science of Bangladesh, University of Dhaka, 1989, 364p.

[3] S. M. Galib, M. A. Samad, A. B. M. Mohsin, F. A. Flowra, and M. T. Alam, Present status of fishes in the Chalan Beel-the largest beel (wetland) of Bangladesh, Int. J. Ani. Fish. Sci., 2009, Vol. 2, No. 3, pp. 214-218.

[4] D. E. Rosen, and P. H. Greemwood, A fourth neotropical species of synbranchid eel and the phylogeny and systematic of Synbranchiformes fishes, Bull. Am. Mus. Nat. Hist., 1976, Vol. 157, pp. 5-69.

[5] J. S. D. Munshi, G. M. Hughes, P. Gehr and E. R. Weibel, Structure of the air-breathing organs of a swamp mud eel, *M. cuchia*, Japanese Journal of Ichthyology, 1989, Vol. 35, No. 4, pp. 453-358.

[6] A. K. Mittal and S. K. Agarwal, Histochemistry of the unicellular glands in relation to their physiological significance in the epidermis of *Monopterus cuchia* (Synbranchiformes, Pisces). J. Zool. London, 1977, Vol. 182, pp. 429-439.

[7] N. Mishra, P. K. Pandey and J. S. D. Munshi, "Haematologica parameters of an air-breathing mud eel, *Monopterus cuchia* (Ham.) (Amphipnoidae: Pisces)", J. Fish Biol., 1977, Vol. 10, pp. 567-573.

[8] J. S. D. Unshiand and B. R. Singh, On the respiratory organs of *Monopterus cuchia* (Hamilton 1822), J. Marph, 1968, No. 124, pp. 423-444.

[9] S.S.T. Nasar, Backyard eel culture, International Institute of Rural Reconstruction, Silang, Cavite, Philippines,1997, pp 88.

[10] B.F. McPherson, R.L. Miller, K. H. Haag, Bradner and Anne, Water quality in Southern Florida, 1996-98: U.S. Geological Survey Circular 1207, 2000, pp. 32

[11] N. T. Narejo, S. M. Rahmatullah, and M. M. Rashid, Reproductive biology of air-breathing freshwater mud eel, *Monopterus cuchia* (Hamilton) from Bangladesh. Indian J. Fish. 2003, pp. 395-399.

[12] F. M. Miah, F. Haque, M. R. Mia, E. Jannat, H. Ali, M. M. A. Quddus, and M. K. Ahmed, "Molecular Identification and Sexual Differentiation of Freshwater Mud Eel, *Monopterus cuchia*". Universal journal of Agriculture Research, 2013, Vol. 1, No. 3, pp. 54-58.

[13] D. A. Jahan, J. Rashid, M. M. Khan, and Y. Mahmud, Reproductive biology and gonad histology of mud eel, *Monopterus cuchia* (Hamilton, 1822), Int. J. Life Sc. Bt & Pharm., 2014, Vol. 3, No. 1, pp. 231-239.

[14] M. F. Miah, H. Ali, E. Zannath, T. M. Shuvra, M. N. Naser and M. K. Ahmed, Breeding biology and induced breeding status of freshwater mud eel, *Monopterus cuchia.* World Academy of Science, Engineering and Technology International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, (2015), Vol. 9, No. 6, pp. 633-637.

[15] M. M. Hasan, B. S. Sarker, K. M. S. Nazrul, M. M. Rahman and A. Al-Mamun, Marketing channel and export potentiality of freshwater mud eel (*Monopterus cuchia*) of Noakhali region in Bangladesh, Int. J. Life Sc. Bt and Pharm. Res., 2012, Vol. 1, No. 3, pp. 226-233.

[16] M. S. Hossian, and M. A. Mazid, A manual on development of floodplain fisheries, Fisheries Research Institute, Mymensingh, 2, 1995.

[17] IUCN-Bangladesh, Red Book of Threatened Fishes of Bangladesh. The World Conservation Union, Bangladesh, 2000, pp. 116.

[18] M. F. Miah, H. Ali, E. Jannat, M. N. Naser and M. K. Ahmed, Rearing and production performance of freshwater mud eel, *Monopterus cuchia* in different culture regimes. Advances in Zoology and Botany, 2015, Vol. 3, No. 3, pp. 42-49.

[19] M. Zaher and M.A. Mazid, Aquafeeds and feeding strategies in Bangladesh, p. 161-180. In M.B. New, A.G.J. Tacon and I. Csavas (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992, Bangkok, Thailand. FAO-RAPA/AADCP, Bangkok, Thailand. 1993, 434 p.

[20] M. M. A. Quddus, A. K. Banerjee, F. Parveen, R. Ara, and M. P. Costa, "Development of social fishery technology using small indigenous fishers of Bangladesh". Dhaka University J. of Biol. Sci., 2000, Vol. 9, No. 2, pp. 131-138.

[21] M. S. Alam, M. S. Islam and M. S. Alam, DNA Fingerprinting of the Freshwater Mud Eel, *Monopterus cuchia* (Hamilton) by Randomly Amplified Polymorphic DNA (RAPD) Marker. International Journal of Biotechnology and Biochemistry, 2010, Vol. 6, No. 2, pp. 271-278.

[22] S. L. He, X. W. Liu, Z. L. Guo, H. Jin. And J. P. Zhang, The genetic diversity of three species of *Monopterus.* J. Hunan Agric. Univ., 2004, Vol. **30,** 145-147

[23] G. U. Ahmed, M. N. Akter, S. A. Nipa, and M. M. Hossain, "Investigation on health condition of a freshwater eel, *Monopterus cuchia* from Ailee beel, Mymensingh, Bangladesh". J. Bangladesh Agril. Univ. 2009, Vol. 7 No. 2, pp. 421–426.

[24] G. M. Hughes, B. R. Singh, and R. N. Thakur, Areas of the air-breathing surface of *Amphipnous cuchia* (Ham.), Proc. Natl. Acad. Sci. India, 1974, Vol. 40, No. B, pp.379-392.

[25] M. F. Miah, P. Guswami, R. Al Rafi, A. Ali, S. Islam, M. M. A. Quddus and M. K. Ahmed, Assessment of Genetic Variability among Individuals of Freshwater Mud Eel, *Monopterus cuchia* in a Population of Bangladesh. American International Journal of Research in Science, Technology, Engineering & Mathematics, 2013, Vol. 3, No. 2, pp. 176-181.

[26] M. F. Miah, M. N. Naser and M. K. Ahmed, The freshwater mud eel, *Monopterus cuchia*-a review. Journal of Global Biosciences, 2015, Vol. 4, No. 3, pp. 1780-1794.

[27] M. Shafi, and M. A. Quddus, Bangladesh Matshyo Sampad. Bangla Academy, Dhaka, 1982, pp. 245-246.

[28] P. K. Talwar, and V. G. Jhingran, Inland Fishes of India and Adjacent Countries. Oxford and IBH Publishing Co. Pvt. Ltd. Calcutta, 1991, Vol. I, pp. 541.

[29] K. Schwenk, A. Sand, M. Boersma, M. Brehm, E. Mader, D. Offerhaus and P. Spaak, Genetic markers, genealogies and biogeographic patterns in the cladocera. Aquatic Ecology, 1998, Vol. 32, pp. 37–51

[30] R. B. Wei, G. F. Qiu and R. Song, Genetic diversity of rice field eel (*Monopterus albus*) in China based on RAPD analysis. Asian Fish. Sci., 2006, Vol. 19, pp. 61-68.

[31] S. Yin, J. Li, G. Zhou, Y. Liu, Population genetic structure of rice field eel (*Monopterus albus*) with RAPD markers. Chin. J. Appl. Environ. Biol., 2005, Vol. 11, pp, 328-332 (in Chinese with English abstract).

[32] W. Li, W. Sun and J. F. C. Zhang, Genetic diversity of wild and cultured swamp eel (*Monopterus albus*) populations from central China revealed by ISSR markers. Biologia, 2013, Vol. 68, No. 4, pp. 727-732.

[33] W. T. Li, X. L. Liao, X. M. Yu, L. Cheng and J. Tong, Isolation and characterization of polymorphic microsatellites in a sex-reversal fish, rice field eel (*Monopterus albus*). Molecular Ecology, 2007, Notes 7.

[34] L. Lei, L. Feng, T. Rong Jian and G. H. Yue, Characterization and multiplex genotyping of novel microsatellites from Asian swamp eel, *Monopterus albus*. Conservation Genet Resour., 2012, Vol. 4, pp. 63-365.