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RAPD based genetic diversity of freshwater snail, *Pila polita* in Bangladesh

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Abstract

Bangladesh is enriched with fish and other aquatic organisms and the freshwater snail *Pila polita* is one of them. Like other snails *Pila polita* is eco-friendly and economically important species, however, study related to genetics particularly diversity and relationships of this snail is very limited. This was the first genetic research of Bangladesh on this species. In this research Random Amplified Polymorphic DNA (RAPD) technique was performed for evaluating the genetic variability among individuals of *Pila polita*. Seven individuals of *Pila polita* were analyzed with three random primers such as B 03, C 04 and OPB 12. A total of 11 polymorphic loci were found in experimental samples. In this analysis, highest polymorphism (35.29%) was showed by the primer B03 and lowest polymorphism (25%) was showed by the primer OPB12. The highest and lowest inter-individual pair wise similarity was recorded 4 and 1 respectively. Genetic distance was observed with values highest 0.944 and lowest 0.556 among these experimental individuals. The highest and lowest linkage distance among individuals was observed 17.0 and 5.0 respectively. Nei's genetic similarity was observed whereas highest and lowest values were recorded 0.500 and 0.111 respectively. In the cluster analysis, it was observed that most of individuals were distinctly related with each other.

Keywords: *Pila polita*, Genetic diversity, RAPD, Bangladesh

1. Introduction

Bangladesh is enriched with aquatic biodiversity and snails are one of the important gastropod which is available in the freshwater habitat and eco-friendly in aquatic environment [1]. Among 450 species of snails in Bangladesh [2] the freshwater apple snail, *Pila polita* is one of the abundant and commercially valued molluscs. They have enormous important role in the ecosystem help to maintain healthy aquatic environments by acting as a bio filter, a pre-requisite for conserving biodiversity [3]. They can also be used as food for many other animals and can graze vast amounts of algae and detritus particularly observed in rivers [4]. People of Muslim community of Bangladesh do not consume snails [5], however, identified 29 groups of tribal people that consume this *Pila polita* and other snails flesh.

Snail meat can be used as a prawn and shrimp feed in the south-western part of the Bangladesh [6], as it is major exports sectors of Bangladesh [7]. The snails shell is used for the production of lime and fertilizer in this country [8]. The chemical composition of this snail shell can play an important role in waste water treatment either as a coagulant or adsorbent [9]. It is also used as a traditional medicine in Thailand for the treatment of a skin disease locally called "sedge" [10]. The freshwater snail *Pila polita* come vary in shape and size [11], [12], however, a variety of habitats and environmental conditions can influence the external characteristics [13]. As Bangladesh blessed with rich biodiversity but in recent years several species have become endangered and many can be extinct in few years due to environmental cues [14]. A drastic reduction of snail diversity has been occurred in Bangladesh due to over fishing, siltation, dam construction and other channel modifications, industrial and agricultural pollution and so on [15]. Therefore, in order to make suitable management schemes for any species together with establishing the conservation programs, it is essential to have basic knowledge on the presence of a certain populations or the numbers of a particular species in a specific area [16]. Genetic diversity is rapid changing process while presence of high genetic variation in a population is indicated that some individuals have a better chance of surviving and reproducing. However, several research have conducted on their abundance but genetic based research is still relatively low and also estimating genetic diversity of *Pila polita* in this country are completely new. So, its various importance taking into account, *Pila polita* were selected for the study. Therefore, the aim of this study was to obtain a definite genetic status particularly genetic diversity of this apple snail in Bangladesh based on RAPD data.

2. Materials and Method

2.1 Collection of sample and identification of species

Snail sample was collected by hand and a small fishing net from the experimental fish pond which is under the department of Genetic Engineering and Biotechnology (GEB) at Shahjalal University of Science and Technology (SUST), Sylhet. Collected snails were brought into the general laboratory of GEB through a plastic container. The species of the *Pila polita* was morphologically identified according to the shell characteristics [11], [12], [13].

2.2 Tissue isolation and preservation

The snail shell was crushed gently by a hammer and a sterilized forceps was used to pry the shell from the soft tissue. The muscles were kept in seven different eppendorf tubes containing 100% ethanol and preserved them at – 20 degree Celsius until DNA extraction.

2.3 DNA extraction

DNA was extracted with a long protocol from adductor muscles of the samples while visceral tissues were used in original protocol [17].

2.4 Checking quality of extracted DNA

DNA quality was checked by electrophoresis on 1% agarose gel with 3µl DNA where 1kb plus ladder was used to compare migration of DNA. The gel was run at 70 volt for 40 minutes and photograp was taken by gel documentation system. Clear bands were found from each of the individuals where medium concentration of DNA was present.

2.5 PCR amplification

In this experiment, three decamer RAPD primers such as B 03 (5'- CAT CCC CCT G-3'), C 04 (5'- CCG CAT CTA C-3') [18] and OPB 12 (5'-CCT TGA CGC A-3') [19] were adopted for studying genetic diversity. PCR reactions were performed in a 15µl reaction mixture for each sample with 8µl of master mix (Promega Hot Start), 1µl of primer, 2µl of template DNA and 4µl deionized distilled water. PCR reaction was conducted for pre heating 94°c 3 minutes, denaturation at 94°c for 1 minutes; annealing temperature for this PCR was 34˚c (for B 03 and OPB 12) and 35 °c (for C 04) in 1 minute and 2 minutes for elongation or extension at 72˚c. A final step of 7 min for 72°c was added to allow complete extension of the amplified fragments. The PCR was run for 35 cycles.

2.6 Checking PCR products

PCR products were checked by gel electrophoresis on 2% agarose with 3µl DNA where 1kb plus ladder was used to compare migration of DNA. The gel was run at 70 volt for 40 minutes and photograph was taken by gel documentation system.

2.7 Data Analysis

Using different software and equations, RAPD data of this experiment was interpreted. The software AlphaEaseFC 4.0 was used for measuring molecular weight of bands. Pair wise similarity was calculated by D = 1- Nxy / Nx+Ny- Nxy, where, D = the genetic distance between sample x and y, Nxy = number of band shared by sample x and y, Nx =the number of bends in sample x, Ny =the number of bends in sample y. Nei's genetic similarity among individuals were measured by F= 2Nxy/ Nx+Ny, where, F= Nei‟s genetic similarity, Nxy= Number of shared Band between X and Y, Nx= Number of bend in X, Ny = Number of band in Y. Polymorphism information content (PIC) was measured as

$$PIC = 1 - \sum_{j=1}^{n} Pij2$$

Where, Pij is the frequency of the ith allele for the marker summed over 'n' alleles. Linkage distance based on new.sta and Intra-individual relationship through dandogram was analyzed by using software "Statistica".

3. Results

3.1 DNA profiling and band scoring

Data Scoring and band scoring was observed by three arbitary primers to study genetic diversity of *Pila polita*. 1kb plus ladder was used as molecular weight marker that ranges from 75bp to 20000 bp (General TM). Each amplified band profile was defined by either the presence (1) or absence (0) of bands at particular positions on the gel which was done separately for each individual and each primer. Analysis showed that primer B 03 and C 04 produce different band and in case of B 03 maximum 4 bands were observed in each individual and size was ranged from 75-1000 bp. Maximum 4 bands were observed in each individual in case of primer C 04 from 75-500 bp length. However, in case of primer OPB 12 bands were found 1-4 between 75-1000 bp lengths.

3.2 Bands Summary

A total of 55 bands were detected among 7 *Pila polita* individual genotypes out of which 16 were polymorphic bands (Table 3.4) and polymorphism was observed in all markers. The highest number of bands were amplified by the primer OPB12 (20) and the lowest number of bands (17) were amplified by the primer B03. Highest polymorphism (35.29%) was showed by the primer B03 and lowest polymorphism (25%) was showed by the primer OPB12. The highest numbers of bands (2.85) per individual was amplified from the primer OPB12 and the lowest numbers of bands (2.42) per individual was amplified from the primer B03. The primer C04 amplified 2.63 numbers of bands per individual sample. Primer OPB12 showed highest Polymorphism Information Content (PIC) (0.915) while an average PIC was 0.949.

Table 1: Summary of the bands revealed from three primers based on RAPD band analysis

Primers	Size of DNA bands (bp)	*P. polita*				
		Total number of DNA bands	Number of polymorpic loci	Percentage of polymorpic loci (%)	Number of bands per sample	Polymorphism information content
B03	143-1105	17	6	35.29	2.42	0.8997
C04	85-485	18	5	27.78	2.63	0.7347
OPB12	126-1670	20	5	25	2.85	0.915
Total		55	16			
Average		18.33	5.33	29.36		0.949

3.3 Inter-individuals pair wise similarity

Inter-individual pair wise similarity was found highest 4 but maximum was recorded 2 (Table 2). Lowest value was observed 1.

Table 2: Inter-individual pair wise similarity of *Pila polita*

----	Individual 1	Individual 2	Individual 3	Individual4	Individual 5	Individual 6	Individual 7
Individual 1	---	3	1	3	3	2	1
Individual 2		--	3	3	2	2	1
Individual 3			---	1	1	2	4
Individual 4				--	2	2	2
Individual 5					--	4	1
Individual 6						---	4
Individual 7							---

3.4 Genetic Distance

Genetic distance was found from 0.556 in between individuals 5 and 6 to 0.944 in between samples 1 and 3. Overall much higher genetic distance was recorded in the other individuals (Table 3.6).

Table 3: Genetic distance among individual of *Pila polita*

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
Individual 1	--	0.800	0.944	0.727	0.727	0.867	0.938
Individual 2		--	0.813	0.727	0.833	0.867	0.938
Individual 3			--	0.929	0.929	0.889	0.714
Individual 4				--	0.750	0.818	0.818
Individual 5					--	0.556	0.917
Individual 6						--	0.667
Individual 7							--

3.5 Measuring Nei's genetic similarity (F)

Highest Nei's genetic similarity was found between individual pair 6 and 7 (0.500) while lowest similarity (0.111) was observed between 2 other pairs (1 and 2, 3 and 6). However, diverse similarity was found in between themselves (Table 4).

Table 4: Nei's genetic similarity among individual of *Pila polita*

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
Individual 1	--	0.111	0.105	0.429	0.429	0.235	0.118
Individual 2		--	0.316	0.429	0.286	0.235	0.118
Individual 3			--	0.133	0.133	0.111	0.444
Individual 4				--	0.400	0.308	0.308
Individual 5					--	0.308	0.154
Individual 6						--	0.500
Individual 7							--

3.6 Linkage Distance

In linkage distance analysis, the values ranged from 5.0 to 17.0 whereas relatively higher linkage distance was observed between all other individual pairs (Table 5).

Table 5: Squared Euclidean distances of *Pila polita*

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
Individual 1	0						
Individual 2	13	0					
Individual 3	17	14	0				
Individual 4	8	9	13	0			
Individual 5	8	11	13	6	0		
Individual 6	13	14	14	9	5	0	
Individual 7	15	16	10	9	11	8	0

3.7 Cluster Analysis

The UPGMA clustering system was generated six clusters (Figure 1) in which individual 5 and 6 was formed one genetic clusters (Cluster 1) at a linkage distance between 4 and 6 which means they are most closely related than the others. These two individuals form another cluster (cluster 2) with individual 4 at a linkage distance was slightly lower than 8. Individual 1 form another cluster (cluster 3) at a linkage distances was found higher than 8 and closely related with above three individuals. At linkage distance higher than 10, another cluster (cluster 4) was seen that connects individuals 7 with first four individuals mentioned above. Individual 2 was also formed another cluster (cluster 5) at linkage distance higher than 12 and lastly, the rest were connected by another cluster (cluster 6) with individual 3 at linkage distance lower than 12.

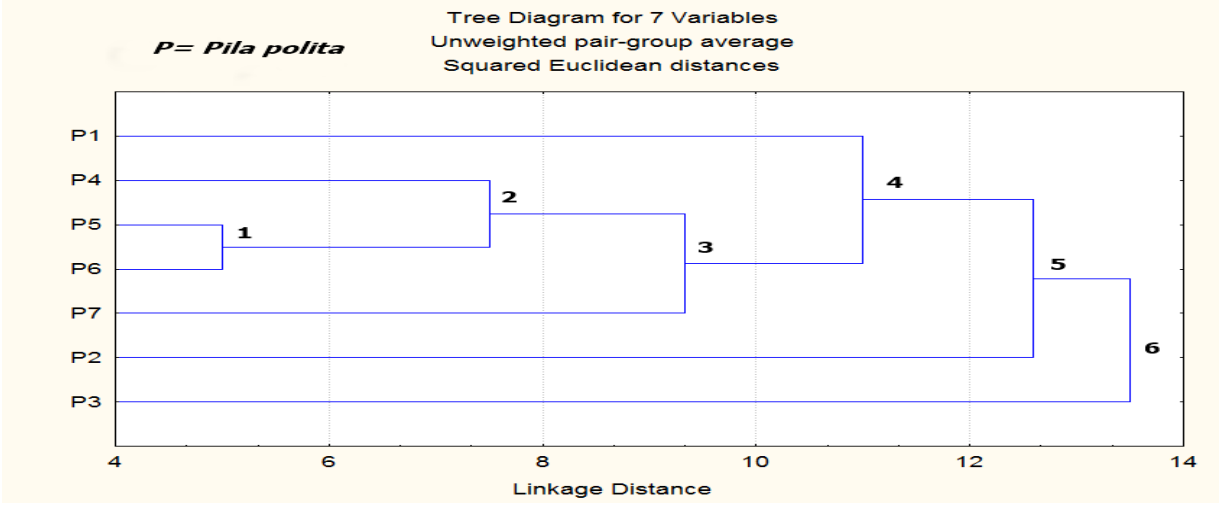

Fig 1: Cluster analysis of *Pila polita* genotypes

4. Discussion

The freshwater apple snail, *Pila polita* have valuable advantages in terms of environmental friendly, food and other purposes. Assessing genetic variation is a key tool for understanding biological potency of an organism. Due to the limitations of lab facilities, as laboratories of GEB is a new department, this research was considered only 7 individual of *Pila polita* with RAPD assay. However, it would be used as an important study for future investigation on this species in Bangladesh.

A study was conducted in Thailand by RAPD technique based on analysis of the genetic diversity of introduced *Pomacea canaliculata* and native (*Pila*) apple snails [10]. From the research in Thailand, all the species e.g., *Pomacea canaliculata,* and four native apple snails; *Pila ampullaceal, P. angelica, P. pesmei* and *P. polita* were polymorphic and the average number of polymorphic bands of each species was nearly identical although a lower level of polymorphism was observed in *P. angelica* and it is suggested the potential of RAPD analysis for determination of inter- and intraspecific genetic differences of apple snails in Thailand. But in this research higher genetic variability were found. Another important notification is that, genetic diversity and molecular diagnostic markers of exactly these species in Thailand were also studied by PCR-RFLP of cytochrome oxidase subunit I and COI surprisingly the result was contradictory to that from RAPD analysis. This should have resulted because of the limited samples of native apple snails [10].

During this experiment, three random primers were applied to measure the genetic variability among 7 individuals of freshwater snails. The primers amplified total 55 DNA bands with an average of 18.33 bands where the size of the band ranges from 85- 1670 bp with a very high specificity. All the primers showed total 16 polymorphic loci with an average of 5.33 by each of the primers. Experimented data indicate that the intra-specific polymorphism was recorded in primer B-03 was 35.29 %, CO-04 was 27.78 % and OPB-12 was 25 % respectively with an average of 29.36 % by each. With the same primers two different apple snails were also studied at the same time on *Pila gracilis* [20] and *Pila globosa* [21] respectively and almost similar results were found.

5. Conclusion

Snails have significant importance in various sectors and are very much needed for social and economic development of the country. The research focused on the analysis of the genetic diversity of *Pila polita* that's ultimate goals is to develop breeding, culture, production and conservation of this species. Overall a relative higher genetic diversity was observed among the 7 individuals of *Pila polita* and this is undoubtedly a good result but due to only seven individuals this result may be unsatisfactory.

6. Acknowledgement

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7. References

1. Nahid S.A.A. Henriksson PJG, Wahab MA. Value-chain of freshwater apple snail (*Pila globosa*) used for on farm feed in the

freshwater prawn farming sector in Bangladesh. ISSN: 2224-0616 Int. J. Agril. Res. Innov. And Tech 2003; 3(2): 22-30.
2. Gain P. Chanda beel. Shrimps attacks snais and environment. In: P. Gain (ed.), Earth Touch. The Society for Environment and Human Development (SEHD), Dhaka, Bangladesh.1998; P 17-20.
3. Jahan MS, Akter MS, Sarker MM, Rahman MR and Pramanik MN. Growth ecology of *Pila globosa* (Swainson) (Gastropoda: Pilidae) in simulated habitat. Pakistan J. Biol. Sci 2001; 4 (5): 581-584.
4. Johnson PD. Sustaining America's Aquatic Biodiversity Freshwater Snail Biodiversity and Conservation. Publication 2009; 420-530.
5. Saha BK. Ecology and bio-eonomics of the freshwater edible snails of Bangladesh.PhD dissertation. Rajshahi University, Bangladesh. 1998; 162 p.
6. Baby RL,Hasan I, Kabir KA And Naser MN. Nutrient analysis of some commercially important molluscs of Bangladesh. J. Sci. Res 2010; 2 (2): 390-396.
7. DoF. National Fish Week Compilation. Department of Fisheries (DoF), Ministry of Fisheries and Livestock, Dhaka, Bangladesh. 2011; 136 p.
8. Ahmed N. Socio-economic aspects of freshwater prawn culture development in Bangladesh. PhD dissertation. Institute of Aquaculture, University of Stirling, Scotland, UK.
9. Jatto EO, Asia LO, Egbon EE, Otutu, JO. Chukwuedo M, Ewansiha CJ. Treatment of Waste Water from Food Industry Using Snail Shell 2010; 10 (3): 5031-5043.
10. Thaewnon-ngiw B, Lauhachinda N, Sri-Aroon P, Lohachit C. Distribution of *Pila polita* in a southern province of Thailand 2003; 2:128-30.
11. Bombeo-Tuburan I, Fukumoto S. and Rodriguez KM. Use of the golden apple snail, cassava, and maize as feeds for the tiger shrimp, *Penaues monodon* in ponds. Aquaculture 1995; 131 (1-2): 91-100.
12. Sing R. Seasonal variation in calorific values of apple snail and *Bellaniya bengalensis*. J. Ecobiol 1991; 3 (2): 98- 101.
13. Keawjam R. The apple snails of Thailand: distribution, habitats and shell morphology. Malacol Rev. 1986; 19:61-81.
14. Jahan MS and Rahman MR. Environment and Agriculture: At the Crossroad of the New Millennium, pp 2000; 522-526.
15. Jahan MS, Rahman MR and Biswas L. Comparative morphometrical indices of freshwater Lymnaeid snails of Bangladesh. Bangladesh Journal of Zoology 1999; 27 (2): 165-168.
16. 16 Klinbunga B, Thaewnon-ngiw K, Phanwichie N, Sangduen N, Lauhachinda, P,Menasveta. Genetic diversity of introduced *(Pomacea canaliculata)* and native *(Pila)* apple snails in Thailand revealed by Randomly Amplified Polynorphic DNA (RAPD) Analysis 2003; 20 (4): 289-306.
17. Krause MK. Molecular Approaches to Zoology-Molecular Zoology: Advances, Strategies, and Protocols Joan D. Ferraris Stephen R. Palumbi. Bioscience 1997; 47 (3):194-196.
18. Schwenk K, Sand A, Boersma M, Brehm M, Mader E, Offerhaus D and Spaak P. Genetic markers, genealogies and biogeographic patterns in the cladocera. Aquatic Ecology, 1998; 32: 37–51.
19. 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; **6**(2): 271-278.
20. Mahzabin S. RAPD based genetic diversity of freshwater snail, *Pila gracilis* in Bangladesh. B.Sc. (Hon's) thesis, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh, 2014.
21. Rahman SE. Assessment of diversity of *Pila globosa* by using RAPD analysis. B.Sc. (Hon's) thesis, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh, 2014.

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