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Population Genetic Structure of Paradise Threadfin *Polynemus paradiseus* (Linnaeus, 1758) Revealed by Allozyme Marker

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ABSTRACT

To elucidate genetic differentiation in three river populations (Tentulia, Paira and Kirtonkhola) of *Polynemus paradiseus*, ten enzymes encoded by seventeen presumptive loci were screened using allozyme electrophoresis marker, where five were polymorphic (Est-1*, Gpi-1*, Gpi-2*, G3pdh-2* and Mdh-1*). The mean proportions of polymorphic loci were observed 17.65, 29.41 and 11.76% in Tentulia, Paira and Kirtonkhola populations, respectively. The highest mean number of allele per locus and mean proportion of heterozygous loci per individual were observed in the Paira population (1.294 and 16.667%, respectively). The highest observed heterozygosity (H_o) and average expected heterozygosity (H_e) were 0.078 and 0.050, respectively found in Tentulia population. The highest pair-wise population differentiation (F_{ST} = 0.148) and lowest gene flow (N_m = 1.443) were found in Tentulia-Kirtonkhola indicate the close relationship among them. Based on genetic distance, UPGMA dendrogram showed that the three river populations of *P. paradiseus* constructed two clusters. Paira and Kirtonkhola populations made one cluster (D = 0.001) and separated from Tentulia population by the genetic distance of 0.014. The results suggested that the considerable genetic variation is maintained among the natural *P. paradiseus* populations.

Key words: Genetic structure, *Polynemus paradiseus*, allozyme electrophoresis

INTRODUCTION

Polynemus paradiseus (Linnaeus, 1758) commonly known as Paradise threadfin belongs to the family Polynemidae (Perciformes) has been widely distributed in the Indo-pacific Ocean including the Bay of Bengal (Rahman, 2005; Motomura *et al*., 2002; Ullah *et al*., 2012; Rashed-Un-Nabi and Ullah, 2012). It is locally known as Ramsosh or Tapasi and considered one of the most important indigenous perch in the coastal region of Bangladesh (Talwar and Jhingran, 1991; Rahman, 2005). This species is fetching high market price due to their good taste and deliciousness. A few years ago, *P. paradiseus* was available almost all round the year in coastal waters, estuaries, mighty rivers like Padma and Meghna and in the Gangetic river system of India and Bangladesh (Talwar and Jhingran, 1991; Rahman, 2005). But now this fish is not available in those water bodies and is going to be endangered day by day like other indigenous species (Allendorf and Phelps, 1980; Sarkar and Bhattacharya, 2003; Siddik *et al*., 2013). There is an ever declining tendency in this fishery in recent years due to apparent deterioration of the habitat, over-exploitation and indeed lack of proper management (IUCN., 1998). The increasing water pollution and destruction of breeding grounds for various reasons restricted the natural breeding of *P. paradiseus*. Consequently, the wild populations become genetically poor and hence there will be no option for betterment of the aquaculture stocks through artificial propagation in future. Thus there is an urgent need for knowing the genetic status of the wild stocks of the species.

Allozyme electrophoresis is a molecular marker that is used as an effective tool for fish population studies and fishery management (Utter, 1991; Aziz *et al*., 2011; Islam and Hossain, 2012). The present study was a preliminary investigation of the genetic status of the three wild samples of *P. paradiseus* population using horizontal starch gel electrophoresis method. Such knowledge would help monitoring the genetic quality as a bid to take appropriate management measures for the populations in nature.

MATERIALS AND METHODS

Samples collection: Samples of *Polynemus paradiseus* were collected directly from the local fishers living adjacent to the southern coastal rivers of Bangladesh (Fig. 1). A total of 30 individuals were collected from each river of Paira, Kirtonkhola and Tentulia on 25 May, 22 June and 27 July, 2013, respectively. Once collected, fish were iced, transported to the laboratory and muscle samples were taken and stored at -80°C until electrophoretic analysis.

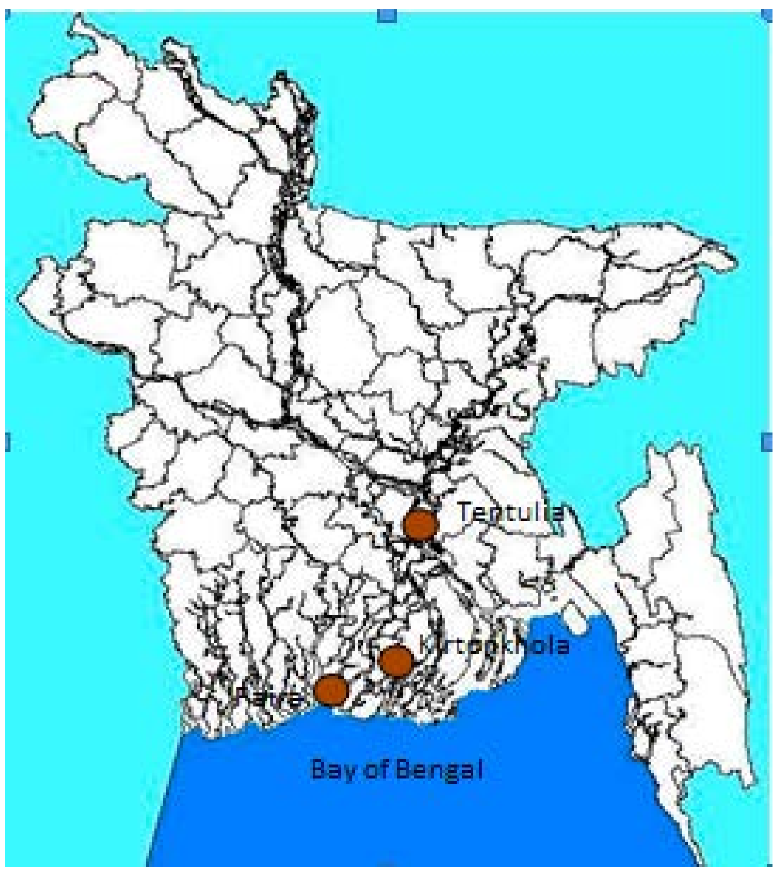

Fig. 1: Sampling site of *Polynemus paradiseus*

Table 1: Ten enzymes examined and nine showed clear banding using muscle tissue

Enzymes	Locus	Enzyme patterns	EC No.
Alcohol dehydrogenase (ADH)	Adh-1*	Dimer	1.1.1.1
	Adh-2*		
Esterase (EST)	Est-1*	Monomer	3.1.1.1
Glucose-6-phosphate isomerase (GPI)	Gpi-1*	Dimer	5.3.1.9
	Gpi-2*		
Glycerol-3-phosphate dehydrogenase (G3PDH)	G3pdh-1*	Dimer	1.1.1.8
	G3pdh-2*		
Glucose-6-phosphate dehydrogenase* (G6PDH)	G6pdh-1*	Dimer	1.1.1.49
Isocitrate dehydrogenase (IDHP)	Idhp-1*	Dimer	1.1.1.42
	Idhp-2*		
Lactate dehydrogenase (LDH)	Ldh-1*	Tetramer	1.1.1.27
	Ldh-2*		
Malate dehydrogenase (MDH)	Mdh-1*	Dimer	1.1.1.37
	Mdh-2*		
Phosphoglucomutase (PGM)	Pgm*	Monomer	5.4.2.2
Sorbitol dehydrogenase (SDH)	Sdh-1*	Tetramer	1.1.1.4
	Sdh-2*		

*G6PDH did not show clear resolution

Allozyme electrophoresis: This experiment was performed by using allozyme markers through horizontal starch gel electrophoresis method of Shaw and Prasad (1970). The electrophoresis was conducted using muscle tissue with amine-citrate buffer (CA 6.1) (Clayton and Tretiak, 1972) and Tris citric acid buffer (TC-1) (Shaw and Prasad, 1970). The enzymes analyzed, E.C. numbers, abbreviation of enzymes and enzyme patterns used for horizontal starch-gel electrophoresis are shown in Table 1. After electrophoresis, the gel slices (about 1 mm thickness) were histochemically stained for different enzymes with some modifications. Loci were numbered consecutively from the anodal to cathodal end. Thus, the most anodal locus was designated as '1'. The electrophoretic bands corresponding to multiple alleles at each locus were alphabetically named as *a, *b, *c etc. in order to detection.

Genetic data analysis: The allele frequencies were calculated simply by direct count of the proportion of different alleles. The mean proportions of polymorphic loci per population, the mean number of alleles per locus and the mean proportion of heterozygous loci per individuals were determined with the assistance of POPGENE (version 1.31) (Yeh *et al.*, 1999) computer package program. Expected heterozygosity (H_e) and observed heterozygosity (H_o) were also examined according to Nei and Roychoudhury (1973), with the help of POPGENE (version 1.31) (Yeh *et al.*, 1999) computer package program. Genetic differentiations (F_{ST}) and gene flow (N_m) were performed using GeneAlEx (version 6) (Peakall and Smouse, 2005) computer package program. Based on the Nei's genetic distance (D) (Nei, 1972); a dendrogram was constructed by the UPGMA (unweighted pair group method using arithmetic average) method (Nei, 1978), with the assistance of POPGENE, (version 1.32) (Yeh *et al.*, 1999) computer package program.

RESULTS

Ten enzymes were studied and seventeen putative loci were identified in three river populations of *P. pangasius* (Table 2). Among the 17 loci, five were showed polymorphic

Table 2: Allele frequency at 17 presumptive loci of *Polynemus paradiseus* populations

	Allele frequency			
Locus	Allele	Tentulia	Paira	Kirtonkhola
Adh-1*	a*	1.000	1.000	1.000
Adh-2*	a*	1.000	1.000	1.000
Est-1*	a*	0.817	0.850	0.817
	b*	0.183	0.150	0.183
P		0.244^{ns}	0.563^{ns}	0.182^{ns}
Gpi-1*	a*	1.000	0.933	1.000
	b*	-	0.067	-
P		ns	0.737^{ns}	ns
Gpi-2*	a*	1.000	0.900	1.000
	b*	-	0.100	-
P		ns	0.581^{ns}	ns
G3pdh-1*	a*	1.000	1.000	1.000
G3pdh-2*	a*	0.533	0.967	1.000
	b*	0.467	0.033	-
P		0.000***	0.895^{ns}	ns
G6pdh-1*	a*	1.000	1.000	1.000
Idhp-1*	a*	1.000	1.000	1.000
Idhp-2*	a*	1.000	1.000	1.000
Ldh-1*	a*	1.000	1.000	1.000
Ldh-2*	a*	1.000	1.000	1.000
Mdh-1*	a*	0.983	0.967	0.917
	b*	0.017	0.033	0.083
P		1.000^{ns}	0.895^{ns}	0.659^{ns}
Mdh-2*	a*	1.000	1.000	1.000
Pgm*	a*	1.000	1.000	1.000
Sdh-1*	a*	1.000	1.000	1.000
Sdh-2*	a*	1.000	1.000	1.000

P: Probability of chi-square value, ***,*Significant at $p<0.01$ and $p<0.10$, ns: Non-significant

characteristics. Four loci, Mdh-1*, G3pdh-2*, Gpi-1* and Gpi-2* were produced two genotypes (*aa and *ab) by two alleles (*a and *b), one locus Est-1* produced three genotypes (*aa, *ab and *bb) by two alleles (*a and *b). Other twelve loci viz. Ldh-1*, Ldh-2*, Mdh-2*, Pgm*, Adh-1*, Adh-2*, Idhp-1*, Idhp-2*, Sdh-1*, Sdh-2*, G6pdh-1* and G3pdh-1* produced homozygous genotype (*aa) with fixed allele *a. Among the 17 loci, Tetulia population showed three (Mdh-1*, Est-1* and G3pdh-2*), Kirtonkhola population showed two (Mdh-1* and Est-1*) and Paira population showed five (Mdh-1*, Est-1*, Gpi-1*, Gpi-2* and G3pdh-2*) polymorphic loci (Table 2). The Tentulia population showed significant variation only in allele frequency of G3pdh-2* locus whereas no significant variation occurred in allele frequencies in other populations (Table 2).

The mean proportion of polymorphic loci in Tentulia, Paira and Kirtonkhola populations were 17.67, 29.41 and 11.76%, respectively. The mean proportion of heterozygous loci per individuals for all populations was 11.113% in average and ranged from 6.667 (Kirtonkhola) to 16.667% (Paira). The observed heterozygosity (H_o) was 0.048 in average and ranged from 0.024 (Kirtonkhola) to 0.078 (Tentulia). The average expected heterozygosity (H_e) was 0.039 and ranged from 0.027 (Kirtonkhola) to 0.050 (Tentulia) (Table 3).

Table 3: Genetic variabilities at 17 loci of *Polynemus paradiseus* populations

Population	Mean proportion of polymorphic loci* (%)	Mean No. of alleles (Na) per locus	Mean No. of effective alleles (Ne) per locus	Mean proportion heterozygous loci of per individual (%)	Heterozygosity H_o	H_e	H_o/H_e
Tentulia	17.65	1.177	1.085	10.000	0.078	0.050	1.578
Paira	29.41	1.294	1.047	16.667	0.041	0.041	1.000
Kirtonkhola	11.76	1.118	1.039	6.667	0.024	0.027	0.867
Average	19.61	1.196	1.057	11.113	0.048	0.039	1.148

Table 4: Pair-wise and overall population differentiations (F_{ST}) and gene flow (N_m) in three *Polynemus paradiseus* populations

Populations	F_{ST} Pair-wise	F_{ST} Overall	N_m* Pair-wise	N_m* Overall
Tentulia-Paira	0.118		1.86400	
Paira- Kirtonkhola	0.017	0.131	14.877	1.661
Tentulia-Kirtonkhola	0.148		1.44300	

*N_m: Gene flow estimated from $F_{ST} = 0.25(1-F_{ST})/F_{ST}$

Table 5: Original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated among 3 populations of *Polynemus paradiseus* based on 17 loci

Population	Tentulia	Paira	Kirtonkhola
Tentulia	***	0.988	0.987
Paira	0.013	***	0.999
Kirtonkhola	0.014	0.001	***

Source: Nei (1972)

The summary of the genetic differentiation (F_{ST}) and gene flow (N_m) are given in Table 4. The Nei (1972) analysis of gene diversity within populations estimated the genetic differentiation (F_{ST}) and the gene flow (N_m) overall three populations are 0.131 and 1.661, respectively. In pair-wise analysis, comparatively higher N_m value (14.877) was estimated between the Paira and Kirtonkhola populations corresponding lower level of F_{ST} value (0.017) (Table 4). The genetic distance (D) values among three populations ranged from 0.001-0.014. The minimum genetic distance (D = 0.001) was observed between Kirtonkhola and Paira populations, while the maximum value (D = 0.014) was found between the Tentulia and Kirtonkhola populations (Table 5). The UPGMA dendrogram constructed from Nei (1972) genetic distances (Fig. 2) showed that Paira and Kirtonkhola populations formed a cluster by the genetic distance of 0.001 and separated from Tentulia population by the genetic distance of 0.014.

DISCUSSION

In the present study, ten enzymes (ADH, EST, G3PDH, G6PDH, GPI, IDHP, LDH, MDH, PGM and SDH) were used and 17 putative loci were identified where two loci (Est-1* and Mdh-1*) showed high variation in all three populations examined. In the present study, both the alleles *a and *b of Gpi-1* and Gpi-2* loci were present in Paira population, whereas these two loci had single allele (*a = 1.00) in Tentulia and Kirtonkhola populations. Only G6PDH enzyme did not show clear resolution, which might be due to buffer system, tissue and/or species specificity; the assumption also agrees with that of Khan (1999).

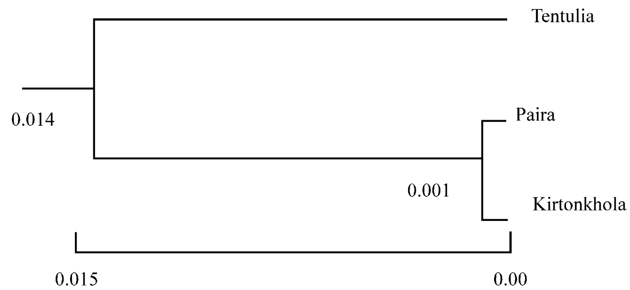

Fig. 2: UPGMA dendrogram showing the genetic distance (D) among three river populations. Source: Nei (1972)

The observed proportion of polymorphic loci per population ranged from 11.76-29.41% (average 19.61%) which was similar (average 18%) to that reported for 20 pangasiid catfish species by Pouyaud *et al.* (2000) but much lower (65.22%) than that of Yellow catfish, *Mystus nemurus* in wild and hatchery populations reported by Leesa-Nga *et al.* (2000) and 36.36% for four populations of Thai pangas, *P. hypophthalmus* reported by Eunus (2004). In this study, the mean proportion of polymorphic loci (19.61%) was similar to that obtained 19.4% by Barua *et al.* (2004) for *P. hypophthalmus*. Nevo *et al.* (1984) estimated polymorphic loci (P) as 15.2% (p = 0.95) for polymorphism in fish in general. Therefore, the studied *P. paradiseus* populations showed a lower level of polymorphism with comparison to the above mentioned fishes. The average heterozygous loci per individual obtained 11.113%, which was similar to the results of 11.25% obtained by Barua *et al.* (2004) and relatively lower than 13.33% obtained by Eunus (2004) for four hatchery populations of *P. hypophthalmus*. The observed heterozygosity (H_o) obtained in the present study ranged from 0.024-0.078 (average 0.048) was much lower than that (0.091) reported by Pouyaud *et al.* (2000). This value (0.048) was lower than that obtained by Barua *et al.* (2004) (0.059) and Eunus (2004) (0.072) for *P. hypophthalmus*. The present H_o values indicated that those corresponding to the Tentulia and Paira populations of *P. paradiseus* were closer to the average values of $H_o = 0.055$ obtained for teleosts (Kirpichnikov, 1992). Nevo (1978) reported that an average observed heterozygosity (H_o) value for bony fishes was 0.051. The expected heterozygosity (H_e) obtained in the present study ranged from 0.027-0.050 (average 0.039) in three populations. However, the H_e values obtained in the present study do not fall in the range of 0.02-0.03, which are generally considered as the lower margins of genetic variability for fishes (Nevo *et al.*, 1984; Kirpichnikov, 1992). The higher observed and expected heterozygosity ($H_o = 0.0784$ and $H_e = 0.0497$) exhibited by the Tentulia population indicated that the gene pool of the Tentulia river was maintained effectively.

The co-efficient of gene differentiation (F_{ST}) in all *P. paradiseus* populations examined (Nei, 1975) for all loci was 0.131, indicated the presence of population with a slight genetic differentiation and the number of individuals that migrate from one population to another is high ($N_m = 1.661$). The pair-wise population gene flow was higher (14.877) between the Paira-Kirtonkhola populations than all other between population comparisons with corresponding lowest F_{ST} value of 0.017. The F_{ST} value (0.131) of *P. paradiseus* populations as obtained in the present study was lower than that obtained (0.792) for *P. hypophthalmus* (Barua *et al.*, 2004).

Based on the Nei (1972) genetic distance (D-value), the UPGMA dendrogram showed that the three river populations can be grouped into two (Fig. 2). First group is comprised alone with

Tentuliar population and separated by the D = 0.014 from second one whereas second group comprised of Paira and Kirtonkhola populations. In second group, the Paira population was differentiated from Kirtonkhola population by D = 0.001. The observed genetic distances among the three populations of *P. paradiseus* in the present study are much lower than the findings of Pouyaud *et al.* (1998) who found the average distances within the species pangasiid catfish (D = 0.106) between population of Kalimantan and the population of Chao Phraya (D = 0.145) between population of Teluk Kuantan in Sumatra and population of sole in Japan. The present D values were much lower than that species (D = 0.366-1.181) reported by Na-Nakorn *et al.* (2002). Leesa-Nga *et al.* (2000) mentioned that the D-values of *Mystus nemurus* ranged from 0.005-0.164 and suggested that the highest genetic distance among them was the subspecies level. On the other hand, the genetic distance (D = 0.109) between interspecies of *P. nasutus* and *P. conchophilus* and also similar value (D = 0.158) shown in the distance between *P. bocourti* and *P. djambal* (Pouyaud *et al.*, 1998). The observed genetic distance differs between Tentulia and other populations might be due to geographical isolation because Tentulia river is geographically isolated from Paira and Kirtonkhola rivers. On the other hand, the Paira river is connected to the Kirtonkhola river in Barisal district. But in the present study, Paira sample was collected from the Patuakhali (near about to the Bay of Bengal) where it might less possibility of mixing of Kirtonkhola population.

CONCLUSION

The present study concludes that although there is considerable genetic variation exists among the wild populations of *P. paradiseus* but probably special care should be taken when taking management options. A broader scale study of *P. paradiseus* population differentiation would be useful as to investigate the population structure of this species over its whole distribution range and possible causes. The existing differentiation seems to be weak; it becomes of great importance the use of molecular markers with a higher polymorphism, such as microsatellites, which have been able to detect a greater degree of population diversity than allozymes.

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