

Genetic diversity of Ganges River sprat, *Corica soborna*, from Kaptai Lake and Kirtankhola River in Bangladesh

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Abstract. Ganges River sprat, *Corica soborna* Hamilton, is an indigenous fish species and an excellent source of animal protein with medicinal values in Bangladesh. Samples from two populations of this fish species were collected from Kaptai Lake and Kirtankhola River in Bangladesh to elucidate their genetic diversity based on Random Amplified Polymorphic DNA (RAPD) analysis. Ten primers were selected for this analysis: OPA-04; OPAL-04; OPA-03; OPF-01; OPG-05; OPG-04; OPA-09; OPAK-04; OPAW-09; OPA-02. A total of sixty-eight bands were found of which thirty-two were polymorphic and indicated a moderate level as 51.07% polymorphisms between these two fish populations. The size of the bands ranged between 200 to 1300 bp in their analysis. Based on the specific primer banding patterns, a maximum polymorphism of 100% was found for primer OPF-01; however, the lowest of 14.29% polymorphism was found for primer OPG-04 for these two populations. The genetic distance and genetic identity of 0.8210 and 0.4400, respectively, also indicated that these two populations were moderately distant from each other. A dendrogram based on Nei's (1972) genetic distance was constructed using the Unweighted Pair Group Method of Arithmetic Means (UPGMA), and it segregated these two populations into one major cluster C1. Therefore, the present study revealed that these two Ganges River sprat populations are moderately genetically diversified. An effective management program and policy should be undertaken to conserve and manage these indigenous fish species in Bangladesh.

Keywords: *Corica soborna*, Ganges River sprat, genetic characterization, Kaptai Lake, Kirtankhola River, RAPD

Introduction

Ganges River sprat, *Corica soborna* Hamilton, is categorized as a small indigenous species (SIS) of Bangladesh, and it is one of the dominant fish species in southwestern Bangladesh (Hossain et al. 2008) with a wide distribution in Bangladesh, India, Thailand, Malaysia, Brunei, and Indonesia (Talwar and Jhingran 1991, Kibria and Ahmed 2005, Froese and Pauly 2021). This fish is easily digestible and an excellent source of protein, essential nutrients, minerals, vitamins A and D, calcium, phosphorus, iron, iodine, etc. and has some medicinal properties (Felts et al. 1996, Thilsted et al. 1997, Galib et al. 2009). It is found commonly in rivers and estuaries, ponds, pools, smaller streams, and rivers (Bhuiyan 1964, Talwar and Jhingran 1991) and also in Chalan Beel (Galib et al. 2009) and Halti Beel (Imteazzaman and Galib 2013). Due to extreme exploitation and pollution, this species is vulnerable with a high risk of extinction (Galib et al. 2009). However in Kaptai Lake its production along with Indian river shad, *Gudusia chapra* (Hamilton) is increasing in comparison with other major carps, and they have become the

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dominant species (Suman et al. 2021). According to Chaudhry (2010), they are least concern (LC) species on the IUCN list. So, different types of research on these species are urgently needed.

In Bangladesh, Kaptai Lake (Latitude 22°29"N and Longitude 92°17"E) is one of the largest artificial freshwater lakes in Southeast Asia (Fernando 1980). It was created for hydro-electricity by damming the Karnaphuli River at the town of Kaptai in the district of Rangamati in Chittagong at 1961. This water body is a unique water reservoir for some freshwater fishes (Rahman et al. 2017). Fisheries, flood control, navigation, drainage, and irrigation were considered as secondary options for Kaptai Lake. This lake constitutes about 46.8% of the total lake area of Bangladesh (Ahmed 1999). The Ganges River sprat from Kaptai Lake have exhibited an increasing pattern of production, and since they inhabit a unique freshwater body, they might also be genetically diverse in relation to Ganges River sprat inhabiting Kirtankhola River, which is the other main source of this species in Bangladesh. Information regarding this possible genetic diversity is important for conservation biology and species viability and potentially for

evolutionary responses to environmental changes (Meffe and Carroll 1997). Species extinction and vulnerability increase when there is a loss of genetic diversity (Milligan et al. 1994). Ecological, geographical, and evolutionary factors could have effects on the genetic diversity of these populations. The characters assessed through RAPD are useful for genetic studies of data-taxonomic populations and inheritance patterns of various organisms including fishes (David and Pandian 2006).

The genetic diversity of Ganges River sprat from Kaptai Lake in comparison to other sources has yet to be done. Therefore, the aim of the present study was to analyze the genetic diversity of Ganges River sprat from Kaptai Lake and the population from the Kirtankhola River, which is the other main habitat of this species in Bangladesh.

Materials and methods

Sampling sites and samples collection

Ganges River sprat were collected from two different locations *viz.* Kaptai Lake (Latitude 22°29"N and Longitude 92°17"E) and Kirtankhola River (22°71" N; 90°38" E) in Bangladesh in September 2016 (Fig. 1).

Isolation of genomic DNA

Fish muscles were collected from approximately 20 specimens from each population and the samples were pooled to extract their genomic DNA. Total genomic DNA was extracted from a 20mg pooled muscle sample using a commercial DNA extraction kit (Maxwell 16 MDx Research Instrument, Promega, USA) following the manufacturer's instructions.

DNA quantification

The extracted DNA was quantified with a NanoDrop spectrophotometer (Model: ND2000), Thermo Fisher

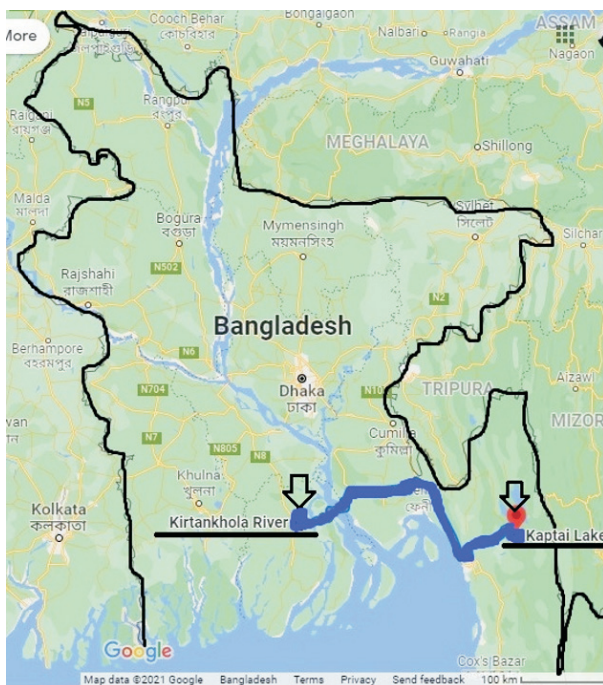


Figure 1. Fish sampling sites in Kaptai Lake and Kirtankhola River, Bangladesh.

Scientific Inc., USA), and the light absorbance of the extracted DNA at 260/280nm was approximately 1.8.

RAPD (Random Amplified Polymorphic DNA) analysis

RAPD was conducted with ten primers *viz* OPA-04, OPAL-04, OPA-03, OPF-01, OPG-05, OPG-04, OPA-09, OPAK-04, OPAW-09, and OPA-02 from Operon Technologies (Alameda, CA, USA). Twenty µl PCR reactions were prepared with 10 µl of Hot Start Green Master Mix (M7432, USA), 8 µl of template DNA at a concentration of 2–20 ng/µl, and 2 µl of Primer at Concentration 10–20 pMol for RAPD analysis. PCR amplification was conducted in an oil-free thermal cycle for 42 cycles after initial denaturation at 95°C for 3 min, denaturation at 95°C for 30 s, annealing at 30°C for 30 s, extension at 72°C for 60 sec, and final extension at 72°C for 5 min. After PCR, the PCR products were separated on 1% agarose gel.

Gel electrophoresis, scoring bands, and data analysis

The successful amplifications were visualized by resolving PCR products in 1% agarose gel (w/v) with

the DNA ladders of 100 bp. Photographs were examined on the basis of presence (1) or absence (0), band size, and polymorphism. The value of pairwise genetic distance was analyzed with POPGENE 32 (version 1.31) (Yeh et al. 1997) and MEGA7 (Kumar et al. 2016).

RESULTS

RAPD analysis

A total of sixty-eight bands were found in these two fish populations of which thirty-two were polymorphic, which indicated a moderate level of 51.07% polymorphism between them. The amplicon sizes ranged from 200 bp to 1300 bp (Figure 2 and Table 1). Based on specific primers, the highest polymorphism at 100% was found in the primer analysis of OPF-01, while the lowest polymorphism was found at 14.29% in the primer analysis of OPG-04. Based on the banding pattern of primer OPF-01, they were totally different from each other, but based on the OPG-04 primer analysis banding pattern, they were very genetically close to each other (Table 1 and Figure 2).

Table 1

Compilation of RAPD analysis in two populations of Ganges River sprat with ten different primers combinations

Primer codes	Sequence	Size ranges (bp)	Total bands	Number of Polymorphic bands	Polymorphism (%)	Mean Polymorphism
OPA-04	AATCGGGCTG	300-1100	6	4	66.67	51.07
OPAL-04	ACAACGGTCC	800-1300	4	2	50	
OPA-03	AGTCAGCCAC	500-1050	4	2	50	
OPF-01	ACGGATCCTG	400-1220	5	5	100	
OPG-05	CTGAGACGGA	200-1070	7	5	71.43	
OPG-04	AGCGTGCTG	350-1080	7	1	14.29	
OPA-09	GGGTAACGCC	600-1180	6	4	66.67	
OPAK-04	AGGGTCGGTC	400-1300	12	4	33.33	
OPAW-09	ACTGGGTCGG	500-1220	8	2	25	
OPA-02	TGCCGAGCTG	500-1180	9	3	33.33	
		Total	68	32		

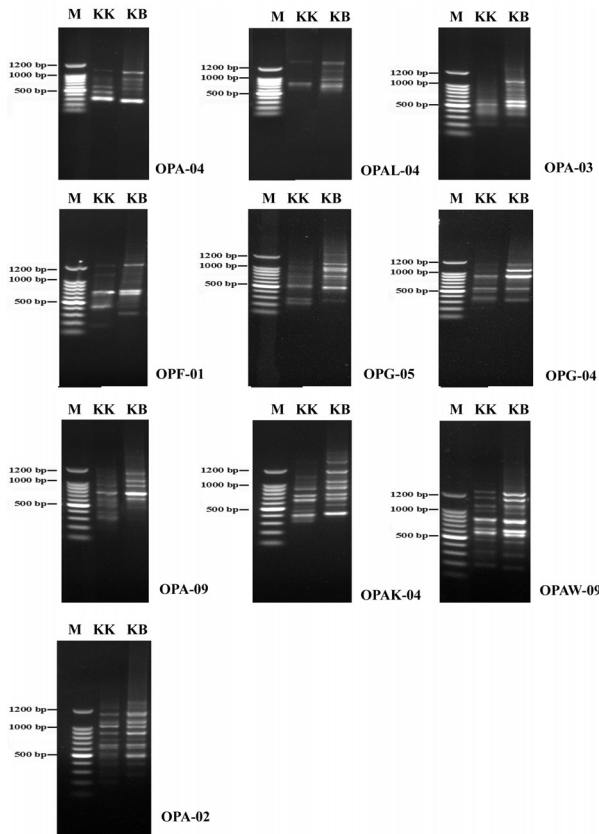


Figure 2. RAPD analysis with 10 primers on two populations of Ganges River sprat from Kaptai Lake = KK and Kirtankhola River = KB. M= 100b DNA ladder.

Genetic distances and genetic identity

The genetic distance was 0.8210 and the genetic identity was 0.4400 indicating that the Ganges River sprat from Kaptai Lake and Kirtankhola River in Bangladesh were moderately diversified, and that they were moderately distant in both genetic identity and genetic distance (Table 2).

Table 2

Genetic distance and genetic identity of the Ganges River sprat from Kaptai Lake and Kirtankhola River in Bangladesh

Population ID	Ganges River sprat from Kaptai Lake	Ganges River sprat from Kirtankhola River
Ganges River sprat from Kaptai Lake	****	0.4400
Ganges River sprat from Kirtankhola River	0.8210	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Cluster analysis

Cluster analysis between these two populations based on banding pattern was performed with POPGENE 32 (version 1.31). The dendrogram based on Nei's (1972) genetic distance was constructed using the Unweighted Pair Group Method of Arithmetic Means (UPGMA) and segregated these two different Ganges River sprat populations from Kaptai Lake and Kirtankhola River in Bangladesh into one major cluster C_1 (Figure 3).

Discussion

RAPD polymorphism

Successful conservation, optimal resource management, avoidance of inbreeding problems, and effective species management depend on the level of genetic diversity within and among species and developing effective strategies to maintain natural genetic divergence (Lakra et al. 2009). Highly diversified species are the best choices for improved breeding programs and their conservation in changing environments since they adapt readily to different environments and demographic fluctuations in the short and long terms (Milligan et al. 1994, Fuchs et al. 1998). This study is the first step to elucidate the present status of the genetic diversity of this Ganges River sprat fish species from Kaptai Lake that is most commonly found in Kirtankhola River in Bangladesh. Although average polymorphism was at a moderate level of 51.07%, the Ganges River sprat from Kaptai Lake showed moderate similarity with populations from Kirtankhola River in Bangladesh (Figure 2).

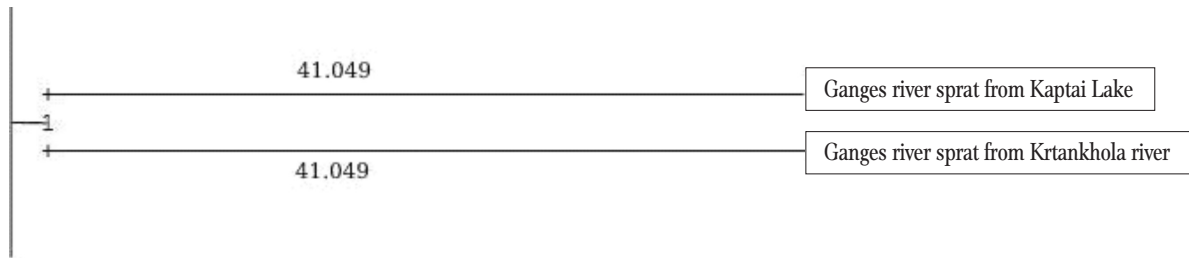


Figure 3. UPGMA dendrogram constructed based on Nei's (1972) genetic distance summarizing the data on the differentiation between two samples of Ganges River

While Kaptai Lake is a unique water reservoir for some freshwater fishes due to its creation (Rahman et al. 2017), pollution from different natural and anthropogenic sources could have led to a decline in the genetic diversity of fishes in this lake. Pollution can affect genetic diversity (Mahboob et al. 2019). According to Carvalho and Hauser (1995), geographical isolation, the uniqueness of ecological parameters of habitats, limited dispersal, and phylopatric behavior of populations should promote genetic differentiation, particularly in freshwater habitats. Based on this concept, the Ganges River sprat from Kaptai Lake should be distant in comparison with other populations found in Bangladesh especially the most commonly found population from the Kirtankhola River in Bangladesh.

The geographical distance between these two Ganges River sprat populations from these freshwater bodies is around 293 km, which is neither close nor distant. Geographical isolation has an impact on genetic diversity. These two populations are not distinctly different from each other in geographical location, so this geographical closeness could affect on their moderate diversification. On the other hand, the pollution in Kaptai Lake could also influence lowering the genetic diversity of fishes in comparison with other populations. Different anthropogenic pressures such as over-fishing, the presence of dams, pesticide runoff from agriculture or urban areas can result in low levels of species genetic diversity (Mukhopadhyay and Bhattacharjee 2018).

Some previous studies of RAPD polymorphisms of the SIS family focused on *Eutropiichthys vacha* (Hamilton) from the Ganga and Kosi rivers and indicated 51.1 and 55.6% genetic diversity, respectively

(Chandra et al. 2010), which strongly supports the present study. Polymorphism was 100, 88.75, 87.33, and 76.12% in different populations of tilapia fish (Mahboob et al. 2019). Garg et al. (2014) reported a moderate degree of genetic diversity of 72.94% in three populations of *Notopterus notopterus* (Pallas), in Madhya Pradesh in India. Garg et al. (2009) also noted 64.98% polymorphism among two populations of *Mystus vittatus* (Bloch) in Madhya Pradesh, India. The mean genetic diversity found in three wild populations of stinging catfish, *Heteropneustes fossilis* (Bloch) was 25, 25, and 12.5%, and the average polymorphic loci was 20.83%, which indicated a lower level of genetic diversity in these different populations (Hossain et al. 2013). Lal et al. (2003) reported genetic diversity in *Clarias gariepinus* (Burchell) at 50%, which also indicated a moderate level of polymorphism. These findings strongly support those of the present study. The polymorphism determined by RAPD and ISSR analysis was 78.44% in three different populations of *Badis badis* (Hamilton) from the three major river systems of Mahananda (Terai), Teesta, and Jaldhaka (Dooars) in India (Mukhopadhyay and Bhattacharjee 2018). The genetic diversity among three riverine populations of *Ompok bimaculatus* (Bloch) was 73.53% with 26.47% genetic variation within the population (Malakar et al. 2013). The genetic diversity of catfish, *Clarias batrachus* (L.), populations collected from three regions of an Indian riverine system were identified by RAPD, and the polymorphism range among them was from 26.5 to 30.5%, while within them it was from 25 to 35.7% (Khedkar et al. 2010).

These previous research results of different SIS species strongly support the findings of the present

study that indicates most SIS members have a moderate level of genetic diversity among them. This research was conducted to elucidate the genetic diversity of Ganges River sprat from Kaptai Lake with a comparison of those in Kirtankhola River in Bangladesh, which is the other source of this species. Since geographical distance, pollution, and ecological parameters are directly correlated with the genetic diversity of different populations (Rahman et al. 2017), these two Ganges River sprat populations showed a moderate level of genetic diversity between them.

Phylogenetic relationships of these two Ganges River sprat populations

Geographical constraints lower gene flow between the populations, thus increasing genetic distances markedly (Wright 1943); however, the geographical distance of these two populations was not great. This could have influenced making them moderately distant based on their genetic relationship. In the present study there was no distinct geographical barrier between these two populations; moreover both are from freshwater bodies, but the pollution in Kaptai Lake could have led to lower genetic diversity between them. The findings of the present study and of previous studies showed that these Ganges River sprat populations have a moderate level of genetic variability between them. Therefore, based on their genetic diversity, the current study indicated that these two populations are likely not good choices for an improved breeding program, and they could cause inbreeding depression.

Conclusion

For the first time, the genetic diversity of the Ganges River sprat from Kaptai Lake was compared with that of the population from Kirtankhola River in Bangladesh to identify whether the former population is genetically diversified or not. The moderate level of genetic diversity indicated that these two Ganges River sprat populations are neither similar nor


distant from each other. Thus, they are likely unsuitable for an improved breeding program. Moreover, an effective management policy for pollution control should be developed and a conservation plan should be implemented to conserve this indigenous fish species in Bangladesh that is of least concern.

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Author contributions. A.K. designed the experiment with data analysis, samples collection, paper writing, literature review along with publication process; G.R. corrected the manuscript with the final approval and critical review.

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