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## EDITED BY

Samuel A. Cushman,  
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## REVIEWED BY

Matias Leonel Giglio,  
The University of Utah, United States  
Bismay Sahoo,  
Central Institute of Freshwater Aquaculture  
(ICAR), India

## \*CORRESPONDENCE

Md Alamgir Kabir

✉ makabir@du.ac.bd

Md Golam Rabbane

✉ rabbane@du.ac.bd

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# Genetic variation of three wild zebrafish population (*Danio rerio*) in Bangladesh

Md Khademul Islam<sup>1</sup>, Md Badruddoza<sup>1</sup>, Sumaiya Binte Salam<sup>1</sup>,  
Mohammad Shamimul Alam<sup>2</sup>, Md Alamgir Kabir<sup>3\*</sup>  
and Md Golam Rabbane<sup>4\*</sup>

<sup>1</sup>Department of Fisheries, Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh,

<sup>2</sup>Department of Zoology, Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh,

<sup>3</sup>Aquaculture Genomics Laboratory, Department of Fisheries, Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh, <sup>4</sup>Fisheries Genetics and Biotechnology Laboratory, Department of Fisheries, Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh

Morphological and genetic diversity of three wild zebrafish populations in Bangladesh (Rangpur, Netrokona, and Cumilla) were elucidated using DNA barcoding and Random Amplified Polymorphic DNA (RAPD). Morphometric variation was minimal therefore, RAPD profiling and DNA barcoding were also used. Total 236 bands, including 29 polymorphic bands and 2 unique bands in RAPD showed 14.09% polymorphism indicating a low genetic diversity among them. Genetic distances were ranged from 0.0048 to 0.1031, with the highest genetic distance between Rangpur and Netrokona populations. DNA barcoding showed 99% similarity to *Danio rerio* from the GenBank, and multiple sequence alignment confirmed high genetic similarity (99.41) among these populations. Phylogenetic analysis showed these three zebrafish populations were grouped into distinct clusters, supporting the presence of genetic structure across populations. These information provides an essential reference data for the conservation, management, and potential selective breeding of zebrafish. The findings of this research also emphasize the efficacy of DNA barcoding and RAPD as complementary methods in the study of population genetics.

## KEYWORDS

*Danio rerio*, zebrafish, morphometric difference, genetic diversity, RAPD, DNA barcoding

## 1 Introduction

*Danio rerio*, commonly known as zebrafish, is a widely used natural ornamental model species in fisheries research around the world (Rabbane et al., 2016; Rahman et al., 2024). The zebrafish are generally found in floodplains, streams, paddy fields, ditches, ponds, and rivers of India, Bangladesh, Nepal, Pakistan, and Myanmar (Rabbane and Rahman, 2017). They are mostly found in floodplains comprising almost entire part of Bangladesh (Lee et al., 2020). The diversity and abundance of zebrafish are influenced by climate and human

activities such as farming and abuse of agrochemicals, raising industrial pollution, destruction of natural stock etc (Thomas, 2010; Kabir et al., 2020). So, there is a possibility of appearing genetic and morphometric variation among different wild populations of zebrafish collected from different sources. It was reported from a study that in the aspect of expected heterozygosity and allelic richness, there was a higher genetic variability in wild zebrafish while compared to strains from laboratory. This study also represented that geographical distribution influences the genetic variation of zebrafish and this genetic variation can be decreased due to a small size of effective population with respect of every breeding program (Coe et al., 2009). The geographical distribution of zebrafish causes genetic variation among their populations. So, it is necessary to determine the genetic variability and structure of zebrafish populations from different geographically isolated areas. This genetic information can provide a proper measure of biodiversity which would be useful for the conservation and management of a wild fish population (Affonso and Galetti, 2007).

The measurements of morphology within species have been used to comprehend genetics, population dynamics, and differences in populations brought on by environmental or genetic variables (Suresh et al., 2023). So, the classical morphometric measurements can help to identify diversity of zebrafish populations collected from geographically isolated areas. The diversity of zebrafish population comparing strain and wild had already been determined using various techniques such as microarray, single nucleotide polymorphism (SNP), simple sequence length polymorphism (SSLP), Random amplified polymorphic DNA (RAPD) etc (Azad et al., 2022). Among all these techniques, RAPD along with DNA barcoding have been considered as a vital tool for the identification of diversity in the species level (Weigt et al., 2012).

RAPD technique had already been used for determining genetic diversity of various species and sub-species of fishes such as in sea bass (Allegrucci et al., 1995); in tilapia (Bardakci and Skibinski, 1994); in red mullet (Mamuris et al., 1998) etc. RAPD marker has been used to determine the variance in genetic aspects among three geographically isolated wild fish populations of *Catla catla* in the Padma, Halda and Jamuna rivers and an artificially-reared population of a Hatchery in Bangladesh (Rahman et al., 2009). So, this marker can also help to determine genetic variance among the wild zebrafish populations collected from different areas. Similarly, DNA barcoding is an effective method which has already been proven in several studies. In fact, the species can be identified 90% accurately to species level using DNA barcoding method (Tsoupas et al., 2022). DNA barcoding method has been used in studies on different kinds of animals, like fishes (Ward et al., 2005), birds (Hebert et al., 2004), gastropods (Meyer and Paulay, 2005) etc. Zebrafish population diversity or variation between strain and wild had been identified by many researchers using various techniques like whole genome sequencing (Balik-Meisner et al., 2018), single nucleotide polymorphisms (SNPs) (Stickney et al., 2002), microarray hybridization (Stickney et al., 2002), single strand conformational polymorphism (SSCP) markers (Woods et al., 2005), simple sequence length polymorphism (SSLP) markers (Shimoda et al., 1999), single nucleotide polymorphism (SNP) (Guryev et al., 2006) etc. Among them, DNA barcoding and RAPD have gained

considerable validation as a tool for species identification and discovery (Weigt et al., 2012).

In Bangladesh, a study has already been directed to know the genetic diversity of three geographically isolated wild zebrafish populations which were collected from Mymensingh, Faridpur, Khulna, and Munshiganj (Azad et al., 2022). But there is no research available for identifying genetic diversity of zebrafish collected from Rangpur, Netrokona, and Cumilla. Therefore, this study intended to assess the genetic diversity of three geographically isolated wild zebrafish populations of Bangladesh which are collected from Rangpur, Netrokona, and Cumilla.

## 2 Materials and methods

### 2.1 Ethical statement

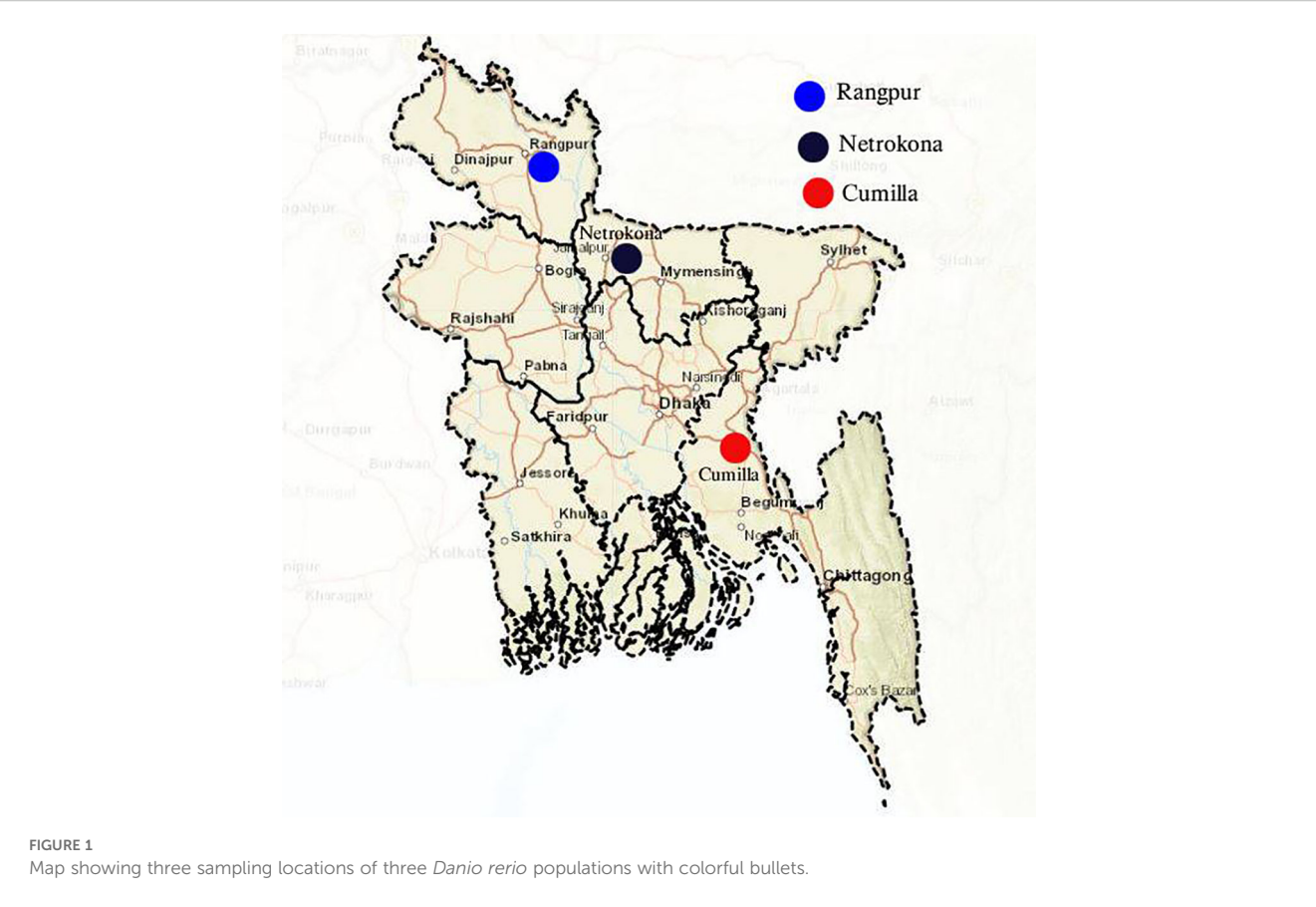
This research was approved by the Ethics Committee of Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh with the reference number 285/Biol. Scs.

### 2.2 Fish sampling and morphometric measurements

Three zebrafish populations were collected from three different locations of Bangladesh, viz: Rangpur (25.7494° N, 89.2611° E), Netrokona (24.8821° N, 90.7231° E), and Cumilla (23.4498° N, 91.1847° E) based on their availability (Figure 1). Total 9 freshly dead fish as three fish for each population were collected from the local fishermen for morphometric and RAPD technique. It would be so interesting if we could do the sampling focused on life stages, specific sizes, specific sexes, etc. but we could not do that due to scarcity of zebrafishes in the wild source. We collected them randomly and focused on their morphometric characteristics where, a total of 16 classical morphometric parameters were measured. The 16 classical morphometric parameters include the total length, the standard length, the body width, the body weight, the body depth, the head length, the length of five fins (dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin), and number of five fin rays. Using taxonomic keys, fish specimens were identified morphologically (Jayaram, 1999). For further study, samples of whole fish were kept at -20°C.

### 2.3 Extraction of genomic DNA

The genomic DNA was extracted from the fish sample's muscle tissue for this investigation. A brief process was used in accordance with a modified methodology to isolate whole genomic DNA (Wu et al., 1995). An automated extractor of DNA (Model: Maxwell 16, Origin: Promega, USA) was utilized for the isolation process. Using a spectrophotometer, the ratio of the optical density recorded at 260/280 nm was applied to calculate the concentration and purity of the extracted genomic DNA. Before being used for PCR



amplification, DNA samples from members of each community were diluted with nano pure water to around 25 ng/ $\mu$ L.

can cover a more comprehensive picture of genetic diversity and can provide something new for their genetic diversity. From the comparison of newly used primer and previous primers, selection of best primers and better understanding can be done in future zebrafish research.

2.4 PCR primers

Two barcode primers, one forward F1 primer and another reverse R1 primer, were selected for DNA barcoding. For PCR amplification, 4 commercially available RAPD primers viz. OPA-11, OPC-05, OPG-03, and OPD-05 were used (Table 1). Here the different primers from Azad et al., 2022 were used for better identification of unique bands or polymorphic loci that can be different from previously used primers. Additionally, new primers

2.5 PCR amplification for RAPD profiling and DNA barcoding

The PCR amplifications of DNA were performed in 20  $\mu$ L volume containing Hot Start Green Master Mix of approximately 10  $\mu$ L. The master mix contains dNTPs, MgCl<sub>2</sub>, Taq DNA polymerase (Cat:

TABLE 1 Parameters of random primers used in this study.

| Sl. No. | Primer codes | Sequences (5' to 3')       | Nucleotide length |
|---------|--------------|----------------------------|-------------------|
| 1       | ForwardF1    | TCAACCAACCACAAAGACATTGGCAC | 26-mer            |
| 2       | ReverseR1    | TAGACTTCTGGGTGGCCAAAGAATCA | 26-mer            |
| 3       | OPA-11       | CAATCGCCGT                 | 10-mer            |
| 4       | OPC-05       | GATGACCGCC                 | 10-mer            |
| 5       | OPG-03       | GAGCCCTCCA                 | 10-mer            |
| 6       | OPD-05       | TGAGCGGACA                 | 10-mer            |

M7432, Promega, USA), Buffer, 2  $\mu$ L of genomic DNA, 6  $\mu$ L of Nuclease-Free Water, and 2  $\mu$ L of primer. The PCR reactions for RAPD and DNA barcoding were carried out with selected primers in a thermal cycler (Applied Bio systems<sup>®</sup> 2720 Thermal Cycler). The thermal cycler was programmed as initially 95 °C for 3minutes, total 40 cycles of 94 °C for 30 seconds, 48 °C for 30 seconds, 72 °C for 90 seconds and finally 72 °C for 5 minutes. The amplified DNA products were segregated by gel electrophoresis on 1% agarose gel. After segregation, they were observed on UV-transilluminator and the segregated bands were photographed. The pictures of the gel containing segregated DNA products were applied for the analysis of amplified products for RAPD analysis. The purified PCR products of DNA barcoding samples were sequenced from Malaysian FIRST BASE sequencing company.

## 2.6 Data analysis

The amplified fragments got from PCR amplification were scored using binary data where 1 is scored for presence of bands and 0 is scored for absence of bands. For further statistical analysis, only data derived from reproducible bands which were present were utilized. Then the polymorphic loci (Nei, 1978) was calculated. Additionally, an Un-Weighted Pair Group Method of Arithmetic means (UPGMA) dendrogram was also built using POPGENE computer software version 1.31. The similarity index (SI) values were calculated between any 2 individuals on the same gel using

following formula:

$$\text{Similarity Index(SI)} = 2 \text{ NAB}/(\text{NA} + \text{NB})$$

Where, NAB represents total number of bands in RAPD shared by individuals A and B, and NA and NB represent the number of bands scored for individuals A and B, respectively (Lynch, 1990).

In DNA barcoding, the results of sequencing of selected samples were expounded by Finch TV software. After that, the sequences were blasted in NCBI BLAST to find out the similarity. Then sequences were aligned by a multi sequence alignment (MSA) tool named CLUSTALW. Finally, a phylogenetic tree was built by MEGA 6.1 software.

## 3 Results

In this study, 16 classical morphometric measurements of three populations of zebrafish were analyzed for assessing morphometric variation. The morphometric measurements of the three zebrafish populations are mentioned in Table 2. The morphometric measurements were nearly similar among the three zebrafish populations. Some measurements of morphometric characteristics of the three zebrafish populations showed little variation from one population to another. However, the result of morphometric measurements found to be insignificant to set up the variation among the three zebrafish populations. So, this experiment was

TABLE 2 Range of mean, variation, and standard deviation of morphometric measurements of *Danio rerio*.

| Sl.No. | Measurements                | Rangpur |      |                   | Netrokona |      |                   | Cumilla |      |                   |
|--------|-----------------------------|---------|------|-------------------|-----------|------|-------------------|---------|------|-------------------|
|        |                             | Min.    | Max. | Mean $\pm$ SD     | Min.      | Max. | Mean $\pm$ SD     | Min.    | Max. | Mean $\pm$ SD     |
| 1.     | Total length(cm)            | 3.40    | 3.50 | 3.45 $\pm$ 0.05   | 4.00      | 4.12 | 4.077 $\pm$ 0.066 | 3.51    | 3.6  | 3.533 $\pm$ 0.045 |
| 2.     | Standard length(cm)         | 3.25    | 3.45 | 3.36 $\pm$ 0.102  | 3.81      | 3.90 | 3.846 $\pm$ 0.047 | 3.33    | 3.45 | 3.39 $\pm$ 0.06   |
| 3.     | Body width(cm)              | 0.90    | 1.10 | 1.01 $\pm$ .100   | 0.94      | 1.20 | 1.083 $\pm$ 0.132 | 0.63    | 0.72 | 0.673 $\pm$ 0.045 |
| 4.     | Body depth(cm)              | 0.46    | 0.62 | 0.54 $\pm$ 0.08   | 0.33      | 0.46 | 0.4 $\pm$ 0.065   | 0.44    | 0.53 | 0.483 $\pm$ 0.045 |
| 5.     | Head length(cm)             | 0.48    | 0.69 | 0.577 $\pm$ 0.106 | 0.55      | 0.62 | 0.583 $\pm$ 0.035 | 0.44    | 0.58 | 0.493 $\pm$ 0.075 |
| 6.     | Body weight(gm)             | 0.21    | .44  | 0.34 $\pm$ 0.121  | 0.42      | 0.48 | 0.453 $\pm$ 0.031 | 0.33    | 0.36 | 0.343 $\pm$ 0.015 |
| 7.     | Length of dorsal fin(cm)    | 0.40    | 0.60 | 0.513 $\pm$ 0.103 | 0.40      | 0.51 | 0.45 $\pm$ 0.056  | 0.45    | 0.56 | 0.496 $\pm$ 0.056 |
| 8.     | Length of caudal fin(cm)    | 0.72    | 0.91 | 0.82 $\pm$ 0.095  | 0.52      | 0.63 | 0.567 $\pm$ 0.057 | 0.47    | 0.64 | 0.556 $\pm$ 0.085 |
| 9.     | Length of pelvic fin(cm)    | 0.32    | 0.41 | 0.37 $\pm$ 0.045  | 0.36      | 0.44 | 0.393 $\pm$ 0.041 | 0.38    | 0.45 | 0.416 $\pm$ 0.035 |
| 10.    | Length of pectoral fin (cm) | 0.51    | 0.62 | 0.563 $\pm$ 0.055 | 0.34      | 0.4  | 0.363 $\pm$ 0.032 | 0.43    | 0.52 | 0.476 $\pm$ 0.045 |
| 11.    | Length of anal fin(cm)      | 0.42    | 0.51 | 0.467 $\pm$ 0.045 | 0.36      | 0.42 | 0.383 $\pm$ 0.032 | 0.36    | 0.41 | 0.383 $\pm$ 0.025 |
| 12.    | Dorsal fin                  | 7       | 8    | 7.67 $\pm$ 0.577  | 9         | 12   | 10.33 $\pm$ 1.528 | 8       | 11   | 9.33 $\pm$ 1.528  |
| 13.    | Caudal fin                  | 21      | 24   | 22.33 $\pm$ 1.528 | 22        | 26   | 24.00 $\pm$ 2.00  | 19      | 24   | 21.00 $\pm$ 2.646 |
| 14.    | Pectoral fin                | 7       | 12   | 9.33 $\pm$ 2.517  | 11        | 16   | 13.33 $\pm$ 2.517 | 6       | 9    | 7.33 $\pm$ 1.528  |
| 15.    | Pelvic fin                  | 5       | 9    | 7.00 $\pm$ 2.00   | 8         | 9    | 8.67 $\pm$ 0.577  | 9       | 13   | 11.33 $\pm$ 2.082 |
| 16.    | Anal fin                    | 11      | 14   | 12.33 $\pm$ 1.528 | 15        | 16   | 15.33 $\pm$ 0.577 | 15      | 19   | 16.67 $\pm$ 2.082 |

further carried out to evaluate the genetic variation and similarities among the fish populations.

### 3.1 RAPD polymorphism

The RAPD profiles of three zebrafish populations from three geographically distant areas of Rangpur, Netrokona, and Cumilla district were generated using four primers which are OPA-11, OPC-05, OPG-03, and OPD-05 having the ability of producing reproducible and well-defined bands. The results were described as RAPD profiles of 9 samples (3 from each population) of the three different zebrafish populations. From Figure 2, it is clearly visible that the data produced using the selected primers in the RAPD profiles of the three different zebrafish populations were somewhat distinct.

In RAPD analysis, total 236 bands were generated using these four primers. Among the 236 bands, 29 bands were polymorphic resulting 14.09% polymorphisms among three geographically distant zebrafish populations. This result indicates a low level of genetic variation and high level of similarity among the three zebrafish populations. The amplified DNA fragments varied in size between 250–1500 bp. Besides the 29 polymorphic bands, 2 unique bands were found in this study. Among the 2 unique bands, one band was observed in a zebrafish sample of Cumilla district for OPD-05 primer and another unique band was observed in a zebrafish sample of Rangpur district for OPA-11 primer (Table 3).

### 3.2 Genetic identity and genetic distance

According to the Nei's original measure of genetic identity and genetic distance, the genetic identity among three zebrafish populations ranged between 0.9020 and 0.9952. The highest genetic identity (0.9952) was found between two fishes of Rangpur district which are Rangpur2 and Rangpur3. On the other hand, the lowest identity was found between the fishes of Rangpur and Netrokona district mentioned as Rangpur3 and Netrokona1 respectively. The genetic distances among three populations were between 0.0048 and 0.1031. The lowest genetic distance (0.0048) was found between two fishes of Rangpur denoted as Rangpur2 and Rangpur3 respectively. On the other hand, the highest genetic distance (0.0970) was found between the fish of Rangpur and Netrokona denoted as Rangpur3 and Netrokona1 respectively (Table 4).

### 3.3 UPGMA dendrogram

The unweighted pair group method of arithmetic means (UPGMA) dendrogram was built applying Nei's (1972) original measures of genetic distance (D) of *Danio rerio* populations of the three geographically distant areas of Bangladesh (Figure 3). The UPGMA dendrogram divided all the 9 individuals collected from three different locations into 4 clusters. The maximum intra-specific genetic resemblance with

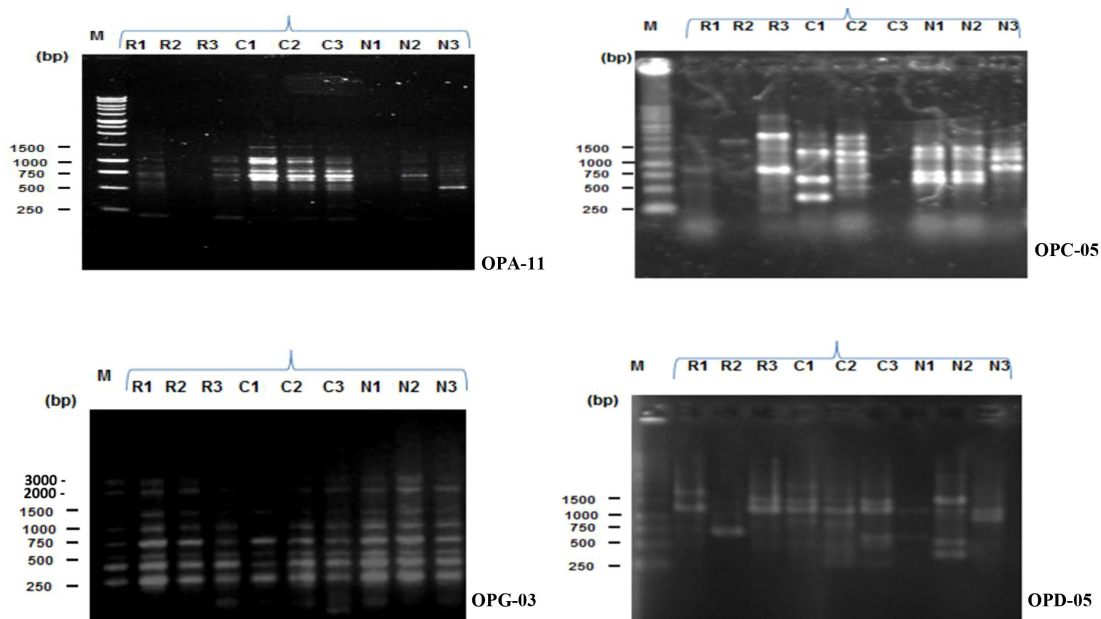


FIGURE 2  
RAPD profiles of three zebrafish populations (R = Rangpur, C = Cumilla, N = Netrokona) produced by four primers (OPA-11, OPC-05, OPG-03, OPD-05). Here, 1, 2, 3 indicate sample number.

TABLE 3 Pattern of polymorphism and uniqueness in 4 primers (primer wise) of 9 individuals from 3 *Danio rerio* populations.

| Sl. no. | Primers code | Size range (bp) | Total bands | Unique bands | Polymorphic bands                            | Polymorphism (%) | Avg. polymorphism (%) |
|---------|--------------|-----------------|-------------|--------------|----------------------------------------------|------------------|-----------------------|
| 1       | OPA-11       | 250-1500        | 82          | 1 (R3)       | 8(900,850,800,750, 650,600,500,240)          | 9.75             | 14.09                 |
| 2       | OPC-05       | 250-1500        | 51          | –            | 8(1500, 1000,900, 850,750,700,650,250)       | 15.68            |                       |
| 3       | OPG-03       | 250-3000        | 54          | –            | 9(3000, 2000,1100,1000,900, 750,700,700,650) | 16.66            |                       |
| 4       | OPD-05       | 250-1500        | 49          | 1 (C3)       | 7(1200,1150,1100,1050,850, 800,700)          | 14.28            |                       |
| Total   |              |                 | 236         | 2            | 32                                           |                  |                       |

TABLE 4 Genetic identity and genetic distance among the three populations of *Danio rerio*.

| Pop id     | Rangpur1 | Rangpur2 | Rangpur3 | Cumilla1 | Cumilla2 | Cumilla3 | Netrokona1 | Netrokona2 | Netrokona3 |
|------------|----------|----------|----------|----------|----------|----------|------------|------------|------------|
| Rangpur1   | ***      | 0.9828   | 0.9827   | 0.9656   | 0.9469   | 0.9457   | 0.9239     | 0.9513     | 0.9436     |
| Rangpur2   | 0.0173   | ***      | 0.9952   | 0.9656   | 0.9262   | 0.9434   | 0.9075     | 0.9491     | 0.9363     |
| Rangpur3   | 0.0175   | 0.0048   | ***      | 0.9616   | 0.9309   | 0.9346   | 0.9020     | 0.9406     | 0.9352     |
| Cumilla1   | 0.0350   | 0.0350   | 0.0392   | ***      | 0.9800   | 0.9663   | 0.9528     | 0.9853     | 0.9696     |
| Cumilla2   | 0.0546   | 0.0766   | 0.0716   | 0.0202   | ***      | 0.9680   | 0.9716     | 0.9811     | 0.9814     |
| Cumilla3   | 0.0558   | 0.0583   | 0.0677   | 0.0343   | 0.0325   | ***      | 0.9568     | 0.9748     | 0.9571     |
| Netrokona1 | 0.0791   | 0.0970   | 0.1031   | 0.0483   | 0.0288   | 0.0441   | ***        | 0.9575     | 0.9730     |
| Netrokona2 | 0.0499   | 0.0523   | 0.0613   | 0.0148   | 0.0191   | 0.0255   | 0.0435     | ***        | 0.9762     |
| Netrokona3 | 0.0580   | 0.0658   | 0.0670   | 0.0308   | 0.0188   | 0.0439   | 0.0273     | 0.0241     | ***        |

Nei's original measure of genetic identity (above diagonals) and genetic distance (below diagonals).

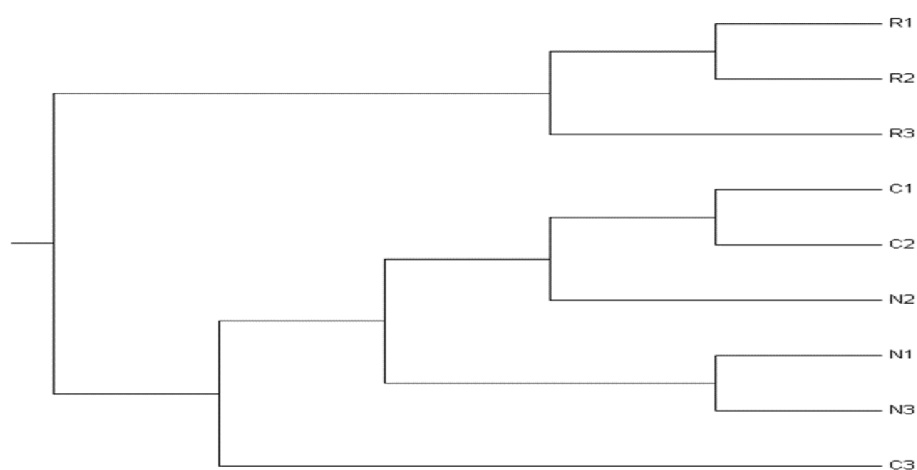


FIGURE 3

UPGMA dendrogram based on Nei's (1972) original measures of genetic distance among *D. rerio* populations according to RAPD analysis. Dendrogram was conducted in POPGENE. Here, R = Rangpur, N = Netrokona, and C = Cumilla.

minimal variation was observed among the fishes of Rangpur (R1, R2, and R3) than the other fish populations. So, they were grouped together, forming one distinct cluster. The genetic distance was higher between the populations of Netrokona and Rangpur. It is clearly evident from the dendrogram that two

individuals of Cumilla (C1, and C2) and one individual of Netrokona (N2) have more similarity. Other two individuals of Netrokona (N1 and N3) showed similarity forming a distinct cluster. C3 is the most distinct individual of all, as seen by its outlier appearance at the base of the dendrogram.

3.4 Genetic diversity analysis through DNA barcodes

Total six samples (two samples from each study area) were successfully amplified through PCR and were subsequently sequenced bi-directionally to assemble a 675 bp length COI barcode accurately. Then all the sequences were blasted against NCBI database and the results showed that sequences were almost similar to COI gene of *Danio rerio* (Table 5). No insertions, stop codons and deletions were detected in any of the sample sequences. The GenBank (NCBI) database was used for evaluating genetic variation and species identification based on the sequence similarity. All the six samples selected for DNA barcoding showed about 100% similarity with the available sequences of *D. rerio* in the GenBank.

In addition, an evolutionary relationship tree was constructed with the sequence data of the samples. The evolutionary tree in Figure 4 showed that the three populations formed three distinct

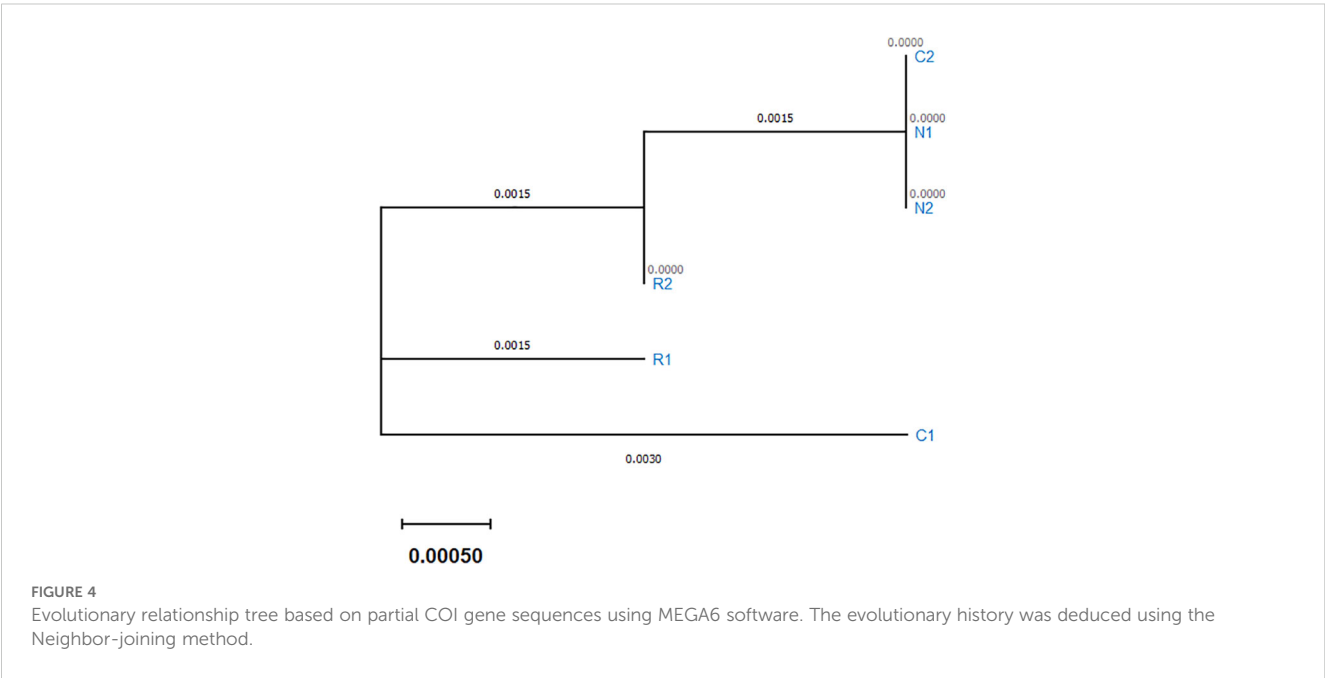
clusters. According to the clusters, the highest similarity was found among Cumilla (C2) and Netrokona (N1 and N2) and the population of Rangpur found to be closer to them. But one fish sample collected from Cumilla (C1) seemed to be more distant from other samples.

3.5 Multiple sequence alignment

The assessment of genetic variations among the three fish populations was done by mitochondrial COI gene based multiple sequence alignment. Multiple sequence alignment revealed that the length of nucleotide alignment was 675 bp. Among these 675 bp, 671 bp found to be similar indicating the similarity about 99.41% based on the alignment data. There was a little dissimilarity as only 4 bp seemed to be different according to the alignment data and the polymorphism of the nucleotide is only 0.59%. The result of multiple sequence alignment showed that there was high

TABLE 5 Results of sequence blast.

| SL | Sample ID | Description     | Max. score | Total score | Query cover | E value | Identification | Accession no. |
|----|-----------|-----------------|------------|-------------|-------------|---------|----------------|---------------|
| 1  | C1        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | KT624626.1    |
| 2  | C2        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | PP963510.1    |
| 3  | N1        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | PP963510.1    |
| 4  | N2        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | PP963510.1    |
| 5  | R1        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | MW518813.1    |
| 6  | R2        | <i>D. rerio</i> | 1247       | 1247        | 100%        | 0.0     | 100%           | MW518814.1    |



similarity among the three zebrafish populations which indicates that the three zebrafish populations were strongly close to each other.

Multiple combination of pair wise alignments among six zebrafish individuals of the three populations was done to evaluate the intra-species genetic diversity of these three zebrafish populations. Intra-population pair wise alignments showed that two individuals from Netrokona (N1 and N2) have the highest similarity, 100% (675 bp out of 675 bp) and individuals of Cumilla population (C1 and C2) have the lowest similarity, 99.56% (672 bp out of 675bp). Moreover, inter-population pairwise alignment revealed that the highest, 100% (675 bp out of 675 bp) was found between Rangpur (R2) and Netrokona (N1 and N2) fish populations and Cumilla (C2) and Netrokona (N1 and N2) populations. The lowest similarity, 99.56% (672bp out of 675 bp) was found between Cumilla (C1) and Netrokona (N1 and N2) fish populations. So, according to the alignment result, there is a high similarity among all the three fish populations (Figure 5).

## 4 Discussion

The integrity of the fishery stocks is weakened by aquaculture, accidental cross-breeding, excessive exploitation, and improper fishery management. This deterioration may cause the loss of the entire gene pool (Smith et al., 1991). So, the assessment of genetic variation among fish populations are required for the conservation of different fish stocks. Classical morphometric assessments in this study showed minimal morphological variations between the three distinct populations of zebrafish. Fish morphometric traits were generally similar and frequently intermingled among the three populations. There is frequently taxonomic confusion since these classical morphometric data are insufficient to support the variations of the population's known genetic structure (Daniels, 1997). Baseline data regarding the genetic diversity and the gene pool of fish populations are required to preserve genetic integrity. So, the genetic similarity and variation of the zebrafish species was assessed in this study by analyzing the genetic diversity of three distinct wild zebrafish (*Danio rerio*) populations from various geographic locations using the RAPD and DNA barcoding techniques. This was done because there are currently a few data on wild zebrafish in Bangladesh, despite the fact that the zebrafish is an important research model fish (Rabbane et al., 2016).

According to the findings of RAPD technique, the present study revealed that the average percentages of polymorphism is 14.09% in all the bands obtained from the four primers. In this study, a wide range of polymorphic loci (9.75–15.68%) was found among these three zebrafish populations. Primer OPC-05 showed the highest polymorphism (15.68%) whereas the lowest polymorphism (9.75%) was exhibited by primer OPA-11. It is possible to identify any potential mixing of these populations using these population-specific distinctive bands, particularly during selective breeding initiatives (Ferguson et al., 1995). Mishra et al. (2009); Nagarajan et al. (2006); Tassanakajon et al. (1998); Mostafa et al. (2009); Kabir et al. (2017) and Saad et al. (2012) also observed population specific

bands in *Metapenaeus dobsoni*, *Channa punctatus*, *Penaeus monodon*, *Labeo calbasu*, *Labeo rohita* and *Plectropomus leopardus* respectively. Numerous uses for RAPD markers have been discovered in the fields of molecular evolutionary genetics, gene mapping, population genetics, and breeding of plant and animal (Bardakci and Skibinski, 1994; Ertaş and Şeker, 2005).

Nei's original measure of genetic identity and genetic distance was calculated using the data derived from RAPD-PCR profile and genetic identity (GI) ranged from 0.9020 to 0.9952 and the genetic distance varies from 0.0048 to 0.1031. Several RAPD primers were used to investigate intra-population genetic diversity in tilapia (Bardakci and Skibinski, 1994). This method seems to be more sensitive than mitochondrial DNA analysis, which was unable to identify differences among populations of tilapia (Seyoum and Kornfield, 1992; Capili, 1990). On the other hand, the RAPD approach was used to examine genetic variation among four distinct populations of Hilsa Shad from the Indian rivers Ganga, Yamuna, Hoogly, and Narmada (Brahmane et al., 2006). Consequently, RAPD has been applied to study genetic variation of fish populations considering it as a useful tool for analyzing variance among populations with varying levels of geographic isolation. The RAPD approach has also been used to investigate the evolutionary relationships between cichlid and tilapia species in a previous study (Bardakci and Skibinski, 1994). Another study also showed that RAPD technique can be an effective tool for assessing the genetic structure of Kalibaus fish species (Mostafa et al., 2009). In this study, a dendrogram emerged from Nei's genetic distance was built to illustrate the phylogenetic relationships among the populations under investigation. The UPGMA dendrogram divided all the 9 individuals (3 individuals from each population) in 4 clusters but the samples were collected from three geographically isolated areas. This observation represented that; all the populations have distinct gene pool. Highest similarity was found among the individuals of Rangpur. The population of Netrokona and Rangpur showed the lowest similarities. Conversely, the individual of Rangpur2 and Rangpur3 had the smallest genetic distance, while the zebrafish population of Netrokona and Rangpur had the highest genetic distance. The zebrafish of Rangpur shared more intra-specific genetic traits than the other two zebrafish populations. Thus, the dendrogram showed genetic variation among and within the three zebrafish populations.

The sequences of the cytochrome oxidase c subunit 1 (COI) gene of six individuals where two from each of the three populations, gave an insight to this study by giving a better understanding of the genetic variation among the populations. The sequences were then compared with reference sequences from GenBank. The results showed a 100% sequence similarity with *Danio rerio* references when compared to the NCBI blast. Multiple sequence alignment (MSA) of the sequences revealed 99.41% similarity and 0.59% dissimilarity of nucleotides among the three zebrafish populations. This MSA indicated that all zebrafish in all geographical areas in Bangladesh are very little diversified. According to other researches, DNA barcodes seemed to be able to identify genetic variation within a number of fish species (Ardura et al., 2010; Ivanova et al., 2007; Rosso et al., 2012; Saad et al., 2012; Waugh, 2007). Lakra et al. (2011) and Zhang et al.

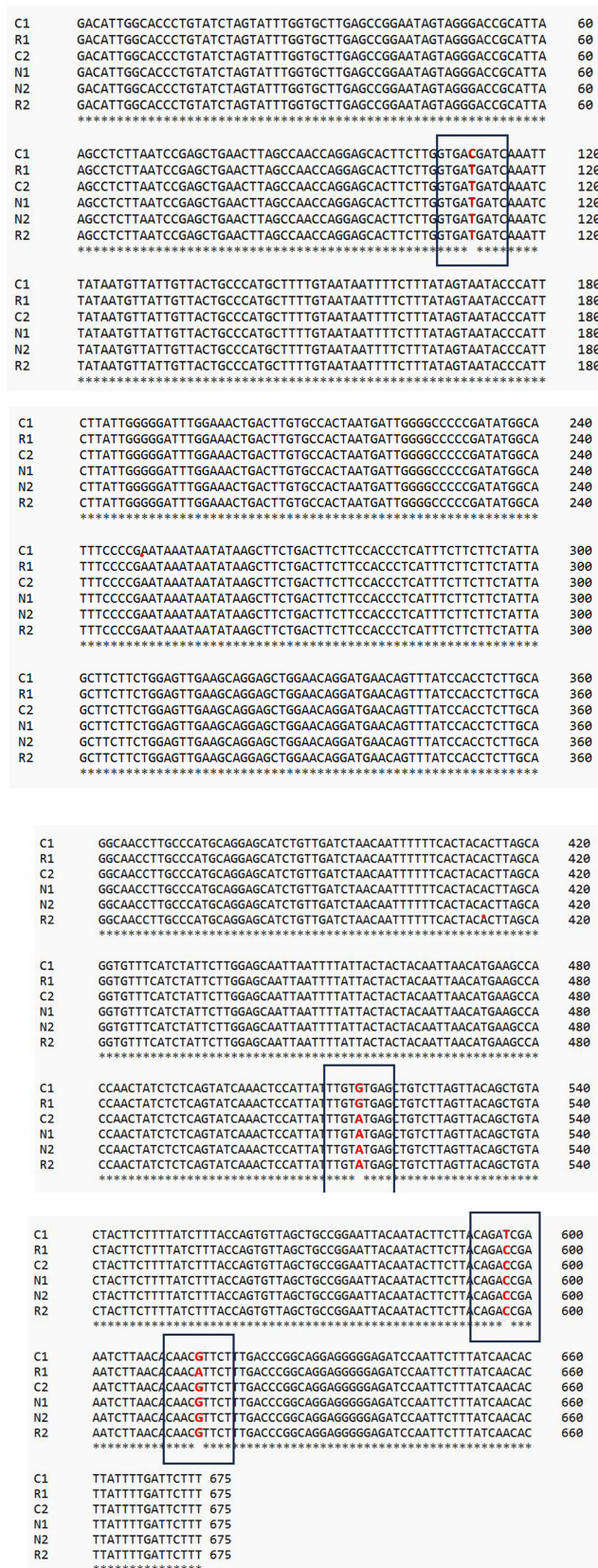


FIGURE 5

Multiple sequence alignment of COI gene fragment from 6 samples of three *Danio rerio* populations. Polymorphic sites are denoted by nucleotide positions illustrated by dark blue box. Alignment length: 675bp; Similarity (\*): 671bp (99.41%); Dissimilarity: 4bp (0.59%). Black color = Similarity and Red color = Dissimilarity.

(2017) also used DNA barcoding technique to establish genetic diversity among fish species in India and China respectively. Thus, it is considered as one of the most important methods for identifying species is DNA barcoding, which provides a simple identification process and efficiently illustrates genetic variation both within and between species. By establishing genetic similarity and dissimilarity, it can also display the relationships between and within species (Cross and King, 1983).

Using DNA barcodes and RAPD markers, the current study discovered a baseline understanding of genetic variation within and between three distinct *Danio rerio* populations in Bangladesh. For populations to survive and effectively adapt to changes in their environment, there must be variety both within and between populations (Ryman et al., 1995). As a result, this study has a lot of potential for the use and development of molecular markers for zebrafish population identification. This is because future conservation and management of commercially harvested endangered inland wild populations depend on a clear understanding of the population's genetic makeup (Allendorf, 1983). The genetic structure of *Danio rerio* populations resulted in this research by RAPD primers will be useful for establishing stock-specific management strategies for the conservation of the species, as well as in creating superior strains for aquaculture practices through selective breeding. Furthermore, a thorough understanding of the genetic diversity of zebrafish will significantly enhance its study value as a vertebrate model.

## 5 Conclusions

The data of genetic diversity has various applications in studies conducted on conservation, evolution, and management of natural resources. To gather genetic variance within and between populations, the genetic diversity study used RAPD analysis and DNA barcoding. As a result, the current study can be used as a reference for future investigations of genetic variations among commercially significant fish populations, and the potential application of DNA markers may open up new management options for fisheries resources in fish molecular biology research in Bangladesh.

## 6 Limitations and recommendation

Sample size was one of the important limitations of this research. More samples should be used for further study. Additionally, further advanced molecular technique should be used for in depth analysis for their genetic variation.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

## Ethics statement

The animal studies were approved by the Ethics Committee of the Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh with the reference number 285/Biol. Scs. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

## Author contributions

MI: Formal Analysis, Investigation, Methodology, Writing – original draft. MB: Formal Analysis, Investigation, Methodology, Writing – review & editing. SS: Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing. MA: Formal Analysis, Investigation, Methodology, Writing – review & editing. MK: Conceptualization, Formal Analysis, Supervision, Writing – review & editing. MR: Conceptualization, Formal Analysis, Investigation, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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