Research Article

Evaluation of Molecular Genetic Diversity and DNA Barcoding of *Rita gogra* (Family Bagridae) from Narmada River Revealed through Mitochondrial COX1 Gene Sequencing

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Submission Date: 13-09-2023; Revision Date: 21-10-2023; Accepted Date: 21-10-2023.

ABSTRACT

Background: Since fish have a great deal of variation and phenotypic flexibility, it can occasionally be challenging to identify them just based on morphological features, therefore, various molecular markers are very reliable tools to make identify genetic divergence and differentiations with and between populations. **Materials and Methods:** The morphological characteristics allowed us to identify the fish as a bagrid catfish. *Rita gogra* (Skyes 1839), from Narmada River and sent fresh DNA barcode information to the Barcode Life and mitochondrial COX1 gene sequences on GenBank, NCBI, USA with the length of 615 bp (accessions MF687949, MF687950, MH427908, MH427909 and MH427910). **Results:** Barcoded and NCBI created data showing the highest conserved regions for *Rita gogra* because it gave more than 99% hits similarities and it differentiated with neighbor species like *Rita rita*. The average gene polymorphism as haplotype gene diversity was 0.52381 and nucleotide diversity was 0.00473 among three populations indicates good gene diversity of *Rita gogra* in Narmada River. Genetic differentiation (Gst) was 0.00826, which indicates that highest genetic variation is within populations of Hoshangabad and Mandleshwar and 0.00% was found between Hoshangabad and Maheshwar populations. These results suggest that genetic differentiation among populations of *R. gogra* is impeded by high gene flow. **Conclusion:** This research evaluated the efficacy of a COX1 for genetic differentiation and development of barcode for discriminating of fish species encourages the global effort to build a complete library of COX1 sequences associated with specific specimens, which has just started.

Keywords: Narmada River, Gene diversity, Haplotype diversity, Nucleotide diversity, Genetic differentiation.

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INTRODUCTION

Rita gogra is an endemic catfish found in the rivers of the Deccan Plateau up to the Krishna riverine systems[1] and rare in Godavari. *R. gogra* is also richly found in the Narmada River flowing in Madhya Pradesh. This species is declining rapidly from the Narmada River may be due to pollution, and heavy harvesting of food by local people.[2] Therefore, it is need to conserve them by estimating molecular genetic diversity, differentiation and identifying illegal or over-exploitation through the development of DNA barcodes for *Rita gogra* of Narmada River. Once, we identify the genetic diversity, suitable habitat and comprehensive identification through DNA barcodes which will be available on Barcode Life, the catches of *Rita* will be identified very smoothly.

DNA Barcoding is a powerful tool for studying biological genomics, offering a thorough understanding of

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DOI: 10.5530/ajbls.2023.12.77

biology and helping to screen large-scale genes, identify individuals who are unknown, perform molecular diagnosis on individuals in relation to describing taxa, and accurately identify species using DNA.[3] A portion of the mitochondrial gene cytochrome oxidase c subunit 1 (COX1 or COI) is typically used in the barcoding of DNA.

The genetic sequencing of the barcoding area collected from many individuals is the next step in the DNA barcoding process. The sequences of DNA produced from this process are then used to build a tree of phylogenetic relationships using a distance-based technique called "neighbor-joining". It appears from the name "barcode" that every species is distinguished by a distinct sequence.[4] After the barcode sequencing is acquired, it is added to the database maintained by the Barcode of Life Data Systems (BOLD), a reference collection of DNA barcodes that may be used to identify previously unidentified specimen data and photos. It tracks the quantity of barcode sequence entries and offers a recognition engine based on the existing barcode library.

A cooperative worldwide research project called the Fish Barcode of Life Initiative (FISH-BOL; www.fishbol.org) aims to create a database of references of DNA barcodes for every kind of fish obtained from voucher samples with reliable taxonomy identifications.[5] Once completed, a quick, precise, and economical method for molecular identification of the world's ichthyo-fauna will be made possible by FISH-BOL. Additionally, FISH-BOL will offer a potent tool for a deeper comprehension of the biological relationships and historical background of fish species.

Currently, morphological examination and barcoding of DNA have been combined to provide a taxonomical method for identifying fish species. Essentially, the recent investigation is goal to estimate molecular diversity, and genetic differentiation through DNA barcoding of *Rita gogra* (Sykes, 1839) of Narmada River and to make a DNA sequence database on NCBI, USA and boldsystems for public domain uses and identification of mislabeled samples.

MATERIALS AND METHODS

During this investigation, we have chosen three study sites at the Narmada River flowing in the state of Madhya Pradesh i.e., Sethanighat at Narmadapuram (22.7631° N, 77.7160° E), Mandleshwar (22.1769° N, 75.6601° E) and Maheshwar (22.1773° N, 75.5830° E) of district Khargone, Madhya Pradesh (Figure 1) and delineated the population genetic studies and developed DNA barcodes for *Rita gogra* (Figure 2) as per their presence of locality for database comprehensive identification at molecular level.

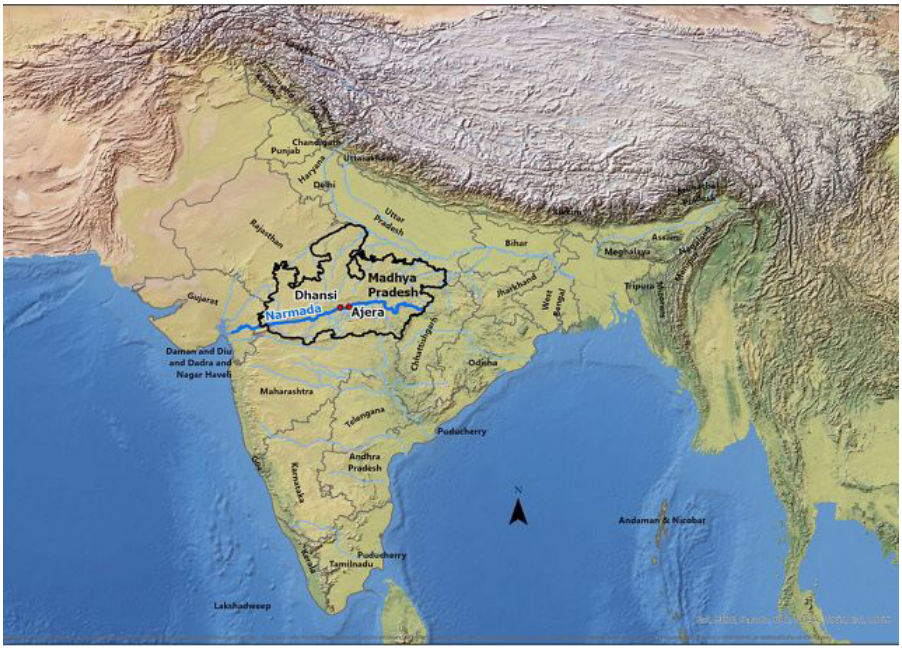

Figure 1: Details of the study sites (Sethanighat, Hoshangabad; Maheshwarghat, Maheshwar and Madleshwarghat, Mandleshwar) of Narmada River, Madhya Pradesh.

Source: https://www.tncindia.in/what-we-do/our-priorities/protect-and-restore/narmada/

Figure 2: Sample specimen of *Rita gogra* collected from Narmada River, Madhya Pradesh.

Study site-A (Sethanighat)

Sethanighat is the largest ghat of Narmada River which is constructed along the banks of the river Narmada at Narmadapuram in Madhya Pradesh. At the time of Narmada Jayanti thousands of people comes and coverage on the ghat. Many public religious infrastructures are built through private funding agencies of India.

Study site-B (Mandleshwar)

Mandleshwar is a town in district Khargone of the state of Madhya Pradesh. It is the bank Narmada River which is found about 8 kilometers east of the Maheshwar. The Mandleshwar is considered as cleanest place of the Narmada River.

Study site-C (Maheshwar)

Maheshwar is a town situated near Khargone city of district of the Khargone of the state of Madhya Pradesh. The Maheshwar town is lies on the north bank of the Narmada River which is a popular place to visit as a part of the Maheshwar tour. The Narmada River flows beside hence this place is very much a picture rescue location and also has Ahilya Fort and Shiva temple is beautiful and can take photos during sundown as magical.

Two (n=02) individuals of *Rita gogra* from each site i.e., study site-A (Sethanighat) and study site-C (Maheshwarghat) was collected, while three (n=03) individuals were collected from Mandaleshwarghat (Table 1). They are frequently captured by professional fishermen and marketed as fish for consumption in Indian markets, while not being protected by the Conservation of Wildlife Act. These fish may be obtained in India without a particular permit, and no laboratory research was done on live individuals. The samples required for molecular work for DNA barcoding and sequencing studies were collected from all three sites with the help of local fishermen as repeated visits were not possible, therefore, liver and muscle tissues were collected simultaneously from the same fish at each location to make it cost as well as labour effective. Molecular experimental work i.e., extraction of genomic DNA, quantification of extracted genomic DNA, PCR amplification, and agarose gel electrophoresis was performed using standard protocols.[6]

Table 1: Details of the samples collection and sampling sites of *Rita gogra*.

Study Site	Samples collection site	Studied individuals and their sample code
Study Site-A	Narmada River	n=02
	(Sethanighat, Hoshangabad, Madhya Pradesh).	(RGA, RGB)
Study Site-B	Narmada River	n=03
	(Mandleshwar, Khargone, Madhya Pradesh).	(RGM1, RGM2, RGM3)
Study Site-C	Narmada River	n=02
	Maheshwar, Khargone, Madhya Pradesh).	(RGNR01, RGNRB01)

Genomic DNA extraction and their quantification

We adopted the genomic DNA extracted from fish tissues using the phenol: chloroform: isoamyl (25:24:1) method with partial modifications:[6] Quantity and purity of the extracted genomic DNA was determined as integrity checking through running of the extracted genomic DNA on 1% agarose and consequently samples were treated with either proteinase-K or RNAase for maintaining the ratios of 1.8 of 260 and 280. Quantified genomic DNA was diluted at 50 ng/μL concentration required for PCR amplification of COX1 gene.

Polymerase chain reaction (PCR) of COX1 gene

The incomplete cytochrome oxidase-I sequence of genes (COX1) was amplified with the help of 96 Well Thermal Cycler (Model: Mastercycler Nexus, Germany) with a pair of COX1 universal primer forward (FishF1: 5-TCAACCAACCACAAAGACATTGGCAC-3) and Reverse (FishR1: 5-TAGACTTCTGGGTGGCCAA AGAATCA-3).[7] Amplification of COX1 gene was conducted in 25 μL reaction volume which contained 12.50 μL of 2X PCR master mix (HiMedia, India), 1.0 μL (10μm concentration) for each forward and backward primer,1.0 μL DNA template and 9.50 μL molecular grade distilled water. The PCR cycling parameters used to amplify COX1gene consisted of one cycle of an initial denaturation of 95ºC for 5 min, 35 cycles of 95ºC for 30sec, 53ºC for 1.0 min, and 72ºC for 1.0 min for annealing, followed by final extension step of 72ºC for 10 min and 4ºC for 10 min as holding temperature.

Agarose gel electrophoresis

PCR amplified products were checked at 1% agarose gel in 1X TAE buffer by adding ethidium bromide (Etbr) for visualization. The amplified DNA pattern was visualized using a UV-Transluminator and captured photograph using a Camera with the support of a Hood attached to the Transluminator (LA1068 Model, Himedia, India). A negative control was included in each round of reactions. The amplified genes were divided and stained with ethidium bromide on a 1.0% agarose gel. During agarose gel electrophoresis a 1 kb DNA ladder (MBT051, Himedia, India) was employed as a standard for the identification of the molecular weight of the targeted COX1 gene.

DNA sequencing and bioinformatics tools used for analysis

The purification of PCR products, cycle sequencing and Sanger sequencing was executed using the commercially available kit of HiMedia, India. Forward chromatograms were checked through Sequencing Analysis 5.2 version software (Applied Biosystems Inc., CA, USA), online nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) and ORF finder (https://www.ncbi.nlm.nih.gov) to trim the low-quality reads and gaps. Clustal-X software was used to align all of the produced and publically accessible database genomes into a final collection.[8] Additionally, the dataset had been prepared for an equal length in order to avoid inconsistent results in the genetic distance and tree analyses. DNA sequences of the COX1 gene of all 07 samples covering three study sites.

The COX1 barcode sequences of *Rita gogra* generated in this study are available in BOLD Systems (www.boldsystem.org) and the accession numbers are given in the NCBI (www.ncbi.nim.nic.gov), USA. Utilizing, alignment of sequences and divergence were performed on MEGA-X software.[9] According to K2P distance, closet neighbour analyses (NN), neighbour joining (NJ) tree, Barcode Index Number (BIN) URI generation, Barcode Gap Analysis as mean intra-specific, Cluster Sequence (RESL) Analysis, and BOLD Systems was used to derive data for nucleotides content (https://v4.boldsystems.org).

RESULTS

A total of 07 specimens (n=07) were collected from all three study sites and were initially identified using taxonomic keys.[10] Consequently, the genomic DNA was extracted from all 07 samples and obtained range of extracted genomic DNA from 30.50 to 316.80 ng/μL concentrations (Table 2). The samples were diluted to maintain a concentration between 40-60 ng/μL required for polymerase chain reaction for the targeted COX1 gene (Table 2). The 260 by 280 ratios of the genomic DNA were obtained between 1.82 to 1.91 as shown in Table 2 which showed good quality of the DNA and qualified for PCR amplification for Sanger's based gene sequencing. A total of 07 samples were targeted for COX1 gene using universal primer set FishF1 (TCAACCAACCACAAAGACATTGGCAC) and FishR1 (TAGACTTCTGGGTGGCCAAAGAATCA) and we obtained the band size of the gene more than 900 bp (Figure 3). During the COX1 gene Sanger's sequencing we used forward primer (FishF1) and obtained sequence length of samples ±800 bp and after trimming we obtained sequenced length ±600 bp were BLAST which demonstrated as same fish species inherent in the samples sequenced. Species *Rita gogra* was identified with a percentage ranging from 98 to 100% across the fish sequences when BLAST with NCBI database. There were 0.0 E-value was found for all 07

Table 2: Quantitative and qualitative status of extracted gDNA from *Rita gogra*, Narmada River.

Sl. No.	Sample code	Quantity of extracted genomic DNA	260/280	Stock of genomic DNA	Molecular grade DW	Final concentration of genomic @40-50 mg/µL
1.	RGA	286.2	1.82	8.82	41.17	50.00
2.	RGB	316.4	1.86	7.90	42.09	50.00
3.	RGM1	300.8	1.83	8.31	41.68	50.00
4.	RGM2	30.5	1.87	30.5	19.54	50.00
5.	RGM3	70.5	1.91	35.46	14.53	50.00
6.	RGNR01	88.0	1.88	25.00	25.00	44.00
7.	RGNRB01	96.0	1.91	25.00	25.00	43.00

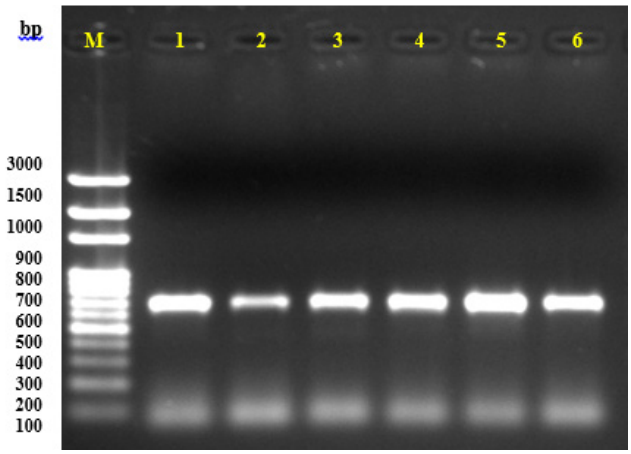

Figure 3: Lane 1: 100bp ladder, Lane 2-5 is 700bp size gene amplicons.

seven sequences when made BLAST with GenBank database of NCBI (Table 3).

All sequences did not show any kind of stop codons and gaps. After trimming all seven sequences were submitted to GenBank with accession numbers MF687949, MF687950, MH427908, MH427909, MH427910, OR533802 and OR533802 (Tables 2 and 4). The generated DNA barcodes of *Rita rita* are available on BOLD systems for reference purposes for comprehensive identification of mislabeled/unidentified specimens. The mean average nucleotide compositions within all 07 specimens were obtained as 30.312 (T), 25.995 (C), 27.092 (A), and 16.599 (G) (Table 5). The maximum distance was obtained between *Rita rita* of Mandleshwar isolates RGM1 and RGM2 as 0.0063 (Table 6). The zero genetic distance was found between RGB and RGA, RGNR01 and RGA, RGB and RGNR01, RGNR01 and RGM1, RGNRB01 and RGM1, RGNR01 and RGM3, RGNR01 and RGNRB01, RGNR01 and RGNRB01. However, RGM1 and RGM2 of the Mandleshwar individuals revealed the highest genetic distance (0.0063) as compared to the other 06 individuals of the *Rita gogra* (Table 6). The genetic differentiation among the population showed that the genetic diversity (Hs) was found lowest as zero between populations of Hoshangabad and Maheshwar,

Table 3: Sequence analysis of COX1 gene of *Rita gogra* and available at GenBank, NCBI.

Sl. No.	Species Name	Species-Voucher	Targeted gene	Accession number	Version	Sequence length	E-value (BLAST)
1.	*Rita gogra*	RGA	mtCOX1	MF687949	MF687949.1	615	0.0
2.	*Rita gogra*	RGB	mtCOX1	MF687950	MF687950.1	615	0.0
3.	*Rita gogra*	RGM1	mtCOX1	MH427908	MH427908.1	630	0.0
4.	*Rita gogra*	RGM2	mtCOX1	MH427909	MH427909.1	636	0.0
5.	*Rita gogra*	RGM3	mtCOX1	MH427910	MH427910.1	639	0.0
6.	*Rita gogra*	RGNR01	mtCOX1	OR533802	OR533802.1	507	0.0
7.	*Rita gogra*	RGNRB01	mtCOX1	OR533802	OR533802.1	551	0.0

Table 4: Universal primer and their conditions used for present investigation.

Name	Sequence	Length	MW	Tm	Nmol	GC (%)
FishF1	TCAACCAACCACAAAGACATTGGCAC	26	7886	73.1	20.93	46.1%
FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	26	8019	71.0	26.3	46.1%

Table 5: Nucleotide composition of sequenced gene of *Rita gogra* of Narmada River.

Sl. No.	Species Domain Data	Species-Voucher	Accession number	T(U)	C	A	G	Total
1.	*Rita gogra*	RGA	MF687949	30.243	26.178	27.479	16.097	615
2.	*Rita gogra*	RGB	MF687950	30.243	26.178	27.479	16.097	615
3.	*Rita gogra*	RGM1	MH427908	30.476	25.873	26.666	16.984	630
4.	*Rita gogra*	RGM2	MH427909	30.031	25.628	26.729	17.610	636
5.	*Rita gogra*	RGM3	MH427910	30.359	25.665	26.447	17.527	639
6.	*Rita gogra*	RGNR01	OR533802	29.783	26.429	27.810	15.976	507
7.	*Rita gogra*	RGNRB01	OR533802	31.034	26.134	27.223	15.607	551
	Average	-	-	**30.312**	**25.995**	**27.092**	**16.599**	**599**

Table 6: Genetic distance matrix among three locations of Narmada River.

Sl. No.	Study Site	1	2	3	4	5	6	7
1.	RGA Narmada River, Hoshangabad.	0.0						
2.	RGB Narmada River, Hoshangabad.	0.00	0.0					
3.	RGM1 Narmada River, Mandleshwar.	0.0048	0.0048	0.0				
4.	RGM2 Narmada River, Mandleshwar	0.0016	0.0016	0.0063	0.0			
5.	RGM3 Narmada River, Mandleshwar.	0.0016	0.0016	0.0015	0.0015	0.0		
6.	RGNR01 Narmada River, Maheshwar.	0.0	0.0	0.0	0.0020	0.0	0.0	
7.	RGNRB01 Narmada River, Maheshwar.	0.0018	0.0018	0.0	0.0018	0.0	0.0	0.0

however, the highest was found between populations of Hoshangabad and Mandleshwar and between Mandleshwar and Maheshwar populations.

The genetic differentiation as H_S between *R. gogra* of Sethanighat and Mandleshwar and Mandleshwar and Maheshwar was 0.66667. However, the H_S G_{ST} and F_{ST} population of Sethanighat (Hoshangabad) and Maheshwarghat (Maheshwar) was 0.00. The G_{ST} between Sethanighat (Hoshangabad) and Mandleshwarghat (Mandleshwar) was 0.00826, and between Mandleshwarghat (Mandleshwar) and Maheshwarghat (Maheshwar) was -0.10082 (Table 7).

During the analysis of molecular genetic identification of *Rita gogra* through DNA barcoding observed that the tree reconstruction of BIN and Nearest Neighbor (BIN-NN) are highly homologous and matched with the same species as the database are available at BOLD systems for *Rita gogra* (Figure 4). All COX1 gene sequences were submitted in BOLD systems for the development of DNA barcodes (https://www.boldsystems.org) and successfully generated barcodes which showed top hits with COI full database from 95.93 to 100% matches (Figure 5).

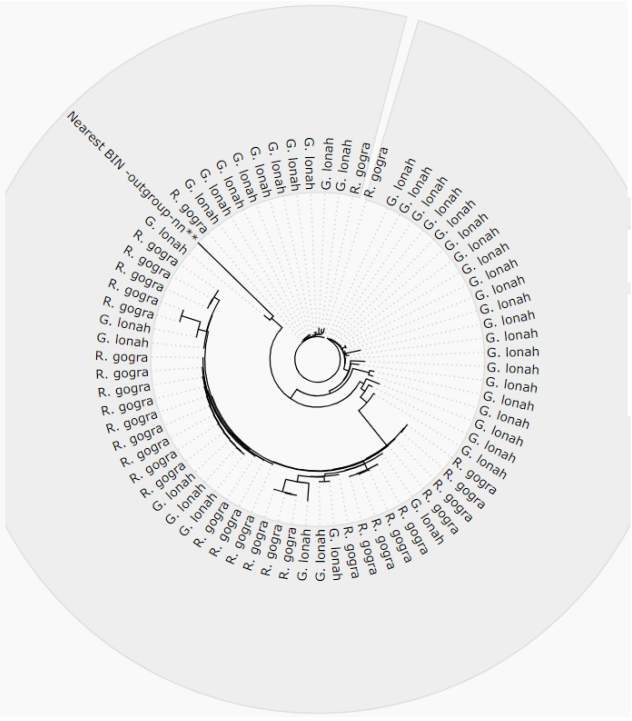

Figure 4: Nearest Neighbor (NN) details tree (All members and members of the nearest BIN) for Barcoding Analysis

Table 7: Genetic differentiation among the populations of the *R. gogra* of Narmada River.

Sl. No.	Population		H_S	G_{ST}	F_{ST}
1.	*R. gogra* Hoshangabad	*R. gogra* Mandleshwar	0.66667	0.00826	0.00
2.	*R. gogra* Hoshangabad	*R. gogra* Maheshwar	0.0	0.00	0.00
3.	*R. gogra* Mandleshwar	*R. gogra* Maheshwar	0.66667	-0.10082	0.00

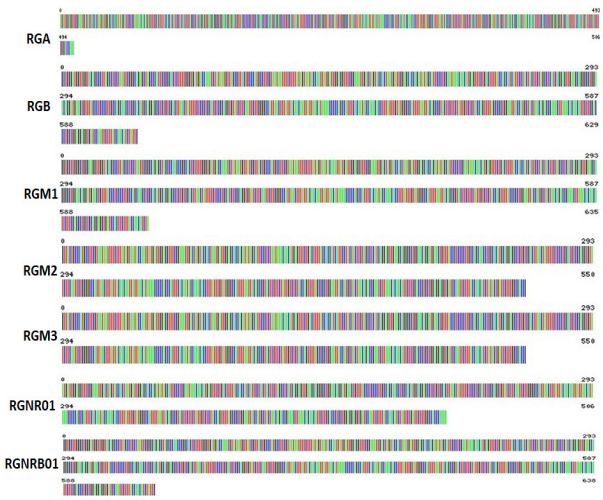

Figure 5: Developed barcodes of *Rita gogra* of Narmada River using COX1 gene and available on BOLD Systems (https://www.boldsystems.org).

DISCUSSION

The utility of genetic diversity and DNA barcoding using mitochondrial DNA COX1 gene sequences have proved their use in phylogenetics and ability to detect polymorphism in the population including fish fauna. This allows for the evaluation of connections between animals that do not share any relevant homologous developmental or morphological characteristics.[11,12] Therefore, this investigation involved seven nucleotide sequences of *Rita gogra* of Narmada River, Madhya Pradesh sampled from three study sites. The partial sequences were found from 507 bp to 639 bp with zero E-values after BLAST at GeneBank, NCBI, USA (Table 4) and there was little variation in the gene at the intraspecific level (Table 9).

The mitochondrial DNA COX1 gene sequences used in this study showed a moderate to high level of polymorphism, highest probabilities, and maximum parsimony, which demonstrated substantial capacity for genetic analysis. The COX1 gene sequences showed two clades in the phylogenetic tree that were highly supported by the bootstrapping values as shown in Figure 6 as RGNR01 and RGNRB01 of Maheshwar and RGM1 and RGM3 of Mandleshwar made a cluster. RGA and RGB of Hoshangabad have made a second cluster, however, only one sample of Mandleshwar RGM2 has created a separate branch in the phylogenetic tree.

The content of percentage of A+T is determined to be greater than G+C content in all seven individuals of *Rita gagra* of the Narmada River (Table 5) which clearly indicated that the species of *Rita gogra* is a stable species in the Narmada River flowing in the state Madhya Pradesh. Thus, it indicated that Mandleshwar habitat is considered a good habitat for *Rita gogra* from considering conservation point of view. Estimated gene flow/ genetic distance and genetic differentiations between two populations were recorded as 5.37, indicating high gene flow in *S. rivulatus* is a separated population[13] which supports our observations carried out on *Rita gogra*. During present investigation, we found highest

Table 8: Genetic polymorphism in three populations of the *R. gogra* of Narmada River.

Sl. No.	Parameters	Population-01 Sethanighat	Population-02 Mandleshwarghat	Population-03 Maheshwarghat	Overall Genetic Polymorphism
1.	Number of Haplotypes (*h*).	1.0	2.00	2.00	3.0
2.	Haplotype (gene) diversity (*Hd*).	0.0	0.66	1.00	0.52381
3.	Average No. of nucleotide differences (*k*).	0.00	6.00	3.80	3.33
4.	Nucleotide diversity (*Pi*).	0.00	0.000621	0.000621	0.00473

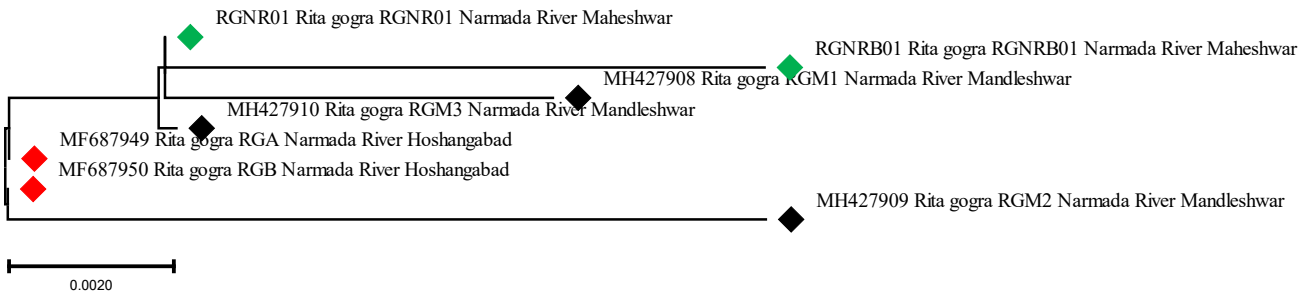

Figure 6: Neighvour joining (NJ) tree constructed using bootstrap and K2P (Kimura-2 Parameter) inferred from DNA sequences of mitochondrial COX1 gene.

overall gene polymorphism as haplotype gene diversity as 0.52381 and nucleotide diversity as 0.00473.

Genetic differentiation (Gst) was 0.00826, which indicates that highest genetic variation is within populations of Hoshangabad and Mandleshwar and 0.00% was found between Hoshangabad and Maheshwar populations. These results suggest that genetic differentiation among populations of *R. gogra* is impeded by high gene flow.

Generally speaking, bigger fish populations should result in larger Ne and therefore more genetic diversity. The actual size of the population (Ne) has a positive relationship with the census number of individuals.[14] During the present investigation, we found the highest overall gene polymorphism as haplotype gene diversity as 0.52381 and nucleotide diversity as 0.00473 which supports the effective population size and census population sizes.[14] Last but not least, greater population numbers imply that the lotic environment might be less susceptible to genetic drift or may undergo less of it.[15,16] The developed DNA barcodes are effectively able to identify species of *Rita gogra* if compared with the database available with BOLD systems such as BIN and Nearest Neighbor (BIN-NN). COX1 gene sequences submitted in BOLD systems for DNA barcodes showed top hits with COI full database *Rita gogra* which clearly reflected that the present investigation may be used for molecular identification purposes.

The use of molecular markers in detecting genetic variations in fish has been fully established.[17,18] Besides, it is also proven that the study of mtDNA can contribute to the identification of the stock and analysis of mixed fisheries. The genetic diversity indices and polymorphism (H=3.0, Hd=0.52381, k=3.33 and Pi=0.00473) were also high for the overall population. The high polymorphism of the COX1 gene suggests that they could be useful for studying the population divergence *R. gogra* of the Narmada River.[19] These findings are consistent with the high level of genetic diversity and relative genetic polymorphism with other populations mentioned above. Therefore, it is important to recognize that this may reflect the current divergence status of all the populations and insufficient time for the loss of genetic diversity after the decline of the census.[20] However, because of the different ecological characteristics, gene flow, and genetic admixture, the gene diversity (*Hd*) values may have always been different and fluctuate among locations in the Narmada River.

CONCLUSION

These results suggest that genetic differentiation among populations of *R. gogra* is impeded by high gene flow. Therefore, this research evaluated a COI efficacy for genetic differentiation and development of barcode for discriminating of fish species backs a global initiative to build a complete library of COI sequences associated with specific specimens, which has just started.

ACKNOWLEDGEMENT

The Authors thank the Director General, M.P. Council of Science and Technology, Government of Madhya Pradesh for their help and encouragement during the research work. The Authors also thank laboratory colleagues and students for their support during the laboratory support. We wish to thank to local fishermen for helping us during sample collections.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORS CONTRIBUTION

R.K. Garg devised the concept, developed the methodology, and supervised the experiment work and **Surbhi Dohre** collected and characterized the samples and performed the majority of experiments during the research work. All authors contributed to the final writing of the manuscript.

ABBREVIATIONS

PCR: Polymerase Chain Reactions; **NCBI:** National Center for Biotechnology Information; **Etbr:** ethidium bromide; **COX1:** Cytochrome-C Oxidase Subunit-1; **BOLD:** Barcode of Life Data Systems; **FISH-BOL:** Fish Barcode of Life Initiative; **NJ:** Neighbor joining; **NN:** Neighbor Analysis; **BIN:** Barcode Index Number; **Ne:** Effective population size; **h:** Number of Haplotypes; **Hd:** Haplotype diversity; **k:** Average No. of nucleotide differences; ***Pi*:** Nucleotide diversity; **ORF:** Open Reading Frame; **GST:** Genetic differentiation.

SUMMARY

Novel DNA barcode data to the Barcode Life and mitochondrial COX1 gene sequences and submitted on GenBank, NCBI, USA with the length of 615 bp (accessions MF687949, MF687950, MH427908, MH427909 and MH427910). Barcoded and NCBI created data showing the highest conserved regions for *Rita gogra* because it gave more than 99% hits similarities and it differentiated with neighbor species like *Rita rita*. Present investigation suggests that

genetic differentiation among populations of *R. gogra* is impeded by high gene flow and effectiveness of a COX1 for genetic differentiation and the development of a barcode for discriminating of fish species supports an international exercise that has recently begun to assemble a comprehensive library of COX1 sequences linked to named specimens.

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Cite this article: Garg RK, Dohre S. Evaluation of Molecular Genetic Diversity and DNA Barcoding of Rita *gogra* (Family Bagridae) from Narmada River Revealed through Mitochondrial COX1 Gene Sequencing. Asian J Biol Life Sci. 2023;12(3):587-95.