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Study on Biometric Parameters and Genetic Diversity of *Labeo rohita* from Harike Wetland-A Ramsar Site

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ABSTRACT

Background: Harike wetland (31°08′N to 31°23′N latitudes and 74°90′E to 75°12′E longitudes) is an internationally important Ramsar site, supports rare, vulnerable and endangered plants, fish and other faunal species. Weed infestation, pollution and encroachment are considered as important threats of its biota thus time series data with respect to fish diversity, catch composition and genetic variability has an utter importance to interpret changes over time. Cyprinidae family of freshwater fishes includes carps is the largest fish family found in Harike wetland comprising around 50% of total fish composition by weight basis and among carps, Rohu (*Labeo rohita*) is one of the commercially important food fish species available throughout the year and preferred by consumers. As river Beas and Sutlej, two major rivers of Indus river system confluence at Harike thus possibility of variation within fish stock is very high. With this background present study was carried out to evaluate the fish biodiversity and catch composition of Harike wetland. Study also emphasized on biometrics, length -weight relationship and genetic diversity based on mitochondrial marker genes of commercially important food fish *Labeo rohita*.

Methods: Assessment of fish catch composition and diversity were conducted in landing centre adjacent to Harike wetland through Rapid Fisheries Assessment by Market Survey (RFAMS) technique. Fin tissue samples were collected for genetic diversity analysis of *L. rohita* by cytochrome oxidase subunit I (COI) gene sequencing, From the fish genomic DNA a partial fragment of approximately 655bp was PCR amplified by FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TCGACTAATCATAAAGATATCGGCAC-3') primer pair. Calculation of intraspecific mean and pairwise distances was performed by MEGA 6.0 software using the K2P parameters

Result: Total 30 species of fishes were recorded from Harike wetland and these belong to 14 families and 21 genera. In *L. rohita* average weight (Wt), total length (TL), standard length (SL) and forked length (FL) were recorded 2600±5.64g (1700-3600 g), 58.2±5.65 cm (51.3-67.6cm), 48.0±8.54 cm (42.5-55.5cm) and 46.0±0.25 cm (38.0-54.5cm), respectively. Biometric study revealed that sufficient numbers of mature *L. rohita* are available in wetland. *L. rohita* established negative algometric growth (b= 2.701); thus species became slender as it increased in length. The pairwise distances ranged from 0.00 to 18.49% with a mean ±S.E value of 4.70% ±0.40. Total of 9 haplotypes were observed in *L. rohita* COI sequences. The diversity in haplotype and nucletide values were observed 0.848 and 0.024, respectively. The present study states that *L. rohita* stocks in Harike wetland are genetically diverse.

Key words: Biometrics, Cytochrome oxidase, Fish diversity, Harike wetland, *Labeo rohita*.

INTRODUCTION

The Cyprinidae family is the largest fish family of vertebrate animals, with about 3,000 species under 370 genera (Nelson, 2006). Because of tremendous diversity in this family, it is difficult to establish a proper phylogenetic relationship. The history related with systematics of Cyprinidae fishes is one of the confusing and debated part and because of that it is impossible to understand the phylogeny only by considering the morphological characters. Thus, mitochondrial and nuclear gene sequences have been routinely used to establish the phylogenetic relationships in this group. The mitochondrial genomic part is mostly carrying the hereditary information. The similarities in sequence serve as important source for functional and structural conservation (Sharma *et al*., 2014) of Harike wetland, an internationally recognized Ramsar site (31°08′N to 31°23′N latitudes and 74°90′E to 75°12′E longitudes) situated at confluence of two major rivers of Indus river system *i.e.* the Beas and Sutlej. It symbolize the fisheries resources of both the rivers and provides a healthy environmental conditions for feeding, breeding and nesting. Over fishing on any stock could imply the extinction of some important genetic characters with loss of intra-specific diversity which ultimately affect the species diversity (Lankau and Strauss, 2017). Ward *et al.* (2005), Hajibabei *et al.* (2007) and Lakra

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et al. (2011) studied the ability of cytochrome oxidase subunit I (COI) gene to differentiate two genera through the phylogram as the different branches. Harike Pattan, the place where river Beas and river Sutlej get mixed, thus possibility of variation within fish stock is very high. Cyprinidae family of freshwater fishes includes carps is the largest fish family found in Harike wetland comprising around 50% of total fish composition by weight basis and among carps, Rohu (*Labeo rohita*) is one of the commercially important fish species available throughout the year and preferred by consumers in this wetland (Datta *et al.,* 2017). Till date no detailed study related with biometric and genetic diversity based on mitochondrial marker genes has been conducted on *L. rohita* as well as Cyprinidae family from this wetland. To fill the gap, the present study was conducted with the objectives to study on biometric characters and genetic diversity based on mitochondrial marker genes of commercially important fish *L. rohita* from Harike wetland and generation of time series data with respect to fish diversity in Harike wetland.

MATERIALS AND METHODS

Taxonomy and biometric parameters

Study was conducted for duration of 6 months (September 2016 – February 2017) on monthly intervals at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Assessment of fish catch composition and diversity were conducted in landing centre adjacent to Harike wetland. The Rapid Fisheries Assessment by Market Survey (RFAMS) methodology described by White *et al*. (2014) was followed for fish catch composition. Catch composition of the landings was divided into major taxonomic families and for each of these major groupings, their percentage contribution to the landings (calculated from estimated weights of each of the families for all groups) and an estimate of the number of species observed was recorded following standard methods of Talwar and Jhingran (1991), Jayaram (1999) and Menon (1999). A total of 250 samples (at least 30 samples from each month) of *L.rohita* were considered for biometric study including 18 morphometric and 5 meristic count. Morphometric measurements were made with measuring board, vernier caliper closest to 0.1mm, magnifying glasses and needles. Counts and measurements were taken as far as possible on the left side of the specimens.

Length-weight relationship

The relationship between length and weight of fish were analysed by measuring length and weight of fish specimens collected from landing centre. Commercially important fish (*L. rohita*) was selected for Length-weight relationship. The statistical relationship between two parameters of fishes were established by using the parabolic equation by Forese (2006).

$$W = aL^b$$

Where,
W= Weight of fish (g),
L = Lenght of fish (mm),
a = Constant
b = An exponential expressing relationship between lenght-weight.

The relationship ($W = aL^b$) when converted into the logarithmic form gives a straight line relationship graphically

$$\text{Log } W = \text{Log } a + b \text{ Log } L$$

Where,
b represents the slope of the line, Log a is a constant.

Condition factor (K)

Condition factor (K) was estimated as per Htun- Han (1978) formula

$$K = W \times 100/L^3$$

Where
W = Weight of fish (9g) and L = Length of fish (cm)

Genetic diversity

During sample collection, a portion of caudal fin was cut by a sterilized scissor and aseptically transferred into a collection vial containing 95% ethanol. The samples were transported to lab on cool gel packs and stored at -80° C till further analysis. Genomic DNA from fish fin tissue was extracted by HiPurA Mammalian Genomic DNA Kit (HiMedia, India) as per supplier's protocol. The quality of genomic DNA was checked by agarose gel electrophoresis. From the fish genomic DNA a partial fragment of approximately 655 bp was PCR amplified by FishF1 (5′-TCAACCAACCACA AAG ACATTGGCAC-3′) and FishR1 (5′-TCGACTAATCATAAA GA TATCGGCAC-3′) primer pair (Ward *et al*., 2005). The 25 µl PCR reactions consisted of 2.5 µl of complete NH_4 reaction buffer (10x) (Bioron GmbH, Germany), 0.4 µM of each primer, 1 unit of DFS-Taq DNA polymerase (Bioron GmbH, Germany), 200 µM of each dNTP (GeNei, India) and 20 - 50 ng of bacterial genomic DNA. The PCR thermal cycler (Bio-Rad, USA) program consisted of initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30s, 55°C for 30s and 72°C for 60s. The final step for extension was performed at 72°C for 10 min. The resulting PCR amplicons were detected on 2% agarose gel as described above and subjected to purification by HiPurA PCR product purification kit as per provided protocols. The purified PCR products were subjected to bidirectional Sanger sequencing (Scigenom, Cochin) using the forward (FishF1) and reverse (FishR1) primers. The sequencing data and chromatograms were inspected and verified by DNASTAR Lasergene software (DNASTAR, USA) for any errors and discrepancies. All DNA sequences were aligned by ClustalW programme (Thomson *et al.,* 1994) in MEGA 6.0 software (Tamura *et al.,* 2011). After alignment, the neighbor joining (NJ) phylogenetic tree was constructed using the Kimura two parameter (K2P) distance model with pairwise deletion for ambiguous base/gap handling. The statistical support to NJ tree was provided by performing bootstrap analysis with

1000 replicates. Sequence data was also used to determine numbers of haplotypes, haplotype diversity index (*h*) and nucleotide diversity index (π) by DnaSP v5 software (Librado and Rozas, 2009). Statistical analysis was performed on SPSS -16 software package.

RESULTS AND DISCUSSION

Total 30 species of fishes were recorded from Harike wetland and these belong to 14 families and 21 genera. Maximum number of species (12) recorded under family Cyprinidae followed by Bagridae (3 species), Siluridae (3 species), Channidae (2 species), Mastacembelidae (2 species each), Notopteridae (2 species), Schilbeidae (1 species), Sisoridae (1 species), Ambassidae (1species), Belontiidae (1 species each), Clupeidae (1 species), Clariidae (1 species), Synbranchidae (1species), Belonidae (1 species) and Nandidae (1 species each). *Labeo rohita, Cyprinus carpio communis,Catla catla*, *Cirrihinus mrigala*, *Labeo calbasu* of Cyprinidae family; *Rita rita* of Bagridae family; *Wallago attu* of Siluridae family; *Channa marulius* and *C. striatus* of Channidae family; *Notopterus notopterus* and *N. chitala* of Notopteridae family were found in all months. Month wise availability of the fish genera and species depicted in the Table 1. Maximum number of genera (21) and species (30) were recorded in the month of January and minimum number of genera (12) and species (17) were recorded during winters This may be attributed to the onset of winter fish might have migrated towards down- stream of the rivers Two species, most abundantly found in the Harike wetland were *L. rohita* and *C. carpio* communis of Cyprinidae family in overall catch. In total Cyprinidae family was predominated in catch composition and accounted about 52.53% of total catch composition followed by Siluridae (14.95%), Channidae (10.90%), Bagiridae (7.68%), Notopteridae (7.53%) contributed significantly (Table 2).

Table 1: Month wise fish genera and species availability in Harike wetland.

Family	Number of genera						Number of species					
	Sep.	Oct	Nov	Dec	Jan	Feb	Sep	Oct	Nov	Dec	Jan	Feb
Cyprinidae	06	05	04	06	04	05	12	8	8	9	6	10
Bagridae	02	02	01	02	01	01	03	03	02	02	01	01
Siluridae	02	01	02	02	02	02	03	01	02	02	03	02
Schilbeidae	01	01	01	-	-	01	01	01	01	-	-	01
Sisoridae	01	01	01	01	01	01	01	01	01	01	01	01
Clariidae	-	-	01	-	-	-	-	-	01	-	-	-
Belonidae	01	01	-	-	-	-	01	01	-	-	-	-
Synbrachidae	01	01	-	-	-	-	01	01	-	-	-	-
Ambassidae	01	01	01	-	01	01	01	01	01	-	01	01
Nandidae	01	01	-	-	01	01	01	01	-	-	01	01
Channidae	01	01	01	01	01	01	02	02	02	02	02	02
Mastacemblidae	02	02	-	-	-	-	02	02	-	-	-	-
Clupidae	01	-	-	-	-	01	01	-	-	-	-	01
Notopteridae	01	01	01	01	01	01	02	02	02	02	02	02
Total	21	18	13	13	12	15	30	24	20	18	17	22

Table 2: Family wise catch composition (%) of fish species from Harike wetland.

Family	Weight basis catch composition (%)						Average
	Sep.	Oct.	Nov.	Dec.	Jan	Feb	
Cyprinidae	46.00	50.20	56.90	54.45	53.50	54.10	52.53
Bagiridae	8.50	9.25	8.50	7.20	5.90	6.70	7.68
Siluridae	16.20	14.30	13.00	15.40	17.02	13.75	14.95
Schilbeidae	0.80	0.60	0.70	0.50	-	-	0.65
Sisoridae	3.90	2.00	2.50	3.75	4.16	4.50	3.47
Clariidae	-	-	0.50	-	-	-	0.50
Belonidae	0.40	0.50	-	-	-	-	0.45
Synbrachidae	0.50	0.60	-	-	-	-	0.55
Ambassidae	0.40	0.30	0.30	-	0.40	0.40	0.36
Nandidae	0.10	0.20	-	-	0.10	0.20	0.15
Channidae	10.50	11.60	10.25	10.20	12.32	10.50	10.90
Clupidae	2.90	-	-	-	-	2.50	2.70
Mastacemblidae	2.50	2.40	-	-	-	-	2.45
Notopteridae	7.30	8.05	7.35	8.50	6.60	7.35	7.53

Among 250 samples studied for biometric analysis of *L. rohita* from Harike wetland average weight (Wt), total length (TL), standard length (SL) and forked length (FL) were recorded 2600±5.64g (1700-3600 g), 58.2±5.65 cm (51.3-67.6cm), 48.0±8.54 cm (42.5-55.5cm) and 46.0±0.25 cm (38.0-54.5cm), respectively. Dorsal fin rays (DFR), Pectoral fin rays (PFR), Anal fin rays (AFR), Caudal fin rays (CFR) ranged from 14-16, 16-18, 5-6 and 18-20, respectively along with Pelvic fin rays (PeFR) recorded 9 in number (Table 3). Biometric study revealed that sufficient numbers of mature *L. rohita* are available in wetland to support the recruitment in subsequent years.

L. rohita established negative algometric growth (b= 2.701); thus species became slender as it increased in length (Table 4). Choudhury *et al.* (1982) reported negative allometric growth (b = 2.347) of *L. rohita* from river Brahmaputra, Assam. Bhat (2011) reported growth coefficient (b) as 2.97 and coefficient of determination (r^2) as 0.98 from Phuj reservoir, Jhansi in *L. rohita.* Prasad *et al.* (2012) also reported negative allometric growth pattern of *L. rohita* from Govindgarh Lake, Rewa. Variation in slope may be attributed due to variation in sample size, life stages and environmental factors (Kleanthids *et al.,* 1999).

The co-efficient of determination (r^2) values explains the proper fit of the model for growth. In the present study, value of r^2 of *L. rohita* was calculated as 0.971 (97% variability) indicating more than 97% variability by the model and good fitness. Condition factor of *L. rohita* recorded greater than one (1.16) (Table 4) indicating fish stocks of the wetland maintaining a healthy condition which may be due to availability of preferred food items as evidenced by the present study during gut content analysis study.This values suggest a state of wellbeing for the species tested. Many factors affect the growth condition of fish including reproductive cycles, availability of food, as well as habitat and environmental factors. The deviation of Kn from 1 reveals information concerning the differences in food availability and consequence of physicochemical features on the life cycle of fish species (Le Cren, 1951).

Table 3: Biometric characteristics of *Labeo rohita* during the study period.

	September	October	November	December	January	February	Average
				Morphometric characters			
Wt (g)	3600^{a}±0.58	3300^{b}±0.25	3200^{c}±11.25	2200^{d}±14.54	1800^{e}±4.58	1700^{f}±0.24	2600±5.64
TL (cm)	67.6^{a}±5.65	63.1^{b}±1.25	60.8^{c}±0.25	53.0^{d}±6.54	53.3^{d}±7.54	51.3^{e}±0.85	58.2±5.65
SL	55.5^{a}±4.25	51.5^{b}±1.65	50.6^{c}±1.35	45.0^{d}±3.25	43.0^{e}±3.25	42.5^{f}±4.51	48.0±8.54
FL	54.5^{a}±9.65	54.6^{a}±0.25	49.0^{b}±1.25	46.3^{c}±1.24	41.7^{d}±1.24	38.0^{e}±1.02	46.0±0.25
HL	26.4^{a}±4.52	23.0^{b}±0.02	23.0^{b}±0.24	11.1^{c}±0.02	10.0^{d}±4.25	9.5^{e}±0.25	15.2±4.15
HD	10.2^{a}±0.58	10.2^{a}±0.84	10.1^{a}±0.45	7.4^{b}±0.65	7.3^{b}±0.25	6.6^{c}±0.24	8.4±0.37
SnL	5.0^{a}±0.31	4.8^{b}±0.57	4.7^{b}±1.25	4.1^{c}±0.24	3.9^{d}±0.35	3.4^{e}±0.65	4.3±0.98
ED	1.8^{a}±0.02	1.7^{b}±0.05	1.7^{b}±0.00	1.4^{c}±0.01	0.9^{d}±0.00	0.9 ^{d}±0.01	1.3±0.01
IOL	8.9^{a}±0.21	8.6^{b}±0.34	8.3^{c}±0.54	7.2^{d}±1.69	6.3^{e}±0.35	5.6^{c}±0.25	7.3±0.97
PDL	24.0^{a}±1.24	23.6^{b}±0.21	22.4^{c}±0.25	18.5^{d}±2.34	17.7^{e}±0.35	16.3^{f}±0.23	20.4±1.87
PPL	12.03^{a}±1.25	11.3^{b}±0.25	11.5^{b}±1.49	9.9^{d}±1.25	10.4^{c}±0.54	9.4 ^{d}±0.65	10.7±0.25
PPeL	28.0^{a}±6.35	28.1^{a}±4.58	27.6^{b}±4.58	22.4^{c}±0.58	21.8^{d}±0.21	21.3^{d}±0.59	24.9±1.23
PAL	42.7^{b}±0.21	43.0^{a}±2.31	41.0^{c}±0.25	34.5^{d}±0.32	33.0^{e}±0.32	32.3^{f}±0.75	37.7±0.27
HOD	6.4^{a}±1.25	6.3^{a}±0.24	6.0^{b}±0.65	5.6^{c}±0.47	5.8^{c}±0.57	5.7^{c}±0.32	6.0±0.35
AL	10.7^{a}±2.35	9.8^{b}±0.25	8.8^{c}±0.65	7.5^{d}±0.46	8.3^{c}±6.32	8.1^{c}±1.58	8.8±2.78
BD	16.0^{a}±1.41	16.8^{a}±0.24	16.3^{a}±2.56	13.3^{b}±1.49	13.2^{b}±4.25	13.5^{b}±5.85	14.8±1.89
CD	7.6^{a}±0.52	7.4^{a}±0.61	7.4^{a}±1.45	7.0^{a}±1.35	6.2^{b}±0.85	6.5^{b}±0.65	7.2±0.97
CL	11.9^{a}±0.85	11.8^{a}±0.94	11.9^{a}±0.54	11.2^{a}±2.62	10.8^{b}±2.58	10.6^{b}±1.65	11.3±2.57
CFS	10.4 ^{b}±1.41	12.3^{a}±0.87	10.3^{b}±2.35	9.6^{c}±0.58	9.0^{c}±0.54	8.8^{d}±0.50	10.1±1.35
				Meristic characters			
DFR	15^{a}±1.00	15^{a}±0.00	15^{a}±2.00	14^{a}±1.00	14^{b}±1.00	14^{a}±1.00	15±1.00
PeFR	9^{a}±0.00	9^{a}±0.00	9^{a}±0.00	9^{a}±0.00	9^{a}±0.00	9^{a}±0.00	9±0.00
PFR	18^{a}±1.00	18^{a}±0.00	18^{a}±0.00	16^{a}±0.00	16^{a}±2.00	16^{a}±1.00	17±1.00
AFR	6^{a}±0.00	6^{a}±1.00	6^{a}±0.00	5^{a}±0.00	6^{a}±1.00	5^{a}±0.00	6±0.00
CFR	20^{a}±1.00	20^{a}±0.00	20^{a}±0.00	19^{a}±2.00	18^{a}±2.00	18^{a}±2.00	19±1.00

*(Weight of fish (Wt), Total length (TL), Standard length (SL), Fork length (FL), Head length (HL), Head depth (HD), Snout length (SnL), Eye diameter (ED), Inter-orbital length (IOL), Pre-dorsal length (PDL), Pre-pectoral length (PPL), Pre-pelvic length (PPeL), Pre-anal length (PAL), Height of dorsal fin (HOD), Anal fin length (AL), Body depth (BD), Caudal depth (CD), Caudal length (CL) and Caudal fin surface (CFS). **Dorsal fin rays (DFR), pelvic fin rays (PeFR), Pectoral fin rays (PFR), Anal fin rays (AFR), Caudal fin rays (CFR).
***The values (mean ± standard error) with different alphabetical superscripts (a, b, c…) differ significantly ($p \leq 0.05$) between the months (in a row).

Table 4: Logarithmic equation of length-weight relationship, coefficient of determination and condition factor of *L. rohita* from Harike wetland during the study period.

Fish Species	Logarithmic equation Log W = log a + b log L	Correlation coefficient 'r'	Coefficient of determination 'r2'	Condition factor 'K'	'b'
L. rohita	Log W = log0.025 + 2.701 log L	0.985	0.971	1.16	2.701

Table 5: Estimation of evolutionary divergence between COI sequences.

Fishes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1_COI	0.00														
2_COI	0.87	0.00													
3_COI	1.01	0.40	0.00												
5_COI	1.25	0.36	0.81	0.00											
6_COI	1.86	0.70	1.01	1.07	0.00										
7_COI	0.84	1.05	1.01	1.43	8.64	0.00									
8_COI	6.65	5.77	5.64	6.09	14.87	10.73	0.00								
9_COI	7.66	7.79	8.42	8.19	13.23	12.12	18.49	0.00							
10_COI	4.66	4.47	4.14	4.01	12.39	10.71	14.88	18.40	0.00						
1J_COI	1.71	0.87	1.01	1.25	1.89	4.86	10.17	9.70	9.24	0.00					
2J_COI	1.70	0.87	1.01	1.25	0.78	4.57	9.87	9.23	8.25	0.31	0.00				
3J_COI	1.69	0.87	1.01	1.25	4.26	7.06	13.06	11.75	11.81	1.09	0.00	0.00			
4J_COI	1.69	0.87	1.01	1.25	0.94	4.54	9.81	9.17	8.20	0.31	0.00	0.00	0.00		
5J_COI	1.71	0.70	1.01	1.07	0.79	4.09	9.77	9.50	8.14	0.16	0.47	0.47	0.47	0.00	
6J_COI	0.00	0.74	1.01	1.14	4.79	7.44	14.30	15.11	12.11	0.67	0.00	1.95	0.00	0.34	0.00

The rate of % base substitution between sequences have been shown.

Genetic diversity in *L. rohita*

Sequence alignment and phylogenetic analysis

The raw sequencing reads generated by Sanger sequencing were subjected to quality trimming. Sequencing bases from both ends with sequencing Quality Value (QV) of <20 were removed. QV >20 is an established metric for determining the quality of sequencing data. It means that probability of a base being miscalled (wrongly sequenced) was not more than 1%. After quality trimming, the sequencing reads ranged from 497-696 bp in length. Sequence alignment by ClustalW and subsequent phylogenetic tree construction revealed the presence of two distinct clades with majority of COI sequences (14 out of 15) getting clustered in one clade (Fig 1). This particular clade further got diverted into two branches. The phylogenetic tree generated in this study was quite reliable as two outgroup COI sequences belonging to *Sperata seenghala* were found to be clustered together and separated from *L. rohita* COI sequences.

COI sequence variation in *L. rohita*

Calculation of intraspecific mean and pairwise distances was performed by MEGA 6.0 software using the K2P parameters. The pairwise distances ranged from 0.00 to 18.49% with a mean±S.E. value of 4.70% ±0.40 (Table 5). During genetic diversity analysis by mitochondrial sequences, numbers of haplotype (h), haplotype div ersity (Hd) and nucleotide diversity (π) are considered as important metrics in any population. A haplotype is a group of genes in an organism that are inherited together from a single parent. Haplotype diversity (also known as gene diversity) represents the

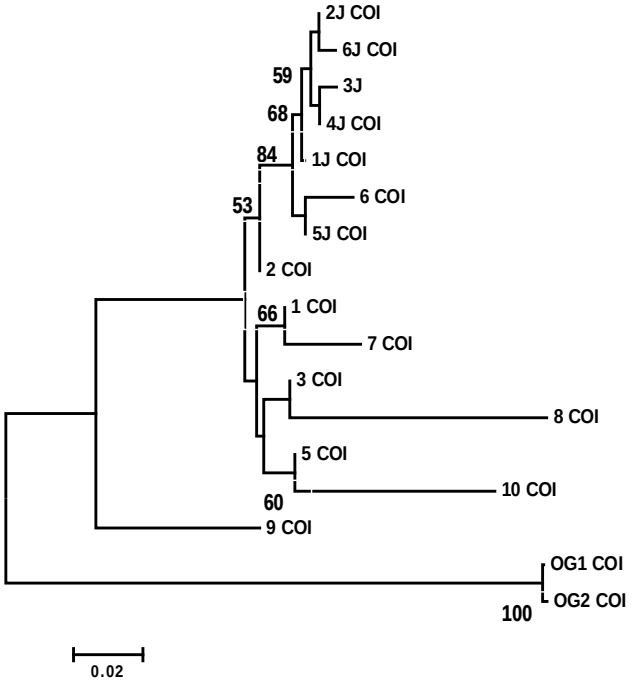

Fig 1: Neighbour joining phylogenetic tree of *Labeo rohita* COI sequences prepared with Kimura two parameter model with 1000 bootstrap replicates. OG1 and OG2 represents the outgroup *Sperata seenghala* COI sequences for testing the reliability of phylogenetic tree. Bootstrap values of >50 have been indicated on nodes. Scale bar represents 0.02 substitution per nucleotide site.

probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences (de Jong *et al*., 2011). Being a probability metric, the highest value of haplotype diversity could be one. In the present study, a total of 9 haplotypes were observed in 15 *L. rohita* COI sequences. The haplotype diversity and nucleotide diversity values were found to be 0.848 and 0.024, respectively. Though no previous data from Harike wetland is available, the genetic diversity of wild populations of *L. rohita* in various Indian rivers systems has been investigated by other researchers. In a study by Luhariya *et al.* (2012), a total of 35 haplotypes with haplotype diversity of 0.751 and nucleotide diversity of 0.005, were observed in 146 samples of *L. rohita* collected from nine distant rivers; Satluj, Brahmaputra, Son, Chambal, Mahanadi, Rapti, Chauka, Bhagirathi and Tons. Comparatively higher (closer to one) value of haplotype diversity in the present study indicated that *L. rohita* stocks in Harike wetland were genetically diverse. Moreover, higher nucleotide diversity values in the present study, also indicated more difference between haplotypes in Harike wetland.

CONCLUSION

Biodiversity study revealed that wetland is supporting a good number of fish diversity of different tropic levels which is a indicator of a healthy ecosystem. Biometric study of *L. rohita* revealed that sufficient numbers of mature fish are available in wetland which could support the subsequent breeding and recruitment in a sustainable manner. Population genetics study on *L. rohita* stocks in Harike wetland revealed that stock are genetically diverse. Though the wetland is still maintaining a good fish diversity but at the same time siltation, weed infestation, pollution and encroachment would be the major threats as observed during the study, for the fish diversity of the wetland in near future which need to be addressed judiciously. The findings pertaining to this study may be utilized for future policy formulation in terms of conservation of fish biodiversity of this Ramsar site.

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