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Morphological and molecular diversity of *Hypophthalmichthys molitrix*, Silver carp (Cypriniformes: Cyprinidae) from Tarbela Dam Khyber Pakhtunkhwa, Pakistan

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ABSTRACT
Hypophthalmichthys molitrix commonly known as Silver carp is fresh water carp that native to Russia and China. The habitat of this fish is large rivers, ponds, slow running water and flood plains but now threatened to natural ecosystem. The present study was conducted from 25 June to 10 August 2024 to investigate morphology and genetic diversity of *H. molitrix* from Tarbela Dam district Haripur Khyber Pakhtunkhwa, Pakistan. We collected 10 fish specimens from Tarbela Dam and identified on the base of morphology as spindled shaped, laterally compressed body with silver coloration, keeled body extended from throat to vent, lower jaw smaller than upper jaw, forked shaped caudal fine with curved lateral line, serrated spins at the front of dorsal and pectoral fin. We analyzed a total of 47 morphological characters and calculated the standard deviation, mean, regression, range difference and Correlation. We submitted Cytochrome oxidase sub unit I (*COI*) gene to GenBank for accession number as PP275112. The reported sequence of *COI* gene from Pakistan shows high similarity almost 99.42% with other sequences of *H. molitrix* reported from China and America. Additionally, we constructed a phylogenetic tree to clarify their phylogenetic relation *H. molitrix* with other related species.

Keywords: Flood plains, genetic diversity, *COI* gene, Haripur, GenBank

INTRODUCTION

Tarbela dam is the world largest earth filled Dam constructed on Indus River located in Tarbela village Hazara division District Haripur Khyber Pakhtunkhwa, Pakistan (Agro-Dev, 2000). The geographical location of the Tarbela Dam is delineated to District Buner in the north, Islamabad the capital territory in the northwest, District Swabi in the east and Punjab Province in the south (Amira, 2020). The average annual flow of this Dam is 101billion cubic meter, the storage capacity is 11.9 billion cubic meter, and surface area is 243 squares kilometer (White, 2001). Tarbela Dam is 12th largest dam in the world in the production of electricity. 70% of Tarbela water not used in the production of electricity and they passed from the dam spillway (Abid et al., 2010). Tarbela Dam is the largest reservoir of fresh water providing balance aquatic ecosystem to support and enhance the fish production. The Fisheries department of Khyber Pakhtunkhwa releasing young fish (fingerlings) into Tarbela Dams to enhance and maintain fisheries. Every year, the Fisheries department of Khyber Pakhtunkhwa stocking 2 Lac (0.2M) fish species of Rohu, Mori, Silver Carp, Grass Carp and Big Head Carp of about 1-2 inch size in Tarbela Dam (Ahmad, 2015).

Hypophthalmichthys molitrix, commonly known as silver carp belonging to subclass

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Actinopterygii (ray-finned fish), is fresh water cyprinid, native to China and Russia (eastern Siberia) (Fishbase). This fish is exotic and exported from China to Pakistan in 1964 to control weeds and eutrophication (Mirza & Sandhu 2007). This fish has specialized feeding organ, the epibranchial organ that secrete mucus and spongy filter with fused gill rakers that trap the minute food particles. Silver carp is usually filter feeder and filter the ditrus, zooplankton and phytoplankton from water (Tumolo, 2019).

H. molitrix is large, elongated, spindle shaped and latterly compressed carp with uniform silver coloration. This fish has 95-103 scales on lateral line. Large mouth without teeth, at the front of pectoral and pelvic fin have strong serrated spins. 12.13 anal fin and 8 dorsal fin rays, 4-4 pharyngeal teeth in pharynx and fused gill rakers with spongy filter (Robison & Buchanan 1988). Fish body is extremely keeled extended from throat to vent with gray black dorsally and silver color in ventral and lateral side. The scales are small cycloid, scale below the lateral line is 28-30 and above the lateral line is 16-17 (Kolar et al., 2007). Silver carp farming is very old and started in 5th century in China. In 1950 the culturing and breeding of this fish was introduced all over the China and exported to 88 countries of the world. This fish attain the maximum length of 140cm and 50kg of maximum weight (Roy et al., 2018). *H. molitrix* is fresh water and brackish water fish usually live in warm water with temperature of 43°- 46°C (Kolar et al., 2007). The average life spin of this fish is 20 years and sexually mature after 6-10 year, in America this fish mature sexually at the age of two years (Nico et al., 2019). For the spawning of this fish required optimum conditions i.e., 15°C temperature, 0.5-1.7M/S water current, high oxygen level and turbid water (Freyhof & Kottelat, 2007).

H. molitrix is economically very important fish that provide fresh meat to local communities and also control the eutrophication and algal bloom. This study provide a baseline and management of *H. molitrix* population across the Khyber Pakhtunkhwa, Pakistan.

MATERIAL AND METHODS

Study area

The selected area for fish sampling was Tarbela Dam Khyber Pakhtunkhwa, Pakistan. Tarbela dam is world largest dam that located in District Haripur on Indus River in village Tarbela with Coordinates 34°05′23″N and 72°41′54″E. The geographical location of the Tarbela Dam is delineated to District Buner in the north, Islamabad the capital territory in the northwest, District Swabi in the east and Punjab Province in the south. The average annual flow of this Dam is 101billion cubic meter, the storage capacity is 11.9 billion cubic meter, and surface area is 243 squares kilometer (**Figure 1**).

Figure 1. Map of Tarbela Dam

Sample collection

A total of 10 fish specimens were collected from Tarbela Dam using Cast net and Trawl

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net. The collected samples were preserved in 70% ethanol and brought back to Aquaculture and Fisheries lab, department of Zoology University of Peshawar for DNA extraction and morphological analysis.

Fish identification

The collected fish samples were morphologically identified by using fish identification standard keys i.e., Key to families of Fishes (Lagler et al., 1977), Fishes of the world (Nelson, 2006), literature of the fishes of Punjab (Mirza & Sendu 2007) and Fish base (online database)**.**

Morphometric Measurements

After the morphological identification a total of 47 morphometric characters were recorded by using digital caliper, digital balance, standard foot, watch glass, thread, pencil and paper. These morphometric characters were Fork length **(FL),** Standard length **(SL)**, Total length **(TL)**, Eye diameter **(ED)**, Width of mouth **(WM)**, Head depth **(HD)**, Length b/w nasals **(LN)**, Inter orbital length **(IOL)**, Pre pelvic Length **(PrPL)**, Pre dorsal Length **(PDL)**, Pre pectoral Length **(PPL)**, Pre anal Length **(PAL)**, Pre orbital Length **(PrOL)**, Post dorsal Length **(PoDL)**, Post pectoral Length **(PoPL)**, Post pelvic Length **(PPeL)**, Post anal Length **(PoAL)**, Snout length **(SnL)**, Post orbital Length **(POL)**, Distance b/w pelvic fin and anus **(DPA)**, Distance b/w pelvic pectoral fins **(DPPF)**, Distance b/w pelvic anal fins **(DPAF)**, Scales below lateral line **(SBLL)**, Scales above lateral line **(SALL)**, Scales on lateral line **(SLL)**, Length of dorsal fin **(LDF)**, Length of pectoral fin **(LPF)**, Length of pelvic fin **(LPiF)**, Length of anal fin **(LAF)**, Length of Caudal fin **(LCF)**, Height of dorsal fin **(HDF)**, Height of pectoral fin **(HPF)**, Height of pelvic fin **(HPiF)**, Height of anal fin **(HAF)**, Height of Caudal fin **(HCF)**, Number of rays in dorsal fin **(NRDF)**, Number of rays in pectoral fin **(NRPF)**, Number of rays in pelvic fin **(NRPiF)**, Number of rays in anal fin **(NRAF)**, Number of rays in Caudal fin **(NRC)**, Lower jaw width **(LJW)**, Body minimum depth **(BMiD)**, Body maximum depth **(BMD)**, Caudal peduncle height **(CPH)**, Caudal peduncle length **(CPL)**, and Weight **(W)**.

Statistical analysis of morphometric characters

For the statistical analysis the morphometric characters were arranged in various columns and rows in Microsoft excel to calculate mean, range, standard deviation and range difference. The regression and correlation were measured with respect of Total length **(TL)**.

Molecular and Phylogenetic analysis

We extracted mDNA from collected fish samples using Ammonium acetate based protocol (Bruford et al. 1998). For the mDNA extraction we taken 20mg fish muscles, gills and liver by applying 20µl proteinase K enzyme and ammonium acetate solution. For the amplification of Cytochrome oxidase sub unit I (*COI*) we used the universal (*COI*) primer i.e., forward and reverse LCO1490: 5'- GGT CAA CAA ATC ATA AAG ATA TTG-3' and HC02198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA -3' (Flomer et al., 1994). The PCR products were sequenced in Rahman medical institute and hospital through next generation sequencing machine. The new available sequence were edited and termed using Bio Edit and submitted to GenBank for accession number. The new sequence were aligned through MEGA 11 for the construction of Phylogenetic tree (Hall, 1999).

RESULTS

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Morphological identification

The *H. molitrix* were elongated, compressed and spindle shaped fish. The maximum total length (TL) were recorded 38.6-44.0cm. Body was sharp keeled that extended from throat to vent. Head was short, the snout was round and without barbells, mouth was terminal, large, obtuse and toothless, upper jaw was larger than lower jaw, gill rakers were found fused, skin covered by small cycloid scales, forked shaped caudal fin and curved lateral line, silver colored skin and red dots on caudal peduncle with black color fins, seriated spin at the front of pectoral and dorsal fin, lateral line scales were 113-120. Fin formula is D. 3/7, P. 1/17, V. 1/7, A. 2-3/12-14, C. 19 (**Figure 2a & 2b**)

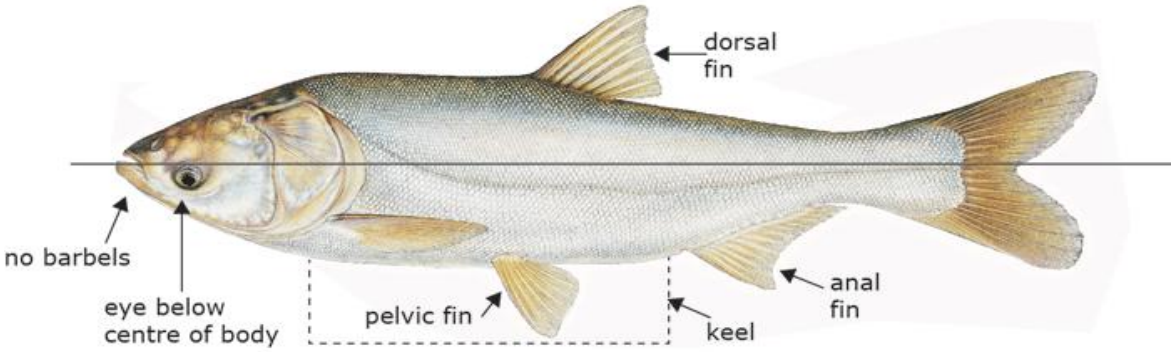

Figure 2a. Dorsal view of labelled edited image of *H. molitrix*

Figure 2b. Dorsal view of fresh collected sample of *H. molitrix*

Morphometric measurements

We collected a total of 10 fish samples from Tarbela Dam district Haripur Khyber Pakhtunkhwa, Pakistan. We recorded 47 morphometric characters of collected fish samples and analyze statistically (**Table 1**).

Table 1.

Morphometric characters of 10 collected samples of H. molitrix

S:No	Morphometric characters	1	2	3	4	5	6	7	8	9	10
1	TL	44.0	42.0	38.6	39.6	39.9	43.6	41.0	39.4	42.8	40.0
2	SL	37.2	35.0	32.0	33.0	33.6	37.6	34.8	32.4	35.0	33.2
3	HL	10.8	9.9	9.8	9.4	9.9	10.0	9.8	9.0	10.0	9.9

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4	FL	37.9	36.9	34.4	35.2	35.8	39.0	37.2	35.4	37.2	36.2
5	WM	2.0	1.8	1.5	1.6	1.8	1.4	1.0	1.8	1.2	1.0
6	ED	1.9	1.5	1.7	1.8	1.8	2.0	1.8	1.6	1.8	1.7
7	LN	3.5	3.0	2.8	2.9	2.8	3.0	2.8	3.8	3.0	3.0
8	PDL	18.2	18.0	17.0	17.8	17.8	18.2	17.8	16.9	18.0	17.0
9	PoDL	15.5	15.0	12.8	13.2	13.0	15.0	14.0	13.0	14.8	13.6
10	PPL	10.4	10.0	9.9	10.0	10.2	10.6	10.0	9.4	10.2	9.6
11	PoPL	24.2	23.9	22.4	22.8	21.9	27.2	24.0	23.0	24.2	23.6
12	PrPL	16.4	15.6	15.0	16.0	15.2	17.0	16.8	16.2	16.6	16.4
13	PPeL	19.1	18.8	18.4	17.8	18.0	21.0	18.9	17.8	18.4	18.0
14	PAL	24.5	23.4	22.0	24.0	23.0	26.0	25.4	23.8	25.4	24.0
15	PoAL	6.4	6.2	6.0	6.0	6.0	7.2	6.0	6.0	5.4	6.0
16	SnL	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
17	PrOL	3.4	3.2	2.9	2.8	3.0	2.0	2.0	2.4	2.4	2.2
18	POL	6.0	5.4	5.4	5.8	5.8	5.8	5.2	5.2	5.8	5.0
19	DPPF	5.0	4.8	4.0	4.8	4.4	6.0	5.4	4.8	5.0	5.0
20	DPA	6.8	6.0	6.0	6.0	6.6	8.0	7.2	6.4	7.0	7.0
21	DPAF	7.4	7.1	7.0	7.0	7.0	9.0	8.2	7.2	8	8.0
22	SLL	115	115	112	116	115	115	114	115	120	113
23	SALL	114	112	113	117	114	115	113	114	117	115
24	SBLL	115	114	114	114	115	114	116	114	114	114
25	LDF	4.2	4.0	3.8	3.0	3.4	4.0	3.6	3.0	4.0	3.9
26	HDF	7.2	7.0	6.5	7.0	7.0	7.1	6.2	6.4	6.8	6.0
27	NRDF	8	8	8	8	8	8	8	8	8	8
28	LPF	2.2	1.8	1.6	1.8	1.9	1.8	1.8	2.0	1.6	2.0
29	HPF	7.6	7.2	6.9	6.8	7.2	6.8	6.4	7.0	6.0	6.9
30	NRPF	16	16	16	16.0	16.0	16	16	16	16	16
31	LPiF	1.1	0.9	0.9	1.0	1.0	0.8	1.0	1.0	1.0	1.0
32	HPiF	5.8	5.4	5.0	4.8	5.1	5.0	5.2	5.4	5.4	5.0
33	NRPiF	8.0	8.0	8.0	8.0	8.0	8	8	8	8	8.0
34	LAF	6.0	5.8	5.4	5.0	5.0	5.0	5.2	5.0	6.0	5.1
35	HAF	4.8	4.5	4.6	4.0	4.0	4.6	4.4	4.4	4.6	4.4
36	NRAF	13	13	13	13	13	13	13	13	13	13
37	BMD	14.4	13.0	12.2	11.9	11.0	11.0	12.0	12.0	12.0	11.9
38	BMD	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
39	CPL	5.0	4.8	4.2	4.4	4.0	5.0	4.8	4.2	4.8	4.6

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40	CPH	5.1	4.8	4.4	4.8	4.2	5.0	4.4	4.6	4.8	4.5
41	LCF	9.4	9.0	8.9	8.8	8.4	7.8	7.9	8.8	8.8	8.0
42	HCF	6.2	6.0	5.0	6.0	6.6	6.4	6.0	6.0	6.0	6.0
43	NRCF	19	19	19	19	19	19	19	19	19	19
44	W	750	701	588	603	628	658	604	576	623	600
45	HD	5.2	5.0	4.9	5.0	5.0	6.2	6.0	5.0	4.8	5.4
46	LJW	3	3	3	3	3	3	3	3.2	3.2	3.0
47	IOL	7.0	6.6	6.8	6.8	6.6	6.4	6.6	6.4	6.0	6.4

Statistical analysis of *H. molitrix*

A total of 47 morphometric characters were analyze statistically which includes mean, standard deviation, range, range difference, regression and correlation. The regression and correlation were calculated with respect of total length. Out of these characters, 5 characters showed high value of correlation coefficient which implies that these characters are directly proportional and hence dependent on each other. While 42 morphometric characters showed moderate correlation coefficient (**Table 2**).

Table 2.

Mean, standard deviation, range, range difference, correlation and regression of the morphometric characters of H. molitrix

S:NO	Name	Range difference	Range	SD	Mean	Regression	Regression
1	TL	5.4	44-38.6	1.9	41.09		
2	SL	5.6	37.6-32	1.91	34.38	Y=0.959+8.114X	**0.964**
3	HL	1.8	10.8-9.0	0.46	9.85	Y=2.952+12.010X	0.712
4	FL	4.6	39-34.4	1.38	36.52	Y=1.278+-5.591X	**0.932**
5	WM	1	2.0-1.0	0.35	1.51	Y=0.479+40.366X	0.089
6	ED	0.5	2.0-1.5	0.14	1.76	Y=6.608+29.458X	0.498
7	LN	1	3.8-2.8	0.33	3.06	Y=1.022+37.961X	0.178
8	PDL	1.3	18.2-16.9	0.51	17.67	Y=3.049+-12.787X	**0.816**
9	PoDL	2.7	15.5-12.8	1.01	13.99	Y=1.823+15.575X	**0.968**
10	PPL	1.2	10.6-9.4	0.35	10.03	Y=3.990+1.068X	0.742
11	PoPL	5.3	27.2-21.9	1.46	23.72	Y=1.007+17.188X	0.774

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12	PrPL	2	17-15	0.67	16.12	Y=1.726+13.265X	0.608
13	PPeL	3.2	21-17.8	0.96	18.62	Y=1.424+14.569X	0.717
14	PAL	4	26-22	1.22	24.15	Y=1.101+14.478X	0.707
15	PoAL	1.8	7.2-5.4	0.45	6.12	Y=1.795+30.103X	0.43
16	SnL	0	1.0-1.0	1	1	Y=0+41.09X	0
17	PrOL	1.4	3.4-2.0	0.5	2.63	Y=0.148+40.699X	0.039
18	POL	1	6.0-5.0	0.34	5.54	Y=3.021+24.353X	0.542
19	DPPF	2	6.0-4.0	0.53	4.92	Y=2.380+29.378X	0.671
20	DPA	2	8.0-6.0	0.64	6.7	Y=1.748+29.374X	0.591
21	DPAF	2	9.0-7.0	0.69	7.49	Y=1.774+27.800X	0.642
22	SLL	8	120-112	2.11	115	Y=0.42+-7.209X	0.467
23	SALL	5	117-112	1.65	114.4	Y=0.116+27.774X	0.101
24	SBLL	2	116-114	0.7	114.4	Y=0.35+1.05X	0.129
25	LDF	1.2	4.2-3.0	0.43	3.69	Y=3.104+29.635X	0.7
26	HDF	1	7.2-6.2	0.4	6.73	Y=2.676+23.078X	0.56
27	NRDF	0	8.0-8.0	0	8	Y=0+41.09X	0
28	LPF	0.6	2.2-1.6	0.22	1.79	Y=2.893+35.911X	0.341
29	HPF	1.6	7.6-6.0	0.6	6.75	Y=0.497+37.730X	0.157
30	NRPF	0	16-16	0	16	Y=0+41.09X	0
31	LPiF	0.3	1.1-0.8	0.08	0.97	Y=-0.868+-41.932X	-0.04
32	HPiF	1	5.8-4.8	0.29	5.22	Y=3.739+21.571X	0.563
33	NRPiF	0	8.0-8.0	0	8	Y=0+41.09X	0
34	LAF	1	6.0-5.0	0.43	5.34	Y=2.573+27.346X	0.587
35	HAF	0.8	4.8-4.0	0.26	4.42	Y=4.516+21.128X	0.623
36	NRAF	0	13-13	0	13	Y=0+41.09X	0
37	BMD	3.4	14.4-11.0	0.98	12.15	Y=0.725+32.280X	0.373

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38	BMD	0	4.0-4.0	0	4	Y=0+41.09X	0
39	CPL	1	5.0-4.0	0.37	4.55	Y=4.640+19.974X	**0.902**
40	CPH	0.9	5.1-4.2	0.32	4.63	Y=4.465+20.413X	0.753
41	LCF	1.6	9.4-7.8	0.5	8.62	Y=0.073+40.459X	0.019
42	HCF	1.6	6.6-5.0	0.42	6.02	Y=2.160+28.082X	0.473
43	NRCF	0	19-19	0	19	Y=0+41.09X	0
44	W	0	0-0	54.9	633.1	Y=0.027+23.714X	0.795
45	HD	1.4	6.2-4.8	0.48	5.25	Y=1.440+33.526X	0.364
46	LJW	0.2	3.2-3.0	0.08	3.04	Y=0.0625+40.900X	0.003
47	IOL	1	7.0-6.0	0.28	6.56	Y=-0.857+46.718X	-0.13

Range difference of morphometric characters of *H. molitrix*

We calculated the range difference of total 47 morphometric characters and classified into vast range, intermediate range and narrow range. Of these 47 morphometric characters 7 characters were vast in range, 23 characters were intermediate in range and 6 were narrow in ranged. While the eleven characters range were equal to zero (**Table 3).**

Table 3.

The Morphometric characters of H. molitrix in vast range, intermediate range and narrow range

S:NO	Range difference	Morphometric characters
1	Narrow range	ED, LPF, LPiF, HAF, CPH, and LJW.
2	Vast range	TL, SL, FL, PoPL, PAL, SLL, and SALL
3	Moderate range	HL, WM, LN, PDL, PoDL, PPL, PrPL, PPel, POL, DPPF, DPA, SBL, LDF, HDF, HPF, HPiL, LAF, BMD, CPL, LCF, HCF, HD and IOL

Molecular and Phylogenetic analysis

We extracted mDNA from the liver and muscle of fish specimen collected from Tarbela Dam Khyber Pakhtunkhwa, Pakistan. The new available Cytochrome oxidase (COI) sequences was submitted into GenBank for accession number. The GenBank accession numbers for the newly generated sequence was PP275112 (695bp; Tarbela Dam). The new sequence was blast through NCBI. The Cytochrome oxidase sub unit I (*COI*) genes of *H. molitrix* from Pakistan showed 99.42% similarity with *H. molitrix* reported from America and China (**Table 4**). A total 14 sequences were downloaded from NCBI and aligned in MEGA11 to construct Phylogenetic tree. The phylogenetic tree consist of two groups i.e., the out-group consist of frog species, *M. agricola* and the in-group were consist of *H. molitrix* (**Figure 3**).

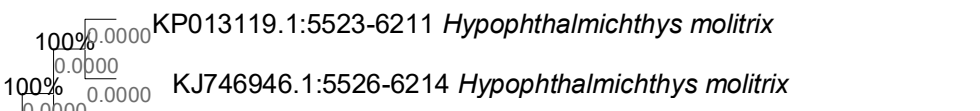

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Figure 3. Phylogenetic analysis of the reported *COI* gene sequences of *H. molitrix*.

Table 4.
Accession number, query cover, base pairs, similarity index and country name of the reported sequence of H. molitrix

S:NO	Accession numbers	Query covered %	Base pairs	Similarity index%	Country name
1	PP275112	100	695	100	Pakistan
2	KJ746947	100	16615	99.42	America
3	KJ746953	100	16615	99.42	America
4	KJ746955	100	16614	99.42	America
5	MT852971	100	1551	99.42	America
6	KJ729093	100	16615	99.42	America
7	KJ729094	100	16615	99.42	America
8	KJ746960	100	16614	99.42	America
9	KJ729076	100	16615	99.42	America
10	KJ746952	100	16615	99.42	America
11	KJ729092	100	16614	99.42	America
12	KJ746951	100	16614	99.42	America
13	KJ746957	100	16614	99.42	America
14	KP013119	100	16612	99.42	China

Discussion

H. molitrix, Silver carp or Silver fin fish is a fresh water carp native to Russia and China and exotic to Pakistan, Africa and Europe. This fish inhabits in large rivers, canals, lakes,

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slow running water and flood plains but usually threatened to natural habitats. The present study was conducted from 25 June to 10 August 2024 to investigate the morphology and genetic diversity of *H. molitrix* from Tarbela Dam district Haripur Khyber Pakhtunkhwa, Pakistan. A total of ten specimens were collected from Tarbela Dam and identified on the basis of morphology having elongated, compressed and spindle shaped body. The Maximum Total body length (TL) was recorded as 38.6-44.0cm. The body was sharp keeled that extended from throat to vent, short head and round snout without barbells, mouth was large, terminal, obtuse and toothless, upper jaw was larger than lower jaw, gill rakers were found fused, forked shaped caudal fin and curved lateral line, silver colored skin, red dots on caudal peduncle with black color fins, seriated spin at the front of pectoral and dorsal fin and the lateral the line scales were 113-120. These findings were similar to the study of Mirza & Sandu (2007), Robison and Buchanan (1988), Koler et al. (2017) and Roy et al. (2018). A total of 47 morphometric characters of the collected 10 samples were recorded and calculated range, range difference, standard deviation, correlation and regression with respect to total length. Of these, 4 morphometric characters had high value of correlation that indicates that all these morphometric characters directly depends on each other, 43 characters had moderate value of correlation. The range difference of morphometric characters was also calculated, of which 7 characters were vast in range, 26 were intermediate and 6 characters were narrow in range. In the morphometric analysis is very important. During growth and development various parts of fish body are changed (Bhuiyan & Islam 1990). For the taxonomic study the morphometric and statistically analysis is important, the standard length, forked length and total length is directly proportional to fish growth (Tandon et al., 1993). For study of the fish taxonomy and evolution the study of fish morphology is very important. The fish morphology consist of meristic and morphometric characters (Cailliet et al., 1986). On the base range difference the morphometric characters are divided into vast range (environmentally control), narrow range (genetically control) genetically and moderate ranged (intermediate range) (Johal et al., 1994). The vast range indicates maximum range while the narrow range shows minimum range of variation (Negi & Nautiyal 2002). In *Garra gotyla,* a total of 18 morphometric characters were analyzed, of these the 2 characters were environmentally controlled, 13 were moderate and 3 character were genetically controlled. Out of these 8 morphometric characters were moderate valve of correction and the 10 morphometric characters were high value of correlation (Barraiche & Akhter 2015). Negi et al. (2010) studied 21 morphometric characters of *schizothorax richardsonii*, out of these the 19 characters were narrow in ranged, 1 was moderate and 1 was vast in ranged. In the percentage of total length 4 morphometric character of *Xenentodon cancila* were studied. All of these characters were vast in ranged. 14 characters were recorded in the percentage of standard length, of these the 2 characters were moderate and 12 characters were vast in ranged (Dhanze et al., 2018). In *Tor putitora* the 2 morphometric characters were vast in range, 5 characters were moderate and 11 characters were found genetically controlled (Johal, 2003). In the percentage of head length a twenty four morphometric characters of In *H. molitrix* were studied, of these five characters were narrow in range, fifteen were vast and four characters were moderate in range (Pant et al., 2018). Molecular literature of *H. molitrix* is very deficient. Jian et al. (2020) sequence the whole mitochondrial genome of *H. nobilis* and *H. molitrix* and studied the evaluation and speciation. The phylogenetic analysis revealed that the *H. nobilis* and *H. molitrix* were diverged 3.6 million years ago; the time when the Zebra fish diverged from Silver carp 50.7 million years ago. Stepien et al. (2019) used two mitochondrial genes i.e., Cytochrome oxidase subunit I and Cytochrome b genes, 10 nuclear DNA microsatellite and nuclear ribosomal protein S7

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gene 1 to resolve the genetic and phylogenetic pattern of *H. molitrix* in North America. Molnar et al. (2021) compere the genetic structure of invasive *Hypophthalmichthys* species populations in Central-European lacustrine and riverine habitats using the genetic marker cytochrome oxidase sub unit I. The genetic information suggests that the species may become invasive similarly in the North American populations. In the present study we provide the first molecular and Phylogenetic record from Tarbela Dam district Haripur Khyber Pakhtunkhwa, Pakistan using genetic marker Cytochrome oxidase sub unit (*COI*) gene. The reported sequence of *COI* gene from Pakistan shows high similarity almost 100% with other reported sequences of *H. molitrix* reported from China and America. This is study provide the base line for further research in *H. molitrix* in Pakistan for their conservation and management.

Conclusion

The present study documented the morphological and genetic identification of *H. molitrix* using a genetic marker, Cytochrome oxidase sub unit I (*COI*) gene. The molecular and Phylogenetic analysis assess the taxonomic status of this species in Pakistan. We provided first genetic record of *H. molitrix* from Tarbela Dam Haripur districts, which provides baseline data for *Hypophthalmichthys* genus and its distribution across of Pakistan.

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