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Revision of gonius subgroup of the Genus *Labeo* Cuvier, 1816 and confirmation of species status of *Labeo rajasthanicus* (Cypriniformes: Cyprinidae) with designation of a neotype

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ABSTRACT

The fish species, *Labeo rajasthanicus* was first described from Jaismund Lake in western Rajasthan region, India, based on a single specimen and has never been reported since its first description in 1970. The taxonomic status of the fish has not been stable due to the conflicting opinion among several authors either as a valid species or as a synonym of *L. boggut*. The present report redescribes the species *L. rajasthanicus* based on the specimen collected from its type and other adjoining localities, with confirmation of its taxonomic status as valid species and designation of a neotype as the holotype specimen is no longer available. Morphometric and molecular data, distinguish this species from its congeners under genus *Labeo*, namely *L. gonius*, *L. boggut* and *L. dussumieri.* Analysis of the morphometric and meristic data as well as truss measurements of all the four species confirmed its identity. Among all the morphometric and meristic characters, anal fin rays showed significant differences ($p<0.05$) between the four species. Divergence in cytochrome oxidase c subunit I (COI) sequences also indicated species level separation of *L. rajasthanicus* from other species of gonius group of the genus *Labeo*. Genetic and morphological evidences support the distinction of *L. rajasthanicus* as a separate species from all related congeners and an identification key has also been proposed.

Keywords: COI gene, Cyprinidae, Gonius group, *Labeo rajasthanicus, L. gonius*, *L. boggut, L. dussumieri*

Introduction

Genus *Labeo* Cuvier, 1816 is represented by 28 species widely distributed throughout South and South-East Asia (Jayaram, 2010). The gonius subgroup of genus *Labeo* with lateral line (LL) scale counts more than 50 includes three species (Jayaram and Dhas, 2000). The distribution of these three species is quite interesting. *L. gonius* (Hamilton, 1822) and *L. boggut* (Sykes, 1839) have wider distribution across all the rivers in India except peninsular India below the river Krishna (*L. gonius*) and excluding rivers of Kerala (*L. boggut*). On the other hand, *L. dussumieri* (Valenciennes, 1842) is endemic to a few westward flowing rivers in Western Ghats including rivers of Kerala (Jayaram and Dhas, 2000; Narayanan *et al.,* 2005).

Records of the Zoological Survey of India (ZSI) (Datta and Majumdar, 1970) which described the fauna of Rajasthan, named a species *Labeo rajasthanicus* having lateral line count of 60, enlisted among the fish species found in Jaisamand Lake, Udaipur, Rajasthan, India. However, limited biological and morphological description is available for the species (Datta and Majumdar, 1970; Froese and Pauly 2013). According to some authors (Talwar and Jhingran, 1991; Jayaram and Dhas, 2000), description of this species and another species from Udaipur area, named as *Labeo udaipurensis* (Tilak, 1968) were based on single type specimen and have not been described since their first report. ZSI undertook detailed revision of genus *Labeo* from India based on examination of the registered and unregistered

type specimens available. The results were published as an occasional paper in the Records of the Zoological Survey of India (Jayaram and Dhas, 2000) and described 28 valid species from India, without any reference to *L. rajashanicus* voucher (ZSI accession no. F.4179/2; Datta and Majumdar, 1970). However, the original descriptions of *L. rajasthanicus* (Datta and Majumdar, 1970) and *L. udaipurensis* were considered and were enlisted under *L. boggut*. Jayaram and Dhas (2000) concluded both these species as synonym to *L. boggut*. Unfortunately, the specimen, which was registered as holotype of *L. rajasthanicus* is no more available for comparative examination (e-mail comm., ZSI).

The fish specimen collections during explorations (2010 – 2012) of the river Tidi (district Udaipur), river Chambal at Kota and Jaisamand Lake near Udaipur, in Rajasthan, revealed a species of genus *Labeo* and previously unrecognised from *L. gonius* but closely similar to *L. rajasthanicus* which was not reported after its type description. Detailed comparison with other closely related congeners *viz.*, *L. gonius, L. boggut* and *L. dussumieri* using morphometric, meristic characters and mitochondrial gene cytochrome oxidase c subunit I (CO1) gene sequencing confirmed the existence of *L. rajasthanicus.*

Materials and methods

Fish sampling

Fish samples were collected by experimental fishing using different types of fishing gears such as gillnets and cast nets from June 2010 to June 2012, from the Tidi River, Jaisamand Lake of Udaipur District, Chambal River in Kota, Rajasthan, Penganga River (Adilabad) and Vembanad Lake in Kerala (Fig. 1). The specimens collected during sampling were identified using standard keys (Talwar and Jhingran, 1991; Jayaram, 2010). Specimens were placed laterally on truss sheet, body posture and fins were spread into natural position, photographed and saved in computer for truss morphometry analysis (Karaoglu *et al.,* 2011). The details of samples used for analysis and comparison with their size and locations are presented in Table 1. All specimens except those for molecular analysis were preserved in 10% formalin and transported to the laboratory for further study and then transferred to 75% ethanol for permanent storage in the National repository at ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR;http://nbaindia.org/content/500/55//biodiversityrelatedi.html) for future examination. The specimens meant for molecular studies were preserved in absolute alcohol.

Morphometric and meristic data

For each specimen, a total of 13 morphometric measurements were taken such as SL: standard length; BD: body depth; HL: head length; IOW: inter orbital width; ED: eye diameter; SntL: snout length; HW: head width; PrDL: predorsal length; PrAL: preanal length; PrPlvL: prepelvic length; DFBL: dorsal fin base length; LCPD: length of caudal peduncle; HCPD: height of caudal peduncle. All measurements were taken on the left side of specimens, to the nearest 0.1 mm using digital caliper

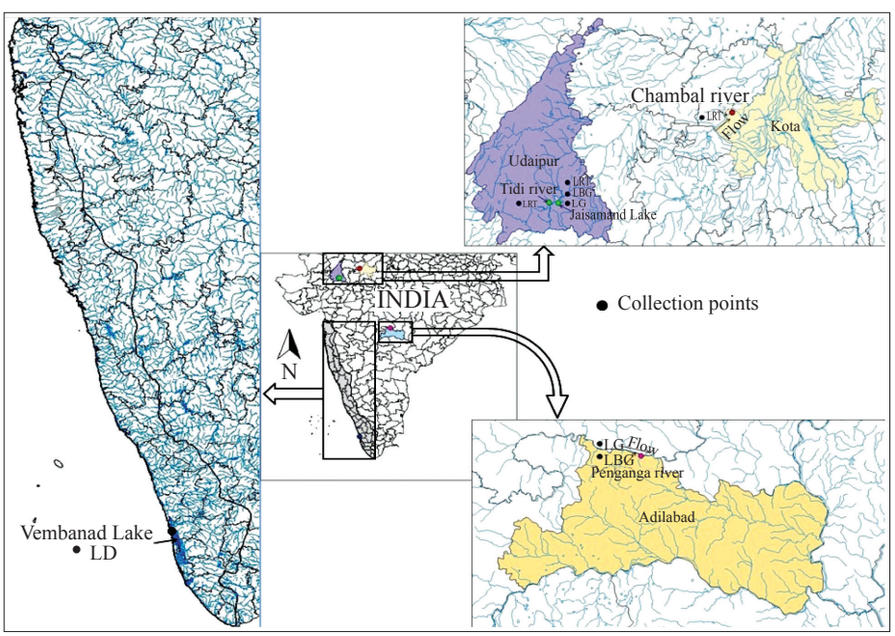

Fig. 1. Map showing the study area

Table 1. Collection locations, sample size and size statistics of four species of *Labeo* used in the study

Species	Sample size for analysis		River/Lake	Locations	GPS coordinates	Size range SL (mm)	Mean SL (mm)±SD
	Truss	Traditional morphology					
L. rajasthanicus	17	12	Jaisamand	Udaipur	N 24° 13.720, E 074° 59.754, Alt: 951 ft	125.71-312.96	185.22±70.31
			Tidi	Udaipur	N 24° 13.654, E 073° 53.691, Alt: 872 ft		
			Chambal	Kota	N 25° 09.29, E 075° 49.07, Alt: 865 ft		
L. boggut	22	8	Jaisamand	Udaipur	N 24° 13.720, E 074° 59.754, Alt: 951 ft	107.49-270.53	168.03±40.17
			Penganga	Adilabad	N 19° 45.987, E 078° 43.058, Alt: 639 ft		
L. gonius	14	12	Jaisamand	Udaipur	N 24° 13.720, E 074° 59.754, Alt: 951 ft	127.59-326.00	221.90±66.21
			Penganga	Adilabad	N 19° 45.987, E 078° 43.058, Alt: 639 ft		
L. dussumieri	5	5	Vembanad lake	Kerala	N 09° 58.23, E 76° 15'29, Alt: 26 ft	211.09-238.45	223.51±11.83

(Tshibwabwa and Teugels, 1995; Tshibwabwa *et al.,* 2006). Ten meristic characters counted were, DFR: dorsal fin rays; PFR: pectoral fin rays; VFR: ventral fin rays; AFR: anal fin rays; LLS: number of lateral line scales, the number of pored scales from beginning of lateral line just behind the head at top of operculum to caudal base; Dorsal fin/Ll: number of scales between dorsal fin rays and lateral line; PevF/Ll: number of scales between pelvic fin rays and lateral line; AF/Ll: number of scales between anal fin rays and lateral line; circum. scales: circum peduncle scales and TVC: total vertebrae counts. Radiography was employed for evaluation of the size and shape of the vertebrae, fin rays and other skeletal elements of fish (Strauss and Bond, 1990). Mammo-radiographs were taken using digital X-ray machine and developed digitally using centricity CR SR100. The first four fused vertebrae (Weberian apparatus) were not included in the vertebral counts.

Truss-based morphometric measurements

Two dimensional Cartesian coordinates of 12 landmarks were recorded on the lateral view of each specimen (Fig. 2) and truss networks were constructed by inter connecting the landmarks. The locations of the landmarks were chosen according to two criteria: reliability in terms of correspondence between specimens, and the ability to best describe the geometry of the form under study. The extraction of the truss distances from

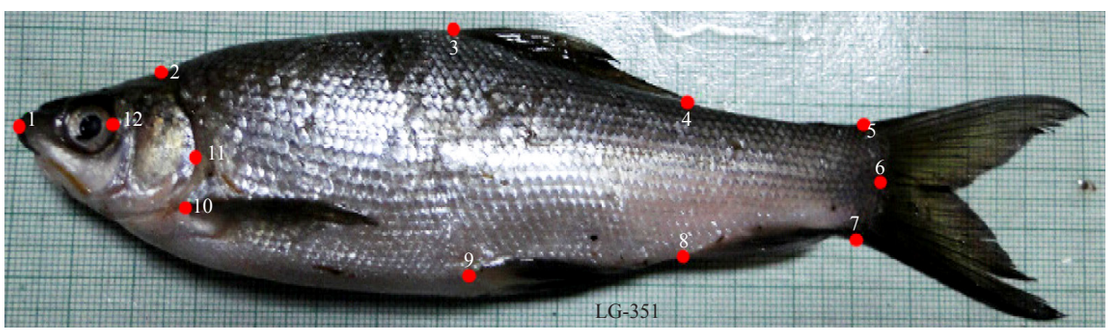

Fig. 2. *Labeo rajasthanicus* showing locations of 12 landmarks used for morphological variations. Land marks refer to: 1. Anterior tip of snout at upper jaw, 2. Most posterior aspect of neurocranium (beginning of scaled nape), 3. Origin of dorsal fin, 4. Position at 90° of the origin of anal fin, 5. Anterior attachment of dorsal membrane from caudal fin, 6. Posterior end of vertebrae column, 7. Anterior attachment of ventral membrane from caudal fin, 8. Origin of anal fin, 9. Insertion of pelvic fin, 10. Insertion of pectoral fin, 11. End of operculum, 12. Posterior end of eye

the digital images of specimens was done using a linear combination of the software platforms, tps Util and tps Dig 2 v2.1 (Hammer *et al.,* 2001; Rohlf, 2006). A total of 66 inter-landmark morphometric characters were extracted by measuring distances between landmarks.

Material examined

In addition to the native species from Rajasthan, (with LL scale count 58-64, having apparent similarity to *L. rajasthanicus*), the members of the gonius subgroup with LL scale count more than 50, (*L. gonius, L. dussumieri* and *L. boggut*) were used for the study. The three species (*L. gonius, L. dussumieri* and *L. boggut*) were collected, studied and compared with putative *L. rajasthanicus* specimens. Among these, *L. gonius* and *L. boggut* were collected from the same study areas from where *L. rajasthanicus* were collected. Details of the material examined are as follows:

Labeo boggut: NBFGR/LBG100-110 (11), 178-270 mm TL; NBFGR/LBG121, TL; 187 mm TL- Jaisamand Lake, Udaipur, Rajasthan.

NBFGR/LBG-122-131 (10), 107-200 mm TL - Penganga River Adilabad, Andhra Pradesh.

Labeo gonius: NBFGR/LG-01-02 (2), 146-259 mm TL; NBFGR/LG-343-346 (4), 176-285 mm TL; NBFG RLG-354-357 (4), 127-321 mm TL - Jaisamand Lake, Udaipur, Rajasthan.

NBFGR/LG-350-353 (4), 146-327 mm TL - Penganga River Adilabad, Andhra Pradesh.

Labeo dussumieri: NBFGR/LD-KR-1 -5 (5), 221-238 mm TL - Vembanad Lake, Kerala.

Labeo rajasthanicus (Neotype): NBFGR/LRT 02, (TL) - 168 mmTL, 137.3 mm SL, collected Tidi River, Udaipur, Rajasthan, 2010 (Fig. 3a) .

Paraneotypes: NBFGR/LRT 01, 145 mm TL, 114.3 mm SL, Chambal River, Kota, Rajasthan, 2011. Rajasthan 2010,; NBFGR/LRT 03-12, Tidi River, and Jaisamand Lake, Udaipur, Rajasthan, 2011.

The specimens of *L. boggut, L. gonius* and *L. dussumieri*, collected from the areas of their known localities, were also examined for morpho-meristic characters. This morpho-meristic data were confirmed with the earlier descriptions of these three species (Jayaram and Dhas, 2000).

Data analyses

To determine inter-specific variations among the species of gonius group of genus *Labeo*, morphometric and meristic characters were used separately in analyses since their allocation abilities are different statistically (Karaoglu and Belduz, 2011). The truss data generated by PAST (PAleontological STatistics) were log-transformed to preserve allometries and to standardise variances (Strauss, 1985). Data were M-transformed to eliminate size effect (Poulet *et al.,* 2005).

$$M\text{-trans} = \log M - b(\log SL - \log SL\ mean)$$

where, M-trans is the transformed measurement, M is the original measurement, b is the within-group slope regression of the log M *versus* log SL, SL is the standard length of the fish (character code: 1-6) and SL mean is the overall mean of the standard length.

Standard length (SL) was excluded from the final analysis as SL was used as a basis for transformation (Mamuris *et al.,* 1998). All statistical analyses were performed for combined sexes since all measurements were transformed and the effect of size also was removed (Karakousis *et al.,* 1993; Mamuris *et al.,* 1998).

The coefficient of variation (CV) for each character was computed as:

$$CV = (100\times SD)/ Xm$$

where, SD is the standard deviation and Xm is the mean of the transformed measurements of characters in each species.

In each species, morphological variability was estimated by the multivariate generalisation of the coefficient of variation (CVp) as:

$$CVp=100x\sqrt{\sum}SDx/\sum Mx$$

where, SDx is the variance of each morphometric variable and Mx is the squared mean.

To identify statistically significant difference if any between the species for each character, one-way analysis of variance (ANOVA) was performed on each variable and significant variables were retained (Snedecor and Cochran, 1980; Zar, 1984). Significant variables were then subjected to principal component analysis (PCA), cluster analysis and forward stepwise discriminant function analysis (DA). All statistical analyses were carried out using SPSS PC ver 10.

Phylogenetic analysis

Total genomic DNA was extracted from blood/tissue samples using the phenol-chloroform protocol modified by Ruzzante *et al.* (1996). Cytochrome oxidase c subunit I (COI) region was amplified with universal primers, WRD-COI-FISH F1 TCA ACC AAC CAC AAA GAC ATT GGC AC, WRD-COI-FISH R1 TAG ACT TCT GGG TGG CCA AAG AAT CA. The amplification comprised 30 cycles with an initial denaturation at 94^{o}C for 5 min,

denaturation at 94°C for 30 sec; annealing at 55°C for 60 sec, extension at 72°C for 90 sec per cycle and final extension at 72°C for 10 min. Amplification was carried out in 50 µl reaction mixture which comprised 7.5 µl distilled water, 5µl 10x PCR buffer, 4 µl template DNA, 2 µl primer (5 picomole), 0.5 µl $MgCl_2$ and 1µl Taq DNA polymerase. PCR products were precipitated using ethanol and ammonium acetate and dissolved in buffer. The purified PCR amplicon was used in setting up sequencing reaction with same set of primers using MegaBace ET Terminator Dye kit. The sequencing PCR was done as per recommendation of GE and comprised of 30 cycles of 95°C for 10 sec; 50°C for 20 sec and 60°C for 2 min.

Amplified COI regions were sequenced in both the directions to check the validity of the sequence data. Sequences were edited using DNASTAR software (DNASTAR, Inc., USA), aligned using ClustalW (Thompson *et al.,* 1997) and submitted to NCBI GenBank. The COI sequences aligned and primers were removed to yield a uniform fragment of 655 bp. Pairwise genetic distance (Kimura 2-parameter), polymorphic sites, nucleotide composition and number of transition/ transversion between species were determined by molecular evolutionary genetics analysis using MEGA 4.0 (Tamura *et al.,* 2007). For the barcode-based identity analysis, we also used a threshold of 2% divergence. NJ clustering analysis were performed using 1000 pseudo replications (Felsenstein, 1993) constructed using MEGA 4.0 (Tamura *et al.,* 2007) along with *Cyprinus carpio* (GenBank Accession No. # KF438027-29) as an outgroup.

Results and discussion

Labeo rajasthanicus Datta and Majumdar, 1970

Neotype: NBFGR/LRT 02: Tidi River, Udaipur, Rajasthan, TL -168 mm, SL – 137 mm, 2010 (Fig. 3).

Paraneotypes: NBFGR/LRT 01: Chambal River, Kota, Rajasthan, 2011. TL-145 mm, SL-114.3 mm, 2010, NBFGRLRT 03-12,36: Tidi River and Jaisamand Lake, Udaipur, Rajasthan, 2011.

Diagnosis: The species of *L. rajasthanicus* is distinguished by a combination of the following characters: Lateral line scales 58-64 without red tinge along the margins; pre-dorsal scales 18-20; dorsal fin rays 14-15 (Fig. 4); ventral fin rays 09; anal fin rays 6-7; dorsal fin lateral line transverse 9.5-12.5; pelvic fin lateral line transverse 8.5; anal fin lateral line transverse 6.5-7.5; circum-peduncular scales 24-26; eye diameter 4.16-5.47 in HL; depth of body 3.40-4.16 in SL (Table 2 and 3).

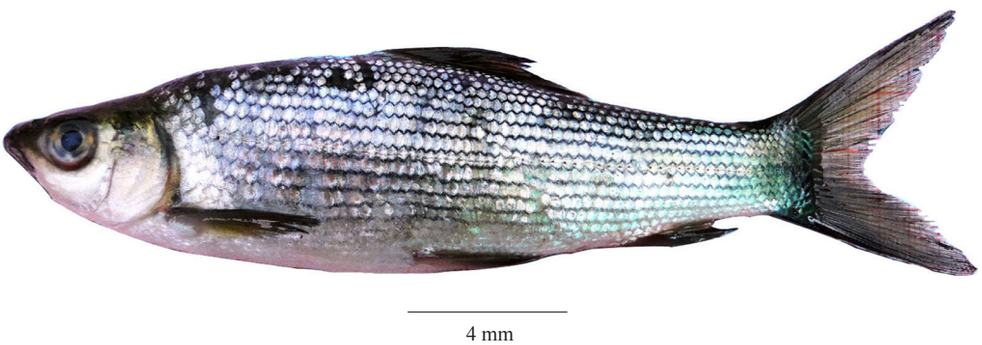

Fig. 3. *Labeo rajasthanicus* Neotype NBFGR-U-LRT02 (TL 168 mm) recorded from river Tidi, Western Rajasthan, India

Fig. 4. *Labeo rajasthanicus* NBFGR-LRT-36 (TL 361 mm) recorded and photographed from river Tidi, Western Rajasthan showing spread of dorsal fin (not used in data)

Table 2. Morphometric measurements in terms of % standard length (%SL) and % head length (%HL) of the holotype and eleven paraneotypes of *L. rajasthanicus* (Total no. = 12)

Characters	Holotype	Paratypes (11)	Mean ± SD
Percentage SL			
Body depth	26.90	24.03-30.52	27.39 ± 1.83
Head length	25.46	22.94-26.07	24.60 ± 0.97
Head width	15.64	14.10-18.42	15.42 ± 1.13
Snout length	9.74	7.78-10.86	9.06 ± 0.88
Dorsal fin base length	21.95	21.08-25.25	23.96 ± 1.23
Eye diameter	5.03	3.99-6.17	4.91 ± 0.50
Inter orbital width	12.61	11.79-13.15	12.45 ± 0.45
Length of caudal peduncle	16.28	12.43-16.28	14.40 ± 1.10
Height of caudal peduncle	12.05	11.15-13.45	12.29 ± 0.65
Predorsal length	46.37	43.57-47.47	45.65 ± 1.26
Preanal length	77.57	73.96-82.48	78.37 ± 2.29
Prepelvic length	52.59	49.87-54.29	52.25 ± 1.23
Percentage HL			
Head width	61.44	56.94-78.08	62.80 ± 5.57
Snout length	38.25	33.14-43.17	36.77 ± 2.74
Eye diameter	19.75	17.18-24.01	19.97 ± 1.78
Inter orbital width	49.53	46.11-54.87	50.68 ± 2.25
Length of caudal peduncle	63.95	51.54-65.56	58.61 ± 4.90
Height of caudal peduncle	47.34	45.65-57.76	50.01 ± 3.13

Table 3. Comparative ratio, mean ±SD (standard deviation) and variance (F) of traditional morphometric (mm) and meristic characters of four *Labeo* species

Variables	*L. boggut* (n=21)			*L. gonius* (n=12)			*L. rajasthanicus* (n=12)			*L. dussumieri* (n=5)			F value
	Ratio		Mean±SD	Ratio		Mean±SD	Ratio		Mean±SD	Ratio		Mean±SD	
	Min.	Max.		Min.	Max.		Min.	Max.		Min.	Max.		
Morphometric characters													
SL/BD	3.96	4.19	4.07±0.09	3.28	3.7	3.5±0.12	3.40	4.16	3.73±0.25	3.51	3.89	3.71±0.14	6.6*
SL/HL	4.04	4.38	4.17±0.15	3.82	4.3	4.1±0.15	3.83	4.16	3.99±0.11	3.31	4.34	3.72±0.23	6.2*
SL/ PrDL	2.04	2.13	2.08±0.04	2.04	2.5	2.2±0.11	2.11	2.21	2.15±0.04	2.28	2.32	2.30±0.02	6.2*
SL/ PrAL	1.28	1.32	1.29±0.02	1.2	1.3	1.2±0.03	1.25	1.29	1.27±0.02	1.27	1.32	1.29±0.02	11.3*
SL/ PrPlvL	1.94	2.04	1.97±0.04	1.8	1.9	1.9±0.04	1.86	2.00	1.91±0.04	2.01	2.05	2.04±0.02	0.2
SntL/ED	2.09	2.24	2.16±0.06	1.3	1.7	1.6±0.09	1.48	2.14	1.89±0.19	0.84	1.02	0.89±0.07	112.6*
IOW/ED	2.25	2.65	2.38±0.18	1.8	2.3	2.1±0.14	2.04	2.92	2.50±0.29	2.25	2.43	2.35±0.07	2.3
HL/ED	5.50	6.51	6.11±0.46	3.3	4.7	4.3±0.44	4.16	5.47	4.98±0.44	3.04	3.37	3.19±0.14	8.2*
HL/SntL	2.63	2.9	2.83±0.15	2.5	3.4	2.8±0.22	2.32	2.87	2.64±0.18	3.29	3.63	3.41±0.25	3.8*
HL/HW	1.73	2.14	1.93±0.18	1.5	1.8	1.6±0.08	1.50	1.75	1.65±0.09	1.32	1.51	1.41±0.08	3.4*
HL/LCPD	1.45	1.72	1.61±0.13	1.7	2.0	1.9±0.08	1.56	1.94	1.77±0.13	0.95	1.12	1.04±0.07	12.3*
HL/HCPD	1.88	2.03	1.96±0.07	1.7	2.1	1.9±0.12	1.87	2.19	2.03±0.11	2.58	3.10	2.81±0.24	13.4*
HL/DFBL	1.18	1.37	1.29±0.08	0.9	1.1	1.0±0.05	0.96	1.16	1.06±0.06	0.68	0.92	0.76±0.09	10.0*
LCPD/HCPD	1.10	1.32	1.22±0.11	0.9	1.1	1.0±0.07	1.00	1.35	1.15±0.13	1.32	1.45	1.10±0.17	137.7*
Meristic counts													
DFR	11	11	11±0.00	14	15	14.9±0.28	14	15	14.8±0.33	14	14	14±0.00	247.49*
PFR	16	17	16.25±0.5	15	16	15.9±0.3	16	16	16.0±0.00	16	16	16±0.00	1.73
VFR	9	9	9±0.00	8	9	8.8±0.38	9	9	9.0±0.00	8	8	8±0.00	19.24*
AFR**	6	6	6±0.00	7	7	7.0±0.00	6	7	6.4±0.53	5	5	5±0.00	57.50*
LLS	61	62	61.7±0.50	66	76	70.4±4.23	58	64	61.1±1.69	52	55	54±1.22	41.59*
D F/Ll	11.5	11.5	11.5±0.00	11.5	14.5	12.8±1.23	9.5	12.5	10.7±0.97	8.5	9.5	9.3±0.45	17.61*
PevF/Ll	8.5	8.5	8.5±0.00	8.5	11.5	9.7±1.14	8.5	8.5	8.5±0.00	5.5	6.5	6.3±0.45	24.43*
AF/Ll	8.5	8.5	8.5±0.00	7.5	10.5	8.5±1.13	6.5	7.5	7.4±0.33	5.5	6.5	6.1±0.55	12.81*
Circum. Scales	26	28	26.5±1.00	24	30	25.7±2.23	24	26	24.8±1.05	21	23	22.4±0.89	5.99*
TVC	32	34	32.8±0.84	35	36	35.5±0.57	34	36	34.9±0.54	34	35	34.8±0.45	19.25*

Data of these species are of specimens collected during present study and verified from the descriptions of type specimens available with Zoological Survey of India (Jayaram and Dhas, 2000).

Description: Body elongate, dorsal profile slightly convex than ventral, body depth 3.4-4.16 in SL, head length 3.83-4.16 in SL (Table 3). Head small, snout with few tubercles and slightly projecting beyond mouth. Eyes medium sized, not visible from underside of head, diameter 4.16 to 5.47 in HL. Mouth narrow, sub-inferior, with thick lips. Dorsal fin inserted nearer to snout than base of caudal fin. Pelvic fin inserted below the middle of dorsal and does not reach to the anal fin. Anal fin short, not reaching to the base of caudal fin. Caudal fin deeply forked with pointed lobes. Two pairs of barbells, both of same length. Scales medium in size, 58 to 64 lateral line scales, lateral transverse scale rows 8.5 between the lateral line and pelvic fin base and circum-peduncular scales 24-26. Genital opening situated distant from anal fin origin, 34 to 36 vertebrae (Fig. 5), maximum size recorded 370 mm.

Colour: Light black on dorsal surface, white on ventral side and scales without red tinged margins. Colour of preserved specimen turned black on the dorsal side and light yellowish on ventral side.

Fin formula: D 2/12-13; P 1/15; V 1/8; A, 1/5-6; C 10 + 9.

*Distribution***:** The species *L. rajasthanicus* is reported from the two isolated rivers, Tidi and Chambal and also from Jaisamand Lake. The Jaisamand Lake and Tidi River are part of Mahi-Som River system flowing south-west into Arabian Sea, on the other hand Chambal River flows into river Yamuna, a tributary of Ganga River system flowing eastward into Bay of Bengal.

*Ecology***:** Occurrence of *L. rajasthanicus* was recorded from rocky substrates with shelter and higher depth (5-20 m), having low water velocity, in the rivers and lakes of Rajasthan, India.

Identity of L. rajasthanicus among the gonius group of Labeo

The detailed comparative distinctness in traditional morphometric characteristics of *L. rajasthanicus* from the other closely related species is presented in Table 3. The limited morpho-metric data available based on a single specimen (holotype) of *L. rajasthanicus* Datta and Majumdar, 1970, show that the species mostly fall in the range of descriptions of *L. rajasthanicus* except for some minor variations in meristic characters such as dorsal fin rays 2/12-13 (vs 2/14), pectoral fin rays 1/15 (vs 1/14), anal fin rays 1/5-6 (vs 2/5) and transverse scales between LI to ventral fin 8.5 (vs 9.5). It is likely that such differences could be within species variation or possibly minor counting error in the original description. Unfortunately, the registered holotype is not available for examination. In the present paper, *L. rajasthanicus* is redescribed and validated with designation of a neotype (NBFGR/LRT 02). The species description here is based on the neotype and paraneotypes (n=12) collected from different localities including the type locality, therefore, this will take precedence over earlier description for the species characteristics of *L. rajasthanicus.*

Truss network system with calibrated coordinates of morphometric locations, or 'landmarks' on the fish body, has been increasingly employed to study inter-specific variations (Bookstein, 1982; Strauss and Bookstein, 1982; Cavalcanti *et al.,* 1999; Mekkawy *et al.,* 2002; Cheng *et al.,* 2005). 'Truss networks' of distances between landmarks coordinates provide more comprehensive coverage of form for greater discriminating power (Jiang *et al.,* 2012). In the present investigation, results of ANOVA showed statistically significant differences ($p<0.05$) among species in all the 65 transformed truss morphometric characters. However, of these 65 characters, Tukey's test extracted 15 characters that showed differences between species, which were used further for multivariate analysis. Low multivariate coefficient of variation of each species (CVp) for the 65 morphometric characters was obtained in all the species. *L. gonius* showed the highest CVp (4.91%) followed by *L. boggut* (4.53%), *L. rajasthanicus* (3.49-%) and *L. dussumieri* (2.88%). The low multivariate generalisation of the coefficient of variation (CVp) observed in our study indicates minimal or very low inter-species variation (CVp<10%), similar to the results obtained by Katselis *et al.* (2006) in the fry of four Mediterranean grey mullet species. The segregation of the species studied, into four groups was also supported

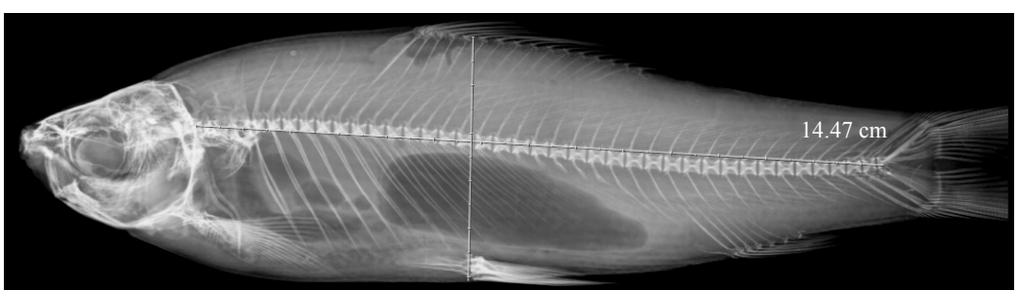

Fig. 5. Mammo-radiograph of *Labeo rajasthanicus* showing the vertebrae

by DA as 100% of individuals were correctly classified into their respective groups, indicating high differentiation between these four species.

Principal component analysis (PCA) using varimax rotation of the 15 significant variables yielded one principal component accounting for 88.35% of the total variation if Jolliffe's rule, which is to retain principal components with Eigen values of at least 0.70 (Dunteman, 1989). Forward stepwise discriminant analysis (DA) of the 15 significant variables produced four discriminating variables. The unstandardised coefficients for the four variables of the morphometric characters for each of the discriminant function (canonical variable) are shown in Table 4. DA extracted three canonical variables (CaV) which contributed to the variance; however the first canonical variable alone contributed 100%, to the total variance. These discriminant functions identified the membership (classification) of individual fish in the data with one of the four species with a success rate of 100%. The graphical presentation of the first and second canonical variables is shown in Fig. 6. The percentage of discrimination per pair of species (PDPS) was also found to be 100% between species. The UPGMA cluster analysis based on the Mahalanobis distance between group centroids showed that the four species produced two major clusters. *L. gonius* and *L. dussumieri* belong to the first cluster (cluster I) while *L. boggut* and *Labeo rajasthanicus* (Fig. 7) belong to the second cluster (cluster II).

All the morphometric character codes showed significant loadings on PC1 (Eigen value 0.70). According to Nimalathasan (2009), factor loading greater than 0.30 is considered significant, 0.40 more important and 0.50 or greater highly significant. The PC1 loading for each sample revealed that the four species were clearly distinct from each other. The character codes of primary importance in distinguishing groups were 1-7 and 2-6 for CaV_1, 1-11 for CaV_2 and 2-7 for CaV_3. The position of each species on the first two canonical variables (CaV_1 and CaV_2) supported a rank based on profile of each species. Considering that all the species examined had equal (statistical) length, the ranking of species on the CaV_1 supported *L. boggut* towards left and *L. dussumieri* towards right.

Table 4. Results of discriminant analysis (DA) based on the transformed data, and Unstandardised canonical discriminant function coefficients of each morphometric variable on three canonical variables (CaVi)

Percentage of variance	CaV_1	CaV_2	CaV_3
	100.00	0.00	0.00
Character code	Discriminant Function Coefficients		
1-7	1926.293	-19.071	-183.042
1-11	18.581	20.464	18.004
2-6	1815.221	-47.863	-50.860
2-7	-1658.259	41.454	209.751
Constant	-2619.072	20.794	31.775

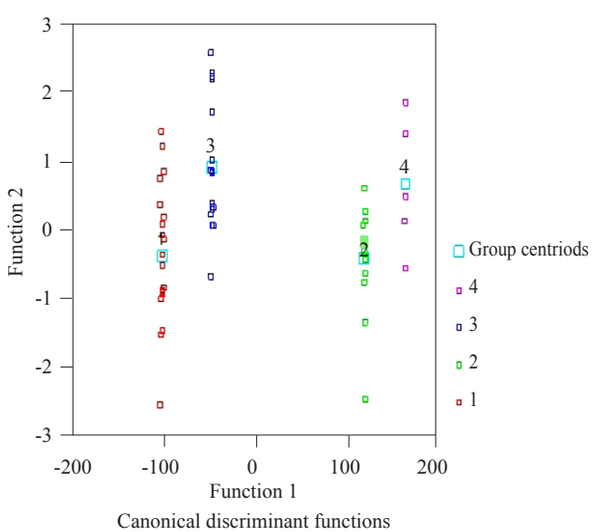

Fig. 6. Discriminant analysis plot from 15 morphometric variables. Group centroids: 1 - *L. boggut*; 2 - *L. gonius*, 3 - *L. rajasthanicus,* 4 - *L. dussumieri*

Conservation value

The present study confirmed that the species *L. rajasthanicus* has restricted distribution, possibly endemic to few rivers of western Rajasthan and so the need to develop management and conservation strategies for this species. The species has good market value as an important food fish in the region and the captive broodstock already raised and produced seeds which can be used for aquaculture and enhancement (Anon., 2014). The taxonomic recognition of the species could establish its identity and aid in preventing accidental hybridisation with its congeneric species.

Phylogenetic analysis

Five species x 655 nucleotides data set of partial COI fragment were used for species identification

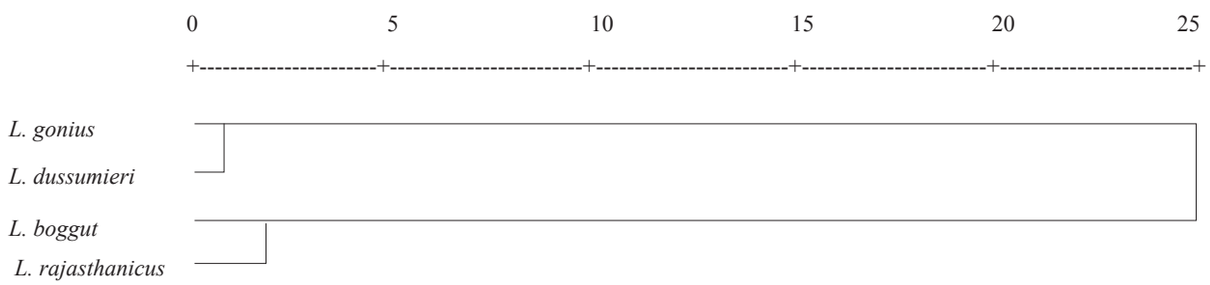

Fig. 7. UPGMA cluster analysis based on the Mahalanobis distance between the species centroids

and phylogenetic analyses. The sequences have been deposited in GenBank (Table 5) and accession numbers for the barcodes, specimen and collection data, sequences, trace files and primers details are available in NCBI. A total of 29 sequences of 655 bp COI were unambiguously aligned without gaps which included 572/655 conserved sites and 83/655 variable positions of which 82/655 were parsimony-informative. Well defined peaks and absence of stop codons indicated that co-amplification of nuclear pseudo-genes did not occur (Zhang and Hewitt, 1996). The sequences were aligned with ease due to the absence of insertions and deletions. Nucleotide composition in these sequences was almost equal in A, C and T contents (mean: 26.4, 27.3, and 28.6%, respectively) and low in G content (mean: 17.8%). The pairwise genetic distance values (K2P) based on COI gene between *Labeo* species using MEGA 4.0 are given in Table 6. The mean genetic distance among all the five *Labeo* species was estimated as 0.052. Intraspecific distances ranged from 0.000 to 0.001 and the interspecific distances ranged from 0.002 to 0.113. The highest interspecific genetic distance (0.113) was between *L. boggut* and *L. rohita* and the lowest (0.002) was between *L. rajasthanicus* and *L. dussumieri*. The three codon positions differed greatly in their base composition. The mean transitional/transversional ratio in *Labeo* species was 6.4 (K2P), which showed that transitional pairs (si= 28) were more than transversional pairs (sv= 4). This level of transition bias is within the range of biases previously reported for other vertebrates and serves as a basis for the transition/transversion weighting ratios used in phylogenetic reconstruction. There is high inter-specific

Table 5. Details of specimen collection and location data of various species of *Labeo* used for molecular analyses along with GenBank accession numbers

Species	Voucher ID	n	Locality	Accession no.
L. gonius	LG-131	05	Chambal, Kota N 25° 11', E 75°50'	JX946409
	LG-221		Chambal, Kota N 25° 11', E 75°50'	JX946410
	LG-337-39		Chambal, Kota N 25° 11', E 75°50'	JX946411-13
L. rajasthanicus	LRT-1-3, 5	04	Jaismand, Jaismand Lake (Udaipur) N 24° 13.720, E 074°59.754	JX946370-73
L. dussumieri	LDU-1 to 5	05	Vembnad Lake, Kerala N 09° 58.23, E 076° 15'29	JX946429-33
L. rohita	LR-3601	05	Penganga River (Adilabad) N 19° 45.987, E 078° 43.058	JX946420
	LR-3602-03		Basar, Godavari River (SRSP) (Adilabad) N 18° 52.078, E 077° 7.670	JX946421-22
	LR-3604		Bakhiya, Tons River (Rewa)	JX946423
	LR-3789		Vaginaka, Banas River (Udaipur) N 24° 54.636, E 073° 5.846	JX946424
L. boggut	LBG-100-03, 105	05	Jaismand, Jaismand Lake (Udaipur) N 24° 13.720, E 074°59.754	JX946387-90, JX946392
Cyprinus Carpio	CYC-01- 02	05	Parel, Ravi River (Chamba) N 32° 35.050, E 076°06.258	KF429972-73
	CYC-05- 07		Sandhara, Ranjeet Sagar Dam, Ravi River (Chamba) N 32° 32.170', E 075°52.11	KF438027-29

Table 6. K2P genetic distances (below diagonal) between *Labeo* species based on the COI gene

Species name	*L. rajasthanicus*	*L. dussumieri*	*L. rohita*	*L. boggut*
L. gonius	0.021	0.020	0.058	0.092
L. rajasthanicus		0.002	0.063	0.089
L. dussumieri			0.065	0.090
L. rohita				0.113

sequence divergence for *Labeo* species as compared to intra-specific sequence divergence.

The single-most parsimonious phylogenetic tree based on 655 bp mitochondrial COI sequences for 5 species of *Labeo* (*L. dussumieri, L. rajasthanicus, L. boggut, L. gonius* and *L. rohita*) and one outgroup according to the neighbor-joining method applied to the K2P distance modal (Fig. 8). Bootstrap values higher than 50% are displayed. The monophyly of the *Labeo* species was strongly supported when outgroup *Cyprinus carpio* were used. In this topology, the *L. rajasthanicus* appeared within a larger monophyletic clade which also contained *L. dussumieri*. Within this clade, *L. dussumieri* was a sister taxon to a group consisting of *L. rajasthanicus*. The other three clades consist of three species of *Labeo viz., L. gonius, L. rohita* and *L. boggut* respectively.

Currently, the 5' segment of the COI gene of mitochondrial genome is being used for phylogenetic reconstructions among closely related species. This gene has been used in various invertebrate and vertebrate taxa (Brown, 1985; Santos *et al.,* 2003; Munasinghe *et al.,* 2004; Vinson *et al.,* 2004; Ward *et al.,* 2005; Khare *et al.*,

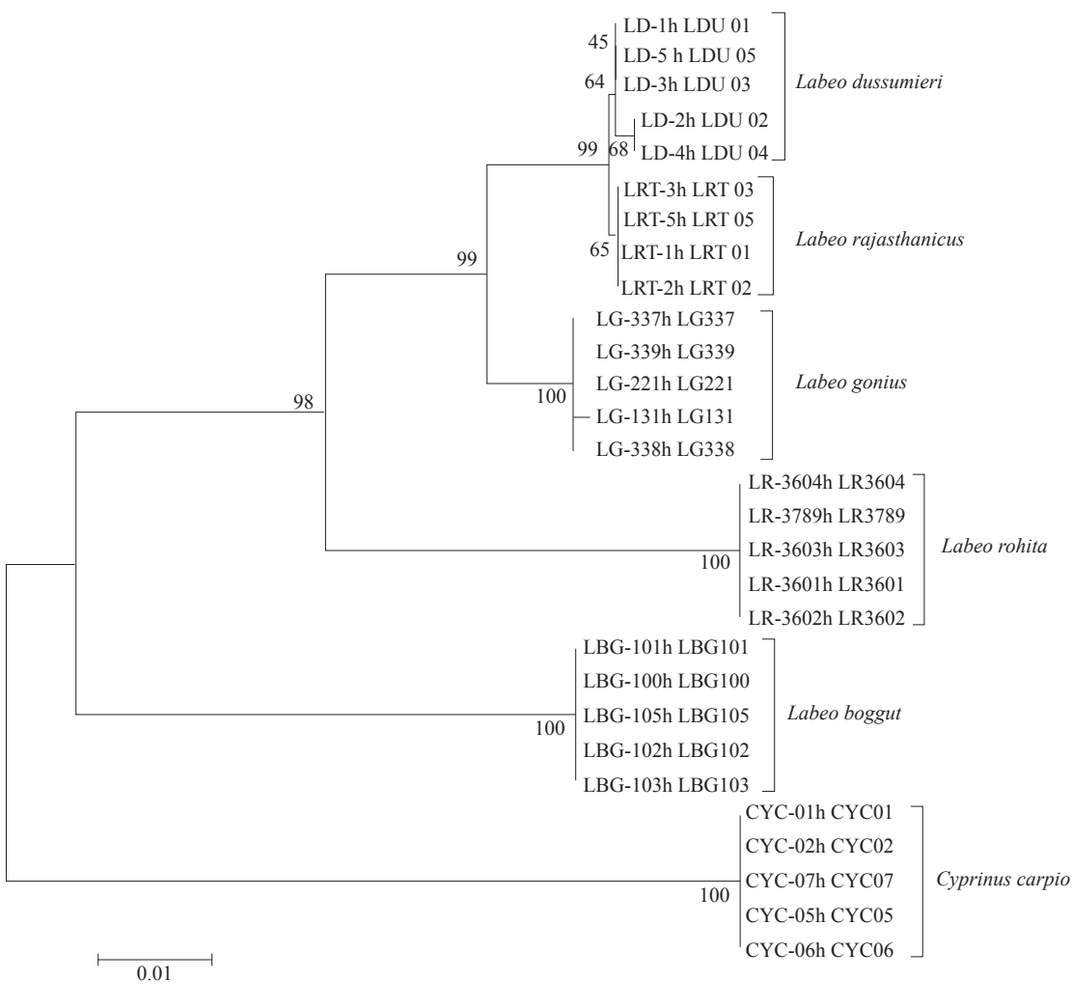

Fig. 8. Neighbor Joining phylogenetic tree of the five morphologically similar species of the genus *Labeo*

2014). In the present investigation, genetic relationship of the five *Labeo* species were determined, based on the tree constructed using COI sequences. Nucleotide sequences in all the taxa were found to be A + T rich (55.90%), which is concordant to many other fishes. Generally, in mtDNA a much larger excess of transitions related to transversion was typically observed (Ward *et al.,* 2005). High transition bias is well known in vertebrate mitochondrial DNA (Meyer, 1993). The average K2P genetic divergence of five *Labeo* species was estimated at 0.2-11.3%. The interspecific sequence divergence observed between *L. rohita* and *L. boggut* is relatively higher than the other *Labeo* congeners studied. Separation of distinct nodes at interspecific values supported with high bootstrap values (99-100%) between *L. rajasthanicus* and other *Labeo* species suggested that *L. rajasthanicus* is a different species.

Our investigation of partial COI gene clearly describes that *L. rajasthanicus* is embedded in a clade, which also includes *L. dussumieri* as a sister taxon. The genetic distance (0.002) between the two species is small however, it is interesting that the node separating the two groups is supported by highly significant (99%) bootstrap value. This indicates the separation of two groups as different species. The low genetic distance found between *L. dussumieri* and *L. rajasthanicus*, make it a suitable point to explore the evolutionary history. The COI is a slow mutating gene and hence, is highly conserved and the species recently diverged from a common ancestor may have low genetic distance (Amaral *et al.,* 2007). UPGMA clustering based on the morphological characters also confirm four distinct species and interestingly, it is also evident that *L. dussumieri* is close to *L. gonius* and *L. rajasthanicus* is closer to *L. boggut*. Therefore, comprehending the evidence derived from morpho-meristics, truss network analysis and the interspecific genetic divergence supported with tree nodes, confirm the existence of the species *L. rajasthanicus*. The major morphometric data from the specimens collected in this study exhibited similarity to that described for *L. rajasthanicus* except some differences in a few characters. The specimens are collected from the type locality of *L. rajasthanicus* and hence, the original name has been retained. A neotype has been designated to replace the missing holotype of *L. rajasthanicus*.

Key to the species under gonius group of Labeo genus (modified after Jayaram, 2010)

1. Lateral line scales 50-85 .....2 (Gonius group)
   Lateral line scales less than 50..... Other groups
2. Dorsal fin rays 11-12, lateral line scales 55-65, one pair of barbels..... *L. boggut*
   Dorsal fin rays 14-18..... 3
3. Lateral line scales 50-55, 8 or 9 parallel brownish stripes above and below the lateral line..... *L. dussumieri*
   Lateral line scales 58-85 4
4. Lateral line scales 58-64, scales light colored, without dark/ red tinge along the margin .....……*L. rajasthanicus*
   Lateral line scales 65-85, scales darkest at their margin with red tinge.....*L. gonius*

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References

Akyol, O. and Kinacigil, T. 2002. Comparative body and otolith morphometrics of Mugilidae in Homa Lagoon (Izmir Bjay, Aegean Sea). *Acta Adriat.,* 42(2): 3-13.

Amaral, A. R., Sequeira, M. and Coelho. M. M. 2007. A first approach to the usefulness of cytochrome *c* oxidase I barcodes in the identification of closely related delphinid cetacean species. *Mar. Freshwat. Res*., 58: 505-510.

Anon. 2014. *Harmonising biodiversity conservation and agricultural intensification through integration of plant, animal and fish genetic resources for livelihood security in fragile ecosystems*. Final project report submitted to NAIP-GEF, 87 pp.

Bookstein, F. L. 1982. Foundation of morphometrics. *Ann. Rev. Ecol. Evol. Syst.,* 13: 451-470.

Brown, W. M. 1985. The mitochondrial genome of animals: In: MacIntyre, R. J. (Ed.), *Molecular evolutionary genetics*. Plenum Press, New York, p. 95-130.

Cavalcanti, M. J., Monteiro, L. R. and Lopez, P. R. D. 1999. Landmark based morphometric analysis in selected species of Serranid fishes (Perciformes: Teleostei). *Zool. Stud.,* 38(3): 287-294.

Cheng, Q. Q., Lu, D. R. and Ma, L. 2005. Morphological differences between close populations discernible by multivariate analysis: A case study of genus *Coilia* (Teleostei: Clupeiformes). *Aquat. Living Resour.,* 18: 187-192.

Datta, A. K. and Majumdar, N. 1970. Fauna of Rajasthan, India. Part 7, Fishes. *Rec. Zool. Surv. India,* 62(1 & 2): 63-100.

Dunteman, G. H. 1989. *Principal components analysis. Sage University paper series on quantitative applications in the social sciences, series no 07-069*, Sage Publi., Beverly Hills, CA, 95 pp.

Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package), version 3.5.* University of Washington.

Froese, R. and Pauly, D. 2013. *Fish base*. http//www.fishbase.org. (Accessed 18 April 2013).

Hammer, O., Harper, D. A. T. and Ryan, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.,* 4(1): 9.

Jayaram, K. C. 2010. *The freshwater fishes of the Indian region*. Narendra Publishing House, New Delhi, 551 pp.

Jayaram, K. C. and Dhas, J. J. 2000. Revision of the fishes of the genus *Labeo* Cuvier from the Indian region with a discussion on its phylogeny and zoogeography. *Rec. Zool. Surv. India (Occasional papers)*, 183: 1-146.

Jiang, W., Ng, H. H., Yang, J. and Chen, X. 2012. A taxonomic review of the catfish identified as *Glyptothorax zanaensis* (Teleostei: Siluriformes: Sisosridae), with the descriptions of two new species. *Zool. J. Linn. Soc.,* 165: 363-389.

Karakousis, Y., Peios, C., Economidis, P. S. and Triantaphyllidis, C. 1993. Multivariate analysis of the morphological variability among *Barbus peloponnesius* (Cyprinidae) populations from Greece and two populations of *B. meridionalis* and *B. meridionalis petenyi. Cybium,* 17: 229-240.

Karaoglu, H. and Belduz, A. O. 2011. Multivariate discrimination among three *Trachurus* species from Turkey. *J. Anim. Vet. Adv.,* 10(1): 121-127.

Katselis, G., Hotos, G., Minos, G. and Kosmas, V. 2006. Phenotypic affinities on fry of four Mediterranean grey mullet species. *Turk. J. Fish. Aquat. Sci.,* 6: 49-55.

Khare, P., Mohindra, V., Barman, A. S., Singh, R. K. and Lal, K. K. 2014. Molecular evidence to reconcile taxonomic instability in mahseer species (Pisces: Cyprinidae) of India. *Org. Div. Evol.*, 14: 307-362.

Mamuris, Z., Apostolidis, A. P., Panagiotaki, P., Theodorou, A. J. and Triantaphyllidis, C. 1998. Morphological variation between red mullet populations in Greece. *J. Fish Biol.,* 52: 107–117.

Mekkawy, I. A. A. and Mohammad, A. S. 2011. Morphometrics and meristics of the three Epinepheline species: *Cephalopholis argus* (Bloch and Schneider, 1801), *Cephalopholis miniata* (Forsskal, 1775) and *Variola touti* (Forsskal, 1775) from the Red Sea, Egypt. *J. Biol. Sci.* 11(1): 10-21.

Mekkawy, I. A. A., Saber, S. A., Shehata, S. M. A. and Osman, A. G. M. 2002. Morphometrics and meristics of four fish species of genus *Epinephelus* (Family Seranidae) from the Red Sea, Egypt. *Bull. Fac. Sci. Assiut. Univ.,* 31(1-E): 21-41.

Meyer, A. 1993. Evolution of mitochondrial DNA in fishes. In: Hochachka, P. W. and Mommsen, T. P. (Eds.), *The biochemistry and molecular biology of fishes*, vol. 2, Elsevier, Amsterdam, p. 1-38.

Munasinghe, H., Burridge, C. and Austin, C. 2004. The systematics of freshwater crayfish of the genus *Cherax* Erichson (Decapoda: Parastacidae) in eastern Australia re-examined using nucleotide sequences from 12S rRNA and 16S rRNA genes. *Inver. Syst.,* 18: 215-225.

Narayanan, S. P., Thapanjith, T. and Thomas, A. P. 2005. A study on the ichthyofauna of Aymanam Panchayat, in Vembanad wetland, Kerala. *Zoos' Print J.,* 20 (9): 1980-1982.

Nimalathasan, B. 2009. Determinants of key performance indicators (KPIs) of private sector banks in Sri Lanka: an application of exploratory factor analysis. *The USV Annals of Economics and Public Administration,* 9(2): 9-17.

Poulet, N., Reyjol, Y., Collier, H. and Lek, S. 2005. Does fish scale morphology allow the identification of fish population of *Leuciscus burdigalensis* (SW France)? *Aquat. Sci.,* 61: 122-127.

Rohlf, F. J. 2006. *TpsDig2, Version 2.1*. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY. http://life.bio.sunysb.edu/morph.

Ruzzante, D. E., Taggart, C. T. and Cook, D. 1996. Spatial and temporal variation in the genetic composition of a larval cod *Gadus morhua* aggregation: Cohort contribution and genetic stability. *Can. J. Fish. Aquat. Sci.,* 53: 2695-2705.

Santos, S., Schneider, H. and Sampaio, I. 2003. Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis. *Genet. Mol. Biol.,* 26(2): 151-161.

Snedecor, G. W. and Cochran, W. G. 1980. *Statistical methods*. 7th edn., The Iowa State University Press, Ames, IA, USA. 593 pp.

Strauss, R. E. 1985. Evolutionary allometry and variation in the body form in the South American catfish genus *Corydoras* (Callichthydae). *Syst. Zool.,* 34: 381-396.

Strauss, R. E. and Bond, C. E. 1990. Taxonomic methods: morphology. In: Schreck C. B. and Moyel, P. B. (Eds.), *Methods for fish biology*. American Fisheries Society, Bethesda, MD, p. 109-110.

Strauss, R. E. and Bookstein, F. L. 1982. The truss: body form reconstruction in morphometrics. *Syst. Zool.,* 31: 113-135.

Talwar, P. K. and Jhingran, A. G. 1991. *Inland fishes of India and adjacent countries, vol 1&2*. Oxford and IBH Publishing, New Delhi, 1097 pp.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.,* 24: 1596-1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.,* 25: 4876-4882.

Tilak, R. 1968. A new species of the genus *Labeo* Cuvier (Pisces) from Rajasthan. *Annales Zoologici* (*Warsaw*) [*Ann. Zool.*], 26(15): 351-353.

Tshibwabwa, S. M., Stiassny, M. L. J. and Schelly, R. C. 2006. Description of a new species of *Labeo* (Teleostei: Cyprinidae) from the lower Congo River. *Zootaxa,* 1224: 33-44.

Tshibwabwa, S. M. and Teugels, G. G. 1995. Contribution to the systematic revision of the African cyprinid fish genus *Labeo*, species from the Lower Zaire river system. *J. Nat. Hist.,* 29: 1543-1579.

Vinson, C., Grazielle, G., Schneider, H. and Sampaio, I. 2004. Sciaenidae fish of the Caete River estuary, Northern Brazil: Mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage. *Genet. Mol. Biol.,* 27(2): 174-180.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. and Hebert, P. D. N. 2005. DNA barcoding Australia's fish species. *Phil. Trans. R. Soc. Lond., B. Biol. Sci.,* 360: 1847-1857.

Zar, J. H. 1984. *Biostatistical analysis*, 2nd edn. Prentine-Hall, Englewood Cliffs, NJ, 718 pp.

Zhang, D. X. and Hewitt, G. M. 1996. Nuclear integrations: challenges for mitochondrial markers. *TREE,* 11: 247-251.

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