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Combined Morphometric and Genetic Approaches for Detecting Hybrids of Indian Major Carps (*Labeo rohita* × *Cirrhinus mrigala*)

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Abstract

Hybridisation happens a lot in Indian major carps, both in hatcheries and in natural water environments. This makes it very hard to maintain brood stock and keep the genetic integrity of native fish. Reliable identification of hybrids is therefore essential in sustainable aquaculture and conservation environments. The present study demonstrates the identification of an interspecific hybrid between rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) using a morphometric approach along with some molecular markers. Fish samples identified as rohu, mrigal, as well as their putative hybrids, were collected from markets across the eastern states of India. Analyses of morphometric measurements were performed using regression and clustering techniques to explore phenotypic differences among the groups. The morphometric analyses showed a marked difference in growth relationships for rohu, mrigal and hybrids, and cluster analysis successfully separated the three groups. Further analyses through sequencing of the mitochondrial and nuclear DNA indicated that mrigal contributed more to mtDNA, reflecting maternal inheritance in hybrids, while the contributions from rohu were greater for nuclear genes, indicating paternal contribution. The combination of morphometric and molecular tools, therefore, could work effectively in identifying hybrids, ultimately improving broodstock management and conservation practices for Indian major carps.

Introduction

About 29 primary carp species are found in India, out of which rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and catla (*Labeo catla*) are by far the most cultured and economically important fishes belonging to the family Cyprinidae. The above-mentioned species are one of the most important species for aquaculture production and can be found in almost all freshwater ecosystems in peninsular India (Pallipuram, 2020).

These carps are often cultured in polyculture systems to optimise fish production owing to their fast growth, adaptability and consumer preference (Jhingran & Pullin, 1985). Carp brood fish of different species are usually kept together in hatchery settings to maximise space usage and facilitate operational efficiency. However, such practices may unintentionally facilitate interspecific hybridisation, leading to the production of hybrid seeds such as rohu–mrigal and rohu–catla (Padhi & Mandal, 1994).

Hybridisation in Indian major carps is observed in hatchery as well as wild riverine environmental conditions (Allendorf et al., 2001; Desai, 1970). Although hybridisation can result in beneficial attributes, including increased growth performance, environmental tolerance and disease resistance (Bartley et al., 2000), there is a threat of genetic introgression from farmed stock into natural populations. Fertile hybrids might cross-breed with native species, potentially altering genetic structures and lowering species purity (Rosenfield et al., 2004; Na-Nakorn et al., 2004; Senanan et al., 2004). Thus, the accurate identification of hybrids is fundamental to maintaining hatchery broodstock quality and protecting native genetic resources.

Morphological traits have historically been used to identify fish species and their hybrids. Rohu generally has a darker grey body and a silvery lateral surface, while mrigal has a darkened dorsal region and a more pronounced silvery belly. Mrigal and rohu also differ in fin colouration and mouth structure, as mrigal has a generally elongated body with a sub-terminal mouth having smooth non-fringed lips, whereas the terminal mouth of rohu has fringed lips adapted for filter feeding (Jhingran, 1982; Talwar & Jhingran, 1991). But hybridisation often gives rise to intermediate morphological features that fall between those of the parental species, making identification difficult—particularly in juveniles.

The use of molecular markers, together with a morphological approach, has been increasingly utilised for species identification and hybrid detection. Restriction fragment length polymorphisms (RFLP), microsatellites, and single-nucleotide polymorphisms (SNPs) have been used in fish genetics for species differentiation and hybridisation events identification (Campton, 1990; Billington et al., 1988; Hallerman & Beckmann, 1988; Padhi & Mandal, 1997). Mitochondrial genes (e.g., cytochrome c oxidase subunit I, COX1) are frequently utilised for species identification and DNA barcoding. However, nuclear markers can provide complementary information on biparental inheritance.

In eastern India, many hybrids of rohu and mrigal are present in fish markets and hatcheries (Bhowmick et al., 1981). As these hybrids tend to possess morphological features that are very similar to those of their parent species, identifying either based on just external features is often nearly impossible. This complexity may inadvertently lead hatcheries to mix hybrid and wild stocks, jeopardising brood stock purity and contributing to the introgression of hybrids into natural populations. Therefore, developing reliable methods to distinguish hybrids from their parental species is important for brood stock management and conservation of native carp genetic resources.

In order to save from this problem, the new study aimed at developing a thorough fusion of morphometric and molecular tools for authenticating hybrids between rohu and mrigal fish. Morphometric traits were analysed to measure phenotypic differentiation between carp groups using regression and clustering techniques. Moreover, we used mitochondrial (COX1) and nuclear (18S rRNA) gene markers to explore genetic variation and infer possible parental lineage of the hybrids. Such an integrative approach is expected to increase the accuracy of hybrid identification and facilitate appropriate management and conservation of Indian major carps.

Materials and Methods

Study Area and Sample Collection

Specimens representing three carp groups, rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), and their putative hybrids, were collected from fish markets located in three districts of eastern India (one district in Jharkhand and two districts in West Bengal) (Figure 1). Five individuals from each group were sampled from each location, providing representative specimens for both morphometric and molecular analyses. Freshly dead fish available in the markets were used for the study, ensuring that no animals were sacrificed specifically for research purposes.

Although the number of individuals analysed was relatively limited, consistent morphometric patterns and diagnostic genetic differences observed across the sampled specimens provided sufficient resolution to distinguish hybrids from their parental species.

Morphometric Measurements and Tissue Sampling

Morphometric and meristic measurements were recorded following standard ichthyological procedures described by Jayaram (1999) and Rainboth (1996). A total of 22 morphometric and meristic characters were measured using a measuring scale and divider. To minimise measurement errors, each parameter was recorded three times, and the average value was used for further analysis.

After completing the morphometric measurements, small pieces of pectoral fin tissue (approximately 1 cm^2) were clipped from each specimen. Fin tissues were gently washed with distilled water and preserved in absolute ethanol before being stored at −20°C until DNA extraction.

DNA Extraction and Quality Assessment

Genomic DNA was extracted from the preserved fin tissues using the phenol–chloroform extraction method described by Wasko et al. (2003). Briefly, fin tissue samples were digested in a lysis buffer containing Tris–HCl, EDTA, NaCl, SDS, and proteinase K to facilitate tissue breakdown. After digestion, the lysate was treated with RNase to remove RNA contamination.

DNA was then purified through phenol–chloroform–isoamyl alcohol extraction and precipitated using sodium acetate and cold ethanol. The resulting DNA pellets were washed with 70% ethanol, air-dried, and resuspended in sterile distilled water.

The quality and integrity of the extracted DNA were assessed using 0.8% agarose gel electrophoresis and spectrophotometric measurement of OD_{260}/OD_{280} ratios.

PCR Amplification of Mitochondrial and Nuclear Genes

Two genetic markers were selected for molecular screening: the mitochondrial cytochrome c oxidase subunit I (COX1) gene and the nuclear 18S rRNA gene. The COX1 fragment was amplified using the universal fish primers FishF1 and FishR1 (Ward et al., 2005), while the 18S rRNA gene was amplified using the universal primers NS1 and NS4 (White et al., 1990).

PCR reactions were carried out in a 10 µL reaction mixture containing approximately 30–50 ng of template DNA, 10 µM of each primer, 1× reaction buffer, 0.2 mM of each dNTP, and 0.5 U of Taq DNA polymerase (New England Biolabs).

To enhance amplification specificity and minimise non-specific products, a touchdown PCR protocol was used (Allu et al., 2014). The cycling conditions consisted of an initial denaturation step at 95°C for 3 minutes, followed by a touchdown phase in which the annealing temperature gradually decreased from 70°C to 54°C (1°C per cycle). This was followed by 34 amplification cycles consisting of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 10 minutes.

PCR products were examined on 2% agarose gels stained with ethidium bromide. In cases where minor non-specific bands were observed, the target amplicons were excised from the gel and purified using the QIAquick Gel Extraction Kit (QIAGEN, USA). This step ensured that only the correct PCR fragments were used for downstream sequencing.

DNA Sequencing

Purified PCR products were sequenced in both directions using an automated DNA sequencer (Applied Biosystems 3730xL Analyser, USA) with the same primers used for PCR amplification.

Morphometric Statistical Analysis

Morphometric measurements were analysed using the statistical software ‘R’. Total length was treated as the predictor variable, while other morphometric parameters were considered response variables. Regression analysis was used to examine relationships

Figure 1. Showing wild rohu, wild Mrigal, and their hybrids in left-to-right order, having little apparent morphological differences.

between total length and the dependent variables, and coefficients of determination (R^2) were calculated.

To evaluate morphological differentiation among the carp groups, K-means clustering analysis was performed using selected morphometric and meristic variables. Principal component analysis (PCA) was used to visualise clustering patterns in a two-dimensional space.

Sequence Analysis

Raw sequence reads were quality-checked using a Phred score threshold of Q>30. Sequences were edited and trimmed using BioEdit software (Hall, 1999), and consensus sequences were generated from forward and reverse reads.

The resulting sequences were compared with reference sequences available in the NCBI database using BLAST to confirm their identity. Multiple sequence alignment was performed using the ClustalX program (Thompson et al., 1997). Polymorphic nucleotide sites and single-nucleotide polymorphisms (SNPs) were identified from the aligned sequences to evaluate genetic differences among rohu, mrigal, and hybrid individuals.

Data Availability Statement

The DNA sequence data generated during this study have been deposited in the NCBI GenBank database. Accession numbers for the COX1 gene sequences include OR965093, OR965088, OR975640, PP430705, and PP430704, while the 18S rRNA gene sequences have been submitted under accession numbers PP439488, PP439491, PP439492, and PP439496. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Results

Morphometric Regression analysis

Regression analysis was conducted using total length (TL) as the predictor variable and several morphometric parameters as dependent variables. Strong correlations between TL and several body measurements were observed across the three carp populations (rohu, mrigal, and hybrids). The coefficients of determination (R^2) for many of these relationships were high, indicating strong linear associations between total length and morphometric traits (Table 1).

Among the measured parameters, standard length (SL), fork length (FL), pre-dorsal length (PDL), body depth (BD), caudal depth (CD), dorsal fin height (DFH), and caudal length (CL) showed clear differences in their regression relationships among the three carp groups. Regression equations ($Y = a + bX$) were calculated for each variable in each population.

Figure 2 shows regression plots for six representative morphometric parameters: SL, PDL, DFH, BD, CD and CL. The slopes of regression lines differ in rohu, mrigal and hybrid individuals, suggesting differences in proportional growth relationships (Figure 2). These differences suggest systematic variability in hybrids when compared with parental species.

Cluster Analysis of Morphometric Traits

To explore morphometric variation among populations of carp, we performed a cluster analysis using selected morphometric and meristic variables. Data were standardised before the analysis for variable comparability.

The clustering involved eight parameters, out of which two were meristic traits, namely the number of caudal fin rays and the number of scales on lateral lines, while six were morphometric variables: snout length, pre-dorsal length, body depth, caudal depth, dorsal fin height, and caudal length.

Results from K-means clustering identified three clusters, corresponding to rohu, mrigal and hybrid samples. Principal component analysis showed that the three groups were clearly separated in this space (Figure 3). The first two principal components accounted for 94.1% of the total variation, indicating that the selected morphometric variables successfully capture differences between carp populations.

SNP-based Sequence Analysis

The genetic diversity of the varieties was assessed by analysing the sequence difference in 18S rRNA and the mitochondrial COX1 gene. Approximately 600 aligned nucleotide positions of the mitochondrial COX1 gene were analysed. In summary, a total of 66 SNPs were detected among the sequences (Figure 4). The comparison of sequences demonstrated a comparatively closer genetic relationship of hybrid samples with wild mrigal sequences than that of wild rohu. More nucleotide differences were found between hybrids and rohu than between hybrids and mrigal.

In contrast, analysis of the nuclear 18S rRNA gene (~780 bp) showed ten single-nucleotide polymorphisms (SNPs) in the sequences examined (Figure 5). Hybrid sequences showed more similarity to rohu than to mrigal. In particular, there were eight SNP differences observed between the mrigal and hybrids, as compared to two differences observed in rohu and hybrids.

Such comparative sequence information indicates that the mitochondrial marker is closer to mrigal than that of the nuclear marker for rohu. Phylogenetic trees based on COX1 sequences clustered the hybrid samples with *Cirrhinus mrigala*. In contrast, the phylogenetic tree generated by using a nuclear 18S rRNA gene placed the hybrids closer to *Labeo rohita*. The bootstrap support values for the top nodes were >70%, indicating that it was a consistent finding (Supplementary Figure).

Table 1. The Correlation (R^2) and Regression equation (Y=a+bX) of Predictor and Dependent Variables of Rohu, Mrigal, and their Hybrid

Variables	Mrigal			Hybrid			Rohu		
	Y=a+bX	R^2	p-value	Y=a+bX	R^2	p-value	Y=a+bX	R^2	p-value
Total Length (TL)									
Standard Length (SL)	Y= 0.558+0.794x	0.998	0.0244*	Y= -5.792+0.951x	0.950	0.1423	Y= -0.972+0.843x	0.996	0.0379*
Forked Length (FL)	Y= -0.023+0.888x	0.998	0.0219*	Y= -11.154+1.133x	0.985	0.0776	Y= 0.544+0.872x	0.974	0.1017
Head Length (HL)	Y= -0.023+0.188x	0.999	0.0002*	Y= 1.353+0.137x	0.998	0.0280*	Y= 9.597+0.085x	0.145	0.7506
Eye Diameter (ED)	Y= 0.723+0.011x	0.999	0.0006*	Y= -1.109+0.054x	0.974	0.1012	Y= 1.547+0.012x	0.723	0.3527
Snout Length (SnL)	Y= 1.047+0.023x	0.999	0.0001*	Y= -1.684+0.088x	0.872	0.2321	Y= 1.168+0.027x	0.276	0.6473
Pre-dorsal Length (PDL)	Y= 1.723+0.311x	0.990	0.0622	Y= -15.220+0.709x	0.9825	0.0844	Y= 4.163+0.253x	0.850	0.2529
Scales on Lateral Line (SLL)	Y=43	-	-	Y=42	-	-	Y=41	-	-
Scales below Lateral Line (SbLL)	Y=6	-	-	Y=6	-	-	Y=6	-	-
Scales above Lateral Line (SaLL)	Y=7	-	-	Y=7	-	-	Y=7	-	-
Dorsal fin rays (DFR)	Y=14	-	-	Y=14	-	-	Y=14	-	-
Pectoral fin rays (PFR)	Y=16	-	-	Y=16	-	-	Y=16	-	-
Pelvic fin rays (PvFR)	Y=9	-	-	Y=9	-	-	Y=9	-	-
Anal fin rays (AFR)	Y=8	-	-	Y=8	-	-	Y=8	-	-
Caudal fin rays (CFR)	Y=22	-	-	Y=20	-	-	Y=21	-	-
Pre-dorsal scales (PDS)	Y=11	-	-	Y=11	-	-	Y=11	-	-
Anal fin base length (AL)	Y= -0.358+0.070x	0.999	0.0002*	Y= -4.868+0.177x	0.872	0.2321	Y= -0.123+0.065x	0.8734	0.2316
Dorsal fin height (DFH)	Y= -0.082+0.158x	0.964	0.1210	Y= -2.325+0.236x	0.872	0.2321	Y= 2.348+0.089x	0.615	0.4259
Body depth (BD)	Y= -1.217+0.241x	0.998	0.0268*	Y= -28.115+0.858x	0.872	0.2321	Y= -0.321+0.239x	0.890	0.2146
Weight (WT)	Y= -358.8+20.59x	0.999	0.0001*	Y= -4776.60+133.15x	0.872	0.2321	Y= -1702.804+63.063x	0.997	0.0315*
Caudal depth (CD)	Y= 0.364+0.082x	0.999	0.0002*	Y= -9.705+0.325x	0.872	0.2321	Y= 0.344+0.092x	0.955	0.1351
Caudal length (CL)	Y= -0.588+0.205x	0.978	0.0938	Y= 5.792+0.048x	0.048	0.8592	Y= 0.988+0.156x	0.952	0.1404

**P*<0.05*

Discussion

In order to preserve natural populations and preserve genetic integrity in aquaculture systems, hybrid detection between closely related fish species is crucial. Indian major carps such as *Labeo rohita* (rohu) and *Cirrhinus mrigala* (mrigal) are commonly cultured together, which increases the probability of unintended hybridisation in hatcheries and natural waters. Because hybrids often exhibit intermediate morphological traits, distinguishing them from parental species using external characteristics alone can be difficult (Allendorf et al., 2001). In this study, morphometric and molecular approaches were integrated to improve the identification of rohu–mrigal hybrids.

Morphometric analysis revealed clear differences in the relationships between total length and several body measurements among rohu, mrigal, and hybrid individuals. Variations in regression slopes for parameters such as standard length, body depth, and caudal depth indicate differences in proportional growth patterns among the three groups. Hybrid fishes often show intermediate or modified morphometric traits as a result of genetic contributions from both parental species (Dwivedi, 2019). The samples were subsequently divided into discrete groups representing rohu, mrigal, and their hybrids using cluster analysis based on certain morphometric and meristic features. These findings show that morphometric analysis can provide an effective preliminary strategy for identifying

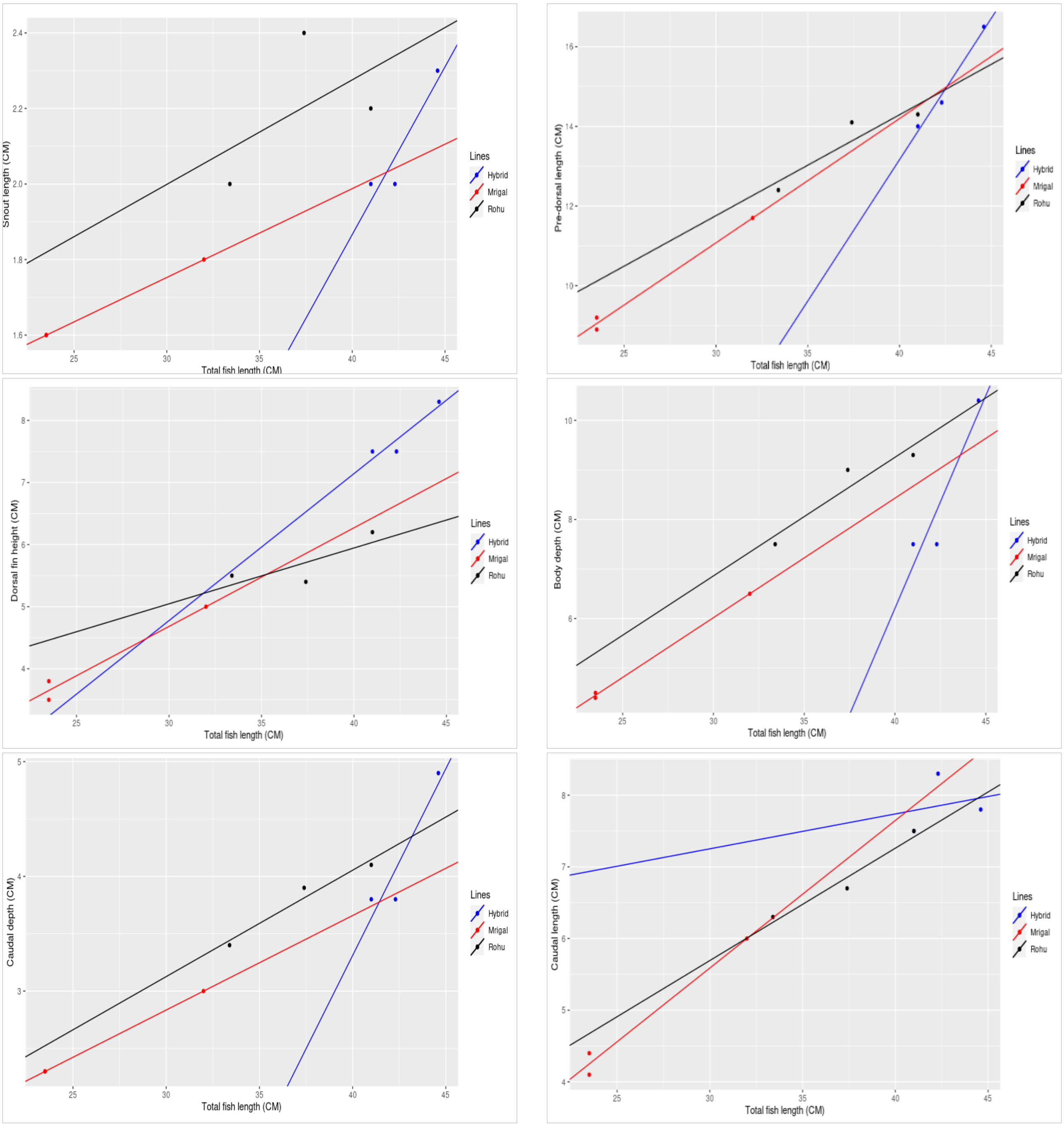

Figure 2. Regression line plots for the six dependent variables (SL, PDL, DFH, BD, CD, CL) on the predictor variable showing difference slope among Rohu, Mrigal, and their Hybrids.

hybrid individuals, provided measurable morphological features are available. However, environmental factors and developmental phases might occasionally affect phenotypic features, which could reduce the accuracy of morphological identification. Molecular markers are therefore frequently utilised to ascertain paternal lineage and validate hybrid status.

The phylogenetic analyses used for the present study also comprised mitochondrial (COX1) and nuclear (18S rRNA) gene markers, which provided complementary genetic information. The hybrid samples showed a closer relationship with *C. mrigala* in terms of mitochondrial COX1 gene sequences, indicating maternal inheritance from this species. Mitochondrial DNA, which is inherited only from the female parent, has played a pivotal role in the development of a universal "DNA barcode" for the identification of species (Ward et al., 2005; Lasker et al., 2013; Lenka et al., 2024) in fishes. However, solely relying on mitochondrial markers does not definitively prove hybridisation, as they represent only maternal ancestry. To further circumvent this limitation, the nuclear 18S rRNA gene was examined as well.

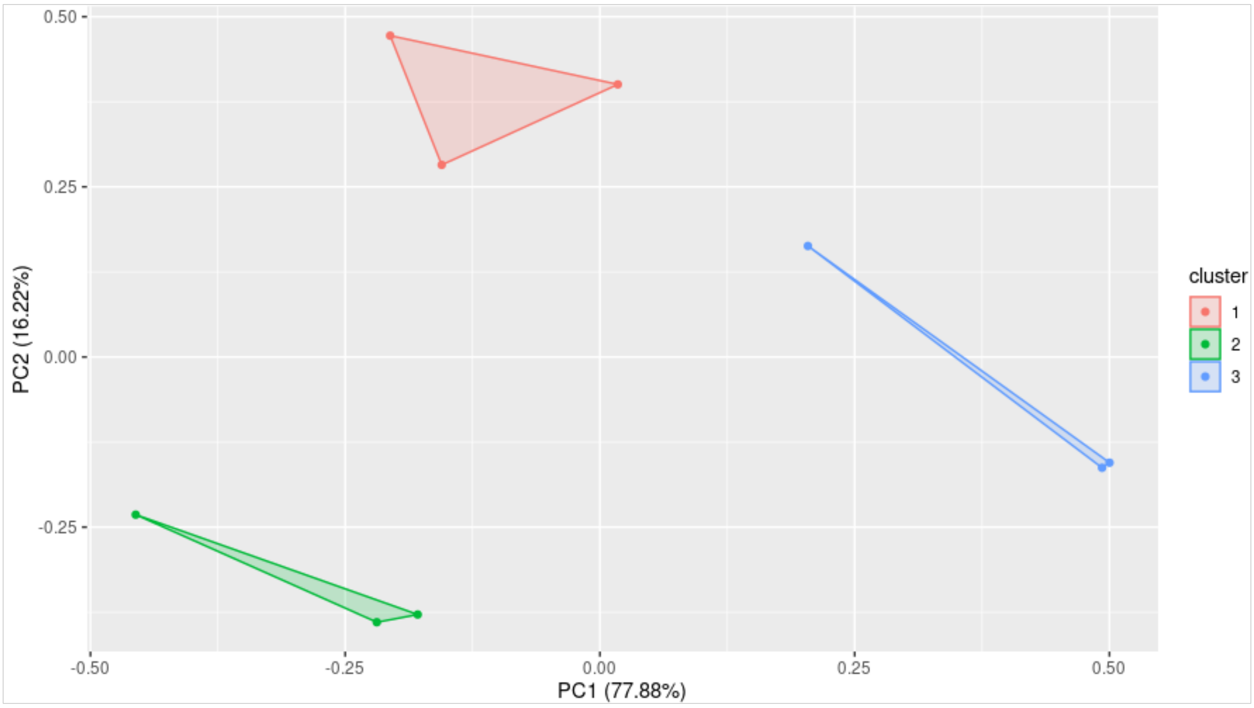

Figure 3. Cluster plot demonstrating how the data points are represented by the first 2 principal components in a two-dimensional plane. Cluster 1 corresponds to the 3 wild types of rohu fish, Cluster 2 corresponds to the 3 wild types of mrigal, and Cluster 3 corresponds to the 3-hybrid (mrigal-rohu) fish.

	18	24	27	54	57	69	75	78	81	87	99	144	147	153	156	160	171	183	219	237	238	244	255	261	276	285	288	297	318	333	366	369	372
PP430704 (Hybrid)	T	C	G	C	C	C	C	C	C	T	G	A	T	T	C	T	G	A	T	G	C	C	T	T	A	A	G	G	C	G	G	T	T
NC 017611.1 (C. mrigala)	T	C	G	C	C	C	C	C	C	T	G	A	T	T	C	T	G	A	T	G	C	C	T	T	A	A	G	G	G	G	G	T	C
NC 017608.1 (L. rohita)	C	T	A	T	T	T	T	T	A	C	A	G	C	C	G	C	A	G	C	A	T	T	C	A	G	G	A	A	C	A	A	A	T

	403	405	411	414	417	423	426	444	453	454	456	462	468	471	474	489	495	498	504	510	513	516	522	525	534	549	552	558	573	582	585	594	597
PP430704 (Hybrid)	A	C	C	C	C	G	C	C	T	C	G	T	C	G	C	G	T	A	G	C	A	T	T	T	C	C	T	C	A	G	A	T	C
NC 017611.1 (C. mrigala)	A	C	C	C	C	G	C	C	T	C	G	T	C	G	C	G	T	A	G	C	A	T	T	T	C	C	T	C	A	G	A	T	C
NC 017608.1 (L. rohita)	G	T	T	T	T	A	T	T	C	T	A	C	T	C	A	A	C	C	A	A	G	C	A	C	T	T	G	T	G	A	G	C	T

Figure 4. The complete alignment of 3 sequences from 3 carp varieties showing a total of 66 SNP positions in the partial COX1 gene sequences (~600 bp). Maximum nucleotide variation was observed between hybrids and wild rohu sequences, while a little with wild mrigal.

	4	27	36	70	82	91	103	450	661	748
PP439496 (Hybrid)	G	G	T	G	G	G	G	G	G	G
GU967674.1 (L. rohita)	G	G	T	G	G	G	G	G	C	C
GU967675.1 (C. mrigala)	A	A	C	A	A	A	A	A	G	G

Figure 5. Showing a total of 10 SNPs located between the positions 4 bp to 748 bp, marked in the partial (~780 bp) 18S rRNA gene sequences.

In contrast, nuclear gene data analysis showed the hybrids were more closely related to *L. rohita,* suggesting a contribution from *L. rohita* (rohu) in the hybridisation events. Because nuclear genes come from both parents, the differences we observe between mitochondrial and nuclear patterns support a hybrid origin of these specimens. Similar approaches have previously been encouraged that integrate mitochondrial and nuclear markers to increase the confidence associated with hybrid identification, along with phylogenetic studies in fish populations (Allendorf et al., 2001; Hebert et al., 2003; Parveen et al., 2024; Singh & Ghosh, 2024).

Overall, this study demonstrates that fixing the genomic loci of mitochondrial and nuclear genetic markers with morphometric analysis viability provides a robust framework to detect hybrids of Indian major carps. This approach will improve hatchery brood stock management and reduce the potential for unintentional hybridisation, which represents a major threat to the integrity of wild fish populations.

Conclusion

These results emphasise the utility of combining morphological data with mitochondrial (COX1) and nuclear (18S rRNA) gene markers to characterise hybrids between *Labeo rohita* and *Cirrhinus mrigala*. Differences in morphometric relationships and clustering patterns enabled the segregation of hybrids from their parental species, whilst molecular markers provided insight into maternal as well as paternal contributions to these interspecific hybrids. The methods, in total, provide a feasible and reliable system for hybrid carp detection. Further, our integrated screening strategy has the potential to improve hatchery broodstock management practices and could also contribute towards the conservation of the genetic integrity of Indian major carp populations.

Ethical Statement

All fish specimens used in this study were obtained from local fish markets after capture for commercial purposes. No animals were sacrificed specifically for this research. Fin tissue samples were collected from freshly dead specimens, and therefore, the study did not involve any experimental handling of live animals.

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Author Contribution

DG and PL contributed equally. PL & NS were involved in sample collection and genomic DNA isolation. DG, PL & SG statistically interpreted, analysed, and designed the manuscript's theoretical framework. SG drafted the work, and with the help of PM, revised it critically. All authors approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: Setting conservation guidelines. Trends in Ecology & Evolution, 16(11), 613–622. https://doi.org/10.1016/S0169-5347(01)02290-X

Allu, P. K., Chakraborty, B., Das, M., Mahapatra, N. R., & Ghosh, S. (2014). PCR-based segregation of one hybrid variety of Labeo rohita and Catla catla from their wild-types. Aquaculture International, 22, 775–782.

Bartley, D. M., Rana, K., & Immink, A. J. (2000). The use of inter-specific hybrids in aquaculture and fisheries. Reviews in Fish Biology and Fisheries, 10, 325–337. https://doi.org/10.1023/A:1008908706052

Bhowmick, R. M., Jana, R. K., Gupta, S. D., Kowtal, G. V., & Rout, M. (1981). Studies on some aspects of biology and morphometry of the intergeneric hybrid Catla catla × Labeo rohita produced by hypophysation. Aquaculture, 23(1–4), 367–371. https://doi.org/10.1016/0044-8486(81)90030-2

Billington, N., Hebert, P. D. N., & Ward, R. D. (1988). Evidence of introgressive hybridization in the genus Stizostedion: Interspecific transfer of mitochondrial DNA between sauger and walleye. Canadian Journal of Fisheries and Aquatic Sciences, 45(11), 2035–2041. https://doi.org/10.1139/f88-237

Campton, D. E. (1990). Application of biochemical and molecular genetic markers to analysis of hybridization. In D. H. Whitmore (Ed.), Electrophoretic and isoelectric focusing techniques in fisheries management (pp. 241–263). CRC Press.

Desai, V. R. (1970). On the occurrence of the natural hybrids of catla–rohu in Madhya Pradesh. Journal of the Zoological Society of India, 22, 35–40.

Dwivedi, A. K. (2019). Detecting natural hybridization among Indian major carps through advance morphometric approach. bioRxiv. https://doi.org/10.1101/553941

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Hallerman, E. M., & Beckmann, J. S. (1988). DNA-level polymorphism as a tool in fisheries science. Canadian Journal of Fisheries and Aquatic Sciences, 45(6), 1075–1087. https://doi.org/10.1139/f88-130

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society B, 270(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Jayaram, K. C. (1999). The freshwater fishes of the Indian region. Narendra Publishing House.

Jhingran, V. G. (1982). Fish and fisheries of India (2nd ed.). Hindustan Publishing Corporation.

Jhingran, V. G., & Pullin, R. S. V. (1985). A hatchery manual for the common, Chinese and Indian major carps. Asian Development Bank / International Center for Living Aquatic Resources Management.

Laskar, B. A., Bhattacharjee, M. J., Dhar, B., Mahadani, P., Kundu, S., & Ghosh, S. K. (2013). The species dilemma of northeast Indian mahseer (Actinopterygii: Cyprinidae): DNA barcoding in clarifying the riddle. PLOS ONE, 8(1), e53704.

Lenka, P., Singh, N., Ghosh, D., Mahadani, P., & Ghosh, S. (2024). Integrated morphometric and molecular approaches to screen hybrid carp populations. Molecular Biology Reports, 51(1), 738.

Na-Nakorn, U., Kamonrat, W., & Ngamsiri, T. (2004). Genetic diversity of walking catfish (Clarias macrocephalus) in Thailand and evidence of genetic introgression from introduced farmed Clarias gariepinus. Aquaculture, 240(1–4), 145–163. https://doi.org/10.1016/j.aquaculture.2004.03.017

Padhi, B. K., & Mandal, R. K. (1994). Improper fish breeding practices and their impact on aquaculture and fish biodiversity. Current Science, 66, 624–626.

Padhi, B. K., & Mandal, R. K. (1997). Inadvertent hybridization in a carp hatchery as detected by nuclear DNA RFLP. Journal of Fish Biology, 50(4), 906–909. https://doi.org/10.1111/j.1095-8649.1997.tb01973.x

Pallipuram, J. (2020). Hybridization in carps and early detection of carp hybrids using PCR-based kit. In Emerging technologies, environment and research for sustainable aquaculture. IntechOpen. https://doi.org/10.5772/intechopen.91946

Parveen, S., Abbas, K., Tayyab, M., Hussain, M., Naz, H., & Shafique, L. (2024). Microsatellite and mtDNA-based exploration of inter-generic hybridization and patterns of genetic diversity in major carps of Punjab, Pakistan. Aquaculture International, 1–28.

Rainboth, W. J. (1996). FAO species identification field guide for fishery purposes: Fishes of the Cambodian Mekong. FAO.

Rosenfield, J. A., Nolasco, S., Lindauer, S., Sandoval, C., & Kodric-Brown, A. (2004). The role of hybrid vigor in the replacement of Pecos pupfish by its hybrids with sheepshead minnow. Conservation Biology, 18(6), 1589–1598. https://doi.org/10.1111/j.1523-1739.2004.00347.x

Senanan, W., Kapuscinski, A. R., Na-Nakorn, U., & Miller, L. M. (2004). Genetic impacts of hybrid catfish farming (Clarias macrocephalus × Clarias gariepinus) on native catfish populations. Aquaculture, 235(1–4), 167–184. https://doi.org/10.1016/j.aquaculture.2004.02.031

Singh, N., & Ghosh, S. (2024). Segregation of white-morphotype and wild mrigal on molecular basis. Journal of Aquatic Biology & Fisheries, 12(1), 54–58.

Talwar, P. K., & Jhingran, A. G. (1991). Inland fishes of India and adjacent countries. Oxford & IBH Publishing.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The Clustal_X Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 25*(24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876

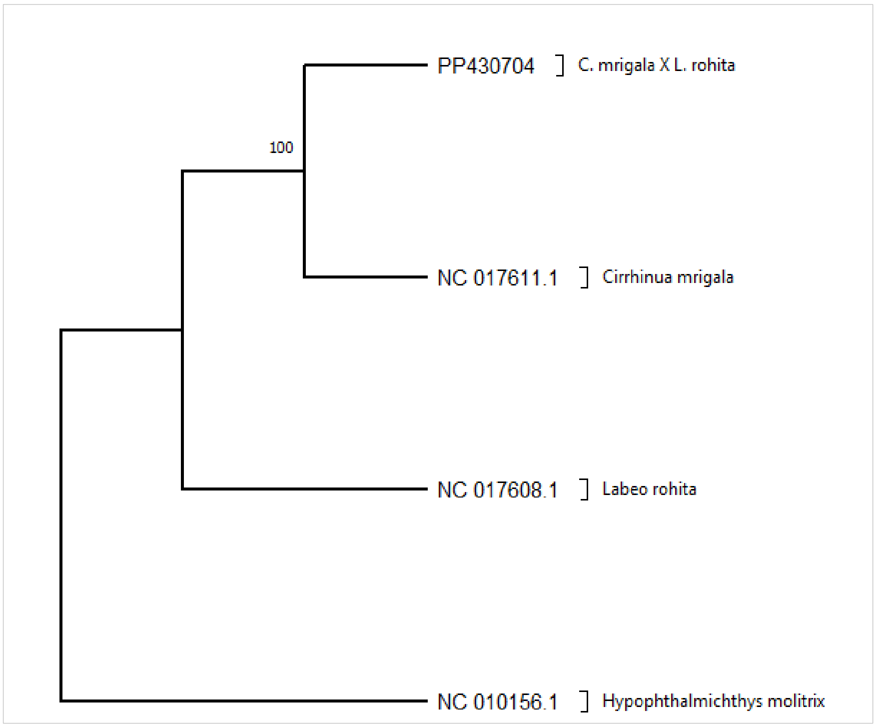
Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B, 360(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wasko, A. P., Martins, C., Oliveira, C., & Foresti, F. (2003). Non-destructive genetic sampling in fish: An improved method for DNA extraction from fish fins and scales. *Hereditas, 138*(3), 161–165. https://doi.org/10.1034/j.1601-5223.2003.00150.x

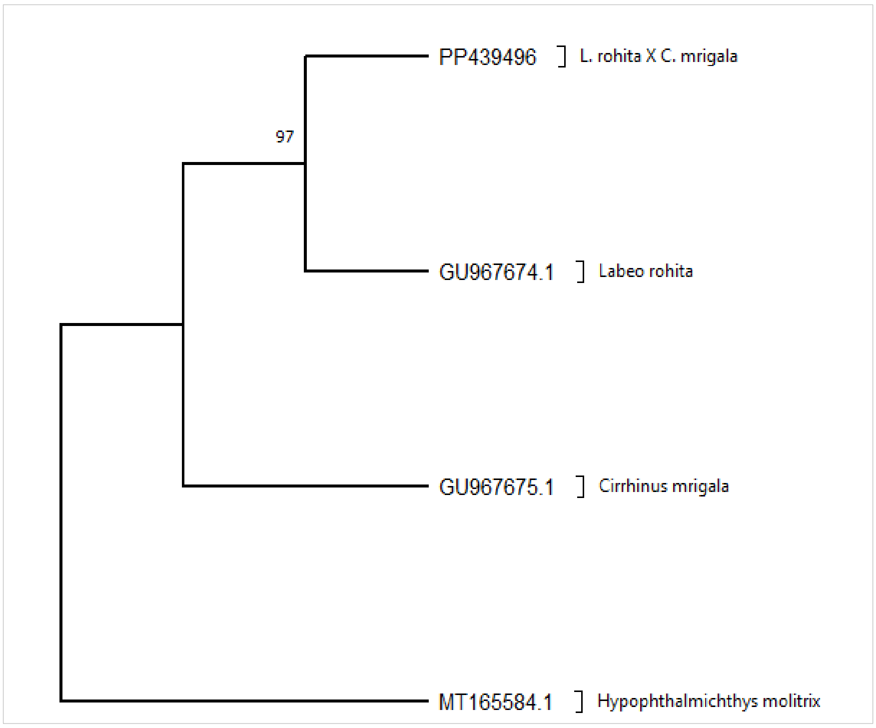
White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. Innis, D. Gelfand, J. Sninsky, & T. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Supplementary Figure

Phylogenetic Relationship



Supplementary Figure 1: The Phylogenetic tree was developed using the UPGMA_K2P method based on 4 sequences of the partial COX1 gene [1 hybrid sequence (PP430704) representative of all hybrids (OR965093, OR965088, OR975640, PP430705), 1 wild Mrigal parent (NC 017611.1), another wild Rohu (NC 017608.1) parent including the outgroup sequence (Accession number NC010156.1)]. A bootstrap value of 100 at the topmost nodal point confirms the strong association of both the hybrid sequence with this nodal group and its exclusion from other related carp sequences.



Supplementary Figure 2: The phylogenetic tree was developed based on 4 sequences of partial 18S rRNA gene, including the outgroup sequence (Accession number MT165584.1), using the UPGMA_ K2P method. A 97% bootstrap value at the topmost nodal point confirms the strong association of both the hybrid sequence with this nodal group and its exclusion from other related carp sequences.