

# Polyphyletic Origins of Schizothoracinae Fishes (Cyprinidae) in Respect to Their Mitochondrial Protein-Coding Genes

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## ABSTRACT

Whole mitochondrial DNA (mtDNA) *S. plagiostomus* was 16569bp with 2 rRNAs genes, 22 tRNA genes, 13 protein-coding genes, and 2 non-coding region. For estimating association of phylogenetic of a species based on Protein coding mitochondrial DNA genes are measured to be a great value. We sequenced protein-coding genes of *Schizothorax plagiostomus* in present study, based upon these genes we compared with *S. plagiostomus* the 48 *Schizothoracinae* fishes by phylogenetic association. We analysed that *S. plagiostomus* to be closely related with *Schizopyge niger*, *Schizothorax labiatus*, *Schizothorax nepalensis* with high-bootstrap values. For understanding phylogenetic relationship of *Schizothorax* species such empirical data would be important.

**Keywords:** Phylogeny; Snow trout; High-altitude adaptation

## MAIN BODY

The Cypriniformes comprises of 5 families (Catostomidae, suckers; Cyprinidae, minnows; algae eaters; Gyrinocheilidae, Balitoridae, river loaches Cobitidae, loaches), depending on investigators every one with subfamilies poorly separate (e.g., 426 genera and 2 to 12 subfamilies recognized Substantial amounts of amino acid and DNA data involved with mtDNA can be provided for phylogenetic analyses [1]. Previous researcher resolved the phylogenetic relationship of Schizothoracinae fishes only for the one species comparison with the other 21 species [2]. Increasing the number of mitochondrial genome sequences of Schizothoracinae and reconstructing the Schizothoracinae phylogenetic tree based on a more comprehensive dataset are necessary to address the remaining problematic clades So analysis of Phylogenetics of Cyprinid taxa based on the functionally vital genes can help to understand the functional divergence and speciation [2]. Majority of the whole mitochondrial genomic sequence occupied by 13 protein-coding genes. In the present study, we our recent study based on monophyletic origin of the Schizothoracinae fish in respect to mitochondrial protein-coding genes, to find out the phylogenetic relationship of Schizothoracinae fishes from the available genome upto date on the basis of mitochondrial protein coding genes.

## MATERIALS AND METHODS

Present study was conducted in Panjkora River, it is reflected as the chief life line of lower Dir, and it is Malakand division part

and situated in the province of Khyber Pakhtunkhwa Pakistan. It lies in the range of Hindu Kush region between Latitude: 34° 39' 59.99" N, Longitude: 71° 45' 59.99" E. Fish samples of *Schizothorax plagiostomus* were collected from River panjkora using different types of nets namely hand nets, cast nets and hooks. Transferred the specimen to the lab of department of zoology were the samples were stored. For the amplification of the mtDNA of *Schizothorax plagiostomus* we used standard high salt extraction method, we extracted the mtDNA from the preserved muscle tissues in 95% ethanol [3]. For polymerase chain reaction (PCR) amplification sixteen sets of primers were designed which were based on original mitochondrial genomes DNA sequences of cyprinid fish. Cocktail reaction included The 25 µL to 6 µL of 10 × buffer, every nucleotide of 1.5 µL (dNTP), every primer of 1 µL, Taq DNA polymerase about 1.5 unit, template DNA of 1–2 µL. The process of thermocycling was started for 5 min at 94°C, followed through 20 cycles at 94°C for 30 s, 56°C for 50 s, and 72°C for 1 min 30 s, with 0.1°C reducing the temperature for annealing to every cycle, with the annealing temperature at 54°C Then 12 other cycles used to, there was an end of final cycle at 8 min extension. On 1.2% of Agarose gel in 1×Trisacetate-EDTA buffer for all samples, PCR product was electrophoresed about 1 µl at 80 V for 30 min then staining with ethidium bromide, as well as in the Gel-Doc system visualized under Ultra violet illumination. The purified PCR products through standard protocols then for sequencing sent to the Sangon biotech company (Sangon Biotech Company Shanghai).

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## Analysis of sequence

Using the program Clustal W the DNA sequences were aligned [4]. The DNA sequences was edited and analysed with Auto Assembler (Applied Bio systems) and DNASIS (Hitachi Software Engineering Co. Ltd) [5]. The locations of the 13 protein-coding genes were determined by comparisons of the amino acid sequences. The phylogenetic association was inferred by MEGA 6.0 [6]. For the phylogenetic association, the mtDNA, 13 sequenced protein coding genes of *Schizothorax plagiostomus* were used, and the sequences of other cyprinids were used for infringe the phylogenetic relationships there sequences retrieved from NCBI.

## Phylogenetic analyses

In addition to the newly obtained sequence of *S. plagiostomus*, we obtained sequences of 13 protein coding genes for another 31 species of 8 genera in Schizothoracinae to elucidate among members of the subfamily Schizothoracinae the phylogenetic relationships. Sequences of 13 protein coding to serve as out-groups genes of four species in the subfamily Barbinae and three species in Cyprininae were obtained from GenBank in the subsequent phylogenetic analyses. In Table 1 all species and the respective GenBank numbers used in the present study are listed. All 13 protein coding genes of all species the alignments were combined,

**Table 1:** List of species and sequences used in this study.

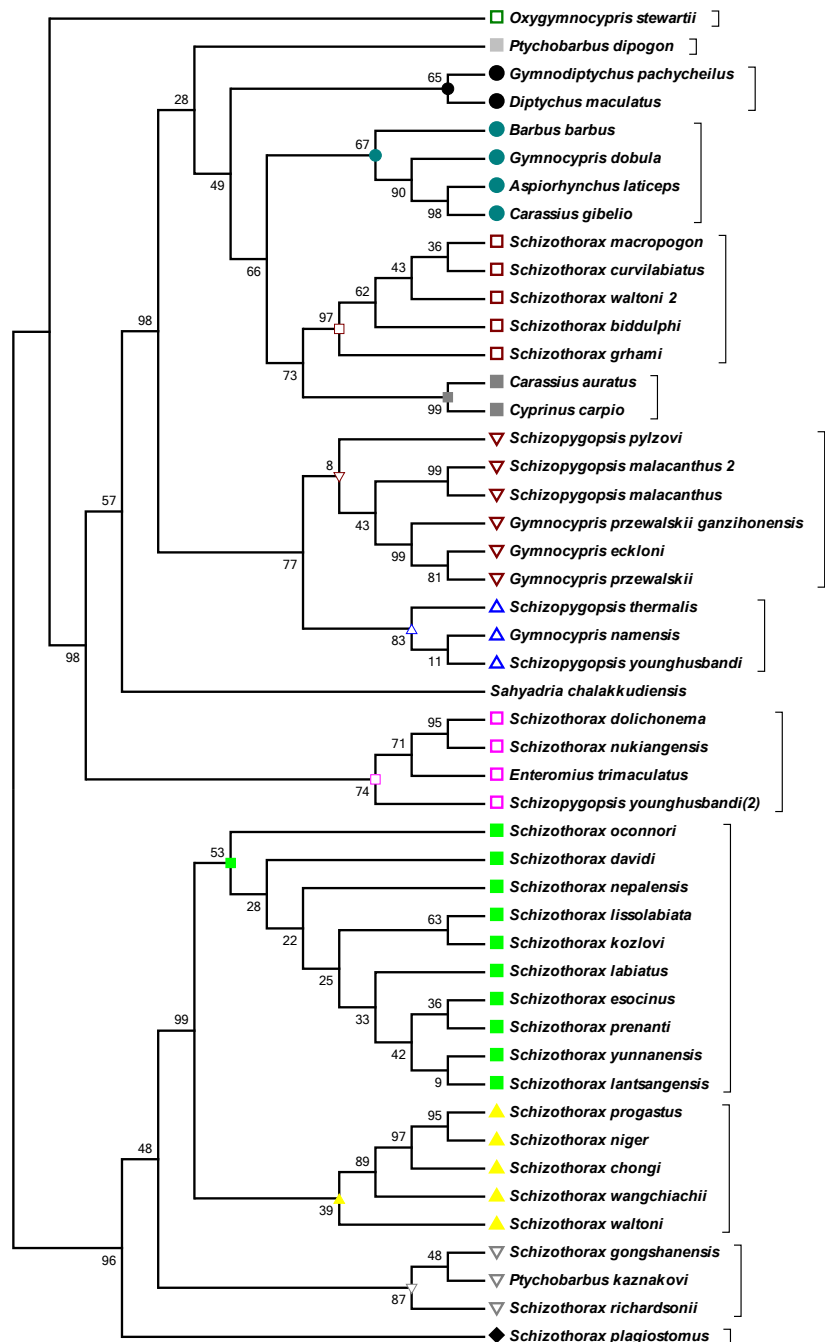
Family	Subfamily	Genus	Species	GenBank no			
Ingroup	Schizothoracinae	<i>Schizothorax</i>	<i>Schizothorax esocinus</i>	KT210882.1			
Cyprinidae			<i>Schizopyge niger</i>	NC_022866.1			
			<i>Schizothorax progastus</i>	NC_023366.1			
			<i>Schizothorax kozlovi</i>	NC_027670.1			
			<i>Schizothorax yunnanensis</i>	KP892531.1			
			<i>Schizothorax lantsangensis</i>	NC_026294.1			
			<i>Schizothorax chongi</i>	NC_024621.1			
			<i>Schizothorax biddulphi</i>	NC_017873.1			
			<i>Schizothorax nepalensis</i>	NC_031537.1			
			<i>Schizothorax davidi</i>	NC_026205.1			
			<i>Schizothorax nukiangensis</i>	KT223584.1			
			<i>Schizothorax prenanti</i>	NC_023829.1			
			<i>Schizothorax oconnori</i>	NC_020781.1			
			<i>Schizothorax waltoni</i>	KC513574.1			
			<i>Schizothorax waltoni 2</i>	JX202592			
			<i>Schizothorax macropogon</i>	NC_020339.1			
			<i>Schizothorax graham</i>	NC_029708.1			
			<i>Schizothorax lissolabiatus</i>	NC_027162.1			
			<i>Schizothorax dolichonema</i>	KJ577589.1			
			<i>Schizothorax richardsonii</i>	NC_021448.1			
			<i>Schizopyge gongshanensis</i>	NC_031803.1			
			<i>Schizothorax pseudoaksaiensis</i>	NC_024833.1			
			<i>Schizothorax labiatus</i>	KT944287.1			
			<i>Schizothorax wangchiachii</i>	NC_020360.1			
					<i>Schizopygopsis</i>	<i>Schizopygopsis malacanthus</i>	KR527479
						<i>Schizopygopsis malacanthus baoxingensis</i>	KM593242
						<i>Schizopygopsis thermalis</i>	KC558499
						<i>Schizopygopsis younghusbandi</i>	KC351895
						<i>Schizopygopsis younghusbandi 2</i>	JX232379
						<i>Schizopygopsis pylzovi</i>	KP316067
					<i>Ptychobarbus</i>	<i>Ptychobarbus dipogon</i>	KF597526
						<i>Ptychobarbus kaznakovi</i>	KM268050
			<i>Schizopygopsis malacanthus</i>	KR527479			
		<i>Oxygymnocypris</i>	<i>Oxygymnocypris stewartii</i>	KF528985			
		<i>Gymnodiptychus</i>	<i>Gymnodiptychus pachycheilus</i>	KF976395			
		<i>Gymnocypris</i>	<i>Gymnocypris dobula</i>	KC558497			
			<i>Gymnocypris eckloni</i>	JQ004279			
			<i>Gymnocypris namensis</i>	KC558498			
			<i>Gymnocypris przewalskii</i>	AB239595			
			<i>Gymnocypris przewalskii ganzihonensis</i>	JQ004278			
		<i>Diptychus</i>	<i>Diptychus maculatus</i>	KM659026			
		<i>Aspiorhynchus</i>	<i>Aspiorhynchus laticeps</i>	KF564793			
<b>Outgroup</b>							
Cyprinidae	Cyprininae	<i>Carassius</i>	<i>Carassius auratus</i>	KJ476998			
			<i>Carassius gibelio</i>	GU170401			
		<i>Cyprinus</i>	<i>Cyprinus carpio</i>	AP009047			
		Barbinae	<i>Barbus</i>	<i>Barbus barbus</i>	AB238965		
				<i>Barbus trimaculatus</i>	AB239600		
				<i>Puntius</i>	<i>Puntius chalakkudiensis</i>	JX311437	

and a concatenated alignment was then generated. Using ClustalW thirteen protein-coding gene sequences were translated into their corresponding amino acids and with default settings aligned, and translated into the nucleotide sequence for obtaining the better results. Gaps and missing data determined and was deleted finally we left with datasets the concatenated nucleotide sequences of the 13 protein-coding genes, were generated for the subsequent phylogenetic analyses. Phylogenies were reconstructed using maximum-likelihood tree constructed by using the mega software. Following accession numbers were taken. (KT184924), (KT210882.1), (NC\_022866.1), (NC\_023366.1), (NC\_027670.1), (KP892531.1), (NC\_026294.1), (NC\_024621.1), (NC\_017873.1), (NC\_031537.1), (NC\_026205.1), (KT223584.1), (NC\_023829.1), (NC\_020781.1), (KC513574.1), (JX202592), (NC\_020339.1), (NC\_029708.1), (NC\_027162.1), (KJ577589.1), (NC\_021448.1), (NC\_031803.1), (NC\_024833.1), (KT944287.1), (NC\_020360.1), (KR527479), (KM593242), (KC558499), (KC351895),

(JX232379), (KP316067), (KF597526), (KM268050), (KR527479), (KF528985), (KF976395), (KC558497), (JQ004279), (KC558498), (AB239595), (JQ004278), (KM659026), (KF564793), (KJ476998), (GU170401), (AP009047), (AB238965), (AB239600)(X311437), (NC\_026294.1).

## RESULTS

Combined data set of all the 13 protein-coding genes of 31 species of 8 genera in Schizothoracinae species of *Schizothorax* yielded two clades having out groups and groups species showed closest relationships with each other. *Schizothorax lantsangensis* group showed 99% of the posterior nodal probability with the *Schizothorax waltoni* group and also showed the maximum probability with *Schizothorax chongi* group and *Schizothorax lantsangensis* group. Maximum bootstrap values supports our results showed in Figure 1.



**Figure 1:** Phylogenetic association of *Schizothorax* fishes was constructed by combining 13 protein-coding genes with closely related 23 other *Schizothorax* species using Mega6 Software. The branches showing the numbers are bootstrap values (represented as %).

## DISCUSSION

Current study was conducted to find out the phylogenetic relationship of *Schizothoracinae* fishes. The *S. plagiostomus* mitochondrial genome sequence was found to be 16569 bp length. Like other cyprinids fishes, genome of *S. plagiostomus* consisted of 13 protein coding gene (PCGs), 22 TRNAs gene, two ribosomal RNA (12S rRNAs and 16S rRNAs) genes, one control region and light strand replication origin (OL) consisted of 33 bp. The neighbour joining analysis was performed in MEGA6 with 1000 bootstrap replicates [7,8]. Consequently, in inter-and intra-specific phylogeny in an animal's mitochondrial genome the genetic information offered, in the studies it is widely analysed [9]. Phylogenetic relationships among genera and species under *Schizothoracinae* have been investigated based on morphological characters, RAPD analysis. Cyprinid fishes phylogenetic analysis based on the functionally vital genes can support to know the functional divergence and speciation of these fishes. Consequently, correspondingly it is the set of proteins which have the greatest marked variances between species observed, Monophyly is intensely supported for the of *schizothorax* fishes in the set of protein [10,11]. *Schizothoracinae* fishes showed the closest relationship with the other fishes formed a different cluster. It was found that the species belonging to the northern Himalayas grouped together while species from north-eastern Himalayas remained separate [12]. Maximum bootstrap values supports our results, these results are somewhat similar with the finding earlier [8]. Inferred the phylogenetic relationship on the basis of whole mitochondrial data set.

## CONCLUSION

As the recent developments in molecular techniques based on genes are very much useful for establishing taxonomical and phylogenetic relationships among different species. The present study resolved the phylogenetic relationships among the 48 species of subfamily *Schizothoracinae*, study of phylogenetic relationship on the basis of sequences of protein-coding gene in these species offers useful visions to the phylogenetic status of cyprinid fishes and provide The step for further investigations of issues with taxonomic and conservation and phylogenetic in this vital up of fishes.

## DECLARATION OF INTEREST

The authors alone are responsible for the content and writing of the paper. The authors report no conflicts of interest.

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