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The complete mitochondrial genome and phylogeny of the green chromide *Etroplus suratensis* (Bloch, 1790) from Vembanad Lake, Kerala, south India

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ABSTRACT

The green chromide *Etroplus suratensis* (Bloch, 1790), is a cichlid species which forms an economically valuable food fish and a preferred candidate for brackishwater aquaculture in India. The complete mitogenome of *E. suratensis* collected from Vembanad Lake, Kerala, India has been characterised in the present study. The entire mitogenome was PCR amplified as contiguous, overlapping segments and sequenced. The assembled mitogenome of *E. suratensis* is 16456 bp circle, contained the 37 mitochondrial structural genes, two ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA (tRNA) genes, 13 protein-coding genes and 1 non-coding control region/D-loop, with the gene order identical to vertebrates. In the phylogenetic analysis, *E. suratensis* is clustered with other Indo-Sri Lankan taxa. Among cichlids, the groups from South America and Africa are monophyletic in origin. The mitogenomic information generated in this study will be valuable for further studies on evolution, taxonomy, conservation, environmental adaptation and selective breeding of this species having aquaculture, ornamental and evolutionary importance.

Keywords: Cichlids, *Etroplus suratensis*, Complete mitochondrial genome, Phylogenetic status

Etroplus suratensis (Bloch, 1790), is a euryhaline cichlid species distributed mainly in freshwater, brackishwater and river mouths of peninsular India and Sri Lanka and it is the most abundant species among the genus *Etroplus* (Jayaram, 2010). Wild populations of *E. suratensis* have been recorded from the states of Kerala and Tamil Nadu (Jayaram, 2010) and introduced populations from Andhra Pradesh and Odisha. Cichlids have been considered as model organisms for evolutionary biology, evolutionary genetics and phenotype-genotype relationship studies because of their ecological, morphological and behavioural diversity (Azuma *et al.*, 2008). *E. suratensis* is one of the most sought after candidate species for brackishwater aquaculture in Kerala due to its suitability for culture in confinement along with tolerance to a wide range of environmental conditions (Chandrasekar *et al.*, 2014). *E. suratensis* is designated as the 'State fish of Kerala', with backwaters of Kerala being the major source of the wild population and a potential source of its seed (Padmakumar *et al.*, 2012). Wild populations of *E. suratensis* are facing habitat deterioration due to increasing urbanisation, tourism activities in backwaters/estuaries and threats from proliferation of exotic species like *Oreochromis mossambicus* and *Trichogaster trichopterus* (Padmakumar *et al.*, 2002; Kumar *et al.*, 2009). Attempts have been made for captive breeding aimed at conservation along with the creation of aquatic sanctuaries/no fishing zones, within some of the larger estuaries (Padmakumar *et al.*, 2012).

Comparative mitogenomic information has revolutionised several concepts of molecular phylogeny and evolution across multiple taxonomic levels (Miya and Nishida, 2015). Genetic information coupled with biological and behavioural data is crucial for the conservation and management of endangered species. Mitochondrial Oxidative Phosphorylation System (OXPHOS complex) has been indicated as important for selection and adaptation to different environmental regimes in marine fishes (Garvin *et al.*, 2012; Caballero *et al.*, 2015). *E. suratensis* is distributed widely across environmental clines and hence identifying the signals of positive and diversifying selection in OXPHOS machinery of the mitogenome will provide clues regarding their vulnerability to environmental alterations. Considering all these, we characterised the complete mitochondrial genome structure and organisation of *E. suratensis* collected from Vembanad Lake, Kerala followed by phylogenetic analysis using complete mitogenome.

The complete mitogenome of *E. suratensis* collected from Chilka Lake, Odisha, India has already been characterised by Mohanta *et al.* (2016). However, detailed analysis on structure, organisation, amino acid content and codon usage have not been reported. In the present investigation, we have conducted an extensive investigation on mitogenome content, structure and phylogenetic position of *E. suratensis*. The phylogenetic analysis included all

Table 2. Features of the mitogenomes of *E. suratensis*

Gene	Position		Strand ^a	Codon ^b	
	From (bp)	To (bp)		Start	Stop
tRNA-Phe	1	69	H		
12S rRNA	70	1017	H		
tRNA-Val	1018	1089	H		
16S rRNA	1090	2780	H		
tRNA-Leu	2781	2853	H		
ND1	2854	3828	H	ATG	TAA
tRNA-Ile	3832	3901	H		
tRNA-Gln	3901	3971	L		
tRNA-Met	3971	4039	H		
ND2	4040	5086	H	ATG	TAA
tRNA-Trp	5087	5157	H		
tRNA-Ala	5159	5227	L		
tRNA-Asn	5229	5301	L		
tRNA-Cys	5339	5405	L		
tRNA-Tyr	5406	5475	L		
CO1	5477	7033	H	GTG	TAA
tRNA-Ser	7050	7120	L		
tRNA-Asp	7124	7195	H		
CO2	7201	7891	H	ATG	T--
tRNA-Lys	7892	7966	H		
ATPase 8	7968	8135	H	ATG	TAA
ATPase 6	8126	8808	H	ATG	TA-
CO3	8809	9593	H	ATG	TA-
tRNA-Gly	9594	9663	H		
ND3	9664	10013	H	ATG	TA-
tRNA-Arg	10014	10081	H		
ND4L	10082	10378	H	ATG	TAA
ND4	10372	11752	H	ATG	T--
tRNA-His	11753	11821	H		
tRNA-Ser	11822	11888	H		
tRNA-Leu	11892	11964	H		
ND5	11965	13803	H	ATG	TAA
ND6	13801	14321	L	ATG	TA-
tRNA-Glu	14322	14390	L		
Cyt b	14395	15488	H	ATG	TA-
tRNA-Thr	15535	15606	H		
tRNA-Pro	15608	15677	L		
control region (D-loop)	15678	16456			

^a H and L, respectively, denote heavy and light strands; ^b Codons containing "--" symbols indicate an incomplete stop codon.

The origin of light strand replication (OL) in *E. suratensis* was located between tRNA Asn and tRNA Cys (WANCY region) and it is from 5303 bp to 5338 bp. WANCY region is a region coding for five mitochondrial tRNAs (tryptophan, alanine, asparagine, cysteine and tyrosine). OL sequence has the potential of forming a stable stem-loop structure in its single-stranded form, which is needed for the initiation of replication (Hixson *et al.*, 1986). A major non-coding region, control region (D-loop) located between the tRNA Pro and tRNA Phe genes (779 bp in size) has several characteristic conserved sequence blocks (CSB) like CSB1, CSB2, CSB3 and promoter region (Fig. 2).

In the phylogenetic tree, *E. suratensis* clustered with cichlids present in Indian and Sri Lankan waters along with one species from Madagascar group (*Paretroplus maculatus*). They formed sister group to all other cichlids (Fig. 3). In the family Cichlidae, species from South America and Africa are monophyletic in origin

Table 3. Nucleotide composition of the mitogenome of *E. suratensis*

% Nucleotide composition (GC 46.2)			
A	C	G	T
Complete mitogenome (H- Strand)			
28.2	30.9	15.3	25.6
All protein coding gene concatenated (H- Strand) ^a			
26.0	32.9	13.7	27.4
ND 6 (L- Strand) ^b			
39.7	38.3	9.6	12.3
1 st codon position ^c			
26.4	28.1	24.7	20.8
2 nd codon position ^c			
17.9	28.1	13.5	40.5
3 rd codon position ^c			
31.9	39.3	6.3	22.5

^a Based on the 12 protein-coding genes located on the H-strand; ^b Based on the ND 6 gene located on the L-strand; ^c Based on the 13 protein-coding genes.

Table 4. Amino acid and codon usage in mitogenome of *E. suratensis*

Amino acid	% ^a	Codons	RCSUC ^b	Amino acid	% ^a	Codons	RCSUC ^b		
Alanine (Ala/A)	8.6	GCU	55	Leucine (Leu/L)	17.6	UUA*	74		
		GCC	146			UUG	32		
		GCA*	110			CUU	168		
		GCG	14			CUC	179		
Arginine (Arg/R)	2.0	CGU	11	Lysine (Lys/K)	1.9	AAA*	71		
		CGC	14			AAG	4		
		CGA*	45			Methionine (Met/M)	3.9	AUA	104
		CGG	4					AUG*	42
Asparagine (Asn/N)	3.0	AAU	29	Phenylalanine (Phe/F)	6.3	UUU	101		
		AAC*	88			UUC*	137		
Aspartic acid (Asp/D)	1.8	GAU	18	Proline (Pro/P)	5.8	CCU*	51		
		GAC*	53			CCC	118		
Cysteine (Cys/C)	0.6	UGU	6			CCA	49		
		UGC*	16			CCG	5		
Glutamic acid (Glu/E)	2.6	GAA*	84	Serine (Ser/S)	6.6	UCU	46		
		GAG	14			UCC	97		
Glutamine (Gln/Q)	2.5	CAA*	90			UCA*	47		
		CAG	7			UCG	5		
Glycine (Gly/G)	6.6	GGU	39	Threonine (Thr/T)	4.1	ACU	4		
		GGC	94			ACC	153		
		GGA*	72			ACA*	116		
		GGG	38			ACG	8		
Histidine (His/H)	2.8	CAU	37	Tryptophan (Trp/W)	3.1	UGA*	107		
		CAC*	74			UGG	11		
Isoleucine (Ile/I)	7.2	AUU	137	Tyrosine (Tyr/Y)	3.0	UAU	34		
		AUC*	148			UAC*	75		
						Valine (Val/V)	5.8	GUU	63
				GUC	54				
				GUA*	61				
				GUG	21				

^aPercentage of amino acid based on the 13 protein-coding genes; ^bRSCU relative synonymous codon usage; *Codons complementary to the tRNA genes.

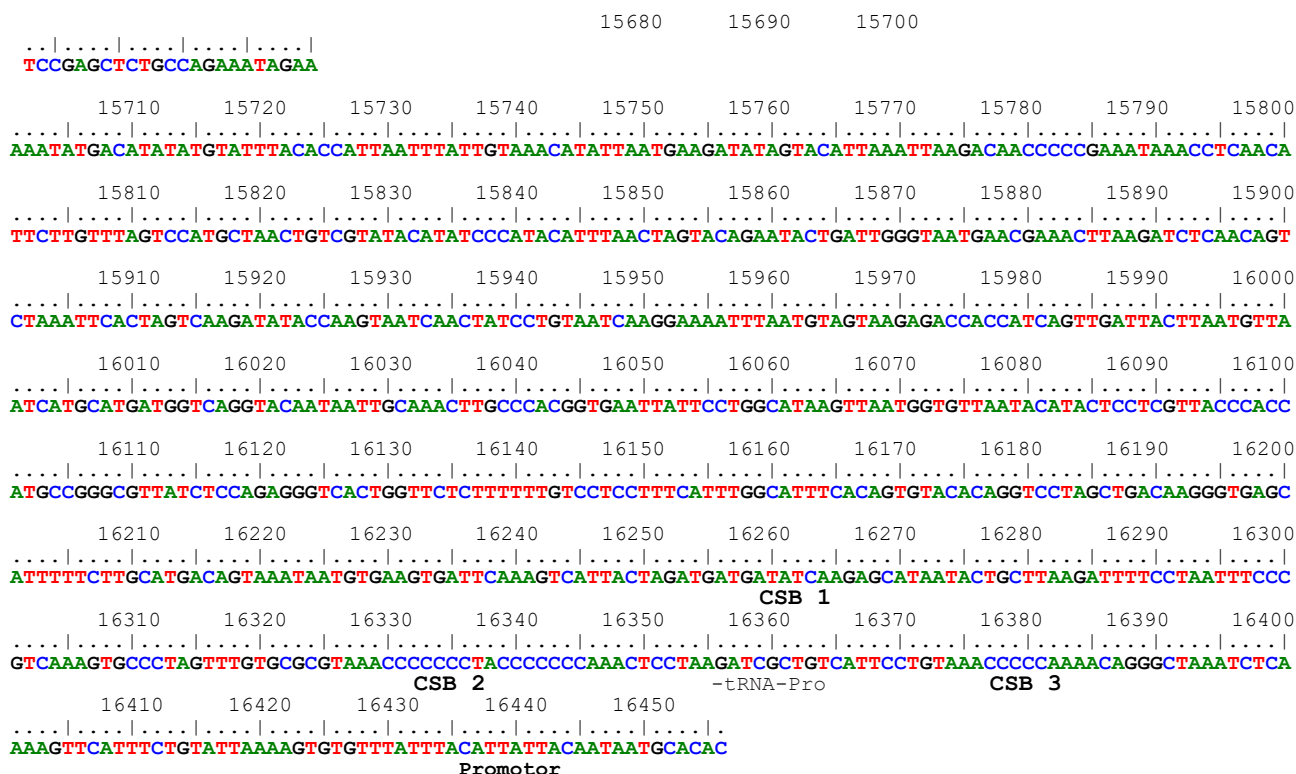


Fig. 2. Characteristic conserved blocks (CSB 1, CSB2, CSB3) and promoter region in the non-coding region (D-Loop) of *E. suratensis* mitochondrial DNA

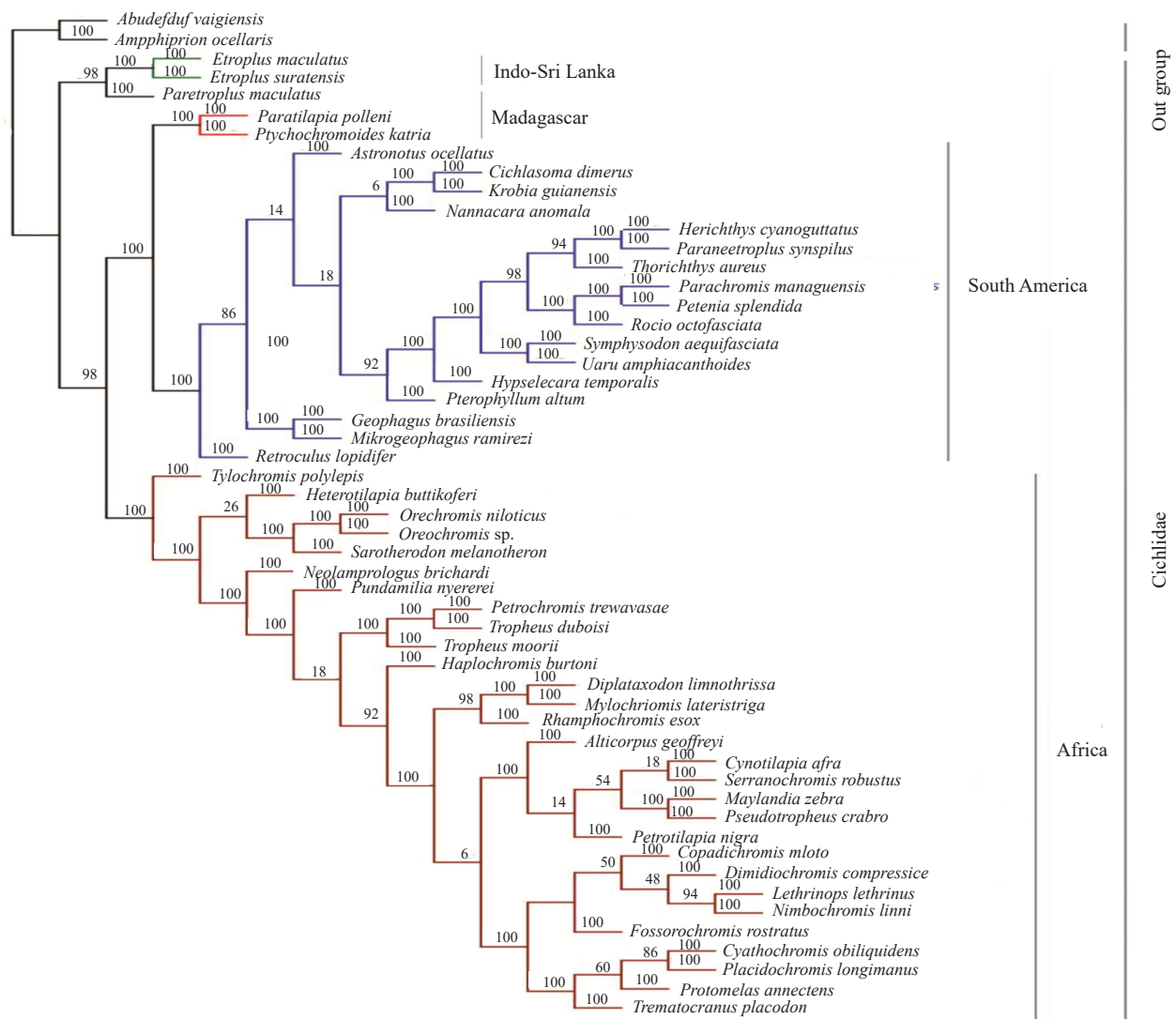


Fig. 3. Neighbor-joining phylogenetic tree generated by alignment of complete mitogenome nucleotide sequences of *E. suratensis* and other cichlids. Cichlid species which are represented from Africa, South America, Madagascar and Indo-Sri Lankan regions were used. *Amphiprion ocellaris* and *Abudehdud vaigiensis* were used as outgroups.

whereas Madagascar and Indo-Sri Lankan groups are not monophyletic. The tree also supported the proposed Gondwanan origin of Cichlidae as the divergence pattern of cichlids belonging to each continent was associated with the geological history of continental drift (Azuma *et al.*, 2008). Results of the complete mitogenome phylogeny of this study also strongly supported early diversification events within Cichlidae as well as Gondwanan origin of cichlid lineages as reported by Sparks and Smith (2004) with mitochondrial and nuclear gene fragments.

The mitogenomic information of *E. suratensis* generated in the present study will provide momentum to further studies on evolution, taxonomy, conservation, environmental adaptation and selective breeding of the species.

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