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), 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, Complete mitochondrial genome, *Etroplus suratensis*, Phylogenetic status

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
the available complete mitogenomes of cichlids to make observations on their divergence.

Genomic DNA was isolated by standard phenol/chloroform method (Sambrook and Russell, 2001). The entire mitogenome was amplified using a long PCR technique with Q5® High-Fidelity DNA polymerase. Primer pairs (Table 1) were designed on the basis of known regions of the *E. suratensis* mtDNA and complete mitogenome was amplified as 5 contiguous, overlapping segments and sequenced with both primers using the BigDye Terminator Sequencing Ready Reaction v3.0 kit (Applied Biosystems). The internal region of large fragments was obtained by sequencing of the PCR products with an internal primer designed from the corresponding sequence obtained in the first sequencing process. The sequence fragments obtained were assembled using Geneious R7 (Kearse et al., 2012), annotated with NCBI-BLAST (National Centre for Biotechnology Information-The Basic Local Alignment Search Tool) and MitoAnnotator (Iwasaki et al., 2013) and deposited in NCBI GenBank (Accession no. KU665487). The phylogenetic status and nucleotide composition of mitogenome were assessed with MEGA 6 (Tamura et al., 2013).

The mitogenome sequence obtained is a 16456 bp circle with 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 (Fig. 1, Table 2). H-strand was the major coding strand but ND6 and eight tRNA genes were encoded on the L-strand. The gene order, gene length as well as heavy (H) and light (L) strand coding pattern are identical to that in other vertebrates (Boore, 1999). The overall base composition of the H-strand was as follows: A (28.2%), T (25.6%), C (30.9%), G (15.3%) and G+C (46.2%). Similar to other vertebrates, low G content and high A+T (53.8%) content were observed in the genome (Table 3).

The 13 protein-coding genes altogether come around 11358 bp. Intergenic overlaps at ATP6 and ATP8 (10 nucleotides), ND4 and ND4L (7 nucleotides) and ND5 and ND6 (4 nucleotides) were observed in their overlapping region which is common within vertebrate mitogenomes and have been reported for several fish species (Boore, 1999; Mu et al., 2015). ATG is used as start codon by all coding genes except CO1 (GTG is the start codon) and TAA was used as stop codon translation terminators for ND1, ND2, CO1, ATP8, ND4L and ND5. The remaining genes used incomplete stop codon TA/T-- (Table 1) and post-transcriptional polyadenylation compensate adenosine nucleotide required for generating the stop codon (TAA) (Ojala et al., 1981). The most frequently used amino acids were leucine (17.6%), followed by alanine (8.6%) and isoleucine (7.2%). The highest estimated RSCU were matched to corresponding tRNAs identified in the mitogenome, with the exception of alanine, glycine, leucine, methionine, proline, serine, threonine and valine (Table 4). In the third codon positions, codons complementary to the tRNAs ending in A and C were the most frequently observed and G nucleotide was the least frequent.

Similar to other vertebrates, *E. suratensis* rRNA genes have high adenine content (52.2%) (Boore, 1999) and 3 of the 22 tRNA genes identified showed overlaps.

![Mitogenome map of E. suratensis (16456 bp) (Gen Bank Accession no. KU665487) generated with MitoAnnotator. Protein-coding genes, rRNAs, tRNAs and D-loop regions are shown in different colours. Genes located within the outer circle are coded on the H-strand whereas the remaining genes are coded on the L-strand.](image-url)
Table 2. Features of the mitogenomes of *E. suratensis*

| Gene     | From (bp) | To (bp) | Strand | Codon |
|----------|-----------|---------|--------|-------|
| 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   |
| tRNA-Ile | 3832      | 3901    | H      |       |
| tRNA-Met | 3901      | 3971    | L      |       |
| ND2      | 3971      | 4039    | H      |       |
| tRNA-Trp | 4040      | 5086    | H      | ATG   |
| 16S rRNA | 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   |
| tRNA-Ser | 7050      | 7120    | L      |       |
| tRNA-Asp | 7124      | 7195    | H      |       |
| CO2      | 7201      | 7891    | H      | ATG   |
| tRNA-Lys | 7892      | 7966    | H      |       |
| ATPase 8 | 7968      | 8135    | H      | ATG   |
| ATPase 6 | 8126      | 8808    | H      | ATG   |
| CO3      | 8809      | 9593    | H      | ATG   |
| tRNA-Gly | 9594      | 9663    | H      |       |
| ND3      | 9664      | 10013   | H      | ATG   |
| tRNA-Arg | 10014     | 10081   | H      |       |
| ND4L     | 10082     | 10378   | H      | ATG   |
| ND4      | 10372     | 11752   | H      | ATG   |
| tRNA-His | 11753     | 11821   | H      |       |
| tRNA-Ser | 11822     | 11888   | H      |       |
| tRNA-Leu | 11892     | 11964   | H      |       |
| ND5      | 11965     | 13803   | H      | ATG   |
| ND6      | 13801     | 14321   | L      | ATG   |
| tRNA-Glu | 14322     | 14390   | L      |       |
| Cyt b    | 14395     | 15488   | H      | ATG   |
| tRNA-Thr | 15535     | 15606   | H      |       |
| tRNA-Pro | 15608     | 15677   | L      |       |

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 |
| ND 6 (L- Strand)*                 | 26.0 | 32.9 | 13.7 | 27.4 |

1st codon position

|                  | 26.4 | 28.1 | 24.7 | 20.8 |
|------------------|------|------|------|------|

2nd codon position

|                  | 17.9 | 28.1 | 13.5 | 40.5 |
|------------------|------|------|------|------|

3rd codon position

|                  | 31.9 | 39.3 | 6.3  | 22.5 |

*Based on the 12 protein-coding genes located on the H-strand; *Based on the ND 6 gene located on the L-strand; *Based on the 13 protein-coding genes.
Table 4. Amino acid and codon usage in mitogenome of *E. suratensis*

| Amino acid | % | Codons | RCSUC |
|------------|---|--------|-------|
| Alanine (Ala/A) | 8.6 | GCU | 55 |
| Arginine (Arg/R) | 2.0 | CGU | 11 |
| Asparagine (Asn/N) | 3.0 | AUA | 110 |
| Aspartic acid (Asp/D) | 1.8 | GAU | 18 |
| Cysteine (Cys/C) | 0.6 | UGU | 6 |
| Glutamic acid (Glu/E) | 2.6 | GAA | 84 |
| Glycine (Gly/G) | 6.6 | GGU | 39 |
| Histidine (His/H) | 2.8 | CAU | 37 |
| Isoleucine (Ile/I) | 7.2 | AUU | 137 |
| Leucine (Leu/L) | 17.6 | UUA | 74 |
| Lysine (Lys/K) | 1.9 | AAA | 71 |
| Methionine (Met/M) | 3.9 | AUG | 104 |
| Phenylalanine (Phe/F) | 6.3 | UUA | 101 |
| Proline (Pro/P) | 5.8 | CCC | 51 |
| Serine (Ser/S) | 6.6 | GCU | 46 |
| Threonine (Thr/T) | 4.1 | ACC | 15 |
| Tryptophan (Trp/W) | 3.1 | UGA | 107 |
| Tyrosine (Tyr/Y) | 3.0 | UAU | 34 |
| Valine (Val/V) | 5.8 | GGU | 63 |

*Percentage of amino acid based on the 13 protein-coding genes; ¹RSCU relative synonymous codon usage; ²Codons complementary to the tRNA genes.*

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

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