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MITOGENOME ANNOUNCEMENT

The mitochondrial genome of an endangered native Singidia tilapia, *Oreochromis esculentus*: genome organization and control region polymorphism

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Abstract

Singidia tilapia (*Oreochromis esculentus*) is a native Cichlid fish of important commercial value, distributed in Lake Victoria, East Africa. Due to its declining population levels in its natural habitat, this species has now been classified as a Critically Endangered by the International Union for the Conservation of nature (IUCN). In the present study the complete nucleotide sequence of the mitochondrial genome (mtDNA) of *O. esculentus* was determined. In addition, polymorphism analysis based on the mtDNA's control region sequence was investigated on two of its remaining populations of Yala and Borabu as well as a phylogenetic consideration using 16S rRNA mtDNA genes to explore its position and relationship within Cichlidae fish. The length of the complete mitogenome of *O. esculentus* is 16 622 bp, containing the same order and an identical number of genes and regions with the other reported Cichlid fishes, which consists of 13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and a putative non-coding region. The phylogenetic analysis shows *O. esculentus* being clustered within the Oreochromini sub-tribe of the Cichlidae. The high genetic diversity and low genetic differentiation between the two populations indicated the need for conservation of both the refuge ecosystems and the fish species under study.

Keywords

Complete mitochondrial DNA, genome structure, *Oreochromis esculentus*, polymorphism

History

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Mitochondrial DNA (mtDNA) has been extensively used as a molecular marker in ecology and evolutionary biology (Buburuzan et al., 2007), and population genetics (Abila et al., 2004). Singidia tilapia (*Oreochromis esculentus*) is an important edible cichlid fish endemic to Eastern Africa's Lake Victoria basin and its satellite lakes and dams. Due to introduction of exotic fish species, environmental degradation and other anthropogenic influences (Ogutu-Ohwayo, 1990), this highly valued fish species has greatly declined in Lakes Victoria and Kyoga, and has now been classified as Critically Endangered species (IUCN, 2014). Most studies on this fish have focused on morphology, ecology, nutrition and conservation (Abila et al., 2004). In the current study, we present the first complete mitogenome sequence of *O. esculentus*, and genetic structure of its remaining two populations through analyzing mtDNA control region polymorphism.

The determined complete mitochondrial genome of *O. esculentus* was 16 622 bp long (NC_025555.1). It contains two ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA genes, 13 protein-coding genes (Cytb, ATP6, ATP8, COXI-III, ND1-6, ND4L), and a putative control region. This

range is close to those of other reported complete cichlid mitogenomes with only a few nucleotide base length variations (He et al., 2011). The overall base composition of the mitochondrial genome was A = 27.7%, T = 25.9%, C = 30.7% and G = 15.7%, and an A + T content (53.6%). Most of the identified 22 tRNA genes were located on the L-strand (tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA^{Ser}, tRNA^{Glu} and tRNA^{Pro}), while the rest on the H-strand. Majority of protein-coding genes had ATG as start codon, with the exception of COXI, which had GTG as the start codon. The mitogenome had one TAG (ND1), and six TAA (COX1, ATPase8, ATPase6, ND4L, ND5, and ND6) stop codons, while the remaining six protein-coding genes had incomplete termination codons, TA– (ND2) and T— (COXII, COXII, ND3, ND4, Cytb). Most of these genes were encoded on the H-strand, except the ND6 gene, which is consistent with bony fishes (He et al., 2011).

The constructed phylogenetic tree based on 16S rRNA gene sequence showed that *O. esculentus* is clustered within sub-tribe Oreochromini of Cichlidae (Figure 1), using maximum likelihood (ML) method in MEGA v5 (Tamura et al., 2011). The result is similar to the early report (Dunz & Schlieven, 2013).

In understanding the genetic diversity of *O. esculentus*, mtDNA control regions of Yala Swamp ($n=31$) and Borabu Dam ($n=38$) populations were sequenced. The number of haplotypes observed from Yala Swamp and Borabu Dam populations were 15, and 21, respectively. These haplotypes were

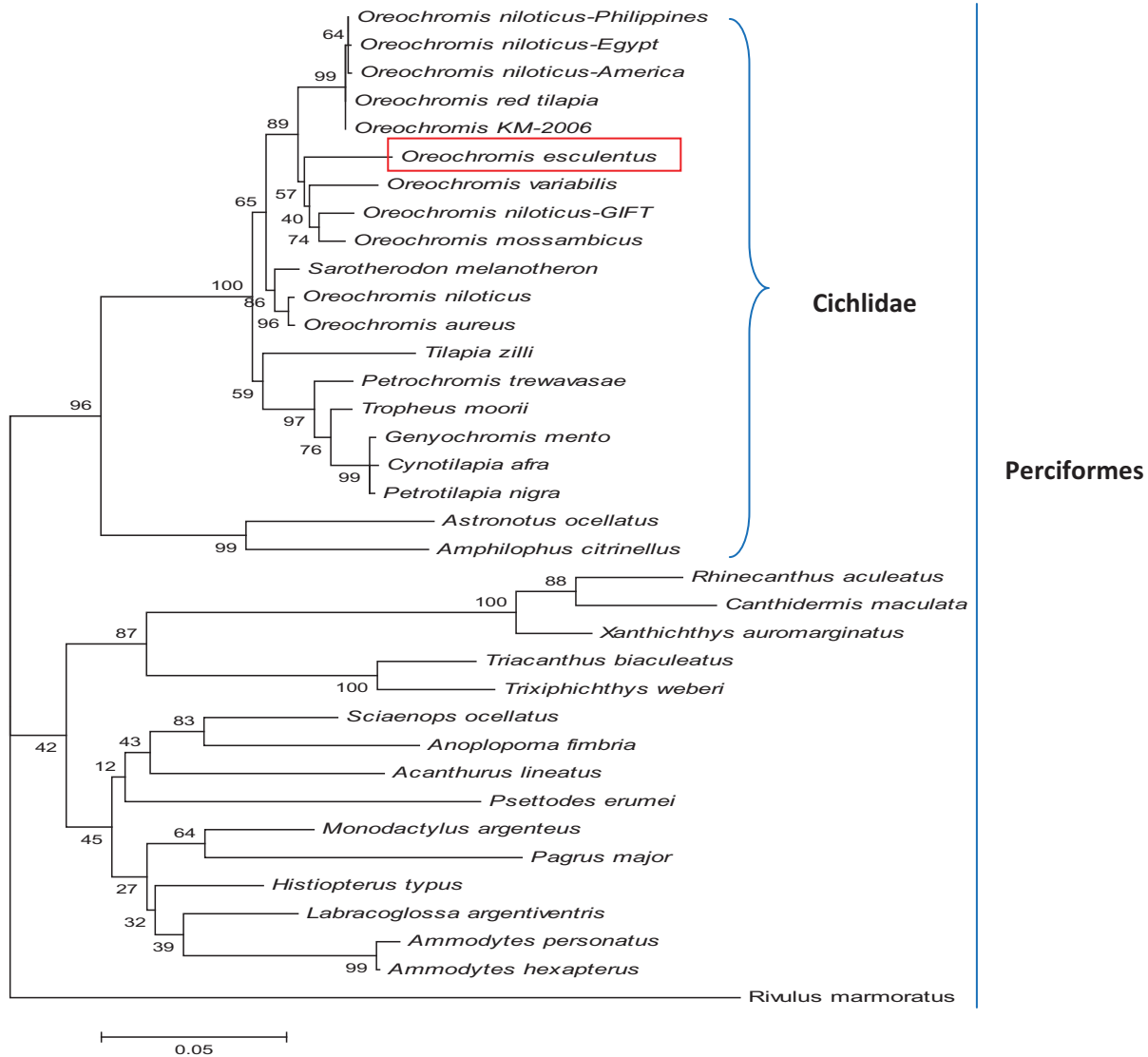


Figure 1. A maximum likelihood (ML) tree constructed by MEGA v5 (Tamura et al., 2011), based on complete 16S rRNA gene sequences retrieved from GenBank database, showing the evolutionary position and relationships of *Oreochromis esculentus* relative to other 35 fish species belonging to order Perciformes. The scale bar is shown under the tree, and the bootstrap value of 1000 replicates is listed on the branches. The analyses included the following species: *Rhinecanthus aculeatus* (NC_011941.1), *Triacanthus biaculeatus* (NC_009863.1), *Psettodes erumei* (NC_020032.1), *Canthidermis maculata* (AP009206.1), *Xanthichthys auromarginatus* (AP009211.1), *Trixiphichthys weberi* (AP009173.1), *Oreochromis niloticus* (NC_013663.1), *Oreochromis niloticus-America* (GU477628.1), *Oreochromis aureus* (NC_013750.1), *Coptodon (tilapia) zilli* (NC_026110.1), *Sarotherodon melanotheron* (NC_015611.1), *Oreochromis sp. 'red tilapia'* (GU477631.1), *Oreochromis sp. KM-2006* (AP009126.1), *Oreochromis niloticus-Philippines* (GU477626.1), *Oreochromis niloticus-Egypt* (GU477625.1), *Oreochromis niloticus-Gift* (GU477624.1), *Oreochromis mossambicus* (NC_007231.1), *Petrochromis trewavasae* (HE961974.1), *Tropheus moorii* (HE961975.1), *Cynotilapia afra* (NC_018564.1), *Oreochromis esculentus* (NC_025555.1), *Genyochromis mento* (NC_018562.1), *Petrotilapia nigra* (NC_018557.1), *Oreochromis variabilis* (NC_026109.1), *Astronotus ocellatus* (AP009127.1), *Amphilophus citrinellus* (KJ562277.1), *Sciaenops ocellatus* (NC_016867.1), *Anoplopoma fimbria* (NC_018119.1), *Acanthurus lineatus* (NC_010108.1), *Pagrus major* (NC_003196.1), *Monodactylus argenteus* (NC_009858.1), *Histioporus typus* (AP006807.1), *Labracoglossa argentiventris* (AP011062.1), *Ammodytes personatus* (KF672362.1), *Ammodytes hexapterus* (NC_020461.1) and *Rivulus marmoratus* (AF283503.1) which was also used as the outgroup taxon.

generally clustered according to their geographical origin. Haplotype diversity and nucleotide diversity were 0.88889 and 0.00768 in Borabu, and 0.86040 and 0.00624 in Yala, respectively, revealing high genetic diversity within two populations. *F_{st}* value was 0.06681, indicating a low level of genetic differentiation between the two populations. Therefore, refuge habitats and genetic diversity therein should be given high priority in management and conservation programs.

Declaration of interest

The authors declare no conflicts of interest. This work was sponsored by K. C. Wong Magna Fund in Ningbo University.

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