E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2022; 10(3): 186-189

www.fisheriesjournal.com
Received: 02-03-2022
Accepted: 05-04-2022

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Phylogenetic position of the Atlantic Mudskipper (*Periophthalmus barbarus*) (Linnaeus, 1766) (Perciformes: Gobiidae): the congruence of the complete mitogenome and the CO1 gene region

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DOI: https://doi.org/10.22271/fish.2022.v10.i3c.2721

Abstract
Mitogenomics is an emerging new area of molecular systematics that has helped resolve controversies in the classification of species that have problematic resolution of species. In our study, we have sequenced the complete mitochondrial genome of the Atlantic mudskipper (*Periophthalmus barbarus*). As it is typical for almost all higher organisms, it has a circular genome which is 16,502 bp long consisting of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and one control region. Phylogenetic analyses strongly support the monophyly of *Periophthalmus* as well as the placement of *P. barbarus* as the earliest diverging lineage within the clade. The resolution of evolutionary history and population genetics of P. barbarus will be better understood within the subfamily Oxudercinae.

Keywords: Mudskipper, mitochondrial genome, fish, *Periophthalmus barbarus*

Introduction
The Nigerian Atlantic Mudskipper (*Periophthalmus barbarus*) (Linnaeus, 1766) (Family: Gobiidae, Subfamily: Oxudercinae) is an amphibious and euryhaline fish that occurs along the tropical Atlantic coasts of Africa including most offshore islands. It is small-sized and important as an indicator for environmental pollution especially in mudflat ecosystems. This study aims at exploring its complete mitochondrial genome (the seventh representative from the 19 species of genus *Periophthalmus* described to date) and the phylogenetic relationships of the mudskippers from genus *Periophthalmus*. A specimen of *P. barbarus* was collected from Abonnema, Nigeria (4.73075, 6.77565) and the specimen was archived at Zoological Museum, Department of Zoology and Environmental Biology, Faculty of Science, Lagos State University, Ojo in Lagos, Nigeria (Olusola Sokefun, olusola.sokefun@lasu.edu.ng) under voucher specimen ZEB/PB/RS 001 and catalogue number PMS/021. Ethical issues did not apply in this research as no further experimentation was performed on the animals. *Periophthalmus barbarus* is commonly consumed as animal protein sources in Nigeria and there has been no concern about its current population status by the IUCN Red List status, thus ethical approval was exempted by the University Ethics Committee, Lagos State University, Nigeria.

Approximately 50 mg of the ethanol-preserved fin sample were homogenized in 500 uL of lysis buffer and extracted using the salting-out method. The DNA was treated with 1 uL of RNAse (10 mg/mL) followed by purification using magnetic bead-bead approach (Oberacker *et al*. 2019) [10]. The purified DNA was measured using Denovix high sensitivity kit giving a concentration of 54.2 ng/uL. Approximately 100 ng of DNA was fragmented to 350 bp using a Bioruptor followed by NEB Ultra II library preparation (NEB, Ipswich, MA) following the instruction of the manufacturer's. Sequencing was performed on an Illumina Nova SEQ 6000 (Illumina, San Diego, CA) using a run configuration of 2 x 150 bp to generate approximately 1 gb of data. Raw reads were trimmed with fastp v0.21 (Chen *et al*. 2018) [4] to remove low-quality bases and Illumina adapter sequences. The trimmed reads were subsequently used for de novo assembly in Mega HIT (default setting) (Li *et al*. 2015) [7].

The mitochondrial-derived contig was identified and circularized with MitoZ (Meng *et al*. 2019) [8]. Orientation and annotation of the circular contig was performed with Mito Annotator (http://mitofish.aori.u-tokyo.ac.jp/annotation/input.html) (Iwasaki *et al*. 2013) [6].

Whole mitogenomes of mudskippers available on the GenBank for close and related to *P. barbarus* (*P. argentilineatus*, *P. magnuspinnatus*, *P. minutus*, *P. modestus*, *P. novemradiatus*, *Periophthalmodon schlosseri*, *Scartelaos histophorus*, *Boleophthalmus boddarti* and *B. pectinirostris*) were accessed and used to infer their evolutionary relationships. The 13 protein-coding genes were extracted from the GenBank files and aligned based on codon position with TranslatorX (Abascal *et al*. 2010) [1]. The 16S and 12S rRNA genes were similarly extracted and aligned with MUSCLE (Edgar 2004) [5]. Together, this generated a total of 41 nucleotide alignments (13 protein coding genes x 3 codon positions + 2 rRNA). Individual alignment was edited with trimAL (Capella-Gutiérrez *et al*. 2009) [3] using the "-automated1" option and subsequently concatenated forming partitioned super-alignment. The best-fit partitioning scheme was calculated using Model Finder in iqtree2 (Minh *et al* 2020) [9]. Subsequently, a maximum likelihood tree was constructed from the best-fit partitioned super alignment using iqtree2 with 1,000 ultrafast bootstraps (Minh *et al*. 2020) [9].

Previously, authors like Yi *et al*. 2016; Qiu *et al*. 2017; Tan *et al*. 2020 [14, 11, 13] had similarly reported a circular genome is 16,502 bp long and consisting of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and one control region for related species of mudskippers. The final nucleotide alignment shows a monophyletic clustering of the genus *Periophthalmus* (Figure 1). Using maximum-likelihood tree reconstruction, a strong support (Bootstrap support (BSS) = 97) for a clade consisting of all *Periophthalmus* species that is sister to *Periophthalmodon* (BSS = 100 was observed. One major but unexpected finding is the placement phylogenetically of *Scartelaos histophorus* (accession number JQ654459) within the *Periophthalmus* clade. Our suspicion of a case of species misidentification is high because of the monophyletic clustering with members of *Periophthalmus*, and a high sequence similarity (>99%) with *P. magnuspinnatus* (GenBank accession numbers KT284931, KT357639). Besides, the COI sequence of *S. histophorus* (accession number JQ654459) showed 85.38% similarity with other *S. histophorus* (AF391346). The specimen is likely to be *P. magnuspinnatus* and sample re-validation is herein recommended. (Tan *et al*. 2020) [13]. for this, it is a critical importance for a voucher deposition to prevent taxonomic errors and ensure reproducibility and legality in genetic studies (Buckner *et al*. 2021) [2]. At the whole mitogenome level, our sample of *P. barbarus* is resolved as the earliest diverging lineage of *Periophthalmus*, possibly indicating a deep evolutionary divergence between this Atlantic species from all other Indo-Pacific species of this clade. Future study should focus on sequencing of more representatives from the genus *Periophthalmus* for a better understanding on its phylogenetic relationships as well as to provide a reliable sequence reference for species identification.

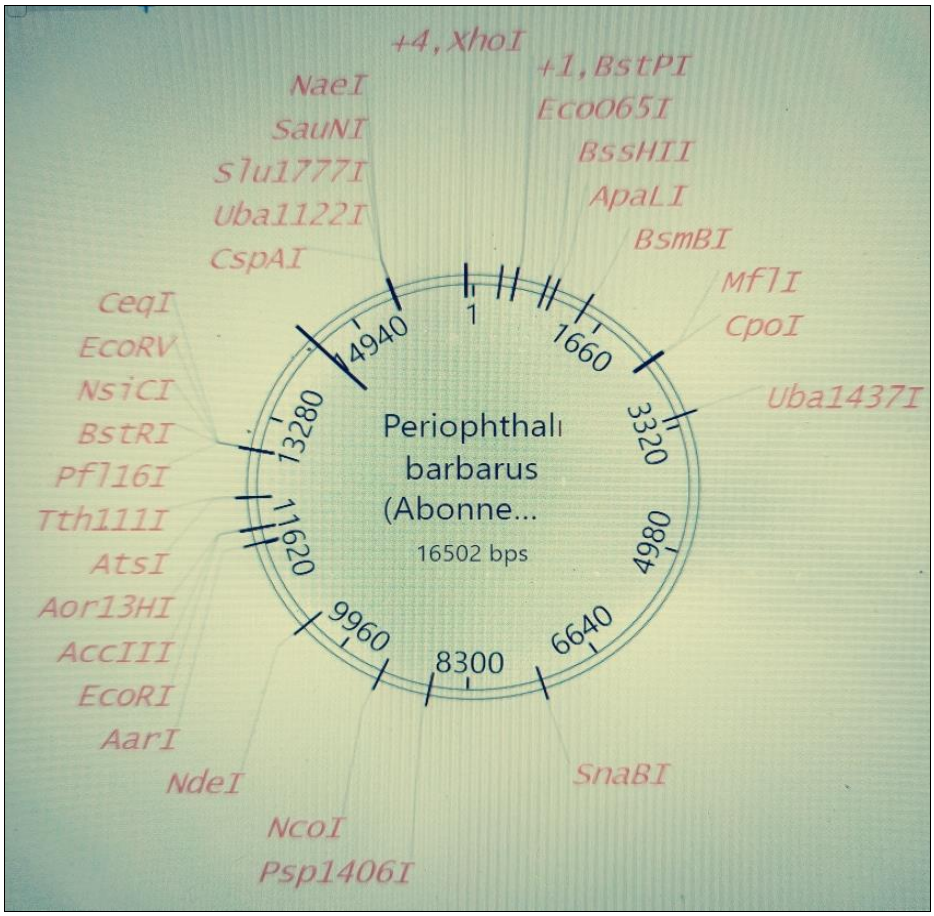

Fig 1: The circular map of the complete mitogenome of *Periophthalmus barbarus*

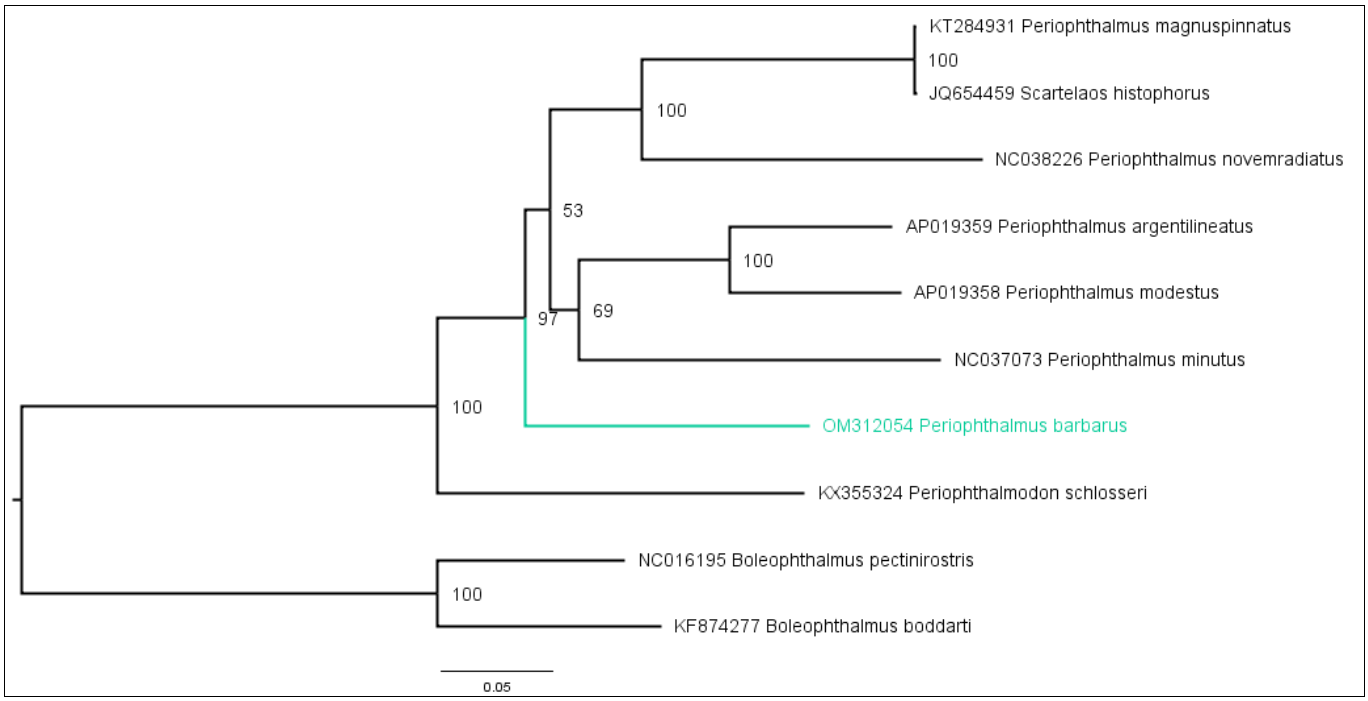

Fig 2: The goby phylogeny based on whole mitogenome using the maximum likelihood criteria. Values at the nodal points represent ultrafast bootstrap support values using 1,000 bootstraps. Branch lengths is equivalent to the mean number of nucleotide substitutions per site.

Acknowledgements
We thank members of our research teams who for reasons on privacy have requested that we do not mention their names.

Disclosure statement
The authors report no conflict of interest and are responsible for the content and writing of the manuscript.

Data availability statement
The data that support the findings of this study is openly accessible at the Zenodo platform at https://doi.org/10.5281/zenodo.5875413 (Sokefun *et al*. 2022) [12]. The data is also deposited at the US National Center for Biotechnology Information (NCBI) database at https://www.ncbi.nlm.nih.gov/. The GenBank accession number is OM312054. The associated BioProject, SRA and Bio-Sample numbers are PRJNA804323, SRX14089601 and SAMN25721908, respectively.

Funding
We funded the research ourselves.

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