
# Molecular Phylogenetic Analyses of Two Ray-Finned Fishes *Acanthurus mata* (Cuvier, 1829) and *Ephippus orbis* (Bloch, 1787) From the Odisha Coast, Bay of Bengal, India

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## Abstract

Two fish species *Acanthurus mata* and *Ephippus orbis* of the order Acanthuriformes were collected from Gopalpur-on-sea, Bay of Bengal, where they were recorded for the first time. Both the fishes were identified using the conventional taxonomic methods followed by the molecular taxonomic method of DNA barcoding using the mitochondrial cytochrome c oxidase subunit I (COI) gene. All COI barcodes produced in this investigation were matched with reference sequences of anticipated species by morphological identification. Based on DNA barcodes, neighbour-joining, and maximum likelihood phylogenetic trees were constructed, and all of the specimens found were clustered according to their species-level taxonomic categorisation. To derive haplotype diversity, a median-joining network was built. The current study additionally looked at the number of variable sites, parsimony informative sites, nucleotide diversity, and variation in amino acids. Significant genetic variations were detected among *A. mata* species from various geographic regions. However, *E. orbis* still has highly conserved natural populations with no significant variations. The findings of this work provide information on the phylogeny, population dynamics, divergence time, and genetic diversity of both fish species as well as substantial validation for the use of DNA barcode sequences for tracking species diversity.

**Keywords:** Gopalpur-on-sea, DNA barcoding, genetic diversity, molecular dating

## Introduction

Fishes have a key role in the generation of income, protein, and dietary supplements for human use (Ugwumba and Ugwumba, 2003; Ward et al., 2005; Rasmussen et al., 2009). Fish have also drawn a lot of interest because of their ecological and economic value, particularly model fish used in scientific study and economic fish in the aquaculture sector (Wang et al., 2024). Biological invasion of non-native fish species into various habitats particularly marine ecosystems has increased significantly during the latter half of the 20th century and become a global problem due to the expansion of world trade, which was accompanied by climate changes, habitat modification, and the development of new trading routes (Vitousek et al., 1996; Hulme, 2009; Havel et al., 2015). The Indian peninsula is surrounded by the Indian Ocean to the south, the Arabian Sea to the west, and the Bay of Bengal to the east. Because it is primarily tropical, this region boasts a broad range of flora, making it one of the world's megadiverse countries (Raghunathan et al., 2012). The Bay of Bengal, a distinct marine ecological system is the biggest triangular basin in the Indian Ocean which has experienced the constant introduction of several marine invasive species, many of which have already developed into local populations (Barua et al., 2001; Barik et al., 2021).

Odisha is one of the coastal states, which is situated on the western bank of the Bay of Bengal on India's east coast with 480 km long coastline (Mohanty et al., 2008). The checklists of marine fish species are available in the marine and estuarine fish fauna of Orissa (Barman et al., 2007) and marine fauna of Odisha, east coast of India (Pati et al., 2018). According

to several studies conducted by Molnar et al. (2008), Vilà et al. (2010), and Katsanevakis et al. (2014), the invasion of non-native species may have detrimental effects on the marine ecosystem, including the displacement of native species that results in the loss of native genotypes, modification of habitats, alteration in community structure, disturbances in food-web properties, and significant economic loss. Many marine fish have reportedly been introduced into the Bay of Bengal, Odisha coast, forcing communities to leave their native habitats (Barik et al., 2017, 2018a, 2018b, 2018c, 2020a, 2020b, 2020c, 2021).

Appropriate fish species identification has a pivotal role in estimating the degree of change in species composition in a particular habitat. Additionally, species identification is essential for maintaining sustainable seafood supplies and fair trade relations, particularly by giving consumers and market regulatory bodies correct information and efficiently overseeing seafood market transactions (Xiong et al., 2018, Dufflocq et al., 2022, Zhang et al., 2023, Jiang et al., 2025). Fishes have unique morphological features that make it difficult to identify them using simply morphological and morphometric data as descriptors (Triantafyllidis et al., 2011; Zhang and Hanner, 2011).

Additionally, the morpho-based characteristics that are altered as a result of convergence and divergence in fishes are what cause debated distinguishability, identification, and classification of fishes (Keskin and Atar, 2013). In the past ten years, advancements in DNA-based methodologies have enabled the practical implementation of DNA analysis in the field of taxonomy. Numerous studies have shown that the highly conserved mitochondrial cytochrome c oxidase-I (COI) gene is widely employed as a barcode marker for the identification of the majority of animal species (Hebert et al., 2003a, b). This method has long been recognised as the most flexible method for delimitation and identification since it consistently examines high rates of sequence divergence at the species level (Ivanova et al., 2012; Vences et al., 2012).

The family of surgeonfishes, tangs, and unicorn fishes is known as Acanthuridae having 84 species distributed in 6 genera. Surgeonfishes are fascinating inhabitants of tropical and subtropical habitats, and they are particularly common on and near coral reefs. With a swift side sweep of the tail, they can slice other fish with their razor-sharp caudal spines. Numerous kinds are well-liked aquarium fishes and have vivid colours. The family Acanthuridae includes the genus *Acanthurus*, which is found in the Atlantic, Indian, and Pacific oceans. They have a pair of dangerously sharp spines, one on either side of the base of the tail, just like other family members. *Acanthurus mata*, also known as the elongate surgeonfish or blue-lined surgeonfish, typically lives on steep slopes among coral reefs (Froese and Pauly, 2025). Several earlier investigations have been conducted to comprehend the evolution of the Acanthuridae family members (Huburt et al., 2010; Sorenson et al., 2013; Nabila et al., 2019; Tao et al., 2020; Yang et al., 2020).

The fishes of the family Ephippidae are aquarium fishes known as spadefishes, batfishes, and scats, which have a total of 15 species distributed in 8 genera. It is a tiny family of tropical and subtropical ocean fishes with deep bodies. They are laterally compressed and shaped like a spade and have extremely symmetrical, triangular dorsal and anal fins. They have vertical brown or black banding and patches of brilliant silver with yellow accents. While juvenile batfish have very long dorsal and anal fins and frequently resemble leaves or other debris, adult batfish have incredibly deep, nearly round bodies. Spadefishes belong to the genus *Ephippus*. This genus presently has two known species *Ephippus orbis* and *Ephippus goreensis* (Froese and Pauly, 2025). *Ephippus orbis*, generally known as orb fishes are recorded from Indo-West Pacific: Persian Gulf to Natal, South Africa, eastward to India and Lesser Sunda Island, Indonesia, north to Japan (Masuda et al., 1984) and Taiwan, south to northern Australia (Lieske and Myers, 1994). Remarkably, few studies were attempted earlier to understand the evolution of members of the Ephippidae family (Cavalluzzi, 2000; Ghanbarifardi and Shahdadi et al., 2022).

The purpose of this study was to elucidate the identification of two species based on both morphological diagnostic characters and molecular features by utilising different approaches to the widely employed mitochondrial COI gene. Further, genetic variation, phylogenetic positioning, and divergence time estimation of the two ray-finned fishes have also been discussed.

## Materials and Methods

### *Ethical approval*

No live animals were used in this study. This study used fish sourced from local fishermen.

### *Study area and specimen collection*

On the Gopalpur-on-sea (19.25°N, 84.90°E), Odisha coast, Bay of Bengal, India, during the year 2020, five specimens of the marine fish species *A. mata* (Family: Acanthuridae) and four specimens of *E. orbis* (Family: Ephippidae) were collected by the local fishermen during their daily fishing activities. Line fishing was used to catch the various fish specimens. The samples were brought to the laboratory under freezing conditions as soon as possible. Additionally, isolated muscle tissue and fin clips were kept in 100 % ethanol at a low temperature.

### *Taxonomic species identification*

Specimens were systematically classified based on the taxonomic characters available in the leading taxonomic guides, e.g., Commercial Sea Fishes of

India (Talwar and Kacker, 1984). The identification of both species was further confirmed by an examination of the taxonomic keys and the meristic characteristics stated in many prominent identification keys (Talwar and Kacker, 1984; Randall, 2001; Rao, 2003). Finally, all the specimens were classified according to Eschemeyer's Catalog of Fishes (Fricke et al., 2022) and designated to their species level.

## DNA extraction, PCR amplification, and sequencing

By using the salting out approach (Sambrook and Russell, 2001), genomic DNA was extracted from the muscle tissue of the two specimens of the species *A. mata* and one specimen of the species *E. orbis* with a final dilution volume of 200 µL. A Nanodrop spectrophotometer was used to check the quality of the isolated DNA. The extracted DNA was further examined using 1.5 percent agarose gel electrophoresis before being put into storage at -20 °C for future usage. Using specific primers, a part of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified (Table 1) (Ivanova et al., 2007). In a 25 µL reaction volume with 100 ng of template DNA, 0.2 M of each primer, 0.2 mM of dNTPs mix, 1 U of Taq DNA polymerase, and 1× PCR assay buffer, the mtCOI gene was amplified. A suitable PCR procedure was used to program the amplification, consisting of an initial step at 95 °C for 2 min, 35 cycles of 94 °C for 30 sec, 54 °C for 30 sec, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR results were electrophoresed on a 1.5 percent agarose gel and the products were purified. Finally, the sequencing of the purified products was outsourced.

Table 1. Primers for the PCR used in DNA barcoding.

| Primer | Primer sequence (5′ to 3′) |
|---|---|
| VF2_t1 | TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC |
| FR1d_t1 | CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA |
| FishF2_t1 | TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC |
| FishR2_t1 | CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA |

Note: COI amplification was carried out using Fish cocktail primers (1:1:1:1) (Ivanova et al., 2007).

## Sequence data analysis

The generated chromatograms' quality was examined with the program CodonCode Aligner (www.codoncode.com), and high-quality continuous sequence reads of bases (Quality value >20) were taken into consideration for additional investigation. Using the software BioEdit, the forward and reverse chromatograms were both evaluated. The accurate barcode sequences were created, aligned, and further consensus sequences were constructed as per the method described by Hall (1999). Using ExPASy (https://www.expasy.org), the protein-coding sequences were converted to amino acids, and no stop codons were discovered. To determine whether the sequence belonged to the locus targeted, the NCBI web server's BLASTN program was used (Altschul et al., 1990). The segment that showed 100 % alignment with no gap or indel (insertion/deletions) was chosen.

## Genetic diversity

The COI gene sequences of both species from all the available locations in the NCBI database were retrieved. The Kimura two-parameter (K2P) distance, nucleotide composition, codon usage bias, and transition/transversion bias of the sequences of both species were determined by MEGA X (Kimura, 1980; Tamura et al., 2004; Kumar et al., 2018). Both a comparison of overall diversity and a pairwise comparison between different places were done to obtain the haplotype diversity. To ascertain the ancestry of haplotypes, a median-joining network (Bandelt et al., 1999) was built in population analysis with reticulate trees PopART v1.7 (Leigh and Bryant, 2015). DNA sequence polymorphism DnaSp v6.12.03 was used to determine the gene sequences' intra-population polymorphism (Rozas et al., 2017). Additionally, analyses were done on the number of variable sites, nucleotide diversity (Jukes and Cantor, 1969), haplotype diversity (Nei and Tajima, 1983), and parsimony informative sites. The Tajima's D test (Tajima, 1989), and Fu and Li's D and F tests (Fu and Li, 1993) were conducted to test the level of significance of the dataset.

## Amino acids variation

The amino acid sequences of respective nucleotide sequences along with the sequences of the same species from different locations were downloaded from the NCBI database. Thereafter, the amino acid sequences were then aligned with ClustalW using MEGA X software (Kumar et al., 2018). Entropy (uncertainty; H(x)) values were determined for each place in BioEdit. Entropy has a value of 0 when there is zero variation, and it rises as variability rises. Based on the resultant values, the variable amino acids were then further separated into four (arbitrary) groups according to increasing entropy: H(x) 0.5–0.7, 0.71–0.9, 0.91–1.1, and >1.1. Entropy values below 0.5 were deemed non-variable amino acid positions and residues with zero variation were defined as conserved (Pentinsaari et al., 2016). We grouped the amino acids into five standard groups in order to identify their chemical characteristics at each site: nonpolar aliphatic (G, A, V, L, M, I); polar uncharged (S, T, C, P, N, Q); aromatic (F, Y, W); positively charged (K, R, H); and negatively charged (D, E). We regarded the site as non-variable and treated it as equal to those sites with entropy <0.5 when the amino acids at a given position exhibited variation exclusively among amino acids within such groups.

## Phylogenetic analysis

Phylogenetic analysis was carried out by constructing the maximum likelihood (ML) tree using MEGA X, (Kumar et al., 2018). The support for the nodes in the two trees was assessed using 1000 bootstrap iterations. The Interspecies and Intergeneric Kimura two-parameter (K2P) distance, a standard for barcoding investigations, was also computed using MEGA X (Kimura, 1980; Kumar et al., 2018). The best-fit model for the development of the maximum likelihood tree was identified.

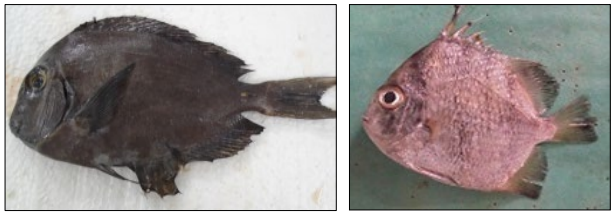
## Molecular dating

The RelTime with ML technique in MEGA X (Kumar et al., 2018) was used to estimate the divergence time of both species. The divergence time was calculated using the sequences of the mtCOI genes from both species as well as a few other sequences obtained from GenBank. Since the RelTime method only needs the minimum and/or maximum calibration boundaries, we selected the following fossil evidence time boundaries from the original studies: (1) fossil evidence of the genera Prionurus and Naso (0.3-33.9 Mya), for the entirety of the group Acanthuridae (Sorenson et al., 2013; Rabosky et al., 2018); and (2) fossil evidence of the genera Platax (7.1-18.2 Mya, for the entirety of the group Ephippidae (Betancur et al., 2015; Sanciangco et al., 2016; Rabosky et al., 2018). The outgroup clade was automatically eliminated from the analysis under the presumption that the equivalent rates of evolution between the in-group and out-group sequences could not be tested (Kumar et al., 2018).

# Results

## Morphology of the specimen

Two marine fish species, *A. mata* and *E. orbis* (Fig.1) were collected for the first time from the Gopalpur-on-sea of Odisha coast, Bay of Bengal. The collected specimens were successfully assigned to their respective species by taxonomic analysis based on their morphological characteristics (Talwar and Cacker, 1984; Randall, 2001; Rao, 2003). Morphometric and meristic characteristics of both the fishes observed were recorded (Supplementary Table 1).



(A) (B)

Fig. 1. (A) *Acanthurus mata* and (B) *Ephippus orbis*, collected from Gopalpur-on-sea, Odisha coast, Bay of Bengal, India.

## Sequence data analysis

COI barcode sequences of both species were successfully amplified. No insertions/deletions, stop codons or heterozygous sites were detected; supporting that the amplified sequence constitutes a functional mitochondrial COI sequence. The lengths of the edited barcodes of two *A. mata* fishes were 703 bp and 699 bp. The length of the edited barcode of *E. orbis* was 672 bp. The sequences of the *A. mata* and *E. orbis* both showed the highest matches to single species in the BLAST database, respectively, with 100 % and >99 % homology of their respective sequences. The accession numbers for the COI gene sequences of the aforementioned specimens are *A. mata*: ON222817, OK345038 and *E. orbis*: ON197105.

## Genetic diversity

For genetic diversity analysis, 12 COI sequences of *A. mata* species and 10 sequences of *E. orbis* species from various locations available in the NCBI database were considered for our study. The frequencies of the nucleotides of *A. mata* were: 26.89 % (A), 31.04 % (T), 25.25 % (C), and 16.82 % (G). The transition/transversion rate ratios were k1 = 7.326 (purines) and k2 = 12.288 (pyrimidines) and the overall transition/transversion bias was R = 5.261. The frequencies of the nucleotide of *E. orbis* were 22.93 % (A), 24.85 % (T), 33.17 % (C), and 19.04 % (G). The ratios of transition/transversion rate were k1 = 14.291 (purines) and k2 = 8.463 (pyrimidines) and R = 5.209 represented the overall bias for transitions and transversions. The distribution of the nucleotides within the codon in the barcode region is represented in Figure 2. The Consortium for the Barcode of Life (CBOL) had recommended the Kimura 2-parameter model for calculating genetic distance (Kimura, 1980; Shen et al., 2016). The Kimura-2-parameter model was used in this work to determine the genetic distances between various organisms. The average K2P distances of the COI sequences of *A. mata* and *E. orbis* at the intraspecies level were 0.017 % and 0.004 %, respectively.

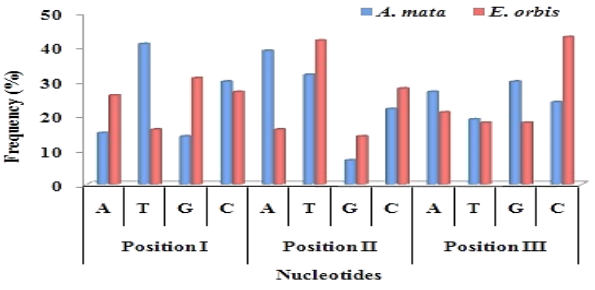



Fig. 2. Distribution of nucleotides within codons within the barcodes of *Acanthurus mata* and *Ephippus orbis*.

The median-joining network's overall topology (Figs. 3A and 3B) of collected fish species corresponded with the phylogenetic trees of both the species. The network was centred on a single widely distributed

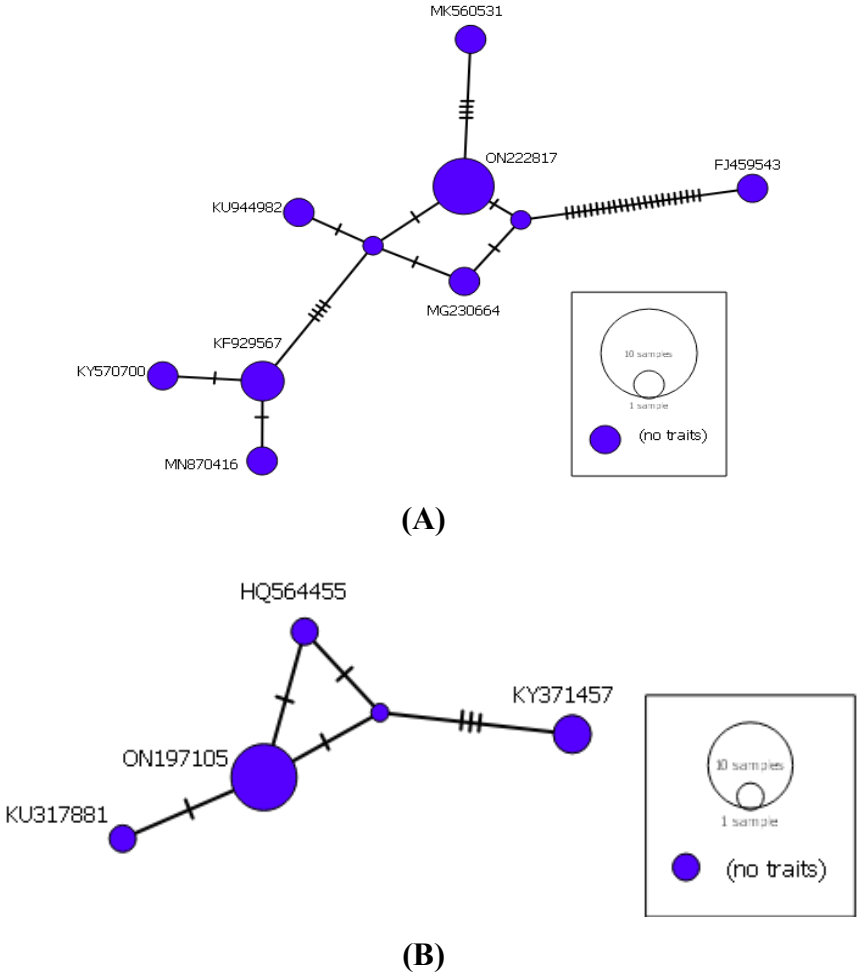



Fig. 3. Median-joining haplotype networks of COI gene of (A) *Acanthurus mata* and (B) *Ephippus orbis*. The size of the circles designates the frequencies of individuals appearing in the sample. Mutations are represented by lines and the rate of mutation is shown by dashes along the lines.

high-frequency haplotype, which was radiated by a few minor low-frequency derived haplotypes.

There were 33 polymorphic sites in the sequences of *A. mata*, of which 25 (75.76 %) were singleton variable sites and 8 (24.24 %) were parsimony informative sites. The dataset had 34 mutations in total. The 12 distinct sequences yielded a total of 8 haplotypes, 6 of which were unique haplotypes (75 %), and 2 of which were shared (25 %). Hap1 was the dominant haplotype with 5 sequences (sequences of Gopalpur, India; South Africa; Pakistan; Indian Ocean, Norway) while the other shared haplotype was Hap4 (sequences of Seychelles: Mahe, Victoria; Tamilnadu, India). In the sequences of *E. orbis*, there were 5 polymorphic sites, of which 4 (80 %) were parsimony informative sites and 1 (20 %) was a singleton variable site. Within the dataset, a total of 6 mutations were discovered. The 10 distinct sequences yielded a total of 4 haplotypes, of which 2 (50 %) were shared haplotypes and the remaining 2 were unique haplotypes (50 %). Hap1 was the dominant haplotype with 6 sequences (sequences of Gopalpur, India; Myanmar; Iran; Bangladesh; Indian Ocean, India; Andhra Pradesh, India) while the other shared haplotype was Hap3 (sequences of China; Taiwan). The nucleotide diversity ($\pi$), haplotype diversity (*Hd*), Tajima's D test and Fu and Li's D and F test values of both species are mentioned in Table 2.

## Amino acid variation

The distribution of amino acids in the COI sequence of the fish species *A. mata* is presented in Figure 4. For

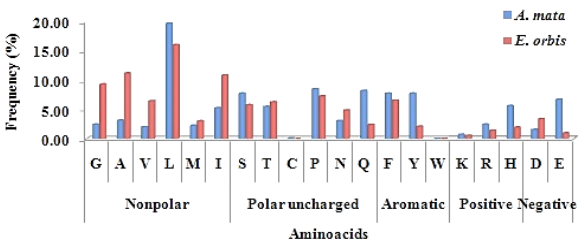



Fig. 4. Distribution of amino acids within the barcodes of *Acanthurus mata* and *Ephippus orbis*.

Table 2. Polymorphism analyses of the COI gene sequences of the fish species *Acanthurus mata* and *Ephippus orbis*

| Fish species | No. of COI gene sequences | No. of haplotypes | No. of shared haplotypes | No. of polymorphic sites (ss) | No. of parsimony informative sites | No. of mutations | $\pi$ | $h$ | Tajima' D (m) | Tajima' D (ss) | Fu and Li's D (m) | Fu and Li's D (ss) | Fu and Li's F (m) | Fu and Li's F (ss) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Acanthurus mata* | 12 | 8 | 2 | 33 | 25 | 34 | 0.019 | 0.894 | -1.4254 | -1.3297 | -1.79669 | -1.76205 | -1.93632 | -1.87854 |
| *Ephippus orbis* | 10 | 4 | 2 | 5 | 4 | 6 | 0.003 | 0.644 | -0.6693 | 0.0235 | 0.20368 | -0.02396 | -0.01194 | -0.01384 |

Note: $\pi$ - Nucleotide diversity, $h$ - Haplotype diversity, m - Test considering mutations, ss - Test considering segregating sites.

the study of amino acid variation in the COI sequences of *A. mata*, 234 amino acids were considered. In evidence of functional constraints influencing diversity in amino acid sequences, 233 out of the 234 amino acids were found to be completely conserved among the *A. mata*. At position 150, only one sequence of *A. mata* was from the Arabian Sea of the coast of India, where the non-polar amino acid leucine (L) was substituted to polar charged amino acid proline (P). Although the substitution of amino acid was from different chemical groups, it was not considered as variable, because the entropy value was less than 0.5. The distribution of amino acids in the COI sequence of *E. orbis* is presented in Figure 5. For the study of amino acid variation in the COI sequences of both *E. orbis*, 231 amino acids were considered. In *E. orbis*, all 231 amino acids were observed to be completely conserved. It is evident from the study that there were no variations in amino acids in the COI sequence of *E. orbis*.

## *Phylogenetic analysis*

To compare the newly created barcode sequences with the sequences available in the NCBI database for other species under the Acanthuridae and Ephippidae families, 70 and 16 COI gene sequences including one outgroup COI gene sequence for each family were included respectively for the phylogenetic reconstruction. The average K2P distance at the interspecies level of the genus *Acanthurus* was estimated to be 0.115. The average K2P distance at the intergeneric level of the family Acanthuridae and Ephippidae were found to be 0.178 and 0.183 respectively. The *Acanthurus* genus had the highest intergenus K2P distance of 0.2 with the genus *Paracanthurus*. It was revealed that *A. mata* had the highest interspecies K2P distance of 0.15 with *Acanthurus tristis* and *Acanthurus polyzona*. The genus *Ephippus* revealed the highest intergeneric K2P distance of 0.21 with *Chaetodipterus*.

DNA barcode sequences from the studied species as well as the retrieved sequences from the related and other species were used to create the Maximum likelihood (ML) phylogenetic tree. The inferred ML trees (Figs. 5 and 6) of both the families displayed highly consistent clustering of sequences that were organized into groups based on their morphological identity and primarily make up monophyletic genera except the *Acanthurus* genus of the family Acanthuridae, which formed a paraphyletic clade with the *Ctenochaetus* genus. According to the model test for the construction of the ML tree, HKY+G+I was identified as the best-fit DNA substitution model for both the datasets with a BIC value of 14322.995 for the Acanthuridae family and 5707.385 for the Ephippidae family. The *A. mata* sequence from Taiwan was closer to the sequences of Gopalpur-on-sea as compared to the sequences of another region. The analysis suggested that the species *Acanthurus maculiceps* and *Acanthurus blochii* were closer to *A. mata* as compared to other species of the genus *Acanthurus*. It

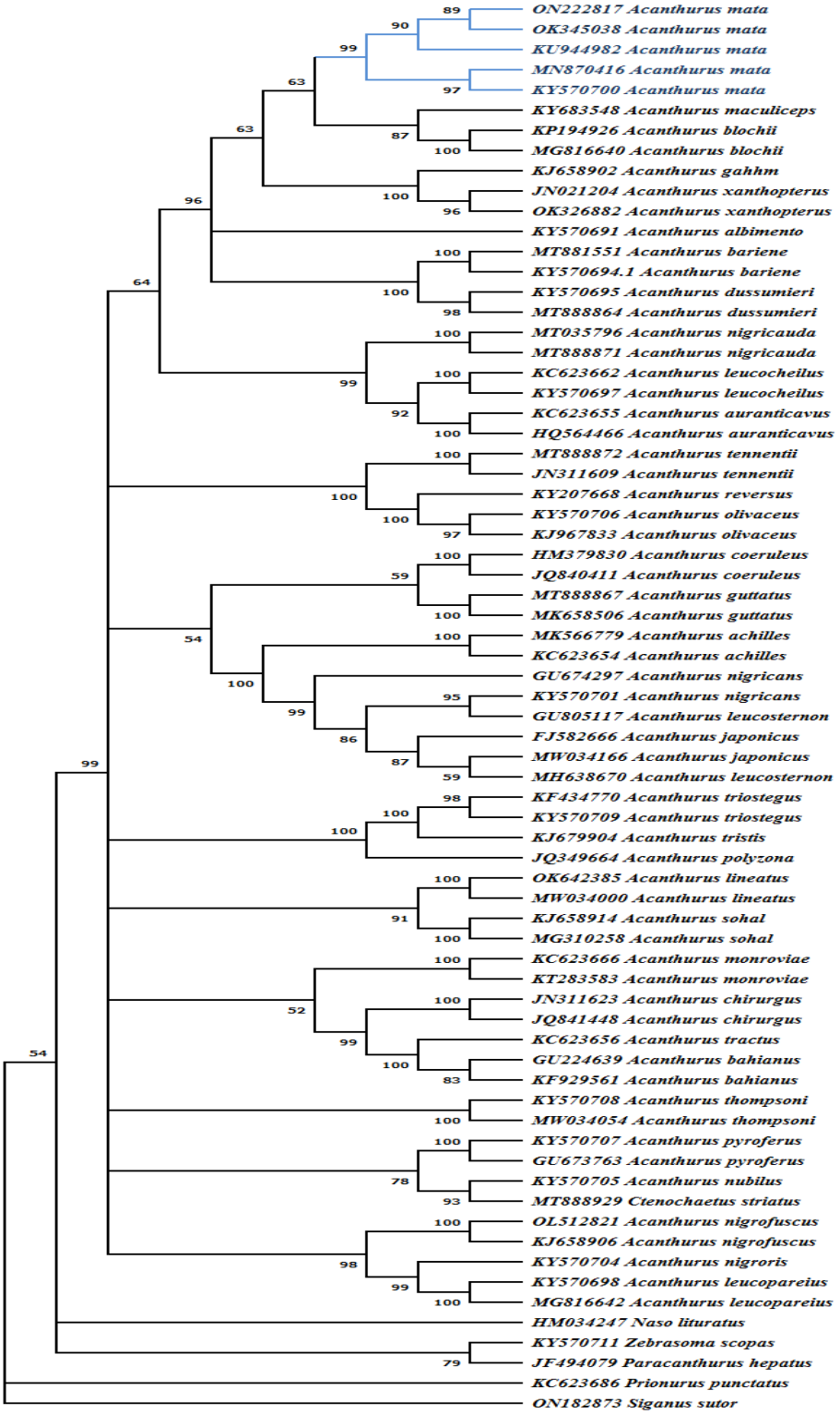



Fig. 5. Maximum likelihood (ML) phylogenetic tree of the family Acanthuridae. ML tree is based on the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Branches resulting in partitions with less than 50 % bootstrap replicates were collapsed. The estimated value of the tree's log likelihood is -6389.78. The variation in evolutionary rates among sites was modelled using a discrete Gamma distribution (5 categories (+G, parameter = 1.422) and Ts/Tv = 7.003). Some sites to be evolutionarily invariable ([+I], 32.79 % sites) were allowed by the rate variation model. Accession No.: ON222817 and OK345038 were generated in the present study.

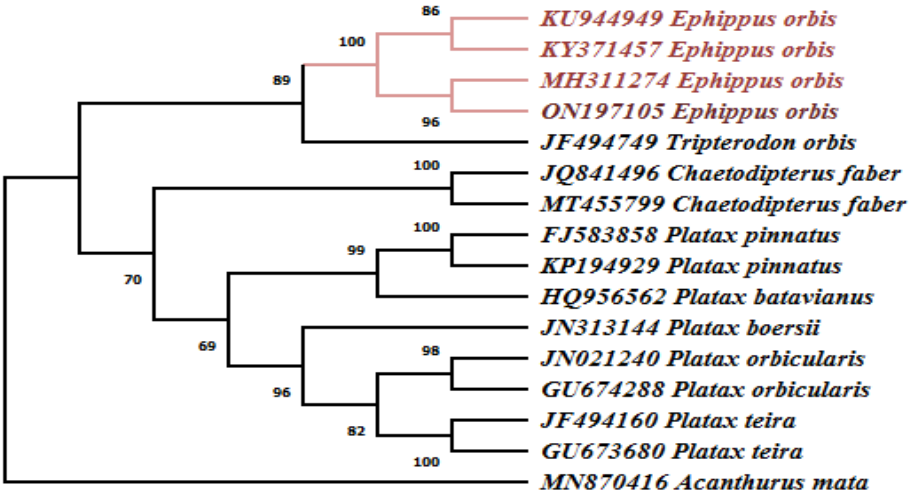



Fig. 6. Maximum Likelihood phylogenetic tree of family Ephippidae: ML tree is based on Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). The percentage of trees in which the associated taxa clustered together was shown next to the branches. Branches resulting in partitions with less than 50 % bootstrap replicates were collapsed. The estimated value of the tree's log likelihood was -2691.82. The variation in evolutionary rates among sites was modelled using a discrete Gamma distribution (5 categories (+G, parameter = 1.213) and Ts/Tv = 4.630). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 34.59 % sites). Some sites to be evolutionarily invariable ([+I], 34.59 % sites) were allowed by the rate variation model. Accession No.: ON197105 was generated in the present study.

is evident that the genus *Prionurus* was the ancestor of the rest of the genera of the Acanthuridae family. The *E. orbis* sequence from Bangladesh was closer to the sequences of Gopalpur-on-sea as compared to the sequences of other regions. It was also observed that the *E. orbis* has a sister lineage with the species *Tripterodon orbis*.

## *Molecular dating*

The families *Acanthuridae* and *Ephippidae* were estimated to have originated around 112.69 Mya and 55.17 Mya during the early Cretaceous period and Eocene epoch respectively. *Acanthurus* and *Ephippus* were two well-established genera, which were estimated to have diverged at the beginning of the Eocene epoch. According to estimates, the separation of the *Acanthurus* clade from its common ancestor took place during the early Eocene sub-epoch around 45.07 Mya whereas the *A. mata* species during the late Miocene sub-epoch around 9.97 Mya. After the origin of *A. mata*, the species *Acanthurus maculiceps* and *Acanthurus blochii* were diverged from the same common ancestor around 7.37 Mya. The species *Acanthurus xanthopterus*, *Acanthurus gahhm*, *Acanthurus olivaceous*, *Acanthurus reversus*, *Acanthus bahianus*, and *Acanthurus tractus* of the genus *Acanthurus* were originated in the recent years which was less than 1 Mya. Similarly, the species *Zebrasoma scopas*, *Zebrasoma flavescens*, *Prionurus laticlavius*, *Prionurus punctatus*, *Naso lituratus*, and *Naso elegans* originated around less than 1 Mya. However, the species *Ctenochaetus flavicauda* and *Ctenochaetus truncatus* originated in the present epoch Holocene, which was less than 0.01 Mya (Fig. 7). *Ephippus* clade's split from its common ancestor was estimated to have happened during the early Eocene sub-epoch around 55.17 Mya and that of *E. orbis* was estimated to have happened during the late Eocene sub-epoch around 36.24 Mya (Fig. 8).

# Discussion

In the present investigation, these two fish species were collected from the Gopalpur-on-sea of Odisha coast, Bay of Bengal which was earlier reported from the Chilika Lagoon (a brackish water reservoir) of Odisha state (Mohapatra et al., 2007; Satapathy and Panda, 2009).

Due to the phenotypic variability of taxa and the relative lack of competence, the conventional morphology-based species identification system has been diverted in recent times, which may ultimately result in misidentifications. DNA barcoding has been utilised in molecular taxonomy for species identification at every stage of life to eliminate these disadvantages. DNA barcoding is supported by an advantageous DNA segment that is shared by organisms with significant interspecies variations. The mtCOI gene is typically used as a standard species barcode due to its high levels of conservation within species and moderate genetic diversity between different species (Bingpeng et al., 2018). Here, the COI barcode sequences of the two species were amplified successfully.

Both the barcode sequences did not contain any stop codons and nuclear mtDNA copies (NUMTs). Typically, the vertebrate NUMTs are less than 550 bp (Zhang and Hewitt, 1996). It is anticipated that the conserved primer pairs used in this investigation amplified

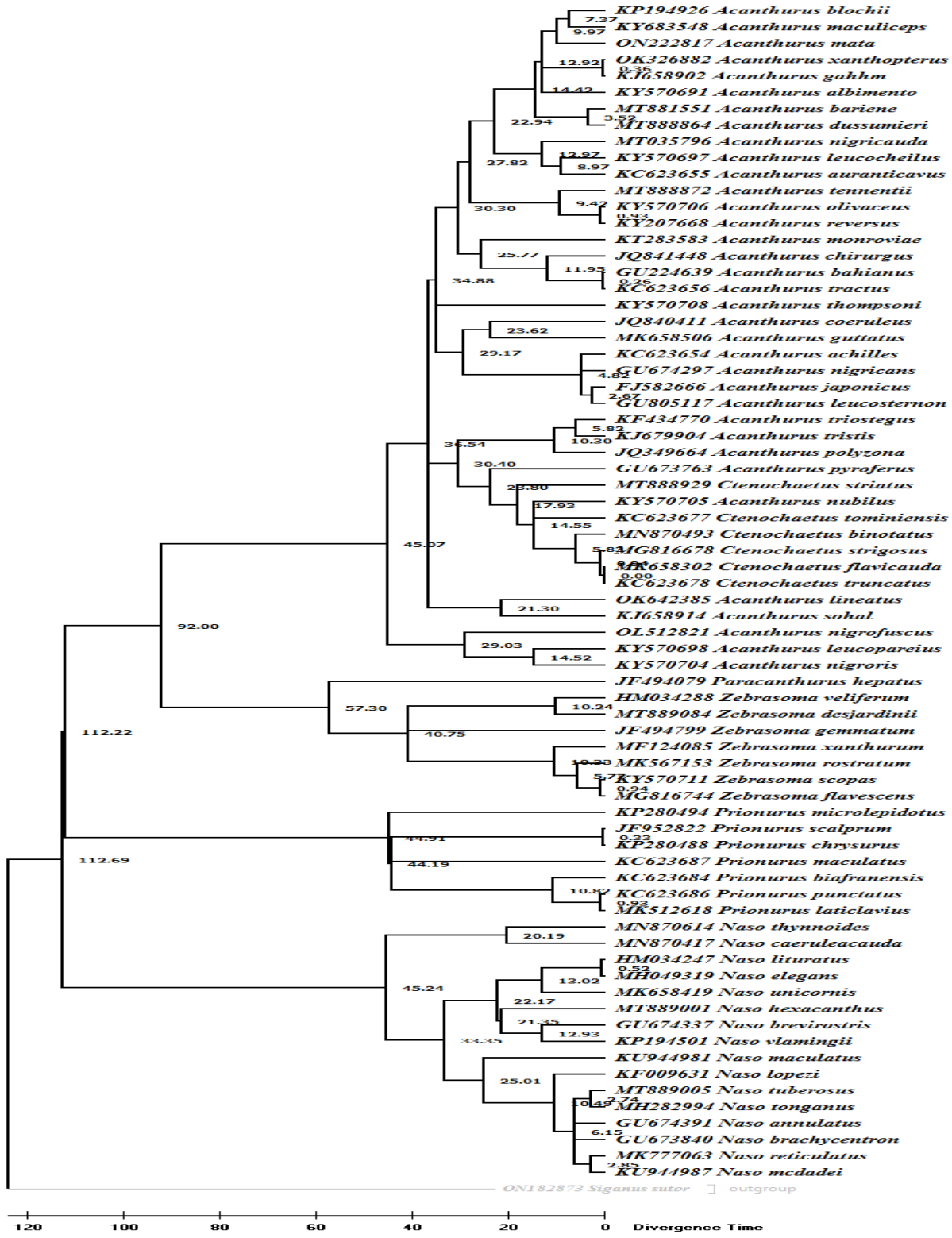



Fig. 7. Time-calibrated phylogenetic tree of family Acanthuridae. The time tree is derived from the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) and the RelTime method (Tamura et al., 2012, 2018). The estimated value of the tree's log likelihood was -8689.36. The variation in evolutionary rates among sites was modelled using discrete Gamma distribution [5 categories (+G, parameter = 1.4191) and Ts/Tv = 7.385)]. The rate variation model permitted some sites ([+I], 31.91 % of sites) to be evolutionarily invariable. The analysis involved 73 nucleotide sequences. Numbers on the branches represent the time (in Mya) of different nodes. Accession No.: ON222817 was generated in the present study.

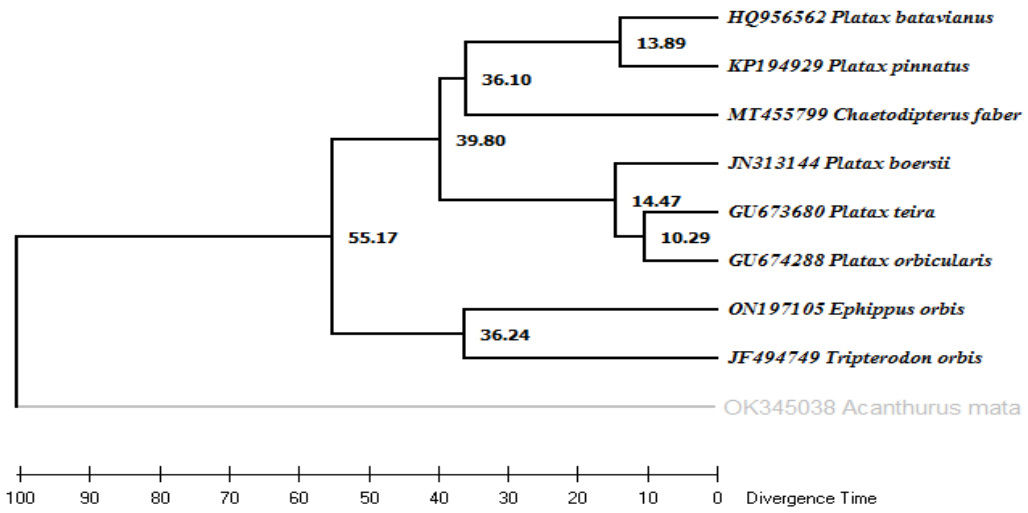



Fig. 8. Time-calibrated phylogenetic tree of family Ephippidae. The time tree is derived from the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) and the RelTime method (Tamura et al., 2012, 2018). Branch lengths of the phylogenetic tree were calculated using the Maximum likelihood (ML) method. The estimated value of the tree's log likelihood was -2666.13. The variation in evolutionary rates among sites was modelled using discrete Gamma distribution [5 categories (+G, parameter = 0.1528)]. The analysis involved 9 nucleotide sequences. Numbers on the branches represent the time (in Mya) of different nodes. Accession No.: ON197105 was generated in the study.

mitochondrial DNA favourably more than NUMTs. The nucleotide composition study of the COI gene sequences showed that in *A. mata*, AT (58 %) content is higher than GC (42 %) content. But in *E. orbis*, this contrasts with *A. mata*, where GC (52 %) content is higher than AT (48 %) content. In *A. mata* species, the utilisation of T (41 %) was the highest as compared to other bases C (30 %), A (15 %), and G (14 %) at the first codon position. G (7 %) was used the least in the second codon position, while the use of other bases C, A, and T was 22 %, 39 %, and 32 %, respectively. The highest percentage of nucleotide used in the third codon position was G (30 %), followed by A (27 %), C (24 %) and T (19 %). However, in *E. orbis* species, T usage at the first codon position was the lowest (16 %), while utilisation of A, G, and C were 26 %, 31 %, and 27 %, respectively. *E. orbis* used T (42 %) the most and G (14 %) the least frequently at the second codon position, whereas the use of other bases was A (16 %) and C (28 %). At the third codon position, the usage of C was the highest (43 %) and the use of other bases was A (21 %), T (18 %), and G (18 %). The first codon position of *A. mata* favours the T bias in contrast with *E. orbis*, which clearly showed anti-T bias supported by earlier studies (Wang et al., 2014; Bingpeng et al., 2018; Barik et al., 2021). The second codon position clearly favours the anti-G bias in *A. mata* and T-bias in *E. orbis* which was also supported by earlier studies (Wang et al., 2014; Bingpeng et al., 2018; Barik et al., 2021). Likewise, the third codon position was anti-T bias in *A. mata*, but high C bias in *E. orbis* which is in contrast with the earlier studies conducted by Wang et al. (2014), Bingpeng et al. (2018) and Barik et al. (2021). The codon positions of mitochondrial genes underwent varied degrees of base-mutation selection pressure during the evolution of species, resulting in base use bias in codon sites. The combinational impact of both neutral and selection pressure on a genome is assessed by the codon usage analysis (Barik et. al., 2021).

From the total 8 haplotypes of *A. mata* found in our study, only 2 haplotypes were shared from which Hap1 was the dominant haplotype with 5 sequences including the derived sequences by us from the Odisha coast near Gopalpur-on-sea. The intraspecies K2P distance of *A. mata* in this study was 0.017 which was much less compared to the previous study by Fadli et al. (2020) which was 0.12. Like *A. mata*, *E. orbis* had also 2 shared haplotypes from the total 4 haplotypes and Hap1 was the dominant haplotype with 6 sequences including our sequence. It was observed that our sequences of both species represent the major haplotype. A high h and low $\pi$ in both the species indicated recent demographic expansion of the populations under study (de Jong et al., 2011; Song et al., 2014; Garg et al., 2018). Tajima's D has a negative value when the population size is expanding due to the bottleneck or selective sweep and purifying selection and a positive value when the population size is declining. Due to the possibility of excess low-frequency mutations in an admixed sample, Tajima's D was not estimated over multiple collections (Stajich and Hahn, 2005). As the Tajima's D and Fu and Li's D and F test values of the species *A. mata* calculated by considering both mutations and segregating sites were strongly negative, which indicated that the population size expansion such as after a bottleneck or a selective sweep or purifying selection of both the species. However, in the species *E. orbis*, Tajima's D values calculated by considering mutations were negative and Tajima's D values calculated by considering segregating sites were positive and

almost equal to 0, which was the indication of neutral evolution. These inferences were also supported by the Fu and Li's D and F test values.

DNA variation in different parts of the COI barcoding region should be related to the functional role of these sections in the protein (Pentinsaari et al., 2016). Many variations were found between the frequencies of amino acids in the COI gene sequences of both the species *A. mata* and *E. orbis* from various geographical areas. Leucine (L), a nonpolar amino acid, was discovered to be most prevalent, and tryptophan (W), an aromatic amino acid, was completely absent. Polar uncharged Cysteine (C) amino acid was found absent from *E. orbis* whereas it was negligible in *A. mata*. Additionally, it was discovered that both the species contained relatively little of Lysine (K). Except for the nonpolar group, where *E. orbis* was found to have substantially higher amino acid frequencies overall, *A. mata* was shown to have higher total amino acid frequencies in all of the amino acid groups. The DNA variation in different portions of the COI barcoding region should be related to the functional role of these sections in the protein (Pentinsaari et al., 2016). It was evident from the study that the species *E. orbis* had still wild populations as the amino acid sequences were completely conserved. There was one amino acid substitution in *A. mata*, however, it was considered a non-variable as the entropy value was less than 0.5.

Phylogenetic analysis offers a thorough understanding of how species evolve through genetic alterations, assesses the route connecting a modern creature with its ancestral origin, and forecasts potential future genetic divergence. The ML trees of both the families Acanthuridae and Ephippidae generated in this study showed the most robust clustering of both the families into respective monophyletic clades of their respective genus and species except the *Acanthurus* genus which formed a paraphyletic clade as the sequence of the *Ctenochaetus* genus clustered inside the *Acanthurus* genus of the family Acanthuridae. In the ML tree of the Acanturidae family, the total species of the genus *Acanthurus* as well as our generated sequence of *A. mata* along with the sequences of the same species came under the monophyletic clade with 100 % support value. The phylogenetic analysis reflects that the *A. mata* species is closer to the *Acanthurus maculiceps* and *Acanthurus blochii* species. The earlier study conducted by Sorenson et al. (2013) lends further evidence to this. Similarly, in both the trees of the Ephippidae family also, our generated sequence of *E. orbis* along with the sequences of the same species came under the monophyletic clade with 100 % support value. The phylogenetic analysis reflects that the *Tripterodon orbis* species is closer to the *E. orbis* species with a 94 % support value. This was supported by the study conducted by Ghanbarifadi and Shahdadi (2022). In the cases of both the family, these inferences are supported by the time tree derived by molecular dating also.

Based on molecular dating phylogeny, the family Acanthuridae originated during the early Cretaceous epoch. However, the *Acanthuridae* family originated during the Eocene epoch according to an earlier study (Sorenson et al., 2013). The family Ephippidae was separated from their ancestors during the Eocene epoch which was evidenced by the earlier studies (Bellwood and Sorbini, 1996; Bellwood et al., 2004). Based on molecular dating phylogeny, the *Acanthurus* genus had radiated from the common ancestor in the early Eocene epoch, ~45.07 Mya which was supported by the earlier study of Sorenson et al. (2013) where the origin of the Acanthurinae group was reported ~42 Mya. Despite different divergence timelines of the taxa, they formed their respective groups. Similarly, the origins of the Ephippidae family and *Ephippus* genus from the common ancestor were in the early of the Eocene epoch, 55.17 Mya approximately which is evidenced by the earlier studies of Bellwood and Sorbini (1996) and Bellwood et al. (2004). This type of situation revealed that the different morphological identifying characteristics like variation in numbers of dorsal spines, soft rays, within the genus or in between the genus probably happened due to the selective environmental pressure. Despite different divergence times, all the genera of both families clustered with their respective groups.

## Conclusion

This finding suggests that DNA barcoding is highly effective at identifying the species. Phylogenetic connections are typically affected by morphological misidentification and nucleotide saturation. Due to phenotypic variability within species, there will inevitably be some instances of morphological misidentification, and this circumstance shows that DNA barcoding may identify these instances. Our findings also provide information on the genetic diversity, divergence time, and phylogenetic relationship of the family Acanthuridae and Ephippidae at the species and genus level. This study evidenced the significant genetic variability in *A. mata* species from different geographical areas. However, the wild populations of *E. orbis* are still present in all the areas. The genetic variation of natural populations was the natural gene pools of species. Therefore, care should be taken to preserve the genetic variation of wild populations. Further, genetic management should be improved by monitoring the genetic variability.

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Author contributions: Bijayalaxmi Sahu: Conceptualisation, data curation, formal analysis, investigation, methodology, software, validation, visualisation; writing - original draft, review and editing. Tapan Kumar Barik: Conceptualisation, investigation, resources, writing – review and editing. Amiya Kumar Patel: Conceptualisation, supervision, writing – review and editing.

# References


Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410. https://doi.org/10.1016/s0022-2836(05)80360-2

Bandelt, H.J., Forster, P., Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2021. Molecular evidence for *Myripristis jacobus* and *Scarus taeniopterus* new to Bay of Bengal: Sporadic appearance or preliminary colonization? Marine Ecology 42:e12632. https://doi.org/10.1111/maec.12632

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2020a. Morphological and molecular evidence supports the first occurrence of two fishes, *Siganus sutor* (Valenciennes, 1835) and *Seriolina nigrofasciata* (Rüppell, 1829) (Actinopterygii: Perciformes), from marine waters of Odisha coast, Bay of Bengal, India. Acta Oceanologica Sinica 39:26–35. https://doi.org/10.1007/s13131-020-1609-x

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2020b. Documenting the first record of the Unicorn leatherjacket filefish *Aluterus monoceros* (Tetraodontiformes: Monacanthidae) from the marine waters of Odisha coast, Bay of Bengal, India. Iranian Journal of Ichthyology 7:85–91. https://doi.org/10.22034/iji.v7i1.328

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2020c. Occurrence of *Pempheris schwenkii* and *Pempheris. mangula* (Teleostei: Pempheridae) from marine waters of Gopalpur-on Sea, Odisha coast, India. Iranian Journal of Ichthyology 7:197–201.

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2018a. First record of *Tylosurus crocodilus* (Péron & Lesueur, 1821) (Beloniformes: Belonidae) from Odisha Coast, Bay of Bengal, India: Exploration of a biological invasion using DNA barcoding. Thalassas 34:209–217. https://doi.org/10.1007/s41208-017-0053-y

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2018b. The first record of *Cephalopholis formosa* (Perciformes: Serranidae) from the marine waters of Odisha coast, Bay of Bengal, India. Journal of Ichthyology 58:751–753. https://doi.org/10.1134/S0032945218050028

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2018c. First record of non-native Bluering angelfish *Pomacanthus annularis* (Perciformes: Pomacanthidae) from the marine water of Odisha coast, Bay of Bengal, India. Iranian Journal of Ichthyology 5:317–321. https://doi.org/10.22034/iji.v5i4.289

Barik, T.K., Swain, S.N., Sahu, B., Tripathy, B., Acharya, U.R. 2017. Morphological and genetic analyses of the first record of longrakered trevally, *Ulua mentalis* (Perciformes: Carangidae) and of the pinjalo snapper, *Pinjalo pinjalo* (Perciformes: Lutjanidae) in the Odisha coast, Bay of Bengal. Mitochondrial DNA Part A 29:552–560. https://doi.org/10.1080/24701394.2017.1320993

Barman, R.P., Mishra, S.S., Kar, S., Mukherjee, P., Saren, S.C. 2007. Marine and estuarine fish fauna of Orissa. Records of the Zoological Survey of India, Kolkata, India, Occasional Paper No. 260. 186 pp.

Barua, S.P., Khan, M.H., Reza, A.H.M.A. 2001. The status of alien invasive species in Bangladesh and their impacts on the ecosystem. In: Alien invasive species, Balakrishnas, P. (Ed.), IUCN Regional Biodiversity Programme Asia, pp. 1–7.

Bellwood, D., Herwerden, L., Konow, N. 2004. Evolution and biogeography of marine angelfishes (Pisces: Pomacanthidae). Molecular Phylogenetics and Evolution. 33:140–155. https://doi.org/10.1016/j.ympev.2004.04.015

Bellwood, D.R., Sorbini, L. 1996. A review of the fossil record of the Pomacentridae (Teleostei: Labroidei) with a description of a new genus and species from the Eocene of Monte Bolca, Italy. Zoological Journal of the Linnean Society 117:159–174. https://doi.org/10.1111/j.1096-3642.1996.tb02154.x

Betancur, R.R., Ortí, G, Pyron, R.A. 2015. Fossil-based comparative analyses reveal ancient marine ancestry erased by extinction in ray-finned fishes. Ecology Letters 18:441–450. https://doi.org/10.1111/ele.12423

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., Jianjun, W. 2018. DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One 13:e0198109. https://doi.org/10.1371/journal.pone.0198109

Cavalluzzi, M.R. 2000. Osteology, phylogeny, and biogeography of the marine fish family Ephippidae (Perciformes, Acanthuroidei), with comments on sister group relationships. Dissertations, Theses, and Masters Projects. Paper 1539616603. https://core.ac.uk/download/pdf/235412456.pdf

de Jong, M.A., Wahlberg, N., van Eijk, M., Brakefield, P.M., Zwaan, B.J. 2011. Mitochondrial DNA signature for range-wide populations of *Bicyclus anynana* suggests a rapid expansion from recent refugia. PLoS One 6:e21385. https://doi.org/10.1371/journal.pone.0021385

Dufflocq, P., Larraín, M.A., Araneda, C. 2022. Species substitution and mislabeling in the swordfish (*Xiphias gladius*) market in Santiago, Chile: Implications in shark conservation. Food Control 133:108607. https://doi.org/10.1016/j.foodcont.2021.108607

Fadli, N., Mohd Nor, S.A., Othman, A.S., Sofyan, H., Muchlisin, Z.A. 2020. DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. Peer J 8:e9641. https://doi.org/10.7717/peerj.9641

Fricke, R., Eschmeyer, W.N., van der Laan, R. 2022. Eschmeyer's catalog of fishes: Genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (Accessed 25 January 2022).

Froese, R., Pauly, D., 2025. FishBase. www.fishbase.org

Fu, Y., Li, W. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709. https://doi.org/10.1093/genetics/133.3.693

Garg, R.K., Mishra, V. 2018. Molecular insights into the genetic and haplotype diversity among four populations of *Catla catla* from Madhya Pradesh revealed through mtDNA cyto b gene sequences. Journal of Genetic Engineering and Biotechnology 16:69–174. https://doi.org/10.1016/j.jgeb.2017.11.003

Ghanbarifardi, M., Shahdadi, A. 2022. Molecular phylogeny of Ephippidae (Actinopteri: Acanthuriformes). Journal of Ornamental Aquatics 9:45–51.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98. https://doi.org/10.12691/ajmr-3-2-1

Hasegawa, M., Kishino, H., Yano, T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174. https://doi.org/10.1007/bf02101694

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S., Kats, L.B. 2015. Aquatic invasive species: Challenges for the future. Hydrobiologia 750:147–170. https://doi.org/10.1007/s10750-014-2166-0

Hebert, P.D.N., Cywinska, A., Ball, S.L., DeWaard, J.R. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B: Biological Sciences 270:313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Ratnasingham, S., DeWaard, J.R. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B: Biological Sciences 270:S96–S99. https://doi.org/10.1098/rsbl.2003.0025

Hubert, N., Delrieu-Trottin, E., Irisson, J.O., Meyer, C., Planes, S. 2010. Identifying coral reef fish larvae through DNA barcoding: a test case with the families Acanthuridae and Holocentridae. Molecular Phylogenetics and Evolution 55:1195–1203. https://doi.org/10.1016/j.ympev.2010.02.023

Hulme, P.E. 2009. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46:10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Ivanova, N.V., Clare, E.L., Borisenko, A.V. 2012. DNA barcoding in mammals. Methods in Molecular Biology 858:153–182. https://doi.org/10.1007/978-1-61779-591-6_8

Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Resources 7:544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x

Jiang, C., Qin, J., Liu, F., Luo, Z., He, X., Yan, Y. 2025. DNA barcoding reveals low-level species substitution, threatened species and cross-border sales in South China seafood products. Journal of Food Composition and Analysis 139:107157. https://doi.org/10.1016/j.jfca.2024.107157

Jukes, T.H., Cantor, C.R. 1969. Evolution of protein molecules. In: Monro HN, editor. Mammalian protein metabolism 3:21–132. https://doi.org/10.1016/B978-1-4832-3211-9.50009-7

Katsanevakis, S., Wallentinus, I., Zenetos, A., Leppäkoski, E., Çinar, M.E., Oztürk, B., Cardoso, A.C. 2014. Impacts of invasive alien marine species on ecosystem services and biodiversity: A pan-European review. Aquatic Invasion 9:391–423. http://dx.doi.org/10.3391/ai.2014.9.4.01

Keskin, E, Atar, H.H. 2013. DNA barcoding of commercially important fish species of Turkey. Molecular Ecology Resources 13:788–797. https://doi.org/10.1111/1755-0998.12120

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120. https://doi.org/10.1007/bf01731581

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Leigh, J., Bryant, D. 2015. PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Lieske, E., Myers, R. 1994. Collins pocket guide. Coral reef fishes. Indo-Pacific & Caribbean including the Red Sea. Harper Collins Publishers. 400 pp.

Masuda, H., Amaoka, K., Araga, C., Uyeno, T., Yoshino, T. 1984. The fishes of the Japanese Archipelago. Vol. 1. Tokai University Press, Tokyo, Japan. 437 pp.

Mohanty, P.K., Panda, U.S., Pal, S.R., Mishra, P. 2008. Monitoring and management of environmental changes along the Orissa coast. Journal of Coastal Research 24:13–27. https://doi.org/10.2112/04-0255.1

Mohapatra, A., Mohanty, R.K., Mohanty, S.K., Bhatta, K.S., and Das, N.R. 2007. Fisheries enhancement and biodiversity assessment of fish prawn and mud crab in Chilika lagoon through hydrological intervention. Wetlands Ecology and Management 15:229–251. https://doi.org/10.1007/s11273-006-9025-3

Molnar, J.L., Gamboa, R.L., Revenga, C., Spalding, M.D. 2008. Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:458–492. https://doi.org/10.1890/070064

Nabila, A., Nuryanto, A., Prabowo, R., Bhagawati, D., Kusbiyanto, K. 2019. Phylogenetic relationships among ornamental Achanturid fish from Ujunggenteng and Taman Manalusu, West Java. Biosaintifika 11:325–331. https://doi.org/10.15294/biosaintifika.v11i3.20671

NCBI Resource Coordinators. 2016. Database resources of the National Center for Biotechnology Information. Nucleic Acids Research 44(D1):D7–D19.

Nei, M., Tajima, F. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105:207–217. https://doi.org/10.1093/genetics/105.1.207

Pati, S.K., Swain, D., Sahu, K.C., Sharma, R.M., Mohapatra, A. 2018. Marine fauna of Odisha, East Coast of India: An annotated checklist of historical data from 135 years. Journal of Aquatic Biology & Fisheries 6:1-115.

Pentinsaari, M., Salmela, H., Mutanen, M., Roslin, T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. Scientific Reports 6:35275. https://doi.org/10.1038/srep35275

Rabosky, D.L., Chang, J., Title, P.O., Cowman, P.F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T.J., Coll, M., Alfaro, M.E. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. Nature 559:392–395. https://doi.org/10.1038/s41586-018-0273-1

Raghunathan, E., Sivaperuman, C., Venkataraman, K. 2012. Recent advances in biodiversity of India. Zoological Survey of India, Kolkata, India. 529 pp.

Randall, J.E. 2001. Acanthuridae. Surgeonfishes (tangs, unicornfishes). In: Species identification guide for fishery purposes. The living marine resources of the western central Pacific. Vol. 6. Bony fishes part 4 (Labridae to Latimeriidae), estuarine crocodiles, sea turtles, sea snakes and marine mammals, Carpenter, K.E., Niem, V.H. (Eds.), FAO, Rome, pp. 3653–3683.

Rao, D.V., 2003. Guide to reef fishes of Andaman and Nicobar Islands. Zoological Survey of India, Kolkata. India. 555 pp.

Rasmussen, R.S., Morrissey, M.T., Hebert, P.D.N. 2009. DNA barcoding of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) from North America. Journal of Agricultural and Food Chemistry 57:8379–8385. https://doi.org/10.1021/jf901618z

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Molecular Biology and Evolution 34:3299–3302. https://doi.org/10.1093/molbev/msx248

Sambrook, J., Russell, D.W. 2001. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York, USA. 999 pp.

Sanciangco, M.D., Carpenter, K.E., Betancur, R.R. 2016. Phylogenetic placement of enigmatic percomorph families (Teleostei: Percomorphaceae). Molecular Phylogenetics and Evolution 94(Pt B):565–576. https://doi.org/10.1016/j.ympev.2015.10.006

Satapathy, D., Panda, S. 2009. Fish atlas of Chilika. Chilika Development Authority, Bhubaneswar, India. 74 pp.

Shen, X.X., Salichos, L., Rokas, A. 2016. A genome-scale investigation of how sequence, function, and tree-based gene properties influence phylogenetic inference. Genome Biology and Evolution 8:2565–2580. https://doi.org/10.1093/gbe/evw179

Song., J., Hou., F.X., Zhang, X.Y., Yue, B.S., Song, Z.B. 2014. Mitochondrial genetic diversity and population structure of a vulnerable freshwater fish, rock carp (*Procypris rabaudi*) in upper Yangtze River drainage. Biochemical Systematics and Ecology 55:1–9. https://doi.org/10.1016/j.bse.2014.02.008

Sorenson, L., Santini, F., Carnevale, G., Alfaro, M.E. 2013. A multi-locus timetree of surgeonfishes (Acanthuridae, Percomorpha), with revised family taxonomy. Molecular Phylogenetics and Evolution 68:150–160. https://doi.org/10.1016/j.ympev.2013.03.014

Stajich, J.E., Hahn, M.W. 2005. Disentangling the effects of demography and selection in human history. Molecular Biology and Evolution 22:63–73. https://doi.org/10.1093/molbev/msh252

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595. https://doi.org/10.1093/genetics/123.3.585

Talwar, P.K., Kacker, R.K. 1984. Commercial sea fishes of India. Zoological Survey of India, Calcutta, India. 997 pp.

Tamura, K., Battistuzzi, F. U., Billing-Ross, P., Murillo, O., Filipski, A., Kumar, S. 2012. Estimating divergence times in large molecular phylogenies. Proceedings of the National Academy of Sciences 109:19333-19338. https://doi.org/10.1073/pnas.1213199109

Tamura, K., Nei, M., Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030–11035. https://doi.org/10.1073/pnas.0404206101

Tao L, Yukai Y, Xiaolin H, Heizhao L, Wei Y, Zhong H, Xidong M. 2020. The complete mitochondrial genome of *Acanthurus mata* (Acanthuriformes, Acanthuridae). Mitochondrial DNA Part B Resources 5:2781–2782. https://doi.org/10.1080/23802359.2020.1719922

Triantafyllidis, A., Bobori, D., Koliamitra, C., Gbandi, E., Mpanti, M., Petriki, O., Karaiskou, N. 2011. DNA barcoding analysis of fish species diversity in four north Greek lakes. Mitochondrial DNA 22:37–42. https://doi.org/10.3109/19401736.2010.542242

Ugwumba, A.A.A., Ugwumba, A.O. 2003. Aquaculture options and the future of fish supply in Nigeria. The Zoologist 2:96–122.

Vences, M., Nagy, Z.T., Sonet, G., Verheyen, E. 2012. DNA barcoding amphibians and reptiles. Methods in Molecular Biology 858:79–107. https://doi.org/10.1007/978-1-61779-591-6_5

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Hulme, P.E. 2010. How well do we understand the impacts of alien species on ecosystem services? A pan-European, crosstaxa assessment. Frontiers in Ecology and the Environment 8:135–144. https://doi.org/10.1890/080083

Vitousek, P.M., Antonio, C.M.D., Loope, L.L., Westbrooks, R. 1996. Biological invasions as global environmental change. American Scientist 84:468.

Wang, S.S.W., Jinxia, J., Xianguang, M., Zhongming, W., Xiaoyu, K. 2014. Applicability of mitochondrial COI and 16S rRNA gene sequences in species identification of sole fish (Pleuronectiformes:Soleidae). Tropical Oceanography 33:57–65. https://doi.org/10.3969/j.issn.1009-5470.2014.03.009

Wang, Y., Yang, Y., Liu, Y., Liu, C., Xu, M., Fang, M., Mu. X. 2024. CoSFISH: a comprehensive reference database of COI and 18S rRNA barcodes for fish. Database (Oxford) 2024:baae038. https://doi.org/10.1093/database/baae038

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B. 360:1847–1857. https://doi.org/10.1098/rstb.2005.1716

Xiong, X., Yao, L., Ying, X., Lu, L., Guardone, L., Armani, A., Guidi, A., Xiong, X. 2018. Multiple fish species identified from China's roasted Xue Yu fillet products using DNA and mini-DNA barcoding: Implications on human health and marine sustainability. Food Control 88:123–130. https://doi.org/10.1016/j.foodcont.2017.12.035

Yang, L., Wang, Y., Ying, N., Song, X., Wu, Y., Qin, B. 2020. The complete mitochondrial genome of *Ctenochaetus flavicauda* (Acanthuriformes, Acanthuridae). Mitochondrial DNA Part B 5:972–973. https://doi.org/10.1080/23802359.2020.1719923

Zhang, D.X., Hewitt, G.M. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology & Evolution 11:247–251. https://doi.org/10.1016/0169-5347(96)10031-8

Zhang, H., Li, D., Zhong, X., Xiong, Y. 2023. Assessment of ecological damage from illegal fishing and judicial practice for damage compensation. Ocean and Coastal Management 246:106909. https://doi.org/10.1016/j.ocecoaman.2023.106909

Zhang, J.B., Hanner, R. 2011. DNA barcoding is a useful tool for the identification of marine fishes from Japan. Biochemical Systematics and Ecology 39:31–42. https://doi.org/10.1016/j.bse.2010.12.017

Supplementary Table 1. Morphological and meristic characteristics of *Acanthurus mata* and *Ephippus orbis*.

| Characters | *Acanthurus mata* | *Ephippus orbis* |
|---|---|---|
| Total length (TL) | 23 cm | 13.7 cm |
| Standard length (SL) | 18.5 cm | 11.4 cm |
| Morphometric measurements, % Total length | | |
| Fork length (*FL*) | 90.87 | 100 |
| Pelvic fin length | 4.35 | 7.3 |
| Anal fin length | 44.35 | 16.06 |
| Dorsal fin length | 58.7 | 48.91 |
| Caudal fin length | 19.57 | 15.33 |
| Caudal height | 14.35 | 21.17 |
| Head length (HL) | 19.13 | 29.92 |
| Pre-dorsal length | 17.82 | 30.66 |
| Pre-anal length | 35.22 | 53.29 |
| Pre-pectoral length | 18.7 | 25.55 |
| Pre-pelvic length | 25.22 | 18.98 |
| Body depth | 36.08 | 65.69 |
| % of Head length | | |
| Eye diameter | 26.13 | 34.15 |
| Snout length | 6.09 | 8.03 |
| Pre-orbital length | 31.81 | 24.39 |
| Meristic counts, Number | | |
| Dorsal fin (Spine+ Soft Rays) | IX+26 | IX+19 |
| Pectoral fin (Soft Rays) | 17 | 18 |
| Anal fin (Spine+ Soft Rays) | III+24 | III+16 |
| Pelvic fin (Spine+ Soft Rays) | I+5 | I+5 |