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SPECIES DOCUMENTATION OF FRESHWATER FISH FROM BANGKA-BELITUNG ISLANDS BASED ON COI DNA BARCODES

DOKUMENTASI SPESIES IKAN AIR TAWAR DARI PULAU BANGKA-BELITUNG BERDASARKAN BARCODE DNA COI

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Abstract

Bangka Belitung Islands are part of the biogeographic region of the Sunda Shelf (Sundaland), which is recognized for its high freshwater biodiversity. Local freshwater fishes are species that inhabit riverine ecosystems across Bangka Island. However, the persistence of these fishes is increasingly threatened by habitat degradation, land-use conversion, and the impacts of open-pit tin mining. Despite escalating environmental pressures, comprehensive documentation of freshwater fish species from Bangka Island—particularly those employing molecular approaches—remains extremely limited. This study aimed to document local freshwater fish species from Bangka Island using a DNA barcoding approach based on the mitochondrial Cytochrome C Oxidase Subunit I (COI) gene. Sampling was conducted through exploratory surveys and random sampling across diverse freshwater habitats on Bangka Island, while molecular analyses were carried out in 2024. The analytical procedures included genomic DNA extraction, PCR amplification of the COI gene, assessment of amplification quality, and DNA sequencing to obtain barcode sequences. Species identification was performed by comparing the obtained sequences using BLAST analysis against the NCBI GenBank database. All validated COI sequences were subsequently submitted to GenBank to enrich publicly accessible molecular reference data. The results of this study provide molecular documentation of several local freshwater fish taxa, including *Betta burdigala, Encheloclarias tapeinopterus, Paedocypris progenetica, Aperioptus pictorius, Barbodes lateristriga, Glyptothorax robustus, Betta foerschi, Betta edithae,* and *Betta uberis*. This study represents the first integrated molecular inventory of local freshwater fishes from Bangka Island and serves as a critical foundation for future taxonomic, phylogenetic, and biodiversity assessments within the Sundaland region. The generated DNA barcoding data are expected to enhance species identification accuracy and support sustainable documentation and conservation of freshwater fish diversity.

Keyword: DNA barcoding; Freshwater fishes; Species documentation; COI gene; Sundaland ; Bangka Island

Abstrak

Pulau Bangka-Belitung merupakan bagian dari kawasan biogeografi Paparan Sunda (*Sundaland*) yang dikenal memiliki keanekaragaman hayati air tawar yang tinggi. Ikan lokal air tawar merupakan spesies yang mendiami ekosistem sungai di Pulau Bangka. Namun demikian, keberadaan ikan-ikan tersebut semakin terancam oleh degradasi habitat, alih fungsi lahan, serta dampak penambangan timah terbuka. Meskipun tekanan lingkungan terus meningkat, pendokumentasian spesies ikan air tawar di Pulau Bangka, khususnya berbasis pendekatan molekuler, masih sangat terbatas. Penelitian ini bertujuan untuk mendokumentasikan spesies ikan lokal air tawar di Pulau Bangka menggunakan pendekatan DNA barcoding berdasarkan gen mitokondria Cytochrome C Oxidase Subunit I (COI). Pengambilan sampel dilakukan melalui eksplorasi dan random sampling pada berbagai habitat perairan tawar di Pulau Bangka, sedangkan analisis molekuler dilaksanakan pada tahun 2024. Tahapan analisis meliputi ekstraksi DNA genom, amplifikasi gen COI menggunakan PCR, evaluasi kualitas hasil amplifikasi, serta sekuensing untuk memperoleh sekuens DNA barcoding. Identifikasi spesies dilakukan melalui perbandingan sekuens menggunakan analisis BLAST terhadap basis data NCBI GenBank. Seluruh sekuens COI yang tervalidasi selanjutnya didaftarkan ke GenBank untuk memperkaya referensi molekuler yang tersedia secara publik. Hasil penelitian ini menghasilkan dokumentasi molekuler beberapa taksa ikan air tawar lokal, antara lain *Betta burdigala, Encheloclarias tapeinopterus, Paedocypris progenetica, Aperioptus pictorius, Barbodes lateristriga, Glyptothorax robustus, Betta foerschi, Betta edithae*, dan *Betta uberis.* Studi ini menyediakan inventaris molekuler terintegrasi pertama untuk ikan air tawar lokal Pulau Bangka dan menjadi dasar penting bagi kajian taksonomi, filogenetik, serta penilaian keanekaragaman hayati di kawasan Sundaland. Data DNA barcoding yang dihasilkan diharapkan dapat meningkatkan akurasi identifikasi spesies dan mendukung upaya pendokumentasian keanekaragaman ikan air tawar secara berkelanjutan.

Kata Kunci: DNA barcoding; ikan air tawar; dokumentasi spesies; cytochrome c oxidase subunit I (COI); Sundaland; Pulau Bangka

INTRODUCTION

The Bangka–Belitung Islands are part of the Sunda Shelf (Sundaland), a region widely recognized for its exceptionally high biodiversity (Kurniawan *et al.,* 2025; Cheng *et al.,* 2024; De Bruyn *et al.,* 2024). Despite long-term geological fragmentation, freshwater fauna in this region continue to exhibit strong biogeographic connectivity and clear signatures of shared evolutionary history (Kurniawan *et al.,* 2022; Pramono *et al.,* 2025). However, over the past few decades, intensive anthropogenic pressures—particularly habitat degradation, land-use change, pollution, and open-pit tin mining—have driven widespread ecosystem fragmentation, increased sedimentation, and substantial deterioration of freshwater quality across the Bangka–Belitung Islands (Valen *et al.,* 2025). The cumulative impacts of these disturbances have resulted in marked declines in freshwater fish populations, including endemic and locally restricted species, thereby elevating the risk of local extinction for several taxa (Syarif *et al.,* 2025a). These conditions underscore the urgent need for biodiversity documentation, science-based conservation status assessments, and more integrated freshwater management strategies to prevent further losses of freshwater fish diversity on Bangka Island (Nazran *et al.,* 2025).

Rapid declines in endemic and local freshwater fish diversity may substantially impair ecosystem functioning, given the pivotal roles of fishes in trophic interactions, nutrient cycling, and energy flow within freshwater ecosystems (Britton, 2023). Consequently, effective conservation and management efforts depend on accurate and up-to-date information on species composition and distribution (Chakona *et al.,* 2022). However, species identification based solely on morphological characters often encounters significant limitations, particularly in the case of cryptic species, juvenile individuals, or specimens with damaged diagnostic features. These challenges highlight the necessity of reliable alternative approaches for comprehensive species documentation (Chac *et al.,* 2023).

DNA barcoding is a molecular approach that employs short, standardized DNA sequences to enable accurate species discrimination (Lindiatika *et al.,* 2026). The mitochondrial cytochrome c oxidase subunit I (COI) gene has been widely adopted as a universal marker for animal species identification and represents a cornerstone of molecular taxonomy and biodiversity research (Valen *et al.,* 2024a). This approach facilitates rapid, consistent, and reproducible species identification, particularly for taxa that are difficult to distinguish based on morphology alone (Syarif *et al.,* 2025b).

Nevertheless, DNA barcoding–based documentation of freshwater fishes from Bangka Island remains extremely limited. Therefore, this study aims to document local freshwater fish species from Bangka Island using a COI gene–based DNA barcoding approach. The molecular data generated are expected to enhance current understanding of freshwater fish diversity on Bangka Island and to provide a robust scientific foundation for zoological and systematic studies, while also supporting biodiversity documentation efforts that are directly relevant to freshwater conservation.

MATERIALS AND METHODS

The study was conducted on Bangka Island, Bangka Belitung Islands, Indonesia. Fish sampling was carried out through exploratory surveys across diverse freshwater habitats on Bangka Island. Molecular analyses, including DNA extraction, PCR amplification, electrophoretic visualization, and sequencing, were conducted in 2024.

Fish specimens were collected from various freshwater locations on Bangka Island using environmentally friendly fishing gear. For molecular analysis, approximately 20–50 mg of tissue was excised from the right pectoral fin or white muscle using sterile scissors and forceps to minimize cross-contamination. Tissue samples were preserved in 96% ethanol, transported at 4 °C, and stored at −20 °C until DNA extraction.

Genomic DNA was extracted from preserved tissues using a commercial DNA extraction kit (gSYNC™ DNA Extraction Kit; Geneaid) following the manufacturer's protocol. PCR amplification of the mitochondrial COI gene fragment was performed using primers FISH-F1 (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FISH-R1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (Ward *et al.,* 2005). PCR reactions were prepared using MyTaq HS Red Mix 2× and conducted under the following thermal cycling conditions: initial denaturation at 94 °C for 3 min; 38 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 60 s; followed by a final extension at 72 °C for 2 min (Wong *et al.,* 2015). PCR products were visualized by electrophoresis on 1% agarose gels in TBE buffer with DNA stain. Positive samples were subsequently subjected to bidirectional Sanger sequencing to obtain COI nucleotide sequences.

Sequence were aligned using the ClustalW algorithm implemented in MEGA version X (Kumar *et al.,* 2018). Species identities were confirmed by comparing sequences against reference data in the NCBI GenBank database using BLAST analysis. Validated sequences were then submitted to GenBank to enrich regional freshwater fish molecular references. We also use some additional sequences to support our research results with the following access codes; OR192539.1; OR166098.1 and PQ675811.1. Phylogenetic analysis was conducted using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987) based on COI sequences.

RESULTS AND DISCUSSION

DNA barcoding analysis using the mitochondrial COI gene successfully identified multiple freshwater fish species from Bangka Island. All obtained COI sequences exhibited high read quality and appropriate fragment lengths for DNA barcoding-based species identification. BLAST matching against the NCBI GenBank database confirmed taxonomic identities to the species level, reflecting the relatively high freshwater fish diversity of Bangka Island.

A total of nine freshwater fish species were molecularly documented, and all COI sequences were formally deposited in the GenBank database. These species belong to several families, including Cyprinidae, Clariidae, Sisoridae, and Osphronemidae, representing the ecological and phylogenetic diversity of freshwater fishes within Sundaland. The presence of these taxa indicates that Bangka Island's freshwater ecosystems continue to support diverse fish communities, despite experiencing intense anthropogenic pressure.

Table 1 presents the list of freshwater fish species from Bangka Island along with their corresponding GenBank accession numbers for the COI sequences generated in this study. These sequences represent valuable molecular contributions that enrich Indonesia's freshwater fish DNA barcoding references, particularly for Bangka Island, where molecular data have previously been scarce.

Table 1. Daftar sekuens DNA COI ikan air tawar lokal Pulau Bangka yang terdaftar di GenBank

No	Species	Family	GenBank Accession Number	Description
1	*Betta burdigala*	Osphronemidae	OR167622.1	Bangka Island isolate
2	*Encheloclarias tapeinopterus*	Clariidae	OQ281707.1	Bangka Island isolate
3	*Barbodes lateristriga*	Cyprinidae	OQ256748.1	Bangka Island isolate
4	*Glyptothorax robustus*	Sisoridae	PQ675810.1	Bangka Island isolate
5	*Paedocypris progenetica*	Cyprinidae	PV833937.1	Bangka Island isolate
6	*Aperioptus pictorius*	Eleotridae	OR144414.1	Bangka Island isolate
7	*Betta foerschi*	Osphronemidae	OR192539.1	Belitung Island isolate
8	*Betta edithae*	Osphronemidae	OR166098.1	Belitung Island isolate
9	*Betta uberis*	Osphronemidae	PQ675811.1	Belitung Island isolate

The COI-based DNA barcoding approach proved effective for rapid and accurate documentation of freshwater fish species (Syarif *et al.,* 2023a). This method is particularly useful for species that are difficult to distinguish morphologically, juvenile individuals, or specimens with incomplete diagnostic characters (Antil *et al.,* 2023). DNA barcoding also enables standardized species identification that can be replicated by other researchers (Yang *et al.,* 2018).

The availability of Bangka-Belitung Islands freshwater fish COI sequences in the GenBank database increases the scientific value of this study and provides an important reference for future taxonomic, phylogenetic, and biodiversity research (Syarif *et al.,* 2023b). The generated molecular dataset can serve as a baseline for monitoring changes in species composition, evaluating conservation status, and developing freshwater fish management strategies (Radinger *et al.,* 2019) for Bangka Island.

Reconstruction of the evolutionary history of Bangka–Belitung freshwater fishes using the Neighbor-Joining (NJ) method based on mitochondrial COI sequences produced a phylogenetic tree consistent with taxonomic classifications at the genus and family levels

(Figure 1). Bootstrap values at several major nodes indicated strong statistical support, confirming the stability of the inferred tree topology.

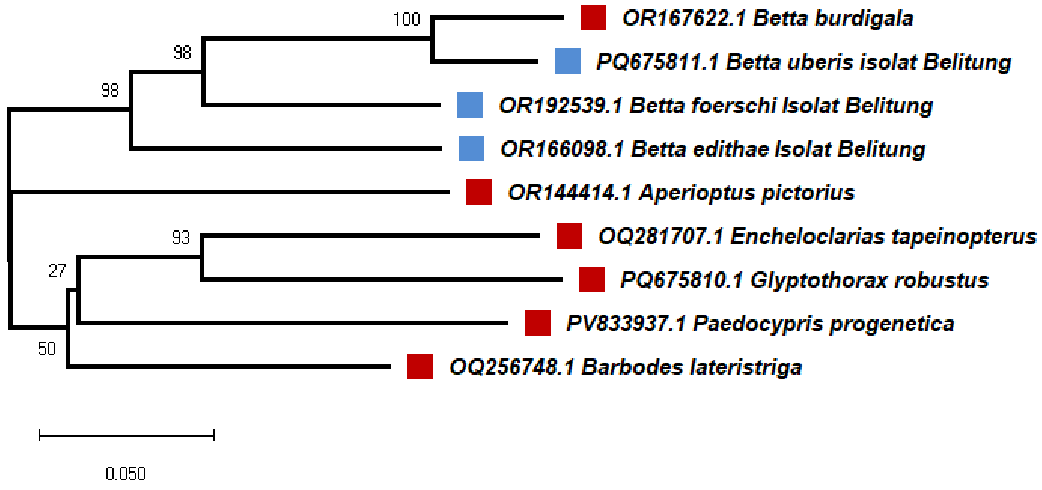

Figure 1. Phylogenetic tree of freshwater fishes from Bangka–Belitung.

However, the genus Betta formed a well-supported monophyletic clade. Bangka Island isolates (*Betta burdigala*) were closely related to Belitung Island isolates (*Betta uberis, Betta foerschi,* and *Betta edithae*) but occupied distinct branches within the clade. This pattern indicates close genetic relationships among Betta species in the Bangka–Belitung region while suggesting genetic differentiation likely influenced by inter-island geographic isolation (Astorga *et al.,* 2022).

Non-Betta species from Bangka Island—*Aperioptus pictorius, Encheloclarias tapeinopterus, Glyptothorax robustus, Paedocypris progenetica,* and *Barbodes lateristriga*—occupied phylogenetic positions consistent with their taxonomic affiliations. *Encheloclarias tapeinopterus* and *Glyptothorax robustus* clustered within the Siluriformes clade with relatively strong bootstrap support, reflecting stable evolutionary relationships among catfishes (Valen *et al.,* 2024b).

Meanwhile, *Paedocypris progenetica* and *Barbodes lateristriga* were positioned within the Cyprinidae clade but on distinct branches, indicating evolutionary divergence among genera within the family. Overall, phylogenetic structure was driven more strongly by evolutionary relationships than by geographic origin alone (Vamosi *et al.,* 2009).

The distribution of Bangka (red squares) and Belitung (blue squares) isolates on the phylogenetic tree indicates that specimens from the two islands do not form geographically exclusive clades. This pattern suggests strong biogeographic connectivity between Bangka and Belitung Islands (Syarif *et al.,* 2025b), likely linked to historical land connectivity during the Sunda Shelf period (Klegarth *et al.,* 2019). Nonetheless, distinct branching in several taxa indicates potential local genetic differentiation warranting further investigation.

CONCLUSION

This study clearly demonstrates that COI-based DNA barcoding is an effective and reliable approach for documenting freshwater fish species in the Bangka–Belitung Islands. Molecular identification of nine freshwater fish species across multiple families, coupled with the submission of COI sequences to the NCBI GenBank database, represents one of the earliest significant contributions toward addressing the molecular data gap for freshwater fishes in this region. Phylogenetic patterns consistent with taxonomic classification, together with close relationships between Bangka and Belitung isolates, underscore the strong biogeographic connectivity of the region within the historical context of the Sunda Shelf.

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