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Molecular evidence-based first DNA barcoding of miniature freshwater fish in Bangka Island, Indonesia

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Abstract. *Sambah AB, Valen FS, Samitra D, Yanuhar U, Kamarudin AS, Mukti AT, Wisudyawati D, Chukwuemeka OM, Hasan V. 2025. Molecular evidence-based first DNA barcoding of miniature freshwater fish in Bangka Island, Indonesia. Biodiversitas 26: 5476-5484.* *Paedocypris* spp., among the world's smallest vertebrates, inhabit Southeast Asian peat swamp ecosystems but remain taxonomically challenging due to their miniature body size and cryptic morphology. These habitats are simultaneously among the most threatened freshwater ecosystems. This study presents the first DNA barcoding assessment of *Paedocypris* from Bangka Island, Indonesia, undertaken to resolve its species identity and phylogenetic placement. Specimens were collected from blackwater peat swamps, and the mitochondrial cytochrome C Oxidase subunit I (COI) gene was sequenced. BLAST analysis revealed 96% similarity to both *Paedocypris progenetica* (AP011287.1) and *Paedocypris micromegethes* (NC_051487.1), but only 89% to *Paedocypris carbunculus* (NC_051488.1). Phylogenetic reconstruction clustered the Bangka specimens with *P. micromegethes* (bootstrap = 85) on a distinct lineage, while pairwise genetic distance analysis indicated 4.18% divergence. This exceeds conventional intraspecific thresholds in fishes, suggesting potential species-level differentiation, possibly driven by geographic isolation, ecological adaptation, or cryptic speciation. Beyond its systematic significance, this finding extends the known distribution of *Paedocypris* beyond Borneo, Sumatra, and Peninsular Malaysia, contributing to Sundaland biogeography and understanding of evolutionary patterns in miniature fishes. The conservation implications are profound: Bangka's peat swamp ecosystems are undergoing rapid degradation due to tin mining, peatland drainage, and large-scale conversion to oil palm plantations. These pressures erode the ecological integrity of blackwater habitats, threatening the persistence of *Paedocypris* and other specialized endemics. This study underscores the urgency of targeted habitat protection, ecological restoration, and sustainable land-use planning to safeguard these fragile ecosystems. Integrating molecular evidence into conservation strategies is vital not only for the survival of *Paedocypris* but also for maintaining freshwater biodiversity and ecosystem resilience across the region.

Keywords: COI, conservation, freshwater fishes, smallest fish, vertebrates

INTRODUCTION

Paedocypris is a genus of tiny cyprinid fish discovered in highly acidic blackwater peat swamps in Southeast Asia. It includes some of the smallest known vertebrates. (Kottelat et al. 2006; Cai et al. 2018). For instance, *Paedocypris progenetica* (Kottelat et al. 2006) (described from Sumatra) has mature females as small as 0.0079 m, making it the smallest recorded fish (Britz and Kottelat 2008; Lumbantobing 2019b). These miniature fish exhibit remarkable paedomorphic traits, retaining larval features such as an incomplete skull roof and larval fin-fold into adulthood (Kottelat et al. 2006). Uniquely, males possess modified pelvic fins with muscular pads that likely function as clasping devices during mating, indicating an unusual reproductive mode (Britz and Kottelat 2008; Lumbantobing 2020). *Paedocypris* species are adapted to the harsh conditions of acidic, dissolved oxygen peat swamps. *Paedocypris* are valuable indicators of ecosystem health. Their survival is threatened by ongoing habitat destruction in Southeast Asia (Britz and Kottelat 2008; Lumbantobing 2019a). The conservation of *Paedocypris* is urgent to protect their unique evolutionary traits and the biodiversity of peat-swamp habitats.

Bangka Island, located off the southeastern coast of Sumatra, Indonesia, is part of the Sundaland region and is known for its rich freshwater biodiversity (Hasan et al. 2023a, 2024a; Nazran et al. 2025; Pramono et al. 2025). The island harbors approximately seven endemic fish species in its rivers and swamps (Conway et al. 2011; Lumbantobing

2019c). Many of these species belong to the Cyprinidae family, reflecting the regional dominance of cyprinids in tropical Asian freshwater ecosystems (Kurniawan et al. 2021, 2022). *Paedocypris* was recently reported in Bangka, possibly representing a new species or a range extension of *P. progenetica*. However, their tiny body size and simplified anatomical features make morphological identification highly challenging. In this context, molecular approaches such as DNA barcoding provide a powerful tool to clarify taxonomic identity, resolve cryptic diversity, and establish reliable baselines that are critical for setting conservation priorities (Kress et al. 2015). Meanwhile, Bangka's freshwater habitats face severe threats from illegal tin mining and land-use changes (Khodijah et al. 2019), leading to pollution and sedimentation that endanger sensitive swamp fishes (Mustikasari et al. 2022; Valen et al. 2025; Syarif et al. 2025a). In this context, clarifying whether Bangka *Paedocypris* represents a known species or an endemic lineage is crucial for establishing conservation priorities.

DNA barcoding has emerged as a powerful tool for species identification and biodiversity assessments. It involves the sequencing of a standard genetic marker in animals, typically a segment of the mitochondrial cytochrome C Oxidase I (COI) gene, to serve as a "barcode" for the species (Chac et al. 2023). Hebert et al. (2003) demonstrated that COI barcodes could reliably assign specimens to the correct species or higher taxonomic groups, thereby forming the core of a global bioidentification system. DNA barcoding is highly effective in fishes, with a clear barcoding gap; intra-species COI divergence averages 0.3-0.4%, while inter-species divergence is around 10%, enabling reliable species identification (Hou et al. 2018; Modeel et al. 2023). Barcoding is especially useful for cryptic or morphologically indistinct organisms and has been applied to detect mislabeled specimens and cryptic species and to assist in cataloging fish diversity (Benson et al. 2013; Muhala et al. 2024).

Although *Paedocypris* is of great interest, no DNA barcode data were available for the Bangka Island population before this study. This represents a knowledge gap in the Bangka biodiversity record. It was unclear whether the Bangka *Paedocypris* belonged to the known Sumatran species (*P. progenetica*) or if they constituted a distinct evolutionary lineage, possibly unique to the island. To address this gap, we conducted the first DNA barcoding study of *Paedocypris* from Bangka Island. The objectives were: (i) to extract and sequence the COI barcode region from *Paedocypris* specimens collected in Bangka's peat swamp habitat, (ii) to compare the obtained sequences with reference sequences of known *Paedocypris* species (e.g., *P. progenetica*, *P. micromegethes* (Kottelat et al. 2006), and others) to determine genetic identity or divergence, and (iii) to analyze phylogenetic relationships and genetic distances to infer the taxonomic and evolutionary status of the Bangka population.

MATERIALS AND METHODS

Sampling site and fish samples collection

Paedocypris specimens were collected from a peat swamp stream in Tugang Village, Bangka Island, Indonesia (1°45'53.0"S 105°26'21.0"E) (Figure 1). A total of 10 diminutive cyprinids were captured and morphologically identified as putative *Paedocypris* (Figure 2). To ensure high-quality DNA preservation, the fish were immediately euthanized and stored in 96% ethanol onsite (Valen et al. 2023a). All necessary research permits were secured prior to fieldwork, including the official research permit No. 503/12/KESBANGPOL/DPMPTSP/2025, and sampling procedures strictly adhered to established ethical guidelines for the use of fish in scientific research.

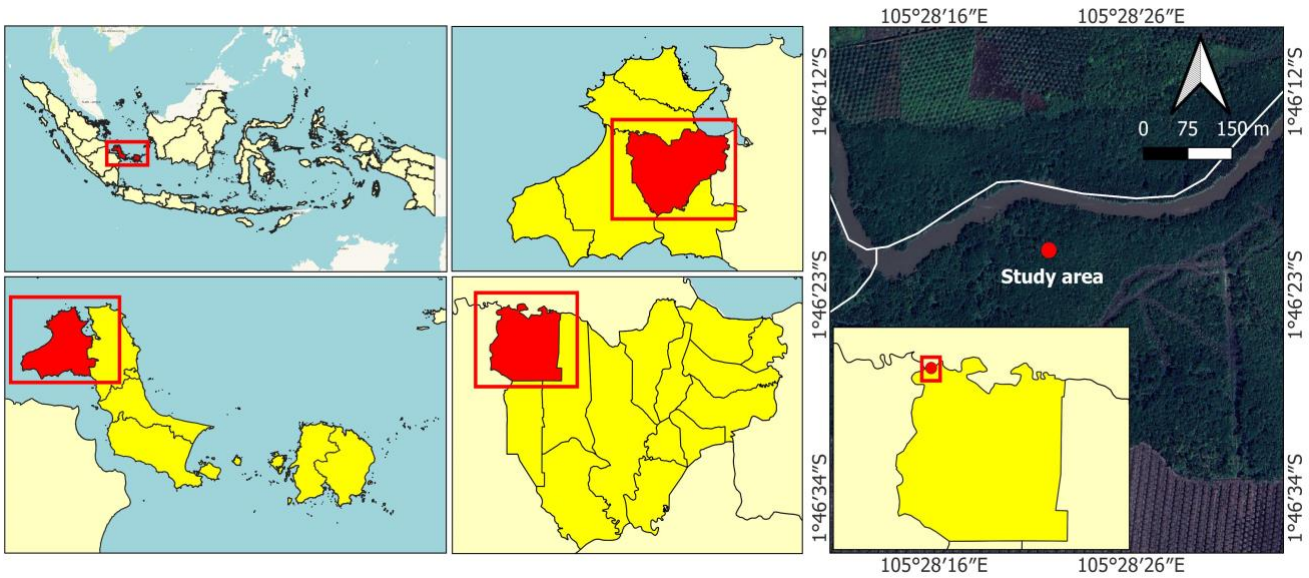

Figure 1. Map of the study area in Tugang Village, Bangka Island, Indonesia

Figure 2. *Paedocypris* sp. specimen from Bangka Island, Indonesia

DNA extraction

Genomic DNA was isolated using the Geneaid™ DNA Extraction Kit, following four primary steps: Cell lysis, DNA binding, washing, and elution. Approximately 20-25 mg of tissue samples was placed into 1.5 mL microcentrifuge tubes and mixed with 200 µL of lysis buffer. The samples were homogenized using a micropestle until complete tissue disruption was achieved. Subsequently, 20 µL of Proteinase K was added, and homogenization was continued. The tubes were incubated at 60°C for 30-45 minutes to facilitate complete cell lysis and protein degradation. After incubation, samples were centrifuged at 12,000 rpm for 2 minutes, and the resulting supernatant was transferred to fresh microcentrifuge tubes. Then, 200 µL of GSB buffer was added, and the mixture was gently mixed by pipetting.

During the binding phase, 200 µL of absolute ethanol was added, and the solution was thoroughly mixed before being applied to a spin column. The column was centrifuged at 14,000 rpm for 1 minute, and the flow-through was discarded. For the washing step, 400 µL of W1 buffer was added to the spin column, followed by centrifugation at 14,000 rpm for 30 seconds. After discarding the flow-through, 600 µL of W2 buffer was added and centrifuged at 12,000 rpm for 30 seconds. A final centrifugation at 12,000 rpm for 3 minutes was conducted to eliminate any residual ethanol. DNA elution was achieved by placing the spin column into a new 1.5 mL tube, adding 100 µL of elution buffer, and centrifuging at 12,000 rpm for 3 minutes, followed by an additional 30-second spin. The eluate containing purified DNA was collected for downstream applications, including PCR amplification and sequencing.

PCR amplification processes

Polymerase Chain Reaction (PCR) amplification was performed targeting the mitochondrial cytochrome C Oxidase subunit I (COI) gene using species-specific primers. Prior to use, primers were diluted by combining 10 µL of stock solution with 90 µL of double-distilled water (ddH_2O) to obtain working concentrations. The primer pair utilized included FishF2 (5′-TCGACTAATCATAAAGATATCGGCAC-3′) and FishR2 (5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′), initially developed by Ward et al. (2005), was selected due to its demonstrated universality across teleosts, high amplification efficiency in minute or degraded tissue samples, and reliable recovery of the standard DNA barcoding region. Their broad validation and specificity for fish taxa ensured optimal performance for *Paedocypris*, a miniature cyprinid with limited DNA yield, while reducing the risk of non-target amplification.

Each PCR reaction comprised a total volume of 50 µL, which included 25 µL of PCR master mix, 15 µL of nuclease-free water, 5 µL of template DNA, and 2.5 µL of each primer. Amplification was carried out in a thermal cycler with the following cycling parameters: an initial denaturation at 94°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 90 seconds, and extension at 72°C for 1 minute. These conditions were optimized to ensure efficient amplification of the COI target fragment. The resulting amplicons were subsequently subjected to electrophoretic analysis and sequencing (Valen et al. 2024a).

Gel electrophoresis

The success of PCR amplification was evaluated via agarose gel electrophoresis. 1% agarose gel was prepared in 1× TAE buffer (Tris-Acetate-EDTA), and 5 µL of PCR product, along with 5 µL of a 100 bp DNA ladder, was loaded into the wells. Electrophoresis was performed at a constant voltage of 100 V for 30 minutes, allowing DNA fragments to separate based on size (Syarif et al. 2023). After electrophoresis, DNA bands were visualized using a UV transilluminator. Successful amplification was confirmed by the presence of a distinct DNA band of approximately 650 base pairs corresponding to the expected size of the COI gene fragment, relative to the molecular weight marker.

Sequence processing and alignment

The amplicons were purified using a Gel/PCR cleanup kit and sequenced by Sanger sequencing in both forward and reverse directions by a commercial sequencing service (1st BASE Laboratories, Malaysia). Sequencing was performed using the BigDye Terminator v3.1 chemistry on an ABI Prism 3730xl Genetic Analyzer. The Raw sequencing chromatograms were examined and edited using Chromas and Geneious software to resolve ambiguous base calls. One High-quality consensus sequence for the COI gene was obtained (one sample was amplified or sequenced). The consensus sequences (~401 bp after primer trimming) were submitted to GenBank with accession number PV833937.1. The collected sequences were aligned with COI reference sequences of known *Paedocypris* species from GenBank, including *Paedocypris progenetica*, *Paedocypris micromegethes*, and *Paedocypris carbunculus*. Alignments were performed using the ClustalW algorithm in MEGA X with default settings. Manual inspection ensured proper reading frames, confirming that all sequences were functional mitochondrial COI without indels or stop codons (Benson et al. 2013).

Phylogenetic analysis and genetic distance

Phylogenetic relationships were inferred using both distance-based and model-based methods in MEGA X (Kumar et al. 2018). A Neighbor-Joining (NJ) tree was built using Kimura 2-parameter distances with 1000 bootstrap replicates to assess branch support. To confirm tree topology, a Maximum Likelihood (ML) analysis was also performed using the Tamura-Nei model, selected as the

best fit. The resulting trees were compared for consistency. Genetic divergence was analyzed using pairwise K2P distances, focusing on average COI divergence between Bangka *Paedocypris* and reference species, and the maximum divergence within Bangka samples (Kumar et al. 2018). In particular, the average COI divergence was measured between the Bangka *Paedocypris* sequences and each reference species, as well as the maximum intragroup divergence among the Bangka samples. All analyses were performed using MEGA X, and the results were recorded for interpretation in the context of species identification and taxonomy (Kumar et al. 2018).

RESULTS AND DISCUSSION

DNA barcode sequence characteristics

High-quality COI barcode sequences (~401 bp) of *Paedocypris* specimens from Bangka Island were obtained (Table 1). Furthermore, the DNA barcode sequence has been submitted to GenBank with access code PV833937.1. The sequences had a high A+T content (approximately 55%, typical for fish mitochondrial DNA) and showed no insertions, deletions, or stop codons, indicating that they were true mitochondrial COI sequences from fish and not nuclear pseudogenes (Weigand et al. 2019).

DNA barcoding is a powerful molecular tool for species identification (Antil et al. 2023), particularly for cryptic and miniature species like the *Paedocypris* genus. Results show a successful sequencing of a mitochondrial DNA fragment from a miniature freshwater fish collected from Bangka Island, and compared it with existing sequences in the GenBank database using BLAST (Basic Local Alignment Search Tool) (Dad et al. 2025). The BLAST analysis revealed that our specimen shared 96% similarity with both *Paedocypris progenetica* (Accession: AP011287.1) and *Paedocypris micromegethes* (Accession: NC_051487.1), with 100% query coverage, suggesting a close genetic relationship (Table 2). Lower similarity (89%) was observed with *Paedocypris carbunculus* (Britz and Kottelat 2008) (Accession: NC_051488.1), indicating a more distant genetic affinity (Valen et al. 2022).

Although the 96% genetic similarity is relatively high, it falls below the 98-99% threshold commonly used for definitive species identification in DNA barcoding studies (Hebert et al. 2003; Ratnasingham and Hebert 2013; Valen et al. 2024b). This suggests that the specimen may represent either intraspecific variation or a potentially new species. It could be a local population or subspecies of *P. progenetica* or *P. micromegethes*, exhibiting minor genetic divergence due to geographic isolation on Bangka Island. Alternatively, the observed genetic divergence may indicate an undescribed species closely related to *P. progenetica* and *P. micromegethes* but with distinct genetic variations. To confirm its taxonomic status, further morphological analyses and additional genetic markers (e.g., the complete mitochondrial genome and nuclear genes) are required (Wang et al. 2020; Liao et al. 2024; Li et al. 2025).

Phylogenetic placement of *Paedocypris* from Bangka Island

The phylogenetic tree (Figure 3) constructed from molecular data provides insights into the evolutionary relationships of *Paedocypris* specimens from Bangka Island, revealing three major clusters. *P. carbunculus* (Borneo clade) formed a distinct and well-supported group with a bootstrap value of 100, indicating strong genetic separation. *P. micromegethes* (Borneo Clade) included the Bangka specimen, which formed a separate branch closely related to *P. micromegethes*, with a bootstrap support value of 85, suggesting a potential genetic connection. *P. progenetica* (Selangor, Malaysia clade) was well defined and separate, with strong bootstrap support (100), and the Bangka specimens did not cluster directly with this group, indicating a more distant genetic relationship.

Table 1. DNA barcoding of *Paedocypris* sp. from Bangka Island, Indonesia

DNA barcoding of *Paedocypris* sp. (GenBank access code PV833937.1)
GGTGCATGAGCTGGCATAGTTGGAACTGCTTTAAGTCTACTAATCCGAGCTGAATTGAGCCAACCCGGGT
CTCTTTTAGGAGACGACCAAATTTACAATGTTATCGTCACTGCACACGCATTTGTTATAATTTTCTTTAT
AGTGATACCAATTTTAATCGGTGGCTTTGGAAATTGACTCCTGCCAATAATAATCGGAGCACCTGATATG
GCTTTCCCCCGAATAAATAATATAAGCTTTTGATTACTACCCCCATCTTTCCTGCTCTTACTTGCATCTT
CTGGGGTAGAGGCTGGAGCAGGAACTGGCTGAACAGTCTACCCACCACTTGCTGGAAACTTAGCCCATGC
AGGAGCGTCTGTAGACCTAACAATTTTTTCACTCCACCTTGCAGGTGTTTC

Table 2. Genetic similarity and species identification of *Paedocypris* sp. from Bangka Island based on COI barcoding

Specimen	**Query coverage (%)**	**Similarity genbank (%)**	**Species outcome**	**Accession number (GenBank)**
Paedocypris sp. (Bangka Island)	100	96	*Paedocypris progenetica*	AP011287.1
(PV833937.1)	100	96	*Paedocypris micromegethes*	NC_051487.1
	99	89	*Paedocypris carbunculus*	NC_051488.1

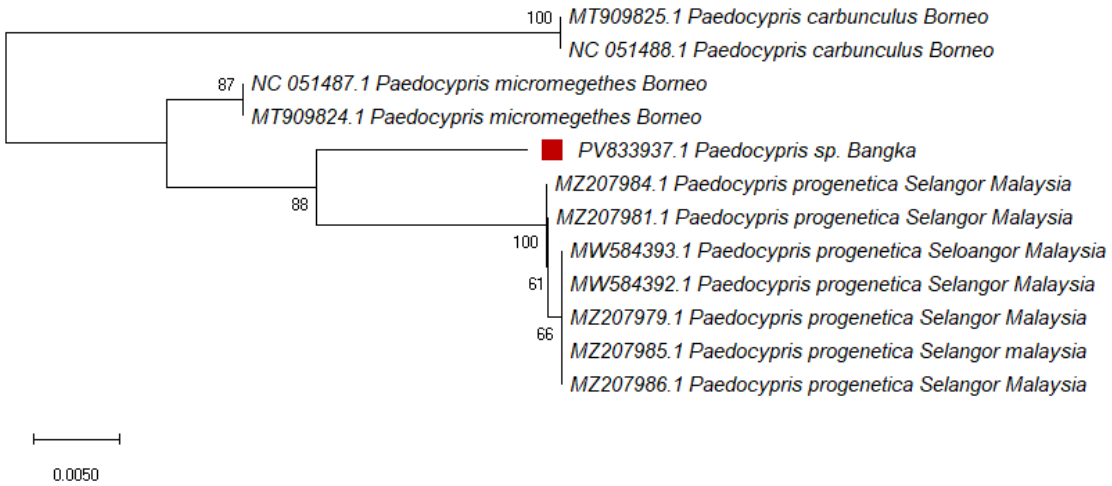

Figure 3. Neighbor-Joining (NJ) tree using Kimura 2-Parameter phylogenetic tree based on COI sequences showing the relationship of *Paedocypris* sp. from Bangka (highlighted in red) with congeners (*Paedocypris micromegethes, Paedocypris carbunculus,* and *Paedocypris progenetica*). Numbers at nodes represent bootstrap support values (1,000 replicates). Scale bar indicates the number of substitutions per site

The placement of the *Paedocypris* Bangka specimen in proximity to *P. micromegethes* rather than *P. progenetica* suggests that it shares a more recent common ancestor with *P. micromegethes* (found in Borneo). However, its independent branching suggests genetic differentiation that may have arisen from several factors: geographically, Bangka Island is separated from Borneo by the Karimata Strait, potentially leading to allopatric speciation due to restricted gene flow and the accumulation of genetic differences over time; microhabitat adaptation (Valen et al. 2023b; Syarif et al. 2025b), as *Paedocypris* species are highly specialized in fragmented peat swamp habitats with varying water chemistry, which may have driven local adaptation in the Bangka population; and the possibility of a cryptic species, since although the Bangka specimen is closely related to *P. micromegethes*, its distinct phylogenetic position indicates it could represent a unique genetic lineage. Additional morphological and genetic studies are required to confirm the taxonomic status of this species (Valen et al. 2024c).

However, the BLAST results supported the phylogenetic findings that the Bangka specimen showed 96% similarity with both *P. progenetica* and *P. micromegethes*. However, the phylogenetic analysis placed it closer to *P. micromegethes*. The lower similarity (89%) to *P. carbunculus* was consistent with its distant placement in the tree.

Moreover, the *Paedocypris* Bangka specimen was genetically distinct but most closely related to *Paedocypris micromegethes* from Borneo. Its unique position suggests a potential taxonomic novelty, although further studies are required. To confirm the species status, future research should include: (i) Comprehensive Morphological Comparison with *P. micromegethes* and *P. progenetica* (Britz et al. 2014). (ii) Multigene phylogenetic analysis using nuclear markers for higher resolution (Zhang et al. 2023; Sun et al. 2025). (iii) Ecological Surveys to understand habitat and population dynamics (Hasan et al. 2023c). Furthermore, this study highlights the importance of molecular tools in biodiversity research and underscores the need for the conservation of Bangka's freshwater habitats, as such unique species are often vulnerable to environmental changes (Cai et al. 2018).

Genetic distance analysis

Table 3 represents the pairwise genetic distances (p-distances) between the Bangka specimen (*Paedocypris sp._Bangka*) and the three other *Paedocypris* species from different geographic regions (*P. progenetica*, *P. micromegethes*, and *P. carbunculus*). These values indicate the level of genetic divergence, with higher values indicating greater genetic differences.

Closest relationships: *Paedocypris* sp. Bangka and *P. micromegethes* (Borneo): The genetic distance between the Bangka specimen and *P. micromegethes* was 0.0418, indicating relatively low genetic divergence. Genetic distance analysis revealed a 4.18% divergence between the Bangka *Paedocypris* and *P. micromegethes*. This level of divergence is well above the commonly accepted threshold for species-level differentiation in fishes (typically >2-3% for COI (Hebert et al. 2003; Ward et al. 2009), strongly suggesting that the Bangka population may represent a distinct evolutionary lineage rather than conspecific variation. This supports the results of the phylogenetic tree, in which the Bangka specimens clustered closely with *P. micromegethes*. This suggests that the Bangka population may be a variant or potentially distinct, but closely related species (Kasayev and Arisuryanti 2022).

Moderate divergence: *Paedocypris* sp. Bangka and *P. progenetica* (Selangor, Malaysia); a genetic distance of 0.0529, which is slightly higher than that of *P. micromegethes*, indicating a more distant relationship and confirming that the Bangka specimen was genetically closer to *P. micromegethes* than to *P. progenetica*.

High divergence: *Paedocypris* sp. *Bangka* and *P. carbunculus* (Borneo); the highest genetic distance (0.1258) was observed between the Bangka specimen and *P. carbunculus*, indicating that they were genetically distinct. This is consistent with the phylogenetic tree, in which *P. carbunculus* formed a separate well-supported clade.

However, the Bangka *Paedocypris* population shows strong evidence of genetic distinctiveness, raising the possibility that it represents a new or divergent species. The observed genetic distance of 0.0418 (4.18%) from *P. micromegethes* is particularly noteworthy, as DNA barcoding studies have demonstrated that a divergence above 2-3% in mitochondrial COI is typically indicative of species-level differentiation in fishes (Hebert et al. 2003; Ward et al. 2009; Insani et al. 2022; Mwita and Chuhila 2023). This level of divergence, well beyond the intraspecific threshold, suggests that the Bangka lineage may either represent a geographically isolated population of *P. micromegethes* or a previously undescribed species.

The close evolutionary affinity between the Bangka specimens and *P. micromegethes* indicates a shared ancestry, yet the 4.18% divergence implies ongoing or incipient speciation. Genetic differentiation in this lineage may have been promoted by historical and contemporary geographic barriers separating Bangka Island, Borneo, and Peninsular Malaysia. Over evolutionary timescales, restricted gene flow among isolated peat swamp populations could have facilitated divergence, consistent with allopatric processes driving cryptic speciation in Sundaland freshwater taxa (de Bruyn et al. 2014; Wang et al. 2019; Liu et al. 2022). Given this evidence, the Bangka *Paedocypris* is best regarded as a candidate cryptic lineage or Evolutionarily Significant Unit (ESU).

Further research is required to validate the taxonomic status of the Bangka *Paedocypris*. This should include comprehensive morphological and osteological comparisons with *P. micromegethes* and *P. progenetica*, multilocus or nuclear marker analyses to refine evolutionary relationships, and ecological surveys to assess habitat specialization and population structure. Such integrative approaches are critical not only for resolving taxonomy but also for informing conservation priorities.

The present study is constrained by its reliance on a single mitochondrial marker (COI). Although COI remains the global standard for DNA barcoding and is highly effective for detecting genetic divergence, mitochondrial data provide only a uniparental perspective. Introgression, selective sweeps, or incomplete lineage sorting may confound them. Consequently, taxonomic inferences based solely on COI must be interpreted with caution. A more robust framework will require the incorporation of multilocus and nuclear genomic datasets (e.g., RAG1, S7 intron, microsatellites, or genome-wide SNPs), complemented by detailed morphological assessments and ecological data. These integrative analyses will be essential to determine whether the Bangka population represents a cryptic species or a distinct evolutionary unit, while simultaneously guiding conservation action.

Ultimately, this study underscores the critical role of molecular evidence in uncovering hidden biodiversity within poorly studied freshwater ecosystems. Protecting the Bangka peat swamps, ecosystems under acute threat from tin mining, peatland drainage, and oil palm expansion, is fundamental to ensuring the persistence of this unique lineage and the broader biodiversity they support.

Ultimately, this study underscores the critical role of molecular evidence in uncovering hidden biodiversity within poorly studied freshwater ecosystems. Protecting the Bangka peat swamps-ecosystems under acute threat from tin mining, peatland drainage, and oil palm expansion- is fundamental to ensuring the persistence of this unique lineage and the broader biodiversity they support.

Utility of DNA barcoding

This study demonstrates the utility of DNA barcoding as a tool for identifying species under challenging groups. Traditional methods struggle to conclusively identify Bangka *Paedocypris* because of their tiny size and subtle diagnostic features (Hussin et al. 2023). By employing DNA barcoding, we obtained objective genetic evidence for comparison with known reference data, enabling us to place Bangka fish in the context of the known *Paedocypris* taxonomy. Apparent clustering of DNA sequences provided a means to detect divergences that were not morphologically obvious. In line with other studies in which DNA barcoding has uncovered cryptic diversity in fish (Weigand et al. 2019), our results suggest that molecular techniques are indispensable for comprehensive biodiversity assessments, especially in understudied regions, such as Bangka Island. We also highlight that building reference barcode libraries (e.g., depositing sequences of *Paedocypris* from various locations into databases, such as BOLD/GenBank) greatly facilitates such comparisons. Before this study, Bangka *Paedocypris* was difficult to classify; however, its genetic barcode is now available for future studies and monitoring. Additionally, our phylogenetic analysis contributes to the understanding of evolutionary relationships within *Paedocypris*. The topology we recovered (Borneo species branching off earlier and the Bangka+Sumatra lineages as sister groups) aligns with the idea that *Paedocypris* species diverged following the geographic fragmentation of peat swamp habitats on the Sunda Shelf (Fujimoto et al. 2019; Cheng and Faidi 2025). This is congruent with the divergence time estimates, suggesting the ancient separation of lineages corresponding to different islands (Short et al. 2022). While our data are limited to a single gene, they provide a foundation for future multigene or genomic studies to delve deeper into the evolutionary history of miniature fish.

The first DNA barcoding investigation of the world's smallest fish from Bangka Island successfully genetically characterized this population and clarified its relationship with known species. Bangka *Paedocypris* has a unique DNA signature that is closely aligned to *P. progenetica* yet distinct, highlighting the rich and potentially endemic biodiversity present in Bangka's aquatic ecosystems. These findings call for heightened attention to conserving peat swamp habitats and encourage further research on the taxonomy and ecology of *Paedocypris* and other small freshwater fishes in Indonesia.

Table 3. Pairwise genetic distances (p-distances) among *Paedocypris* variants based on COI sequences. Distances were calculated in MEGA X using uncorrected p-distances with all codon positions included and pairwise deletion of gaps

	1	2	3
Paedocypris sp._Bangka			
MZ207986.1_*P. progenetica*_Selangor Malaysia	0.0529		
NC_051487.1_*P. micromegethes* Borneo	0.0418	0.0557	
MT909825.1_*P. carbunculus* Borneo	0.1258	0.1213	0.0888

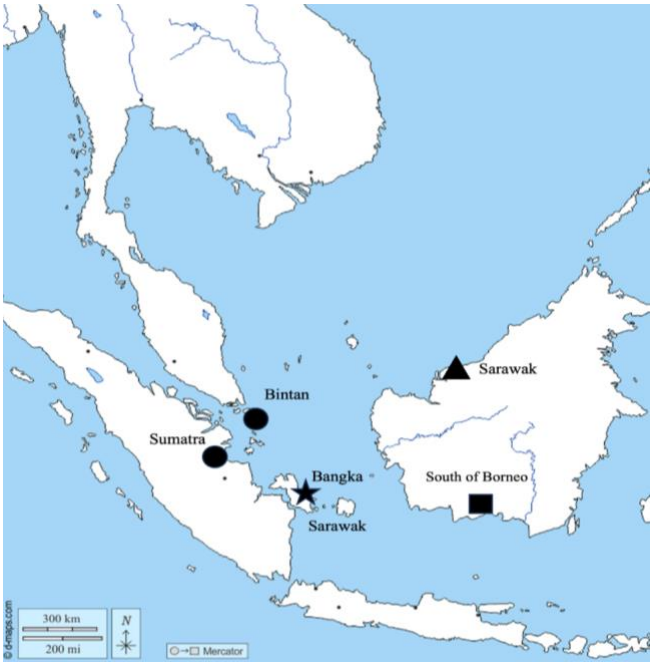

Figure 4. Distribution map of *Paedocypris* in Southeast Asia. Circles: *Paedocypris progenetica* (Lumbantobing 2019b), Triangle: *Paedocypris micromegethes* (Lumbantobing 2019a), Square: *Paedocypris carbunculus* (Lumbantobing 2020), Star: *Paedocypris* sp. Bangka (this study)

Map of distribution

The discovery of *Paedocypris* sp. on Bangka Island represents a significant extension to the known distribution of this enigmatic genus. Until now, *Paedocypris* species had only been documented in Borneo, Sumatra, and Peninsular Malaysia (Figure 4). The presence of *Paedocypris* in Bangka provides new insights into the historical biogeography and dispersal patterns of the genus, suggesting that its distribution is broader than previously understood. This also implies that ecological conditions favorable to *Paedocypris*—such as acidic, tannin-rich, low-oxygen peat swamp environments—persist in Bangka and can support these miniature fishes (Hussin et al. 2023).

This finding underscores several key biogeographic and ecological implications: (i) New population record: This is the first molecular evidence of a *Paedocypris* species in Bangka Island, extending the known range of the genus. (ii) Potential endemism: The geographical separation of Bangka from Borneo and Sumatra suggests that this population could be genetically unique due to long-term isolation. (iii) Ecological continuity: The similarity of peat swamp conditions across the region supports the habitat specialization of *Paedocypris* and may explain its persistence in Bangka despite isolation (de Bruyn et al. 2014; Berghuis et al. 2025).

Bangka Island was historically part of Sundaland, a landmass that connected Sumatra, Borneo, and the Malay Peninsula during the Pleistocene (Hasan et al. 2024). The discovery of *Paedocypris* in Bangka supports the idea that ancestral populations may have been widespread across Sundaland before sea levels rose, isolating populations on different land masses (Hasan et al. 2023b, 2024b). However, the genetic divergence observed in the Bangka population suggests that it may have been isolated from other *Paedocypris* populations for an extended period, leading to potential local adaptation or speciation.

The genetic distinctiveness of the Bangka *Paedocypris* population highlights its elevated conservation importance. Yet, its peat swamp habitat has undergone severe degradation, primarily due to water pollution from intensive tin mining and large-scale deforestation driven by oil palm expansion (Khodijah et al. 2019). These anthropogenic pressures have accelerated habitat loss, reduced water quality, and disrupted ecosystem functioning, thereby placing this lineage at considerable risk of extinction. The convergence of genetic evidence and environmental threats underscores that conservation strategies must integrate both taxonomic recognition and habitat protection.

Effective management should include the enforcement of stricter environmental regulations for tin mining, explicit requirements for peat swamp conservation in land-use policies, and careful monitoring of oil palm development to avoid further habitat conversion. Equally important are ecological surveys to characterize habitat conditions, assess population density, and monitor long-term viability of *Paedocypris* in Bangka. A formal conservation assessment is urgently needed to quantify threats and prioritize actions such as ecological restoration, designation of protected swamp reserves, and community-based stewardship (Liu et al. 2023; Valen et al. 2025. By coupling molecular evidence of genetic uniqueness with targeted management interventions, this study demonstrates that safeguarding *Paedocypris* is inseparable from broader efforts to conserve the biodiversity and ecological resilience of Southeast Asia's peat swamp ecosystems.

In conclusion, this study provides the first DNA barcode reference for *Paedocypris* from Bangka Island and thereby fills a critical gap in the molecular inventory of Sundaland's miniature freshwater fishes. Cytochrome c oxidase I sequencing placed the Bangka specimen closest to *P. micromegethes* (96% similarity), *P. progenetica* (96% similarity), and *P. carbunculus* (89% similarity). Phylogenetic reconstruction placed the Bangka specimen in a clade with *P. micromegethes* (bootstrap = 85) and *P. progenetica* (bootstrap = 92). The Bangka specimen exhibited 4.18% genetic distance (K2P) to *P. micromegethes*, 5.29% to *P. progenetica*, and a substantially greater 12.58% to P. *carbunculus*. The observed interspecific distance exceeds

commonly accepted thresholds (2-3%) for species-level differentiation in teleosts. Collectively, these data indicate that geographic isolation within Bangka's black-water peat-swamp habitats has fostered either incipient speciation or cryptic lineage divergence. These findings, which expand the known distribution of *Paedocypris* beyond Borneo, Sumatra, and Peninsular Malaysia, underscore the potential for geographic isolation in Bangka's peat swamps to drive genetic divergence. Given the ecological specialization and extreme miniaturization of *Paedocypris*, the degradation of Bangka's freshwater habitats—due to tin mining and oil palm expansion—poses a serious threat to its persistence. Future research should include: (i) comprehensive morphological comparisons with *P. micromegethes* and *P. progenetica*, (ii) multigene phylogenetic analyses using nuclear markers for higher taxonomic resolution, and (iii) ecological surveys to assess habitat conditions and population dynamics. This study highlights the importance of molecular tools in biodiversity research. It reinforces the urgency of conserving Bangka's fragile freshwater ecosystems, where unique evolutionary lineages may be lost before they are fully described.

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REFERENCES

Antil S, Abraham JS, Sripoorna S, Maurya S, Dagar J, Makhija S, Bhagat P, Gupta R, Sood U, Lal R, Toteja R. 2023. DNA barcoding, an effective tool for species identification: A review. Mol Biol Rep 50 (1): 761-775. DOI: 10.1007/s11033-022-08015-7.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. Nucleic Acids Res 41: D36-D42. DOI: 10.1093/nar/gks1195.

Berghuis HWK, van den Bergh G, van Kolfschoten T, Wibowo UP, Kurniawan I, Adhityatama S, Sutisna I, Verheijen I, Pop E, Veldkamp A, Joordens JCA. 2025. First vertebrate faunal record from submerged Sundaland: The late Middle Pleistocene, hominin-bearing fauna of the Madura Strait. Quat Environ Hum 3 (2): 100047. DOI: 10.1016/j.qeh.2024.100047.

Britz R, Kottelat M. 2008. *Paedocypris carbunculus*, a new species of miniature fish from Borneo (Teleostei: Cypriniformes: Cyprinidae). Raffles Bull Zool 56 (2): 415-422.

Britz R, Conway KW, Rüber L. 2014. Miniatures, morphology and molecules: *Paedocypris* and its phylogenetic position (Teleostei, Cypriniformes). Zool J Linnean Soc 172 (3): 556-615. DOI: 10.1111/zoj.12184.

Cai Y, Davison G, Chan L, Liong S-Y. 2018. Conservation outputs and recommendations for Nee Soon freshwater swamp forest, Singapore. Gard Bull Singapore 70: 191-217. DOI: 10.26492/gbs70(suppl.1).2018-10.

Chac LD, Thinh BB. 2023. Species identification through DNA barcoding and its applications: A review. Biol Bull 50 (6): 1143-1156. DOI: 10.1134/S106235902360229X.

Cheng S, Faidi MA. 2025. Palaeodrainages of the Sunda Shelf detailed in new maps. J Palaeogeogr 14: 186-202. DOI: 10.1016/j.jop.2024.10.001.

Conway KW, Kottelat M, Hui TH. 2011. Review of the Southeast Asian miniature cyprinid genus *Sundadanio* (Ostariophysi: Cyprinidae) with descriptions of seven new species from Indonesia and Malaysia. Ichthyol Explor Freshw 22 (3): 251-288.

Dad O, Khan MF, Rasool A, Attaullah M, Israr M, Akhtar N. 2025. Molecular phylogeny and cryptic species identification of the genus *Triplophysa* in the freshwater of Malakand division, Pakistan. Zool Anz 315: 97-104. DOI: 10.1016/j.jcz.2025.02.006.

de Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, van den Bergh G, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, von Rintelen, T. 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian Biodiversity. Syst Biol 63 (6): 879-901. DOI: 10.1093/sysbio/syu047.

Fujimoto K, Miura M, Kobayashi S, Simbolon H. 2019. Habitat evolution of a peat swamp forest and belowground carbon sequestration during the Holocene along the coastal lowland in Central Sumatra, Indonesia. Prog Earth Planet Sci 6: 41. DOI: 10.1186/s40645-019-0288-8.

Hasan V, Vieira LO, South J, Ottoni FP. 2023a. First record of *Mastacembelus notophthalmus* (Actinopterygii: Synbranchiformes: Mastacembelidae) for Belitung Island, Indonesia. Acta Ichthyologica et Piscatoria 53: 123-127. DOI: 10.3897/aiep.53.105318

Hasan V, warlanda, Katz AM, South J, Ottoni FP, Nurjirana, Gani A. 2023b. First record of the uncommon spiny eel *Mastacembelus notophthalmus* Roberts, 1989 (Synbranchiformes: Mastacembelidae) for Bangka Island, Indonesia. Cybium 47 (2): 189-191. DOI: 10.26028/cybium/2023-008.

Hasan V, Ottoni, FP, South J. 2023c. First record of the vulnerable freshwater fish *Lobocheilos falcifer* (Valenciennes, 1842) (Teleostei, Cyprinidae) in Sumatra, Indonesia. Check List 19 (1): 51-55. DOI: doi.org/10.15560/19.1.51.

Hasan V, Kusumah W, Purnawan M, Nasidi, Arianto, Wijaya I, Yusnandar F, Baptista Y, South J, Ottoni FP. 2024a. First record of the fire-eyed loach *Barbucca diabolica* Roberts, 1989 (Cypriniformes: Barbuccidae) for Belitung Island, Indonesia. Cybium 48 (1): 71-74. DOI: 10.26028/cybium/2023-042.

Hasan V, South J, Valen FS, Andriyono S, Vieira LO, Sambah AB, Ottoni FP. 2024b. First record of Saddle barb, *Barbodes sellifer* Kottelat & Lim 2021 (Cypriniformes, Cyprinidae), on Belitung, Indonesia, with an update of its geographic distribution. Check List 20 (2): 524-529. DOI: 10.15560/20.2.524.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.

Hou G, Chen WT, Lu HS, Cheng F, Xie SG. 2018. Developing a DNA barcode library for perciform fishes in the South China Sea: Species identification, accuracy and cryptic diversity. Mol Ecol Resour 18 (1): 137-146. DOI: 10.1111/1755-0998.12718.

Hussin NJ, Azmir IA, Esa Y, Ahmad A. 2023. Diversity, distribution and conservation status of the world's smallest fish, *Paedocypris progenetica*. IOP Conf Ser: Earth Environ Sci 1241: 012120. DOI: 10.1088/1755-1315/1241/1/012120.

Insani L, Jatayu D, Valen FS, Widodo MS, Hasan V. 2022. Comparing genetic *Mystacoleucus marginatus* and *Mystacoleucus padangensis* (Cypriniformes: Cyprinidae) based on Cythochrome C Oxidase sub unit I (COI) gene. Iran J Ichthyol 9 (4): 195-203. DOI: 10.22034/iji.v9i4.903.

Kasayev T, Arisuryanti T. 2022. COI-Based DNA barcoding of selais fish from arut river, Central Kalimantan, Indonesia. J Trop Biodivers Biotechnol 7 (1): 66510. DOI: 10.22146/jtbb.66510.

Khodijah NS, Suwignyo RA, Harun MU, Robiartini L. 2019. Phytoremediation potential of some grasses on lead heavy metal in tailing planting media of former tin mining. Biodiversitas 20 (7): 1973-1982. DOI: 10.13057/biodiv/d200725.

Kottelat M, Britz R, Hui TH, Witte K-E. 2006. *Paedocypris*, a new genus of Southeast Asian cyprinid fish with a remarkable sexual dimorphism, comprises the world's smallest vertebrate. Proc Biol Sci 273 (1589): 895-899. DOI: 10.1098/rspb.2005.3419.

Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. Trends Ecol Evol 30 (1): 25-35. DOI: 10.1016/j.tree.2014.10.008.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35: 1547-1549. DOI: 10.1093/molbev/msy096.

Kurniawan A, Hariati AM, Kurniawan A, Kurniawan N, Nugroho TW, Bidayani E, Syarif AF, Wiadnya DGR. 2022. Genetic diversity

analysis and phylogeography of *Osteochilus spilurus* (Cyprinidae: Labeoninae) from Bangka, Belitung, and Kalimantan Islands (Indonesia) using cytochrome b gene. Biodiversitas 23 (9): 4738-4746. DOI: 10.13057/biodiv/d230941.

Kurniawan A, Hariati AM, Kurniawan A, Wiadnya DGR. 2021. First genetic record and the phylogenetic relationship of *Osteochilus spilurus* (Cyprinidae: Labeoninae) originating from Bangka and Belitung Islands, Indonesia. Biodiversitas 22 (2): 794-802. DOI: 10.13057/biodiv/d220233.

Li Z, Yu J, Zhou C. 2025. Complete mitochondrial genome of *Acheilognathus mengyangensis* (Cypriniformes, Cyprinidae, and Acheilognathinae): Characterization and phylogenetic analysis. Ecol Evol 15 (8): e71909. DOI: 10.1002/ece3.71909.

Liao X, Shih Y, Jia C, Gao T. 2024. Complete mitochondrial genome of four Peristediidae fish species: Genome characterization and phylogenetic analysis. Genes 15: 557. DOI: 10.3390/genes15050557.

Liu S, Luo W, Ge L, Wang Y. 2023. An evaluation method for the priority protection of fishes based on multiple ecological dimensions. J Freshw Ecol 38 (1): 2144956. DOI: 10.1080/02705060.2022.2144956.

Liu Y, Yu W, Wu B, Li J. 2022. Patterns of genomic divergence in sympatric and allopatric speciation of three Mihoutao (*Actinidia*) species. Hortic Res 9: uhac054. DOI: 10.1093/hr/uhac054.

Lumbantobing D. 2019a. *Paedocypris micromegethes.* The IUCN Red List of Threatened Species 2019: e.T90332118A90332123. International Union for Conservation of Nature, 2025. www.iucnredlist.org.

Lumbantobing D. 2019b. *Paedocypris progenetica* . The IUCN Red List of Threatened Species 2019. e.T91067386A91067390. International Union for Conservation of Nature, 2025. www.iucnredlist.org.

Lumbantobing D. 2019c. *Sundadanio gargula.* The IUCN Red List of Threatened Species 2019. International Union for Conservation of Nature, 2025. www.iucnredlist.org.

Lumbantobing D. 2020. *Paedocypris carbunculus.* The IUCN Red List of Threatened Species 2020: e.T91067318A91067324. International Union for Conservation of Nature, 2025. www.iucnredlist.org.

Modeel S, Negi RK, Sharma M, Dolkar P, Yadav S, Siwach S, Yadav P, Negi T. 2024. A comprehensive DNA barcoding of Indian freshwater fishes of the Indus River system, Beas. Sci Rep 14: 2763. DOI: 10.1038/s41598-024-52519-0.

Muhala V, Guimarães-Costa A, Macate IE, Rabelo LP, Bessa-Silva AR, Watanabe L, dos Santos GD, Sambora L, Vallinoto M, Sampaio I. 2024. DNA barcoding for the assessment of marine and coastal fish diversity from the Coast of Mozambique. PLoS One 19 (2): e0293345. DOI: 10.1371/journal.pone.0293345.

Mustikasari D, Nuryanto A, Suryaningsih S. 2022. Phylogeography of *Aplocheilus panchax* in Indonesia, with special focus on the Bangka Island population. Biodiversitas 23 (4): 2035-2046. DOI: 10.13057/biodiv/d230439.

Mwita CJ, Chuhila YJ. 2023. Fish DNA barcoding: Advances and challenges. In: Lakra WS, Goswami M, Trudeau VL (eds). Frontiers in Aquaculture Biotechnology. Academic Press, United Kingdom. DOI: 10.1016/B978-0-323-91240-2.00013-0.

Nazran, Syofriani, Ulfauza, Valen FS, Oktaviandi D, Prayoga A, Ilmia M, Czech M, Kamarudin AS, Hasan V. 2025. DNA Barcoding of *Rasbora* sp. from Bangka Island: A genetic identification basis and its potential application in aquaculture. Egyptian J Aquat Biol Fish 29 (3): 213-226. DOI: 10.21608/ejabf.2025.426534.

Pramono TB, Valen FS, Oktaviandi D, Prayoga A, Ilmia M, Purnama F, Hidayat R, Amru TJ, Czech M, Islamy RA, Kamarudin AS, Hasan V. 2025. First record of *Mystus singaringan* from Bangka Island, Indonesia: A new insight into its distribution. Egyptian J Aquat Bi Fish 29 (3): 2707-2719. DOI: 10.21608/ejabf.2025.433795.

Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS One 8 (7): e66213. DOI: 10.1371/journal.pone.0066213.

Short KA, Sands CJ, McInnes SJ, Pisani D, Stevens MI, Convey P. 2022. An ancient, Antarctic-specific species complex: Large divergences between multiple Antarctic lineages of the tardigrade genus *Mesobiotus*. Mol Phylogenet Evol 170: 107429. DOI: 10.1016/j.ympev.2022.107429.

Sun W, Wang Y, Zhang H. 2025. Genetic characteristics and phylogenetic relationships of 18 anchovy species based on mitochondrial genomes in the seas around China. Ecol Evol 15 (5): e71496. DOI: 10.1002/ece3.71496.

Syarif AF, Valen FS, Herjayanto M. 2023. First DNA barcoding and phylogenetics of wild *Betta edithae* (Anabantiformes: Osphronemidae) from Belitung Island, Indonesia. AACL Bioflux 16 (5) : 2626-2636.

Syarif AF, Athar MHF, Manik JDN, Czech M, Valen FS. 2025a. Distribution range update of red wine betta, *Betta burdigala* in Central Bangka with description of different color variants. Egyptian J Aquat Biol Fish 29: 343-357. DOI: 10.21608/ejabf.2025.440352.

Syarif AF, Valen FS, Kurniawan A, Rudiansyah, Herjayanto M, Mamat NB, Ottoni FP, Andriyono S, Hasan V. 2025b. Unveiling the first DNA barcoding of *Betta cf. uberis* fish (Anabantiformes: Osphronemidae) from Belitung Island, Indonesia. Egypt J Aquat Biol Fish 29 (1): 2023-2036. DOI: 10.21608/ejabf.2025.411476.

Valen FS, Widodo MS, Islamy RA, Wicaksono KP, Soemarno, Insani L, Hasan V. 2022. Molecular phylogenetic of silver barb *Barbonymus gonionotus* (bleeker, 1849) (Cypriniformes: Cyprinidae) in Java, Indonesia. IOP Conf Ser: Earth Environ Sci 1036: 012011. DOI: 10.1088/1755-1315/1036/1/012011.

Valen FS, Notonegoro H, Pamungkas A, Swarlanda, Hasan V. 2023a. Revolutionary breakthrough: Unveiling the first DNA barcoding of the endemic wild *Betta burdigala* (Kottelat and Ng 1994) (Anabantiformes: Osphronemidae): A critically endangered wild betta from Bangka Island, Indonesia. Intl J Agric Biol 30 (4): 269-275. DOI: 10.17957/ijab/15.2084.

Valen FS, Bidayani E, Alfian RA, Prananda M, Notonegoro H, Susilo NB, Ardi WD, Swarlanda, Aziz MA, Puryoso, Widodo MS, Faqih AR, Hasan V. 2023b. Unveiling the close relationship between *Betta burdigala* and *Betta uberis* through DNA barcoding based on COI gene. IOP Conf Ser: Earth Environ Sci 1267: 012066. DOI: 10.1088/1755-1315/1267/1/012066.

Valen FS, Hafidz AM, Islamy RA, Faqih AR, WidodoMS, Hasan, V. 2024a. Molecular identification of *Nemacheilus* sp. from Bangka Island, Indonesia Based on the Cytochrome C oxidase Subunit I (COI) Gene. IOP Conf Ser: Earth Environ Sci 1392 (1): 012031. DOI: 10.1088/1755-1315/1392/1/012031.

Valen FS, Syaputra D, Hasmadi M, Swarlanda, Kamarudin AS, Hasan V, Itaya I. 2024b. Unveiling the enigmatic dwarf horseface loach *Acanthopsoides molobrion*: A groundbreaking discovery in Indonesia. Biosaintifika 16: 265-272. DOI: 10.15294/biosaintifika.v16i2.5671.

Valen FS, Mamat NB, South J, Ottoni FP, Vieira LO, Kamarudin AS, Afandi AY, Hasan V. 2024c. Unveiling the occurrence of vulnerable Sisorid catfish (Teleostei: Sisoridae) in Bangka based on morphological and molecular evidence. Biodiversitas 25 (11): 4543-4550. DOI: 10.13057/biodiv/d251153

Valen FS, Salmi, Syarif, AF. 2025. DNA Barcoding Reveals the Genetic Identity of licorice gourami *Parosphromenus deissneri*, an endangered species threatened by tin mining in Bangka. Egyptian J Aquat Biol Fish 29: 573-589. DOI: 10.21608/ejabf.2025.440996.

Wang X, Que P, Heckel G, Hu J, Zhang X, Chiang C-Y, Zhang N, Huang Q, Liu S, Martinez J, Pagani-Núñez E, Dingle C, Leung YY, Székely T, Zhang Z, Liu Y. 2019. Genetic, phenotypic and ecological differentiation suggests incipient speciation in two *Charadrius plovers* along the Chinese coast. BMC Evol Biol 19 (1): 135. DOI: 10.1186/s12862-019-1449-5.

Wang IC, Lin HD, Liang CM, Huang CC, Wang RD, Yang JQ, Wang WK. 2020. Complete mitochondrial genome of the freshwater fish *Onychostoma lepturum* (Teleostei, Cyprinidae): Genome characterization and phylogenetic analysis. ZooKeys 1005: 57. 10.3897/zookeys.1005.57592.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 360 (1462): 1847-1857. DOI: 10.1098/rstb.2005.1716.

Ward RD. 2009. DNA barcode divergence among species and genera of birds and fishes. Mol Ecol Resour 9 (4): 1077-1085. DOI: 10.1111/j.1755-0998.2009.02541.x.

Weigand H, Beermann AJ, Čiampor F et al. 2019. DNA barcode reference libraries for the monitoring of aquatic biota in Europe: Gap-analysis and recommendations for future work. Sci Total Environ 678: 499-524. DOI: 10.1016/j.scitotenv.2019.04.247.

Zhang R, Zhu T, Luo Q. 2023. The complete mitochondrial genome of the freshwater fish *Onychostoma ovale* (Cypriniformes, Cyprinidae): Genome characterization and phylogenetic analysis. Genes 14 (6): 1227. DOI: 10.3390/genes14061227.