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DNA Barcoding of *Rasbora* sp. from Bangka Island: A Genetic Identification Basis and Its Potential Application in Aquaculture

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ABSTRACT

Indonesia is a country with very high freshwater biodiversity, including the fish group of the genus Rasbora. Morphological similarities between species in this genus often make identification challenging without additional methods. This study aimed to identify Rasbora specimens from Bangka Island using a DNA barcoding approach based on the cytochrome c oxidase subunit I (COI) gene. The sequencing results showed that the specimens had the highest similarity to *Rasbora caudimaculata* (93.10%) and *Brevibora dorsiocellata* (94.15%), but did not reach the definitive species identification threshold. Genetic distance analysis (K2P) showed a divergence of 6–8%, with phylogenetic trees grouping the specimens into distinct clades. These findings indicate the possibility of a new species or a genetically isolated local population. Further studies with an integrative approach are required for taxonomic validation. This finding also opens up opportunities for utilization in aquaculture, especially in the development of local species as economically valuable aquaculture candidates with specific adaptations to the aquatic environment of Bangka Island.

INTRODUCTION

Indonesia ranks among the world's megadiverse nations, with an extraordinary richness of freshwater fish species **(Hasan *et al.*, 2022a; Robin *et al.*, 2022; Valen *et al.*,**

2022a). One of the commonly encountered genera of freshwater fish in Southeast Asian waters, particularly in Indonesia, is Rasbora **(Syarif *et al.*, 2023a)**. This genus belongs to the family Cyprinidae and is characterized by high morphological similarity among species, making morphology-based identification often ambiguous.

As part of the Bangka Belitung Islands Province, Bangka Island hosts a variety of river and lake ecosystems that have received limited genetic exploration **(Valen *et al.*, 2024a)**. Research on fish diversity in the region is still scarce, especially in terms of molecular-level investigations. Indeed, molecular techniques like DNA barcoding play a crucial role in accurately identifying species, particularly cryptic species that cannot be reliably distinguished through morphology **(Valen *et al.*, 2024b)**.

DNA barcoding is a method of species identification that uses short fragments of specific genes; in animals, the mitochondrial cytochrome c oxidase subunit I (COI) gene is most commonly used **(Syarif *et al.*, 2025)**. Through this approach, genetic variation between species can be analyzed and visualized through phylogenetic trees, which illustrate the evolutionary relatedness among the studied specimens.

This study aimed to identify Rasbora specimens from Bangka Island using a molecular approach based on the COI gene as well as to evaluate their phylogenetic relationships with reference species from the GenBank and BOLD databases **(Ahmed *et al.*, 2025)**. The results of this study are expected to contribute to the understanding of the genetic diversity of Indonesia's freshwater fish, as well as supporting conservation efforts and more accurate taxonomic classification.

In addition, the genetic information generated from this research has the potential to be used in the development of the aquaculture sector focused on local species **(Yang *et al.*, 2024)**. Identifying the genetic traits of Rasbora specimens from Bangka facilitates the domestication process, the selection of high-quality broodstocks, and the development of strains that are better adapted to local aquatic environments **(Fu & Yuna, 2022; Jiang *et al.*, 2023)**. This not only strengthens the conservation of endemic species but can also support the diversification of economically valuable aquaculture commodities at a local and national levels.

MATERIALS AND METHODS

1. Study area and sampling sites

The research was conducted in several freshwater locations on Bangka Island, ranging from the Upang River to the Payak Benua River, Bangka Belitung Islands Province, Indonesia. Sampling was carried out during a field trip in June 2024. Rasbora specimens were caught using scoop nets and a fish trap, then placed in a container filled with river water for prelimnary morphological identification. Each individual was photographed and coded before being released. A total of 10 live specimens were maintained at the aquaculture hatchery at the University of Bangka Belitung for

domestication efforts. One specimen was euthanized to obtain a tissue sample from the pectoral fin. Muscle tissue from the fins or posterior body was carefully excised using a sterile knife and was stored in a microcentrifuge tube with 70% ethanol **(Syarif *et al.*, 2025)**. The sample was kept at 4°C during transport to the laboratory for genetic analysis. Additionally, one specimen was preserved in 10% formalin **(Valen *et al.*, 2020; Hasan *et al.*, 2020)** and stored at the Bangka Belitung University Ichthyofauna Museum with voucher code BBIM 002.

Fig. 1. Live specimen of Rasbora sp. Bangka (BBIM 002) (11-12 predorsal scale rows; scale margins highlighted by reticulated melanin pattern; caudal fin red with black tips of caudal lobes; elongated body shape)

2. Isolation, amplification and sequencing of COI genes

DNA isolation was conducted using the Chelex 10% method as described by **Walsh *et al.* (1991)**. Following extraction, a partial mitochondrial cytochrome c oxidase subunit I (COI) gene fragment was amplified using the BIONESIA protocol with primer pairs 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′), following the approach of **Ward *et al.* (2005)**. The PCR reaction was prepared in a final volume of 25µL, containing 2µL of template DNA, 1.25µL of each primer (10mM), 4.5µL of distilled water, 1.5µL of 10× buffer, 2.5µL dNTP mix, 2.0µL $MgCl_2$, and 0.125µL of PE Amplitaq polymerase. Amplification was carried out using an Applied Biosystems™ 2720 Thermal Cycler. Thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 45 seconds **(Syarif *et al.*, 2025)**. PCR products were examined by 1% agarose gel electrophoresis using GelRed® for visualization **(Insani *et al.*, 2023)**. Successfully amplified DNA, indicated by visible bands, was sequenced using the Sanger method at PT. Genetics Science, Jakarta.

3. Data analysis

The DNA sequences obtained were edited using BioEdit software. Sequence alignment was performed with ClustalW, while genetic distance calculation was performed using the 2-parameter Kimura (K2P) model in the MEGA X program **(Kumar**

***et al.*, 2018)**. Phylogenetic trees were constructed using the neighbor-joining (NJ) method with 1000 bootstrap replications to assess branch support. The sequences were subsequently compared to the GenBank and BOLD (Barcode of Life Data System) databases for similarity-based species identification.

RESULTS

1. DNA barcoding

The DNA sequence of the cytochrome oxidase subunit I (COI) gene from the Rasbora sp. specimen was successfully amplified and sequenced, yielding a base pair length of 665 bp (Table 1).

Table 1. DNA barcoding of *Rasbora* sp. Bangka

DNA barcoding of *Rasbora* sp. Bangka
GTATTCGGGTGCCTGANCTGGAATAGTTGGAACCGCCCTTAGTCTTCTCATCCGTGCTGA ACTCAGCCAACCGGGATCACTTTTAGGGGATGACCAAATTTATAATGTAATTGTAACTGC CCATGCCTTCGTAATAATTTTCTTTATAGTTATGCCAATACTAATTGGGGGCTTTGGAAA CTGATTAGTCCCACTAATAATCGGGGCACCAGACATGGCATTCCCACGAATAAACAACAT AAGCTTTTGACTTCTTCCCCCATCATTTCTATTACTGTTAGCCTCCTCTGGCGTTGAAGC CGGGGCTGGAACAGGGTGAACAGTTTACCCGCCACTCGCAGGCAATCTTGCCCACGCAGG AGCATCAGTAGACCTAACAATCTTTTCACTCCACTTAGCAGGTGTATCATCAATTTTAGG AGCTATTAATTTTATTACAACAATTATTAATATGAAACCCCCAGCTATCACCCAATATCA AACCCCACTATTTGTATGAGCAGTATTAGTTACAGCTGTCCTACTACTCCTATCCCTACC AGTGCTAGCTGCTGGAATTACAATACTCCTCACAGACCGAAACCTTAACACCACATTCTT CGACCCAGCTGGTGGAGGAGACCCAATTTTATACCAACACCTATTCTGATTCTTTGGCCA CCAAG

2. Species identification based on the COI gene

COI sequences were analyzed using BLAST (GenBank) and Barcode of Life Data Systems (BOLD) database matching. The matching results showed the highest identity with the species *Brevibora dorsiocellata* showing a nucleotide similarity percentage of 94.15% and *Rasbora caudimaculata* (93,10 %) with the same nucleotide similarity percentage (Table 2). However, both matches fall below the commonly accepted species identification threshold ≥97% for COI sequences.

Table 2. Sequence similarity of *Rasbora* sp. Bangka

Specimen	Query Coverage (%)	Simlarity GenBank (%)	Species Outcome	Accession Number (GenBank)
Rasbora sp. Bangka	98	94,15	*Brevibora dorsiocellata*	NC_063865.1
	96	93,10	*Rasbora caudimaculata*	NC_063872.1
	95	92,77	*Rasbora vulgaris*	HM224243.1
	97	92,41	*Rasbora elegans*	MH561387.1
	95	92,77	*Rasbora hobelmani*	HM224229.1

The highest percentage of identity was observed with *Brevibora dorsiocellata* at 94.15%, but it has not reached the definitive species threshold (>97%). This suggests the possibility of a new species or a local variation, potentially a cryptic species.

3. Genetic distances

Genetic distance analysis using the Kimura 2-Parameter (K2P) model supports the BLAST results. The *Rasbora* sp. Bangka specimen shows the closest distance to *Brevibora dorsiocellata* at 0.06, followed by *Rasbora caudimaculata*, *Rasbora vulgaris*, and *Rasbora elegans*, each with a distance of approximately 0.08 (Table 3).

Tabel 3. Genetic distances of *Rasbora* sp. Bangka

		1	2	3	4	5	6	7
1	*Rasbora* sp. Bangka							
2	*Brevibora dorsiocellata*	0,06						
3	*Rasbora caudimaculata*	0,08	0,07					
4	*Rasbora vulgaris*	0,08	0,09	0,09				
5	*Rasbora elegans*	0,08	0,09	0,09	0,01			
6	*Rasbora hobelmani*	0,08	0,09	0,09	0,01	0,01		
7	*Rasbora paviana*	0,08	0,09	0,09	0,00	0,01	0,01	

4. Nucleotide composition

Analysis of the nucleotide base composition of the Cytochrome C Oxidase subunit I (COI) gene fragment showed variations in the proportion of bases between *Rasbora* sp. Bangka and two comparative species, namely *Brevibora dorsiocellata* and *Rasbora*

caudimaculata. The details of nucleotide composition (% total) are shown in the following Table (4).

Tabel 4. Nucleotide composition of *Rasbora* sp. Bangka

Sequence	T(U)	C	A	G
Rasbora sp. Bangka	28,74	26,37	27,56	17,33
Brevibora dorsiocellata	29,29	25,61	27,30	17,79
Rasbora caudimaculata	28,80	26,32	26,46	18,42

5. Molecular phylogeny

The phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap replicates based on COI gene sequence data (Fig. 2)

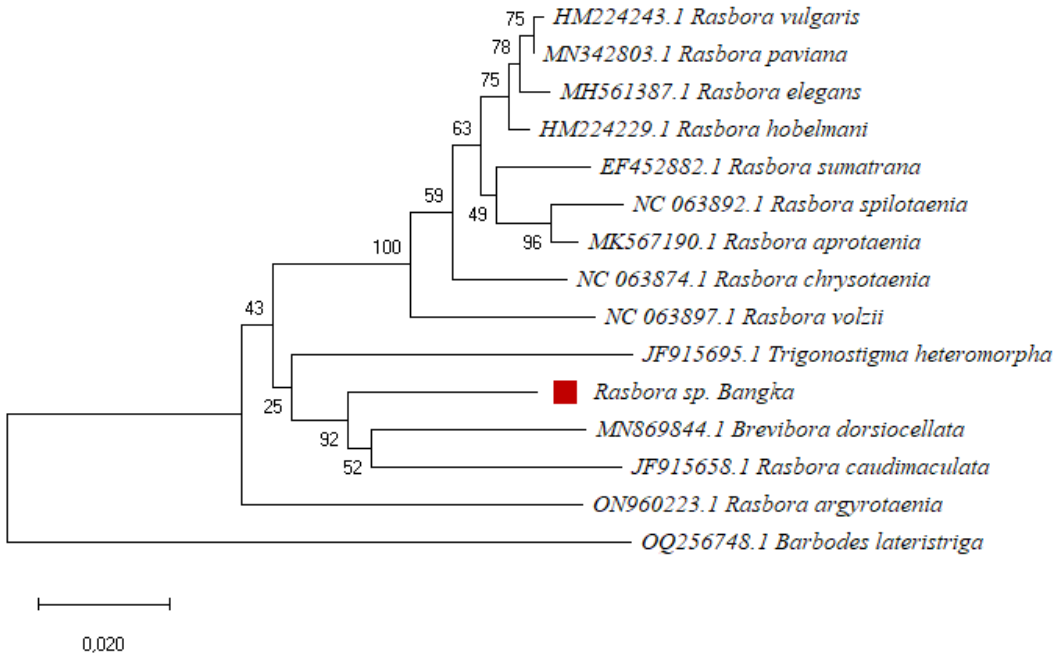

Fig. 2. Evolutionary tree of of *Rasbora* sp. Bangka (red square)

DISCUSSION

The DNA barcoding method using the cytochrome c oxidase subunit I (COI) gene has successfully amplified and extracted genetic information from *Rasbora* sp. specimens from Bangka Island, with a fragment length of 665bp. The results of the sequence matching with the GenBank database via BLAST showed the highest identity at 94.15% with the species *Brevibora dorsiocellata*, followed by other species of the genus Rasbora, such as *Rasbora caudimaculata* (93.10%) and *Rasbora vulgaris* (92.77%).

These identity values have not yet reached the generally accepted threshold of ≥97% for definitive species identification, which is widely used in DNA barcoding studies as a distinguishing criterion between species **(Čandek, & Kuntner, 2015; Valen *et al.*, 2024c)**. Therefore, these data suggest that the specimens collected from Bangka

Island are likely not part of the documented species, but rather represent a new species or, at least, local variants (cryptic species) of closely related taxa.

This is supported by genetic distance analysis using the Kimura 2-Parameter (K2P) model to support the BLAST findings. Specimen *Rasbora* sp. Bangka shows the closest distance to *Brevibora dorsiocellata* at 0.06, followed by *Rasbora caudimaculata*, *Rasbora vulgaris*, and *Rasbora elegans* (around 0.08 each). This value exceeds the interspecies threshold in freshwater fish genera, typically in the range of 0.02–0.03 **(Valen *et al.*, 2022b)**. The smaller the distance value (e.g. 0.00–0.03), the closer the kinship. Values above 0.07–0.11 indicate significant genetic distance, suggesting different species or at least a genetic variant of a geographically isolated species **(Robin *et al.*, 2023)**. The clade consisting *Rasbora* sp. Bangka + *Brevibora dorsiocellata* form the basal group, showing significant kinship proximity compared to other species. Species like *Rasbora chrysotaenia*, *Rasbora sumatrana*, and *Rasbora volzii* are positioned outside the main clade, signaling distant relatives. The divergence value at genetic distance ≥0.07 has consistently been interpreted as an indication of specific differences, which reinforces the argument that *Rasbora* sp. Bangka is a taxon that differs from the reference species. The genetic variation observed in *Rasbora* sp. Bangka in this study may result from the geographical isolation of Bangka Island, which facilitates local evolutionary divergence in line with the concept of allopatric speciation in biogeography **(Hasan *et al.*, 2023a; Valen *et al.*, 2023a)**.

Phylogenetic trees constructed using the neighbor-joining (NJ) method based on COI data, revealed that *Rasbora* sp. Bangka forms its own clade, although it is adjacent to *Brevibora dorsiocellata* and *Rasbora caudimaculata*, suggesting the bootstrap value on the main branch node of *Rasbora* sp. Bangka is 25, indicating weak statistical support for a direct relationship with *Brevibora dorsiocellata*. In contrast, the clades containing *Brevibora dorsiocellata* and *Rasbora caudimaculata* have high bootstrap values (92), reflecting a stronger and more stable relationship between the two species. Other species, such as *Rasbora* sp. Bangka, as well as *Rasbora vulgaris*, *Rasbora paviana*, and *Rasbora hobelmani*, form a more divergent phylogenetic group. This group is positioned at the top of the tree with bootstrap support of ≥75. The inclusion of *Barbodes lateristriga* form a more divergent phylogenetic group, positioned at the top of the tree with bootstrap support ≥75. The inclusion of *Rasbora* sp. belongs to the broader Rasbora clade, but its taxonomic status remains unresolved.

The COI genes of all three specimens showed a relatively high proportion of thymine (T), adenine (A), and cytosine (C) bases, which is characteristic of vertebrate mitochondrial DNA, while guanine (G) consistently showed the lowest representation. *Rasbora* sp. Bangka displayed the lowest guanine content (17.33%) among the compared species. Its cytosin content (26.37%) was slightly higher than that of *Brevibora dorsiocellata* and closely resembled that of *Rasbora caudimaculata*. Overall, the base composition is similar across species, with minor variations that may reflect interspecific

genetic divergence. These subtle differences support the findings from genetic distance analysis and further suggest a degree of evolutionary separation between *Rasbora* sp. Bangka and the reference taxa **(Insani *et al.*, 2022)**.

From a biogeographic perspective, these findings support the hypothesis that Bangka Island – geologically part of the Sundaland region – has the potential to serve as a local center of speciation for freshwater fish **(Ihwan *et al.*, 2020; Hasan *et al.*, 2024)**. The island's ecological isolation, coupled with the diversity of freshwater habitats such as rivers, marshes and lakes, creates environmental selection pressures that can give rise to genetically distinct populations. This is exemplified by the *Rasbora* sp. Bangka specimen. Similar biogeographic patterns have been observed in other small freshwater fish species across Southeast Asia, where morphologically similar populations have been revealed through genetic analysis to be separate taxonomic entities **(Freitas *et al.*, 2020; Insani *et al.*, 2022; Valen *et al.*, 2023b; Syarif *et al.*, 2025)**.

The results of this molecular identification are significant not only for taxonomy and biodiversity but also for their applied value in conservation and local aquaculture development **(Andriyono & Suciyono, 2020)**. Specimens exhibiting high genetic uniqueness, such as *Rasbora* sp. Bangka, warrant prioritization in conservation efforts – both *in situ* (through the preservation of natural habitats) and *ex situ* (via genetic storage in laboratories or hatcheries) **(Valen *et al.*, 2023b)**. This genetic data may inform germplasm conservation policies **(Syarif *et al.*, 2023c)**, particularly in regions facing ecological pressures from anthropogenic activities such as tin mining and deforestration for palm oil plantation **(Kusumah *et al.*, 2023)**. Furthermore, the distinct genetic profile of *Rasbora* sp. Bangka presents an opportunity to develop locally adapted aquaculture strains, suited to the specific environmental conditions of Bangka Island **(Hasan *et al.*, 2023b)**. Such developments are critical for diversifying aquaculture species and reducing reliance on non-native introductions **(Serdiati *et al.*, 2020)**. Additionally, given that several *Rasbora* species are well-established in the global ornamental fish trade **(Syarif *et al.*, 2023a)**, any unique morphological traits of the Bangka specimen could substantially enhance its value as a potential endemic ornamental fish. Supported by genetic evidence, this could offer both ecological and economic benefits.

The discovery of *Rasbora* sp. from Bangka Island, which exhibits significant genetic divergence from known reference species, presents a promising opportunity for the development of local species-based aquaculture (**Kurniawan *et al.*, 2024**). This potential can be evaluated through ecological, economic, and conservation lenses **(Bidayani *et al.*, 2023)**. Ecologically, *Rasbora* sp. Bangka has demonstrated the ability to thrive in the acidic freshwater environments typical of the region, indicating a high level of local adaptation. Such resilience makes it a strong candidate for development as a superior indigenous aquaculture species, better suited to withstand environmental pressures compared to introduced species **(Kurniawan & Syarif, 2023)**. Economically, many species within the Rasbora genus area already well-regarded in the global

ornamental fish market due to their streamlined body shape, vibrant coloration, and agile swimming behavior **(Tarihoran, *et al.*, 2023)**. Should *Rasbora* sp. Bangka exhibit distinct morphological traits, it could be positioned as a high-value export commodity **(Hasan *et al.*, 2022b)**. Its small size and simple feed requirements further enhance its suitability for sustainable cultivation at household and small-industry scales.

From a conservation and bioprospecting perspective, the cultivation of *Rasbora* sp. Bangka offers an effective *ex situ* conservation strategy, while also facilitating domestication and the development of superior broodstock **(Kurniawan & Syarif, 2023; Budi *et al.*, 2024)**. Genetic characterization enables targeted breeding programs for improved traits such as adaptability, growth rate, and disease resistance **(Gjedrem *et al.*, 2012)**. This approach aligns with contemporary genetic resource management in aquaculture, which views local genetic diversity as a strategic asset. Moreover, the molecular identity of *Rasbora* sp. Bangka can serve as a foundation for local strain certification, aiding in geographical indication and policy development for the protection of endemic aquatic biodiversity **(Lind *et al.*, 2012; Kurniawan & Syarif, 2023; Budi *et al.*, 2024; Kurniawan *et al.*, 2024)**. These efforts are crucial in advancing national blue economy strategies based on native species and addressing food security and climate change through the diversification of aquaculture.

Thus, *Rasbora* sp. Bangka holds not only academic relevance as a subject of taxonomic and molecular research but also substantial potential as a novel candidate for sustainable, biodiversity-based, and competitive aquaculture systems.

CONCLUSION

This study successfully idientified fish specimens of the genus Rasbora from Bangka Island using a DNA barcoding approach based on the mitochondrial Cytochrome c Oxidase Subunit I (COI) gene. Sequencing results revealed the highest genetic similarity with *Brevibora dorsiocellata* (94.15%), followed by *Rasbora caudimaculata* (93.10%) and *Rasbora vulgaris* (92.77%). However, none of these matches exceeded the >97% threshold required for definite species identification, suggesting taxonomic ambiguity. Genetic distance analysis using the Kimura 2-parameter (K2P) model indicated divergence values ranging from 6% to 8%, supporting the possibility that the specimen represents either a distinct species or a genetically isolated local population. Phylogenetic reconstruction via neighbor-joining (NJ) method further substantiated this finding, placing *Rasbora* sp. Bangka in a separate clade with low bootstrap support for the comparator species, thereby indicating significant molecular divergence. The nucleotide composition analysis also revealed subtle distinctions, notably a reduced guanine (G) content relative to reference species, reinforcing the evidence of genetic differentiation. The results suggest that *Rasbora* sp. Bangka is a potential candidate for designation as a new species, or at minimum, a taxonomic unit requiring further investigation. An integrative approach combining morphological, ecological, and

molecular data is essential for conclusive classification. Overall, this study underscores the critical role of molecular tools in taxonomic resolution and highlights their value as a foundation for conservation planning and the development of aquaculture strategies rooted in local genetic resources.

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