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DNA barcoding of *Brevibora cheeya* from Bumang River, Bangka Island using the COI gene (cytochrome Oxidase Subunit 1)

DNA barcoding Brevibora cheeya *asal Sungai Bumang, Pulau Bangka menggunakan gen COI* (*cytochrome Oxidase Subunit 1*)

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ABSTRAK

Brevibora cheeya merupakan salah satu spesies ikan yang berasal dari keluarga cyprinidae yang memiliki sebaran di Pulau Bangka, Belitung, Sumatra dan Kalimantan. Masyarakat lokal di Bangka menyebut ikan ini sebagai ikan Seluang. Spesies ini seringkali dianggap sebagai *Brevibora dorsiocellata* karena memiliki kemiripan secara morfologi. Tujuan dari penelitian ini untuk mengetahui pasti spesies ikan tersebut dan menganalisis bagaimana hubungan kekerabatan ikan tersebut dengan spesies yang sama diberbagai wilayah. Untuk pemastian spesies dapat menggunakan metode molekuler yang belum pernah dilakukan pada spesies ini di Pulau Bangka. Penelitian ini berupaya untuk mengidentifikasi ikan dari spesies *B. cheeya* asal Pulau Bangka secara molekuler menggunakan gen COI (DNA *barcoding*) serta menganalisis hubungan kekerabatannya. Metode penelitian ini menggunakan metode deskriptif eksploratif. Proses sampling dilakukan pada bulan Februari 2023 di Sungai Bumang, Pulau Bangka dan identifikasi dilaksanakan di Laboratorium Biologi, Universitas Bangka Belitung. Analisis data menggunakan NCBI BLAST dan MEGA 11. Hasil menunjukkan bahwa sampel ikan asal Pulau Bangka memiliki hubungan genetik intraspesifik spesies terhadap *B. cheeya* asal Belitung, Bangka, Kalimantan Tengah dan Sumatra Selatan dengan jarak genetik antara 0 – 0,9%. Hubungan terdekat terhadap *Rasbora dorsiocellata* memiliki jarak genetik 3,2%. Pohon filogenetik menunjukkan kekerabatan terhadap database dari pulau Bangka, Belitung dan Kalimantan Tengah memiliki nilai bootstrap 91, sementara hubungan terhadap database Bangka dan Sumatra Selatan pada nilai bootstrap 95.

Kata kunci: Bangka, *Brevibora cheeya*, COI, lokal, molekuler

ABSTRACT

Brevibora cheeya was a cyprinid fish found in Bangka, Belitung, Sumatra, and Kalimantan. Bangka locals call this fish Seluang. This species was often considered *Brevibora dorsiocellata* due to its similar morphology. The aimed of this research was to determine the exact species of fish and analyze how the fish are related to the same species in various regions. Molecular methods that have never been used for this species on Bangka Island could be used to confirm this species. This study uses COI genes (DNA barcoding) to molecularly identify *B. cheeya* fish from Bangka Island and analyze their relationships. The sampling process was conducted in Bumang River, Bangka Island, in February 2023, and identification was conducted at the University of Bangka Belitung Biological Laboratory. NCBI BLAST and MEGA 11 were used for data analysis. The results showed that fish

samples from Bangka Island have intraspecific genetic relatedness to *B. cheeya* from Belitung Island, Bangka Island, Central Kalimantan, and South Sumatra, with genetic distances ranging from 0% to 0.9%. The closest genetic relationship to Rasbora dorsiocellata was 3.2%. The phylogenetic tree showed a bootstrap value of 91 for relationships with the Bangka, Belitung, and Central Kalimantan databases and a bootstrap value of 95 for relationships with the Bangka and South Sumatra databases.

Keywords: Bangka, Brevibora cheeya, CO1, local, molecularly

INTRODUCTION

Indonesia is home to three species of the Brevibora family: *Brevibora exilis*, *Brevibora cheeya*, and *Brevibora doriocellata*. he black dots, or eyespots, that these three species share on their dorsal fins are what unites them (Liao & Tan, 2011). Errors in species identification may arise from morphological similarities (Khayra et al., 2016; Cahyono et al., 2018). Cryptic species of fish that have similar physical characteristics cause the misidentification of species (Khedkar et al., 2014).

When the native fish comes to economic species, identification is crucial to conservation and management efforts (Shen et al., 2013; Thu et al., 2019). Species identification needs to be simple and effective in the domains of biology, conservation biology, and ecology (Mitchell et al, 2019). Frankham et al. (2002) explained that ensuring accurate classification based on morphological and molecular traits is crucial for genetic conservation. For several reasons, such as the legal protection of endangered species, distribution maps, endemism, or species status, taxonomic clarity is necessary. Identifying desirable and invasive species is financially significant.

One of them, *B. cheeya*, is found in Bangka, Belitung, Sumatra, and Kalimantan. This species is native to Bangka Island and ussually found in the Bumang River. The inhabitants of Bangka Island are referred to locally as *Seluang*. Species identification is necessary to identify Seluang fish found in the Bumang River using molecular method.

DNA barcoding is effective in distinguishing different fish down to the species level (Thu et al., 2019). Short DNA sequences are used in DNA barcoding, a molecular taxonomy technique, to identify species. A sequence in mitochondrial cytochrome oxidase Subunit 1 (CO1) often codes for the target DNA at the higher animal level (Madduppa et al., 2017. In addition to providing information about a species' condition and genetic diversity, molecular identification can be used to manage fisheries resources through domestication and conservation initiatives (Kurniawan et al., 2022).

The purpose of this study was to employ DNA barcoding to extract the COI gene's nucleotide sequence from *Seluang* fish from the Bumang River in Bangka (Saleky & Merly, 2021). This information may then be utilized to infer the fishes' genetic relationships. The gathered genetic data has the potential to improve the database of native Indonesian fish and serve as the foundation for domesticating them.

MATERIALS AND METHODS

Preparing Research

Two phases of this study were completed in February 2023. First stage involved collecting samples in the field on Bangka Island's Bumang River. *Bubu,* fish trapping equipment, was used to collect samples at various points along the river. Buckets and plastic containers were used as sample containers. One feature of the target fish was that its dorsal fin has black spots on it. The fish was taken to the laboratory for the second step, known as the molecular identification stage, once the target fish sample has been obtained. Identification was done at Bangka Belitung University's Biology laboratory with the execution of multiple steps, such as electrophoresis, PCR amplification, and extraction.

DNA Extraction, PCR Amplification, Electrophoresis and Sequensing

To extract pure DNA was the goal. Lysis, binding, washing, and elution were the steps involved in extracting the sample using the

NEXprepTM Cell/Tissue DNA Mini Kit, according to the instructions included in the kit. Following the acquisition of the DNA sample, primer FISH-2: 5'TCGACTA ATCATAAAGATATCGGCAC3' and reverse primer FISH-2: 5'ACTTCAG GGTGACCGAAGAATCAGAA3' were used to amplify the mitochondrial cytochrome c oxidase subunit I (COI) gene by PCR (Ward et al., 2005).

The steps in the PCR process were predenaturation at 94° for 30 seconds, denaturation at 94° for 30 seconds, annealing at 50° for 60 seconds, extension or elongation at 72° for 60 seconds, and post-extension at 72° for 7 minutes. In order to obtain thousands of DNA, 35 cycles were repeated. Electrophoresis was then used to identify the PCR results. The separation of nucleic acids according to size was the fundamental idea behind electrophoresis. In order to ascertain the nucleotide sequence contained in DNA, positive electrophoresis results (i.e., bands of DNA) were either prepared for sequencing or sent to a sequencing service. The resulting DNA sequences were then examined using the Mega IX and BLAST (*Basic Local Alignment Search Tool*) programs.

Data Analysis

BLAST Analysis

Nucleotide sequences were read using MEGA XI (Molecular Evolutionary Genetic Analysis) software. Using the BLAST (*Basic Local Alignment Search Tool*) program, data from the sequencing analysis results were compared to data from GeneBank on the NCBI (*National Center for Biotechnology Information*) website. By choosing the BLAST menu, users could access the program online through the official NCBI website, which was located at http://www.ncbi.nlm.nih.gov.

Phylogenetic Analysis and Genetic Distance

According to Sogandi (2018), phylogenetic trees were depicted as branching tree diagrams, with the length of the branches corresponding to the evolutionary distance. To make the phylogenetic tree easier to read, genetic distance analysis was done after phylogenetic analysis using MEGA XI software and the maximum likelihood approach.

RESULTS

BLAST analysis

The Seluang Fish from the Bumang River, Bangka Island, has a similarity level of 99.24% with *B.cheeya* fish with accession number NC_063864.1 in NCBI on 98% query cover value, 0.0 E - Value, and 99.24% identity based on the results of identification using BLAST on NCBI (Table 1). The five closest nucleotides in the blast results also lead to *B. cheeya* with query cover of 95% and above.

Genetic and Phylogenetic Distance Analysis

The genetic distance in Figure 1 was measured for the subsequent analysis. Ordering the values from smallest to largest facilitates reading the degree of relationship between species in genetic distance analysis. The *B. cheeya*, which was native in South Sumatra, has the closest genetic distance on 0.2% according to genetic distance analysis.

Figure 2 displays the 1000x bootstrap value based on the outcomes of phylogenetic analysis using the Kimura model 2 parameter. The phylogenetic tree shows that *B. cheeya* from the Bumang River has a close similarity too with species from Belitung Island and the Middle of Kalimantan.

Table 1. BLAST results of *B. cheeya* from Bangka Island

Species	Query cover	E value	Per. Ident
B.cheeya	98%	0.0	99.24%
B.cheeya	95%	0.0	100.0%
B.cheeya	95%	0.0	99.84%
B.cheeya	95%	0.0	99.69%
B. cheeya	95%	0.0	99.37%

	1	2	3	4	5	6
1. Brevibora Cheeya pulau Bangka		0.002	0.002	0.003	0.003	0.007
2. MN869282.1:17-655 Brevibora cheeya Sumatera Selatan	0.002		0.003	0.003	0.004	0.006
3. MN869281.1:17-655 Brevibora cheeya Kalimantan Tengah	0.003	0.005		0.004	0.004	0.007
4. MN869178.1:17-655 Brevibora cheeya Bangka	0.006	0.008	0.009		0.003	0.007
5. MN869044.1:17-655 Brevibora cheeya Belitung	0.006	0.008	0.009	0.006		0.007
6. JF915661.1:7-661 Rasbora dorsiocellata	0.026	0.024	0.029	0.032	0.032	

Figure 1. Genetic distance of *B. cheeya* from the Bumang River, Bangka Island

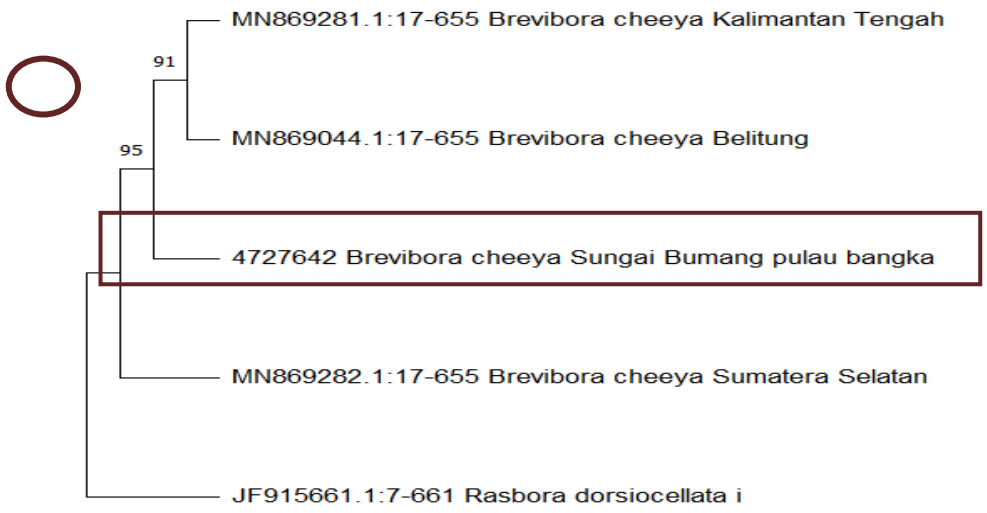

Figure 2. Phylogenetic tree of *B.cheeya* from the Bumang River, Bangka Island

DISCUSSION

Bangka Island has *B. cheeya* as a native freshwater fish according to the analysis of Seluang samples taken from the Bumang River. BLAST alignment at NCBI reached 98% query cover coverage. The value of 0 (zero) for the E (expected) value denotes that the two sequences are the same. Research by Triandiza and Madduppa (2018) supports this, showing that in each database, the most similar BLAST results for sequence data in NCBI GenBank have the same Max score and total score, query coverage that is almost 100%, E (Expect) value that is almost 0, and Per-Ident that is almost 100%. This indicates a high degree of sequence similarity or confidence.

The fish sample from the Bumang River has a related distribution to *B. cheeya* from Belitung Island, Bangka Island, Central Kalimantan, and South Sumatra. Bootstrap values for them on the phylogenetic tree were 91–95%. It is shown that the fish samples in this study and *B. cheeya* have a close relationship. When arranging branches on a phylogenetic tree, the bootstrap values are utilized to verify the accuracy of the sequence data (Dharmayanti 2011). To identify potential species relationships in phylogenetic trees with more accurate data, bootstrap methods are especially useful for figuring out how many repetitions there are in sequence alignments (Hills & Bull 1993). The stability of the tree formation can be determined by a high bootstrap value. Better grouping and branching are formed when the bootstrap value is higher (Saleky et al., 2020). Zein & Sulandari (2009) assert that there has been more evolutionary change in a phylogenetic tree with longer branches.

The genetic distance calculation enhances the phylogenetic tree analysis. A table showing the genetic distances between each individual can be used to analyze the relationships between species (Yuliani et al., 2017). The *Rasbora dirsiocellata* species has the greatest distance at 3.2% from the other species (Sari, 2020). Figure 2 displays the genetic distance of the *B. cheeya* species at less

than 3%. This means the relationship between them is categorized as intraspecific and is at a lower genetic distance (Aminan et al., 2020). Lower genetic distance values between species show a closer relationship between them. Conversely, the closer the relationship, the greater the genetic distance. Bangka Island *B. cheeya* fish and fish from Belitung Island, Central Kalimantan, and South Sumatra have comparatively low genetic distances, indicating that the two species have similar affinities and that genetic distance is the most common relationship between them. This is a considerable distance for the *B. cheeya* species, which is indigenous to Central Kalimantan and South Sumatra. Dharmayanti (2011) echoes this, saying that a relationship is closer the lower the genetic distance and vice versa. Because of shared parental origins and genetic proximity, close kinship relationships can exist between populations (Kusuma et al., 2016).

CONCLUSION

Based on DNA research on the Baroding Brevibora cheeya which originates from the Bumang River on Bangka Island, it can be concluded that the Seluang Fish which originates from the Bumang River in Bangka has genetic similarities at the species level with B. cheeya. The genetic relationship between B. cheeya from Bangka Island and findings from the Belitung, Bangka, Central Kalimantan and South Sumatra areas is between 96-98%.

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