BIODIVERSITAS
Volume 23, Number 9, September 2022
Pages: 4724-4729

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230939

Mitochondrial cytochrome oxidase 1 (CO1) and morphology of Penja fish (*Sicyopterus* spp.) in Budong-Budong River, West Sulawesi, Indonesia

IDA ASTUTI[1,♥], MOHAMAD FADJAR[2], RAHMI NURDIANI[2], TITIK DWI SULISTIYATI[2]

[1]Graduate School Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Jl. Veteran, Ketawanggede, Lowokwaru, Malang 65145, East Java, Indonesia. Tel./fax.: +62-341-551611, ♥email: idaastuti19@student.ub.ac.id

[2]Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Jl. Veteran, Ketawanggede, Lowokwaru, Malang 65145, East Java, Indonesia

Manuscript received: 21 June 2022. Revision accepted: 9 September 2022.

Abstract. *Astuti I, Fadjar M, Nurdiani R, Sulistiyani TD. 2022. Mitochondrial cytochrome oxidase 1 (CO1) and morphology of Penja fish (*Sicyopterus *spp.) in Budong-Budong River, West Sulawesi, Indonesia. Biodiversitas 23: 4724-4729.* Penja fish (local name) is a fish that is often found in West Sulawesi. Penja fish belong to the Goobidae family, which has a habit of migrating from the sea to the river. Penja fish species have not been widely published or in the Gen bank. The objective of this study was to analyze the Penja fish species found in the Budong-Budong river, West Sulawesi, Indonesia. The method used was a measurement of fish morphology and analysis of mitochondrial cytochrome oxidase I (COI) using a species barcode (~700). Samples were taken from the catch of fishermen at the mouth of the Budong-Budong river. The result of morphological measurements obtained an average total length of 25-38 mm, standard length 22-35 mm, head length 2-5 mm, tail length 3-5 mm, pelvic fins 0.5-2 mm, pectoral fins 1-3 mm, dorsal fins 1-3 mm and anal fin 0.1-1 and body width 3-6 mm. Electrophoretic analysis on Penja fish samples showed that the DNA between P1 and P2 samples was not much different and has very small genetic variation in 696 bp fragment length. The analysis on the Basic Local Alignment Search Tool (BLAST) showed the identity percentage reached 99.85%. The Penja fish identified in the Budong-Budong river is of the family Gobiidae, genus *Sicyopterus* with the species name *Sicyopterus pugnans*, which is classified as post larva.

Keywords: Gobiidae, molekuler, morphology, Penja fish, *Sicyopterus pugnans*

INTRODUCTION

Goby fish play a role in ecosystem function and contribute to biodiversity and also have a role in ecology in the water. The ecological role of goby fish is to eat phytobenthic and crustaceans (Sulistiono et al. 2006; Pusey et al. 2017). Goby fish are geographically widely distributed and the species number is abundant (Nugroho et al. 2016). Penja is a group of Gobiidae fish that migrate in groups that are also called amphidromous. It is because starts from larvae that live in the sea and then migrate to freshwater areas (Iida et al. 2017). Hadiaty et al. (2018) also stated that some gobiid species are amphidromous and spawn hatch in fresh waters, but their chicks migrate to the sea, undergo metamorphosis, and return to freshwater.

Penja fish is one of the most important commercial fish that is widely traded in fresh and dried form by people in West Sulawesi (Muthiadin et al. 2020). Penja fishing is routinely carried out, especially in coastal areas with rivers as a migration route for various types of migrant fish, including glass eels (*Anguilla* sp.). Penja fish are distributed across West Sulawesi, especially in Majene, Mamuju, and Central Mamuju regencies. Fishermen in the area carry out fishing activities when Penja migrates. The most widely used fishing gear is the Mini Purse Seine, while some fishermen use small nets such as scoop nets and push nets (Nurjirana et al. 2019).

Penja fish research using morphometric methods and DNA sequences (RAPD and mitochondrial DNA) at different locations with different results has been carried out (Muthiadin et al. 2018) but in Central Mamuju, no research has been conducted on these fish. The mitochondrial gene was chosen because the resolution of the COI gene at the intraspecific level was better than that of other core genes, so it was used to identify species at the intraspecific level (Strüder et al. 2010). These COI primers yield informative sequences for phylogenetic analysis at species and higher taxonomic levels. Several studies are suggested to conduct further research on the genetic variation of these fish in West Sulawesi (Muthiadin et al. 2017; Nurjirana et al. 2019).

Penja fish are a group of several species of fish from the family Gobiidae and Eleotridae, so no conclusions can be drawn about their naming. This is also due to the difficulty of identifying it because Penja fish are thought to be larvae that have not been widely discussed in the form of journals or identification books. The lack of references to the Penja fish species provides its own motivation for academics to conduct research. Several studies have identified morphologically Penja fish species in the Leppangan river, Majene District (Burhanuddin et al. 2019: Nurjirana et al. 2019). The results of this study suggest that Penja fish consist of 9 species, 2 families, and 6 genera (Nurjirana et al. 2019). While in the Karema river, Mamuju District, 1 family, 1 genus, and 2 species were found (Muthiadin et al.

2020). Penja fish that have been identified from the two areas are (Nurjirana et al. 2019; Muthiadin et al. 2020).

In the Pacific area, Sicyopterus is the most diverse Sicydiine genus. It is distributed in the Indo-Pacific from the Western Indian Ocean to French Polynesia in the East-Central Pacific (Hubert et al. 2016). A new species of Sicyopterus, freshwater goby, is described from Sumatra and Java, west Sulawesi and Gorontalo Bay, Indonesia. It differs from other species belonging to the genus by combining characteristics (Keith et al. 2015; Nurjirana et al. 2019). The main characteristics of *Sicyopterus* have two lateral slits on the upper lip which are furrowed, a second dorsal fin with one spine and 10 segmented rays, second and third rays of the first dorsal fin filamentous, more lateral, predorsal, and transverse dorsal scales, and caudal fin reddish in males with few blue lines at the top and bottom. *Sicyopterus* possesses numerous large tricuspid premaxillary teeth in both sexes. The ascending process on the premaxilla is broad at the dorsal tip. The tongue is fused to the floor of the mouth (Keith et al. 2020).

This study aimed to analyze the endemic species of Penja fish found in the Budong-budong river, Central Mamuju District, with the method of measuring the morphology and molecular characteristics of mitochondrial cytochrome oxidase 1 (CO 1).

MATERIALS AND METHODS

Study Area

The sampling location for Penja fish was at the estuary of Budong-budong river, Central Mamuju District, West Sulawesi Province, Indonesia. Fishermen catch fish in rivers and estuaries, which are the most common Penja fishing locations. The Budong-budong river is located in 4 sub-districts, i.e., Tobadak, Topoyo, Salupangkang and Budong-Budong. The upper reaches of the river are located in the village of Batu Parigi, Tobadak, and to have an estuary the village of Babana, Budong-Budong. The map of the Budong-Budong river and the sampling locations are presented in Figure 1.

Material

Penja fish morphology

The sample material used in the morphological measurements was 100 Penja fish taken directly from fishermen caught during the fishing season, namely in July-August 2021 in the Budong-Budong river. The tools used in this research are a cool box, ruler, camera, black cloth, etc.

Mitochondria DNA CO1

Materials used in DNA testing using the Mitochondria Cytochrome Oxidase 1 (CO1) method is Penja fish consisting of 2 samples (P1 and P2, each sample consisting of 2 fish) obtained directly from fishermen. The tools used were: trays, label paper, plastic clips, O'haus analytical balance with 0.01 g accuracy, ruler, parchment paper, cutting board, digital camera, plasticine, and box. Freezer, photo table, GPS, Olympus SZ51 microscope, 1.5 ml microtube, and plastic wrap.

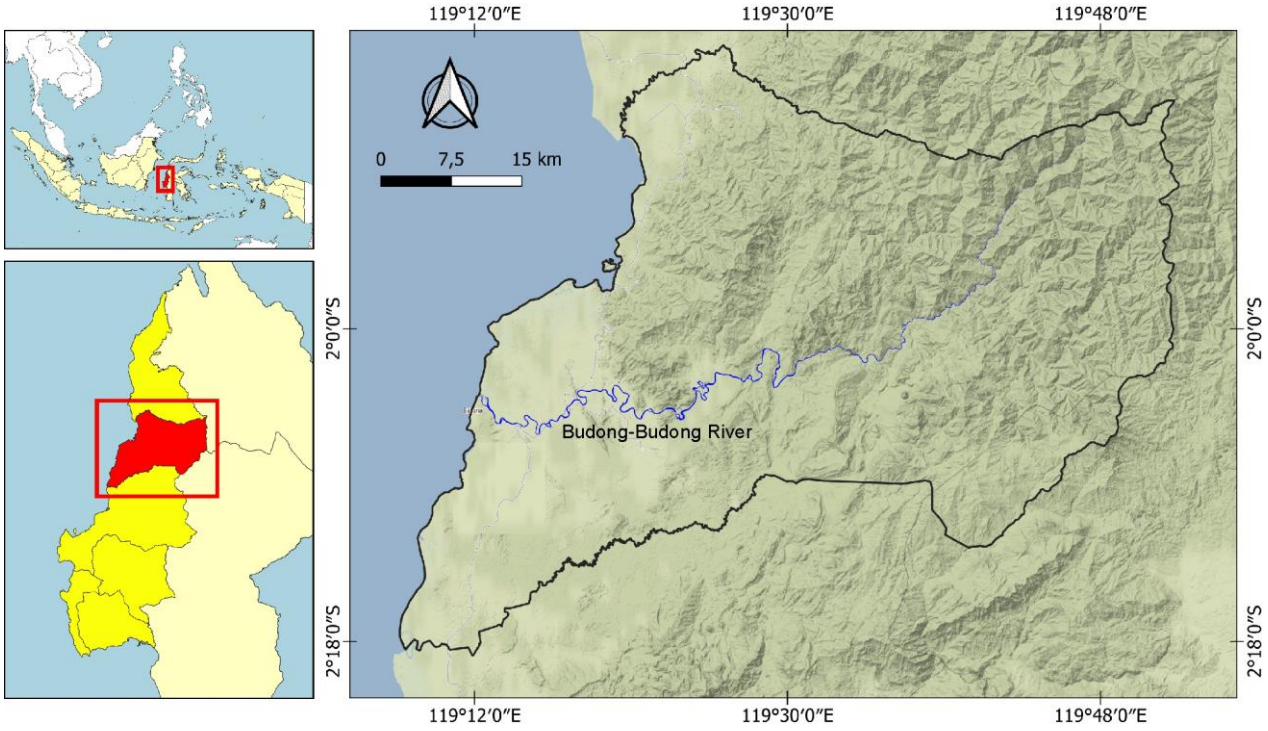

Figure 1. Map of the Budong-Budong River, Central Mamuju District, West Sulawesi Province, Indonesia and sampling location

Procedures

Morphology

Samples that were obtained directly from fishermen were put into plastics measuring 6x8 cm, which then stored in bulk ice to keep it fresh, considering that the distance between the sampling location and the laboratory took ±24 hours of travel time. A total of 70 fish representing the population were measured using a ruler. Parameters measured in morphological measurements were total length, fork length, standard length, head length, and body and fin width. To get the appropriate results, it was necessary to use a digital caliper with an accuracy of 0.01 mm or use a ruler.

Identifying Penja fish species using mitochondria DNA CO1.

The method of taking fish samples was carried out with procedures applied by genetics as a place for DNA analysis. Fish samples were taken directly from the fishermen, then the fish were cleaned and put in a cool box (styrofoam). The sample fish were then put into 1.5 mL microtubes for as many as 2 fish (sized fish), then 70% alcohol was added (Hadiat 2001) and samples were labeled. The sample is then put into a box lined with bubble wrap to prevent damage and then sent to the laboratory for further analysis.

Sample packaging was adjusted to the size and purpose of the inspection. Molecular analysis was carried out through several stages. The isolation method was carried out in accordance with the standard protocol of the product (Pangemanan et al. 2020). The working procedure followed the genomic DNA purification kit animal tissue wizard, which was to add 600 µL of Nuclei Lysis Solution into a 15 mL centrifuge tube and cool it on ice. Adding 10-20 mg of fresh tissue that has been thawed into cold lysis solution then homogenized for 10 seconds using a homogenizer. The lysate was transferred to 1.5 mL microcentrifugation tube.

The lysate was incubated at 65°C for 15-30 minutes. Added 3 L of RNase solution to the core lysate and mixed the sample by inverting the tube 2-5 times, then the mixture was incubated for 15-30 minutes at 37°C. Samples were allowed to cool to room temperature for 5 minutes. Furthermore, extraction of deoxyribonucleic acid (DNA), DNA Polymerase Chain Reaction (PCR), electrophoresis, and DNA sequencing were carried out. The mitochondrial COI DNA gene was further amplified using PCR products Species Barcoding (~700) owned by PT Genetika Science Indonesia. The results of further sequencing are carried out on the Basic Local Alignment Search Tool (BLAST) program at NCBI and Phylogenetic Tree - Neighbor Joining (Unrooted Tree) by NCBI Blast Tree Method.

RESULTS AND DISCUSSION

Result

Morphological measurement results

Morphological measurements of sampled fish obtained an average total length of 25-38 mm, standard length 22-35 mm, head length 2-5 mm, tail length 3-5 mm, pelvic fins 0.5-2 mm, pectoral fin 1-3 mm, dorsal fin 1-3 mm and anal fin 0.1-1 and body width 3-6 mm. Measurements of Penja fish are presented in Figure 2.

DNA isolation results

The results of electrophoresis analysis on Penja fish samples found in the Budong-budong river showed that the DNA between samples P1 and P2 was not much different. The Penja Fish DNA Genome Electrophoresis can be seen in Figure 3. This figure shows that the fragment length of the two samples is 696 bp. This means that P1 and P2 fish have very similar genetic diversity, which causes very small genetic variation. The mitochondrial DNA COI gene sequence was then analyzed in the Basic Local Alignment Search Tool (BLAST) program at NCBI. Following are the results of the BLAST analysis of the two sample sequences in Table 1.

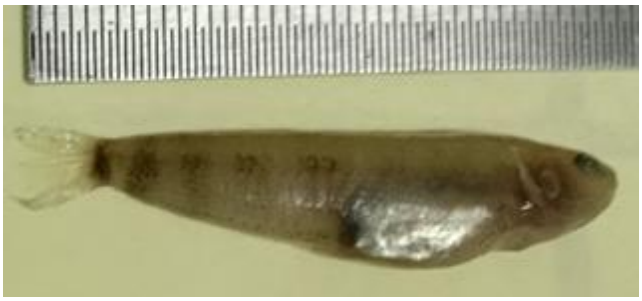

Figure 2. Measurement of Penja fish

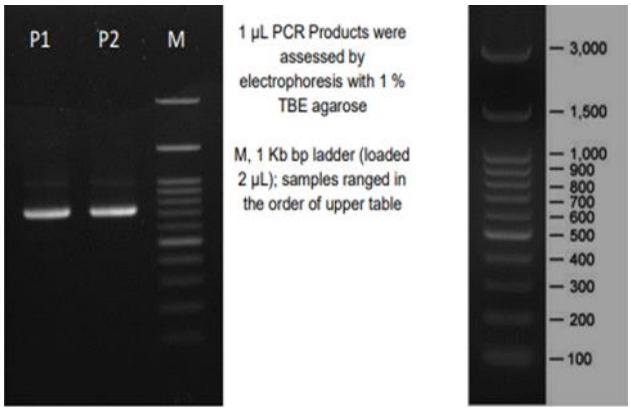

Figure 3. Genome DNA electrophoresis of Penja fish (*Sicyopterus pugnans*). Note: P1: The results of DNA isolation of Penja fish 1; P2: The results of DNA isolation of Penja fish 2

Table 1. Results of BLAST analysis of Penja fish by NCBI database

Sample code	Species	Max score	Total score	Query cover	E value	Ident	Accession
P1	*Sicyopterus pugnans*	1225	1225	95%	0	99.85%	MT227831
P2	*Sicyopterus pugnans*	1225	1225	95%	0	99.85%	MT227831

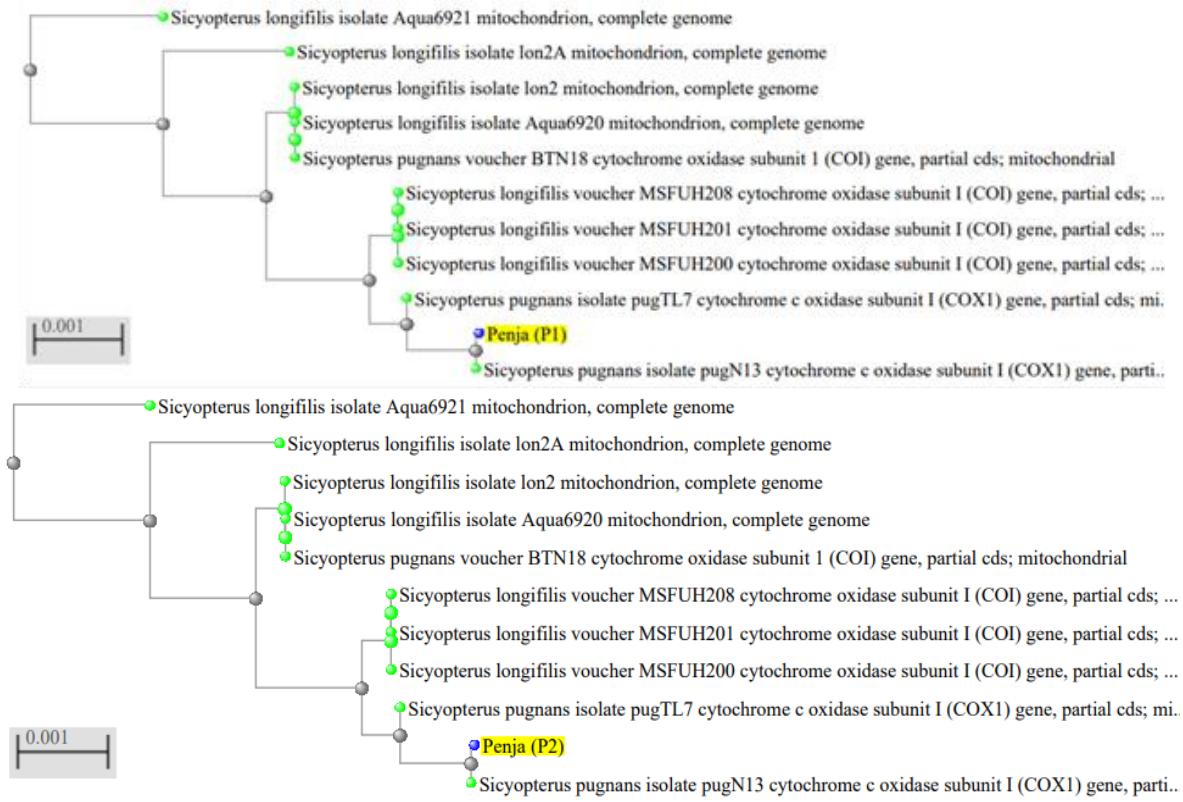

Figure 4. Phylogenetic rods of Penja (*Sicyopterus pugnans*)

Discussion

Morphological measurement

Morphological measurements are standard measurements used in fish. These measurements include standard length, head length, fin length or tail stem height, and body width. This morphological measurement is also an important measurement in describing the type of fish. In general, fish of the *Sicyopterus* genus have almost the same total length. Maeda and Saeki (2018) stated that *S. japonicus* has a length of 60-100 mm for males and 55-100 for females, *S. lagocephalus* has a length of 30-60 mm for males and 40-55 mm for females. While *S. longifilis* has a maximum length of 9.7 cm for males and *S. pugnans* have a maximum length of 7.2 cm for males (fish base) and the female has a length between 44-55 mm (Teichert et al. 2014). When viewed from the average results of morphological measurements, the Penja fish found in the Budong-budong river is classified as post-larval. Iida et al. (2015) stated that the standard length of post-larval *S. japonicus* was 22.5-34.0 mm. Burhanuddin et al. (2019) confirmed that the Penja fish caught by fishermen are still relatively small, with a total size range of 20-40 mm. The peak season for catching Penja fish is in April with sizes ranging from class lengths of 25-30 mm and in July with class lengths of 30-35 mm. The size distribution consists of one peak or one structure of the same age group.

The growth of the Penja fish did not change after 3-7 days in freshwater or at the mouth of a river. Total and standard-length growth showed the same evolutionary pattern over time. This growth does not vary after arrival in fresh water. Fish growth begins after major transformations have occurred (head, pectoral fins, scales, pigmentation, etc.) for approximately 2 weeks after freshwater colonization. Size does not appear to play a significant role in the colonization process (Keith et al. 2008). Schoenfuss et al. (1997) stated that within 48 hours after entering the river, *S. stimpsoni* fish experienced changes in the head and snout, but the total length and head length did not change.

Identification of Penja fish DNA

Based on the results of electrophoresis, Figure 2 shows that the DNA genomes of P1 and P2 fish found in the Budong-budong river are the same species. However, it cannot be concluded that genetic diversity is very low. The

results of the mitochondrial DNA COI gene sequences were then analyzed in the Basic Local Alignment Search Tool (BLAST) program at NCBI. The results of the BLAST analysis showed that the percentage of fish identity in the sample with the database reached 99.85%, which means that Penja 1 and Penja 2 are identical and have a high degree of similarity with *Sicyopterus pugnans* found in the NCBI database. Likewise, with the value of E or the expected value, which has a value of 0 (Table 1). The expected value is a measure of the degree to which this alignment occurs by chance. A good E value is close to zero, so it is impossible for an alignment event to occur by chance.

The use of molecular markers in the phylogenetic studies of various organisms is increasingly important. Phylogenetics explains that living things evolved from one genus. The phylogenetic tree shows species that are more related to one another. The use of the mitogenome brings robust results, and it is compatible with most of the markers already published (Miya and Nishida 2015). The results of phylogenetic analysis (Figure 5) showed that P1 and P2 fish are species that have the same lineage. The Penja fish identified in the Budong-budong river belong to the genus *S. pugnans*, which is still in the same lineage as *S. longifilis.*

Penja fish belong to the Gobiidae family. However, most of the existing taxonomies are not phylogenetic (Thacker 2003). Morphologically, the two basic differences between Gobiidae and Eleotridae are the shape of the pelvic fins and the relative length of the dorsal fin base (Nurjirana et al. 2019). The genus *Sicyopterus* is mostly found in the waters of Sulawesi. *Sicyopterus pugnans* were found in the Leppangan river, Majene, West Sulawesi (Nurjirana et al. 2019), the Karama River, Mamuju, West Sulawesi (Muthiadin et al. 2020), the Poigar river, North Sulawesi (Pangemanan et al. 2020) and, the Biak and Koyoan rivers, Luwuk Banggae, Center Sulawesi (Gani et al. 2020).

In conclusion, based on the results of DNA analysis using the mitochondrial CO 1 method and morphological measurements of Penja fish, it can be concluded that the Penja fish found in the Budong-budong river, Central Mamuju District, West Sulawesi Province, Indonesia belongs to the Gobiidae family, genus *Sicyopterus* and species *Sicyopterus pugnans* and are classified as post-larval fish with a size of 25-38 mm total length.

ACKNOWLEDGEMENTS

The author would like to thank the Brawijaya University, Malang, Indonesia, who has given permission and supported the implementation of this research, and the Regional Government of Central Mamuju, West Sulawesi, Indonesia, as well as all parties who have contributed to this research.

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