

ORIGINAL ARTICLE

Range extension of Spanner barbs, *Barbodes lateristriga* (Valenciennes, 1842) (Cypriniformes: Cyprinidae) to Bangka Island, Indonesia

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Abstract

The Spanner barb, *Barbodes lateristriga*, a Cyprinid, lives as a benthopelagic in the clear mountain streams with rocks and boulders beds, frequently found below waterfalls. This species was previously recorded from Southern Thailand; Malay Peninsula, Singapore and Borneo. In this study, we report from Bangka Island, Indonesia. The Bangka Islands are located on the Sundaland, which has a quite high biodiversity. The specimens of *B. lateristriga* were taken from the Menumbing Hill River, Bangka Island, Indonesia and then identified morphologically and molecularly. The molecular identification was carried out using the DNA barcoding method and the standardized cytochrome c oxidase subunit I (COI) gene. The results of molecular analysis revealed that specimens of the Bangka Islands are *B. lateristriga* with a similarity level of up to 100%. This is also confirmed by the results of phylogenetic tree analysis where samples from the Bangka Islands clustered in the same group as *B. lateristriga* found in the Genbank.

Keywords: COI, Cyprinidae, DNA Barcoding, Gene.

INTRODUCTION

Indonesia is a mega biodiversity country, with the second highest biodiversity of freshwater species after Brazil, with 1266 freshwater fish species (Fishbase 2022). However, the diversity of freshwater species is currently starting to be seriously threatened due to habitat and environmental damage caused by land conversion, pollution, and climate change (Morid et al. 2016; Radkhah et al. 2022). In addition, the presence of non-native fish is also a threat to the native fish (Radkhah et al. 2016; Eagderi et al. 2019; Insani et al. 2020; Mangitung et al. 2021; Ganjali et al. 2021; Bariyyah et al. 2021; Serdiati et al. 2021; Robin et al. 2023). Loss of biodiversity greatly affects ecosystem function (Cardinale et al. 2012). Thus, it requires efforts to quickly collect their ecological features such as species richness, for their conservation and management (Kelly et al. 2014). Every species is valuable, so every detail of information must be collected and reported, including its recent geographic distribution. The collection of this information must be done quickly

in line with the speed of biodiversity loss. Currently, several methods have been used to collect species data, such as analyzing the morphology and genetic markers/DNA barcoding (Hebert et al. 2003). These methods have been able to identify species quickly and accurately and provide up-to-date information about the geographic distribution of a species.

Spanner barb, *Barbodes lateristriga* (Valenciennes, 1842), is one of the freshwater species found in Southern Thailand, the Malay Peninsula, Singapore, and Borneo (Sutin et al. 2007; Li et al. 2015; Halim et al. 2018; Aqmal & Amirrudin 2018; Ng et al. 2019; Ren et al. 2020). This cyprinid species lives as a benthopelagic and feeds on worms, crustaceans, insects, and plant matter in tropical freshwaters (Sutin et al. 2007). The previous presence of the Spanner barb in Indonesia was recorded in West Kalimantan (Kottelat & Widjanarti 2005). In recent years, no new records have been officially disclosed for this species in other areas of Indonesia.

We caught the Spanner barb specimens in the Bangka River, then identified them based on



Fig.1. Menumbing Hill River Flow, Bangka Island, Indonesia.



Fig.2. The Spanner barb from Menumbing hill River flow, Bangka Island, Indonesia (8.5cm).

Kottelat & Lim (2021), Sutin et al. (2007), and Lim et al. (2016). In addition, the DNA Barcoding method (Hebert et al. 2003) was used as a tool for the rapid identification of species with a high degree of accuracy (Hubert et al. 2008; Luo et al. 2011). In this study, we used the cytochrome oxidase subunit I, a gene routinely used for species identification for freshwater fishes (Lutfiatunnisa et al. al. 2020; Valen et al. 2021; Valen et al. 2022b). DNA Barcoding was first proposed by Hebert et al. (2003), using a short sequence of a standardized gene as a tool for species identification. As the aim of this work, we report a new record of the Spanner barb from Bangka Islands, as the second record for Indonesia. The discovery of this species adds to our knowledge about its geographical distribution and

range extension (Ihwan et al. 2021; Hasan et al. 2021; Valen et al. 2022a).

MATERIAL AND METHODS

The study was conducted from 26-28 September 2022 in Menumbing Hill River, Bangka Island, Indonesia (Fig. 1). A total of 19 specimens were collected using a cast net and fish trap (Serdiati et al. 2021) (Fig. 2). Ten specimens were fixed in 10% formalin (Serdiati et al. 2020) and deposited at the Laboratory of Universitas Bangka Belitung, Indonesia, and five specimens were preserved in 95% ethanol (Valen et al. 2019a) for subsequent DNA analysis.

Morphological analysis: The morphological data including, meristic and morphometric characters

Table 1. Meristics of *Barbodes lateristriga* from Menumbing hill river flow, Bangka Island, Indonesia (n=19).

Meristics (counts)	Present study	Alfred (1966)
Pored Lateral line scales	24-25	22-24
Dorsal fin rays	II.8	IV.8
Anal fin rays	iii.6-7	iii.5-6
Pectoral fin rays	i.12-13	i.12-13
Ventral fin rays	i.7-8	i.8

following the Kottelat & Lim, (2021), Alfred (1966), Sutin et al. (2007) and Lim et al. (2016) were measured.

DNA Extraction: The extraction of DNA was performed using the Genomic DNeasy Blood & Qiagen Tissue Kit. 25mg of tissue samples that put into a 1.5ml tube with sterile tweezers. Then, 180µl of ATL buffer and 20µl of proteinase K were added into tube, vortexed, and centrifuged for 20 seconds, and heated in a heating block at 56°C overnight. Then 200µl Buffer AL add, vortexed, and incubated at 56°C for 10min. Afterward, 200µl 96% ethanol was added and vortexed. The sample and reagent mixture was transferred to a DNeasy Mini spin column which was placed in a 2ml collection tube, then centrifuged at 8000 rpm for 1 minute. The liquid was drained into the collection tube, placed in the spin column in a new 2ml collection tube, 500µl of buffer AW1 added to it, and centrifuged at 8000 rpm for 1min. The fluid was discarded, and the collection tube was placed in the spin column in a new 2ml collection tube, added 500µl of buffer AW2, and centrifuged at 14,000 rpm for 3min. The fluid discard and collection tube were transferred to the spin column to a new 1.5ml tube. DNA was eluted by adding 100µl of ddH₂O to the center of the membrane spin column and incubated at room temperature (15-25°C) for 1 minute. Then centrifuged at 8,000rpm for 1 minute. The final step was repeated by adding 100µl of ddH₂O, so a final volume of 200µl was obtained.

Amplification and sequencing: The extracted DNA was amplified using primers of FISH F1 and FISH R1 of COI (cytochrome C oxidase I) (Ward et al. 2005) gene using the Hotstart method. The parameters of this method are pre-denaturation at 94°C for 3min, denaturing at 94°C for 30 seconds,

annealing at 48°C for 30 seconds, and extension at 72°C for 45 seconds. The PCR process was repeated for 38 cycles. The PCR results were visualized in 1% agarose gel via electrophoresis by staining Nucleic Acid Gel Stain (GelRed®) (Valen et al. 2019b). The samples were amplified by the PCR method, then sequenced by a sequencing service facility using the Sanger sequencing method (Sanger & Nicklen 1977).

Data analysis: We identify the species using The BOLD SYSTEM (<https://www.boldsystems.org>) and BLASTn (Basic Local Alignment Search Tool-nucleotide) method in the NCBI (National Center for Biotechnology Information) (<https://blast.ncbi.nlm.nih.gov>) to analyze the sequence homology and similarity. The evolutionary tree was performed based on the Maximum Likelihood method using the Tamura-Nei model (Tamura & Nei 1993). The analysis involved 22 COI sequences each with a total of 639 positions in the final dataset. For this purpose, we downloaded the sequences from the Genbank, including tree sequences of *B. lateristriga*, tree sequences of *B. semifasciolatus*, tree sequences of *B. tumba*, and tree sequences of *B. binotatus*. All sequences were initially aligned using the Clustal W and calculated the genetic distance was calculated using the p-distance method (Nei & Kumar 2000) in MEGAX software (Kumar et al. 2018). Tree reconstruction was done in MEGA X (Kumar et al. 2018).

RESULTS AND DISCUSSIONS

Morphological identifications: The meristic characters of *B. lateristriga* are given in Table 1. Other morphological characteristics of *B. lateristriga* are as follows: Body compressed,

Table 2. DNA Barcoding of the Spanner barb from Menumbing Hill Stream, Bangka Island, Indonesia.

DNA Barcoding
CCCTTTTTCGTATTTGGGTGCCTGAGCCGGAATAGTGGAACCGCCCTAAGCCTTCTTATTTCGGGCTGAATTAAGCC AACCTGGGTCACTTTTAGGTGATGACCAAATTTATAATGTTATTGTTACTGCCACGCCTTCGTGATAATTTTCTTTA TAGTAATGCCTATCCTTATTGGAGGATTTGGAACTGACTCGTACCCTAATAATTGGAGCCCCAGATATAGCATT CCCACGAATAAATAATATAAGCTTCTGACTACTACCTCCATCATTTTTATTACTGCTAGCCTCTTCTGGGGTTGAGG CAGGGGCAGGAACAGGATGAACAGTGTACCCACCTCTCGCAGGAAACCTAGCCCATGCCGGAGCATCAGTAGATT TAACAATTTTTCTACTACCTAGCAGGTGTCTCATCAATTCTAGGGGCAATTAATTTTTATTACCACAACGATTAAC ATAAAACCCCCGGAATCTCTCAATACCAAACACCTTTATTTGTCTGATCAGTACTTGTAAGTGTGTACTACTTCT ATTATCACTACCAGTGTTAGCCGCTGGGATTACAATACTTCTAACAGATCGAAACCTCAACACCACATTCTTTGACC CGGCAGGTGGAGGAGACCAATCCTTTACCAACACTTATTTTGATTCTTTGGCCAC

Table 3. Species Identification and Similarity of The Spanner barb from Menumbing hill river flow, Bangka Island, Indonesia.

Specimen	Similarity GenBank	Species identified	Accession Number (GenBank)	Phylum	Class	Family	Genus
Spanner barb	100%	<i>B.lateristriga</i>	MN342337.1	Chordata	Actinopterygii	Cyprinidae	<i>barbodes</i>
Spanner barb	99%	<i>B.lateristriga</i>	MN342336.1	Chordata	Actinopterygii	Cyprinidae	<i>barbodes</i>
Spanner barb	99%	<i>B.lateristriga</i>	MN483453.1	Chordata	Actinopterygii	Cyprinidae	<i>barbodes</i>

**Fig.3.** Distribution of *Barbodes lateristriga* in Shothest Asia regions. Black circles are the previous record. Red circle is the recent record from Bangka Island, Indonesia.

mouth terminal, teeth villiform; scale ctenoid; tail forked; stomach J-shape; maxillary barbels present; eye moderately large; rostral barbels present; 24-25 scales on lateral line row on body; average total length of 7.5-8.5cm. *Barbodes lateristriga* was larger than ± 8 cm, whereas, in previous findings in Singapore, its size was around 3.2cm (Lim et al. 2016) and identical to the specimens from Southeast Thailand with a size of 5.57-8.24cm (Sutin et al. 2007).

Molecular identifications: COI of Spanner barb

from Bangka Island was sequenced with a base-pair length of 670 bp (Table 2). According to Hebert et al. (2003) and Ward et al. (2005) fragments that have more than 655 base pairs of COI genes can be used as a standard for differentiating between animals. Based on the results, the samples from the Menumbing hill river flow belonged to *B. lateristriga* with a similarity of 99-100% (Table 3). According to Hebert et al. (2003), Species with 99-100% similarity levels are identical.

The discovery of *B. lateristriga* in Menumbing hill river flow, Bangka Island, Indonesia is a new record of this species (Fig. 3). New records of fish contribute to understanding species diversity and biogeography (Valen et al. 2020; Valen et al., 2022c). The previous record of the Spanner barb in Indonesia was from West Kalimantan (Robert 1989; Rachmatika, 2004) and the presence of *B. lateristriga* in Bangka Island is its second record for Indonesia. This species was also previously found in the Malay Peninsula (Halim et al. 2018; Aqmal & Amirrudin 2018; Ng et al. 2019; Ren et al., 2020), Singapore (Li et al. 2015) and Southern Thailand (Sutin et al. 2007). The existence of *B. lateristriga* in the Malay Peninsula, Singapore, Southeast Thailand, West Kalimantan, and the Bangka Islands proves that these islands were

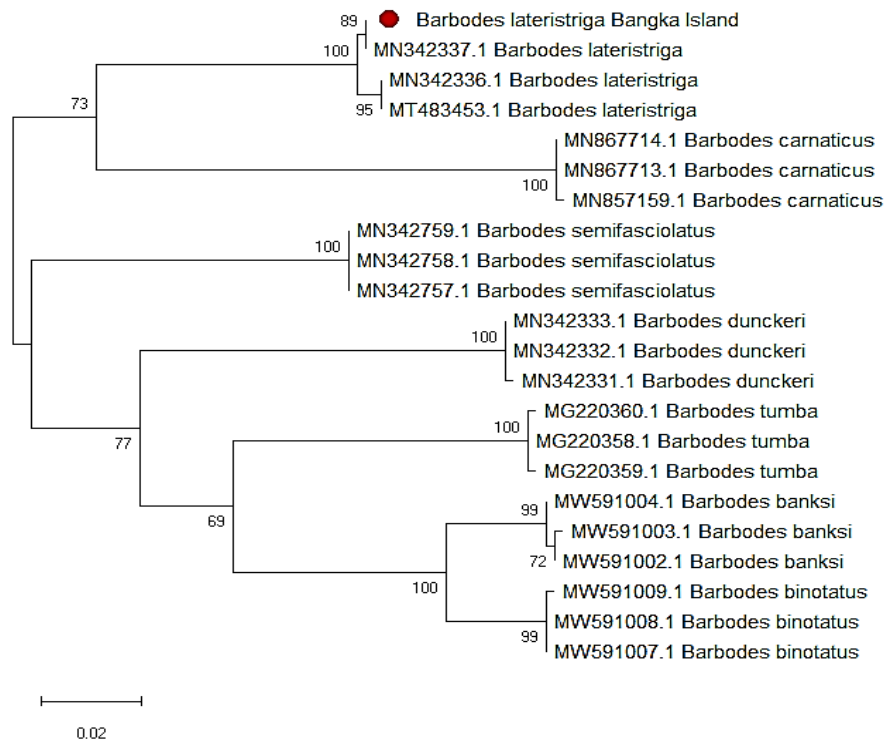


Fig.4. Phylogenetic tree of *Barbodes lateristriga* based on ML using COI Gene.

connected to the Sundaland, the ancient river in the old times. The extent of the Sundaland is approximately 1,800,000 km² including the Malay Peninsula, Sumatra and Java, and the islands of Borneo (Ng & Kottelat 2016). *Barbodes lateristriga* was found in Menumbing hill river flow, Bangka Island on the rocky freshwater with high areas on the hills and clean waters. This ecosystem was natural and far from residential areas. The habitat was the same as the *B. lateristriga* habitat found in previous records, in tropical freshwater, below waterfalls, and usually found in clear mountain streams with rocks and boulders (Sutin et al. 2007). The phylogenetic tree of *B. lateristriga* was drawn to a scale of 0.02 using 22 sequences (Fig. 4). The genus *Barbodes* was monophyletic, and that of Bangka Island clustered with *B. lateristriga* from the Malay Peninsula. They have been separated for a long time during the 2nd glacial. *Barbodes lateristriga* is also found in Kalimantan and the Malay Peninsula (Kottelat 2013; Amirrudin & Zakaria 2014; Fahmi et al. 2015; Aqmal & Amirrudin 2018), *B. carnaticus* inhabits the Cauvery River basin, Kerala, southern India (Ramya et al. 2021), *B. semifasciolatus* is from East

Asia and introduced elsewhere (Dyldin et al. 2020), *B. dunckeri* (Sobri et al. 2021) is from Malaysia, *B. tumba* from the Philippines (Abdulmalik-Labe & Quilang 2019), *B. banksi* from Borneo (Kottelat & Lim 2021; Jamaluddin et al. 2022), and *B. binotatus* from Southeast Asia (Ng & Tan 2021). *Barbodes banksi* and *B. binotatus* have the closest distance since they found the same habitat.

Based on the genetic distance results (Table 4), the closest distance was found between *B. lateristriga* from Bangka Island and *B. lateristriga* from the GenBank database with a distance of 0.000, i.e the two sequences are identical. Even the two samples came from different locations. Bangka Island had been isolated from the second glacial phase from the islands of Sumatra and Sundaland.

In addition, the closest genetic distance found between *B. banksi* and *B. binotatus* as 4.2%. Both species belong to the category of low genetic distance according to Nei (1972). Then the most genetic distance was recorded between *B. binotatus* and *B. carnaticus* as 17.4%, followed by *B. carnaticus* and *B. banksi* with a genetic distance of 16.6%, and *B. dunckeri* and *B. semifasciolatus* with a genetic distance of 14.7% which are included in

Table 4. The genetic distance of *Barbodes lateristriga*.

	1	2	3	4	5	6	7
1 <i>Barbodes lateristriga</i> Bangka_Island							
2 MN342337.1 <i>Barbodes lateristriga</i>	0,000						
3 MW591009.1 <i>Barbodes binotatus</i>	0,138	0,138					
4 MW591004.1 <i>Barbodes banksi</i>	0,127	0,127	0,042				
5 MN867714.1 <i>Barbodes carnaticus</i>	0,141	0,141	0,174	0,166			
6 MN342759.1 <i>Barbodes semifasciolatus</i>	0,124	0,124	0,137	0,140	0,164		
7 MN342333.1 <i>Barbodes dunckeri</i>	0,137	0,137	0,134	0,131	0,163	0,147	

the medium genetic distance category. According to Nei (1972) a genetic distance of 0.010-0.099 is included in the low category, and 0.1-0.99 is included in the medium. In general, the genetic distance between *B. lateristriga*, *B. carnaticus*, *B. semifasciolatus*, *B. dunckeri*, *B. tumba*, *B. banksi*, *B. binotatus* belongs to the medium genetic distance category with the genetic distance range between them is 0.124-0.174. This is the exception for *B. banksi* and *B. binotatus* which fall into the category of a low genetic distance of 0.042.

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مقاله کامل

گسترش دامنه پراکنش *Barbodes lateristriga* (Valenciennes, 1842)

(کیورماهی شکلان: کیورماهیان) به جزیره بانگکا، اندونزی

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چکیده: ماهی *Barbodes lateristriga* از خانواده کیورماهیان، گونه بنتوپلاژیک است که در جویبارهای شفاف کوهستانی با صخره‌ها و بسترهای تخته سنگی زندگی می‌کند و اغلب در زیر آبشارها یافت می‌شود. این گونه قبلاً از جنوب تایلند، شبه جزیره مالایی، سنگاپور و بورنئو گزارش شده بود. در این مطالعه، این گونه از جزیره بانگکا، اندونزی گزارش می‌گردد. جزایر بانگکا در سوندالند واقع شده است که تنوع زیستی بسیار بالایی دارد. نمونه‌های *B. lateristriga* از رودخانه منومینگ هیل، جزیره بانگکا، اندونزی صید شد سپس از نظر ریخت‌شناسی و مولکولی شناسایی شد. شناسایی مولکولی با استفاده از روش بارکد DNA و ژن سیتوکروم اکسیداز I (COI) انجام شد. نتایج آنالیز مولکولی نمونه‌های جزایر بانگکا را با سطح شباهت تا ۱۰۰٪ به‌عنوان *B. lateristriga* نشان داد. همچنین نتایج تجزیه و تحلیل درخت فیلوژنی تایید می‌کند که در آن نمونه‌هایی از جزایر بانگکا در همان گروه *B. lateristriga* یافت شده در بانک ژن قرار گرفتند.

کلمات کلیدی: سیتوکروم اکسیداز I، کیورماهیان، بارکد DNA، ژن.