

Anthropogenic introduction of the Spotted Barb, *Barbodes binotatus*, across the Wallace Line in western Sulawesi, Indonesia

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Manuscript received: 2 March 2023. Revision accepted: 21 March 2023.

Abstract. Astuti SS, Hariati AM, Kusuma WE, Yuniarti A, Kurniawan N, Wiadnya DGR. 2023. Anthropogenic introduction of the Spotted Barb, *Barbodes binotatus* across the Wallace Line in western Sulawesi, Indonesia. *Biodiversitas* 24: 1916-1925. *Barbodes binotatus* (Valenciennes, 1842) is a native freshwater fish species in the western part of Indonesia, ranging from Sumatra, Borneo, Java, and Bali Islands to the east in the Wallacea region as far as Lombok and Sumbawa Islands. We reported the first record of its occurrence in other Wallacea regions, especially in the western part of Sulawesi Island. Thirty specimens were captured from the Salu Salibo and Lantora Rivers during the field survey. Morphological examinations and genetic confirmation by DNA barcoding approach provided accurate and reliable identification for *B. binotatus*. The DNA data suggested that the existence of *B. binotatus* in the western part of Sulawesi Island, across the Wallace Line, is due to human introduction. The human introduction of non-native species, such as *B. binotatus*, may cause a severe decline in native ichthyo-biodiversity in Sulawesi Island. We, therefore, suggest that further studies are important to mitigate the negative consequences of this supposedly introduced species on the island.

Keywords: COI, invasive species, nucleotide BLASTN, phylogenetic tree, Wallacea

INTRODUCTION

Barbodes is a genus of 44 taxonomically-accepted species (Van der Laan et al. 2014; Fricke et al. 2022). Among these number, nine species are naturally distributed in Indonesia: *Barbodes binotatus* (Valenciennes, 1842), *B. bunau* (Rachmatika, 2005), *B. everetti* (Boulenger, 1894), *B. kuchingensis* (Herre, 1940), *B. lateristriga* (Valenciennes, 1842), *B. microps* (Günther, 1868), *B. rhombeus* (Kottelat, 2000), *B. sealei*, and *B. sellifer* (Kottelat 2013; Van der Laan 2018; Kottelat and Lim 2021). *B. binotatus* is economically important in Indonesia as locals consume it as a protein source and its popularity as an ornamental fish (Batubara et al. 2019). Therefore, this species has a wide geographic distribution in Indonesia, from Sumatra, Borneo, Java, and Bali Islands to the east, the Lesser Sunda Island of Lombok and Sumbawa Islands (Froese and Pauly 2019; Chua et al. 2019). This primary freshwater has a relatively small body size, with the largest size reaching 20 cm in standard length. They inhabit various habitats, from fast-moving river streams with clean and highly oxygenated water to non-flowing waters. In addition, it occurs in medium to large rivers, irrigation canals, ponds, lakes, and ditches. Both adults and juveniles feed on various types of zooplankton, insect larvae, and some vascular plants (Rainboth 1996; Froese and Pauly 2019). *B. binotatus*, or the spotted barb, features two large, rounded

spots on its body: one at the anterior base of the dorsal fin and another at the middle of the caudal peduncle. This species can be distinguished from other species in the genus *Barbodes* mainly based on body color pattern: a dark mid-lateral stripe located starting from the upper extremity of the gill opening to the middle of the caudal-fin base, usually wider on the body than on the caudal peduncle (Kottelat 2000; Kottelat and Lim 2021).

The taxonomic history of *B. binotatus* is somewhat complicated. This species was originally described by Kuhl and van Hasselt in 1823 under the name of *Barbus maculatus* before subsequently changing the name to *Barbus striatus* (van Hasselt 1823). However, due to insufficient description and lack of references, both names were invalid and unavailable (*nomen nudum*) (Roberts 1993; Kottelat 2000). Later, in 1842, Achille Valenciennes examined materials from Java Island, provided a sufficient description, and named the specimens *Barbus binotatus* (Valenciennes in Cuvier and Valenciennes 1842). Afterward, this species was categorized into different genera: *Barbodes*, *Systemus*, and *Puntius*, before it was finally assigned to the genus *Barbodes* (Kottelat 1999; Pethiyagoda and Kottelat 2005; Kottelat 2013; Sobri et al. 2021).

Wallacea is a transitional biogeographical region between the Indo-Malayan region on the western side and the Australasian region on the eastern side and has long

been considered a region with complex geological and climate history (Stelbrink et al. 2012; Parenti 2019; Ali et al. 2021; Hasan et al. 2021). The boundary of Wallacea is formed by the deep and wide Lombok and Makassar Straits on the western side and the Australo-Papuan Shelf on the eastern side (Lourie and Vincent 2004; Anderson et al. 2020; Buckley et al. 2020). Wallacea consists of several Islands, including Sulawesi Island, Lesser Sunda, Halmahera, Buru, Seram, and other smaller islands. This region has long been known as one of the world's biodiversity hotspots with a high level of endemism, including freshwater fish (Lohman et al. 2011; Kealy et al. (2016)). Over 300 freshwater fish species can be found in Wallacea, of which 75 are endemic (Tweedley et al. 2013; Fisher et al. 2020). On the other hand, 70 known fish species have been recorded from Sulawesi, of which 53 are endemic to the island (Parenti et al. 2015; Hadiaty 2018; Andriyono and Fitriani 2021). However, the geographic distribution of species in Wallacea is highly influenced by natural and historical events, such as geological and climatic history (Morley 2000; Hall 2009; Lohman et al. 2011; Bacon et al. 2013; Geer et al. 2021). Some reports suggested that non-native freshwater fish species have been inadvertently or deliberately introduced into Sulawesi Island by anthropogenic activities-these fish spp. include *Amphilophus labiatus* (Günther, 1864), *Oreochromis niloticus* (Linnaeus, 1758) (Ohee et al. 2018), *Carrasius auratus* (Linnaeus, 1758), *Cyprinus carpio* Linnaeus, 1758, *Oreochromis mossambicus* (Peters, 1852) (Sultana and Hashim, 2015), *Anabas testudineus* (Bloch, 1792) (Herder et al. 2022).

Several freshwater fish of Asian origin, e.g., *Rasbora lateristriata* (Bleeker, 1854), *A. testudineus*, *Channa gachua* (Hamilton, 1822), and *B. binotatus*, were also found in Lombok and Sumbawa Islands, crossed the hypothetical faunal boundary of the Wallace Line (Briggs 1987; Kottelat et al. 1993; Berra 2001). Natural migration across salt water of a primary freshwater fish from the

western to the eastern side of the Wallace Line is quite impossible. Lombok and Makassar Straits create natural physical dispersal barriers to obstruct migration. However, the phenomenon itself cannot be negligible. Thus, the occurrence of these species in the region due to natural events or anthropogenic activities remains an enigma.

This study aims to report the first occurrence of *B. binotatus* from Sulawesi Island, especially from the western part, beyond its natural range distribution. Therefore, species identification was carefully done by conducting both morphological characterization and genetic confirmation of the collected specimens. For genetic confirmation, the DNA barcoding technique (Hebert et al. 2003; Hubert and Hanner 2015) was applied to verify our morphology-based identification. Furthermore, we used genetic data to infer the source population for the occurrence of *B. binotatus* in the region.

MATERIALS AND METHODS

Specimen collection

During the ichthyological survey in West Sulawesi, Indonesia a total of 30 individuals of *B. binotatus* were captured using nets, traps, and fishing rods from two different rivers on 8-9 February 2020, i.e.: Salu Salibo River of Moso District (3°30'19" S; 119°09'19"E) and Lantora River of Majene District (3°27'10" 'S; 119°02'29"E) (Figure 1). Fifteen individuals were collected from each locality. A small portion of the right pectoral fin was excised from two randomly-selected specimens and preserved in 99% ethanol for DNA extraction and molecular analyses. Afterward, whole specimens were remixed in 10% formalin before being transferred to 70% ethanol for final preservation. All specimens were registered to the Depository Ichthyologicum Brawijaya, Universitas Brawijaya, with voucher numbers in Table 1.

Table 1. Details for the sample collection, together with the top 10 BLASTN results based on maximum scores

Species	Country	Locality	GenBank acc. no.	Voucher number	Max. score	Reference
<i>B. binotatus</i>	Indonesia	Salu Salibo, Sulawesi	MW931716	DIB.FPIK.UB.022021.01	162	<i>This study</i>
<i>B. binotatus</i>	Indonesia	Salu Salibo, Sulawesi	MW931717	DIB.FPIK.UB.022021.02	162	<i>This study</i>
<i>B. binotatus</i>	Indonesia	Lantora, Sulawesi	MW931719	DIB.FPIK.UB.022021.07	162	<i>This study</i>
<i>B. binotatus</i>	Indonesia	Lantora, Sulawesi	MW931720	DIB.FPIK.UB.022021.08	162	<i>This study</i>
<i>B. binotatus</i>	Indonesia	West Nusa Tenggara	MN640073.1	BIF2115	174	Arisuryanti et al. (2017)
<i>B. binotatus</i>	Indonesia	West Nusa Tenggara	MN640074.1	BIF2182	166	Arisuryanti et al. (2017)
<i>B. binotatus</i>	Indonesia	Lumajang, East Java	MG699684.1	BIF2117	162	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Lumajang, East Java	MG699680.1	BIF2116	162	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Lumajang, East Java	MG699645.1	BIF2113	162	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Lumajang, East Java	MG699644.1	BIF2914	162	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Lumajang, East Java	MG699586.1	BIF2915	162	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Buleleng, West Bali, Bali	MG699681.1	BIF2464	158	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Buleleng, West Bali, Bali	MG699678.1	BIF2724	158	Hutama et al. (2016)
<i>B. binotatus</i>	Indonesia	Buleleng, West Bali, Bali	MG699669.1	BIF2725	158	Hutama et al. (2016)

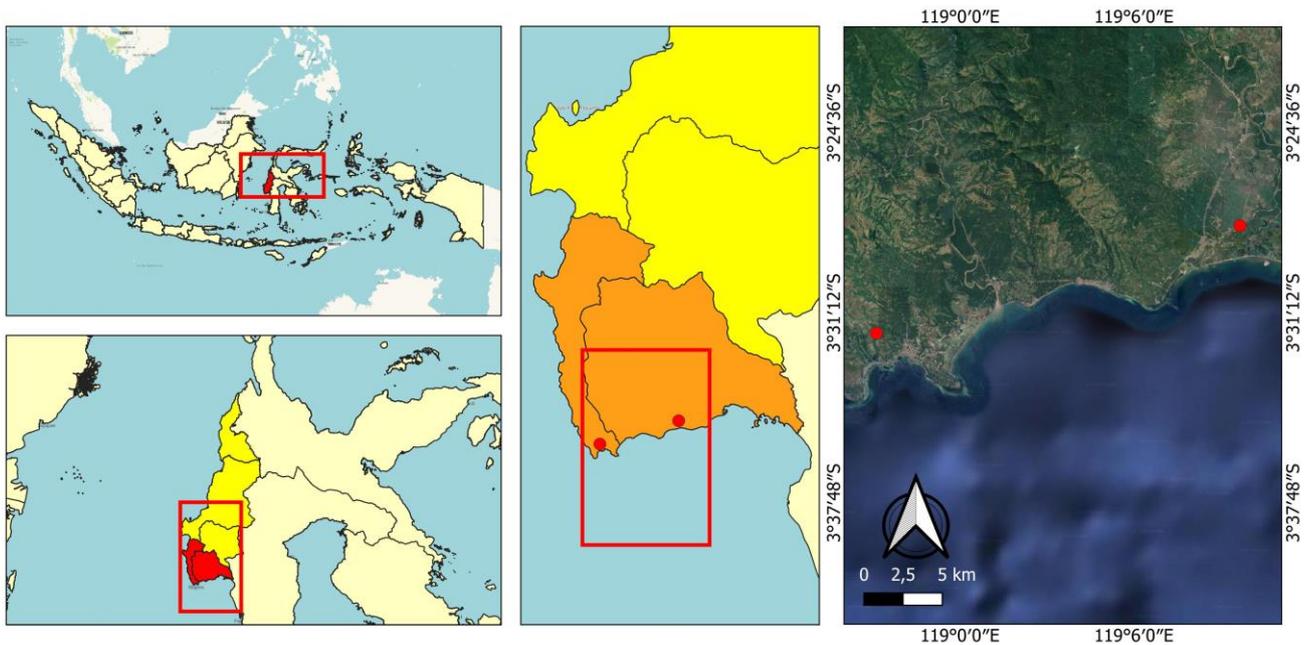


Figure 1. Maps showing research site: A. Indonesian islands with the position of Wallacea region are highlighted with light yellow color. The boundary of Wallacea region is adopted from Gill et al. (2015); B. Map of Sulawesi Island in which the locality of sampling is indicated with a red-dotted circle; C. Sampling localities of *B. binotatus* in West Sulawesi from two different rivers: Salu Salibo, and Lantora Rivers, Indonesia

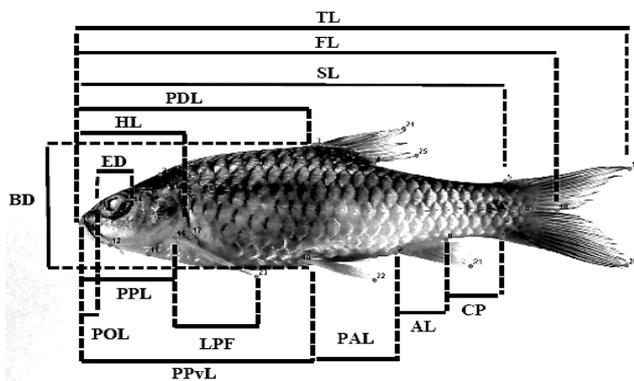


Figure 2. Fourteen morphometric characters of *B. binotatus* were measured in this study (Kottelat et al. 1999). BD: Body Depth; ED: Eye Diameter; TL: Total Length, SL: Standard Length; HL: Head Length; LPF: Pelvic Fin Length; PDL: Pre Dorsal Length; PAL: Pre Anal Length; CP: Caudal Peduncle; POL: Pre Orbital Length; AL: Anal Length; PPvL: Pre Pelvic Length; PPL: Pre Pectoral Length; FL: Fork Length

Morphological identification

Specimens from both sampling locations were identified based on the twenty-two morphological characters of *B. binotatus* (14 morphometric and eight meristic characters). Measurements and counts of morphological characters follow Hubbs and Lagler (1958) and Kottelat and Freyhof (2007). We also examined prominent distinctive characters such as two pairs of barbels, head shape, body shape, and body color patterns (Figure 2). Specimen documentation using digital marking

and a selection of landmark points was carried out using tpsDig2 v.2 (Rohlf 2015). Morphological images were converted to TPS file format using tpsUtil (Rohlf 2015).

DNA extraction, PCR amplification, and DNA sequencing

Total genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, United States) according to the manufacturer's protocol. The barcoding region of Cytochrome Oxidase subunit I (COI) was amplified by Polymerase Chain Reaction (PCR) using the primers Fish F1 and Fish R1 (Ward et al. 2005). The PCR was carried out in a reaction volume of 50 μ L which contained 25 μ L PCR master mix, 5.0 μ L forward primer (10 μ M), 5.0 μ L reverse primer (10 μ M), 5 μ L DNA template, 5 μ L Taq DNA Polymerase, and nuclease-free water. The PCR parameter was set for 5 min of initial denaturation at 94°C. Next, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 52°C for 60 s and extension at 72°C for 90 s, then followed by a 7 min final extension at 72°C. The PCR products were electrophoresed and visualized on a 1.5% agarose gel. Successful amplicons were PCR purified and sequenced using the Sanger sequencing method by Apical Scientific Sdn Bhd, Malaysia.

Molecular analysis

DNA contigs were assembled and validated based on the forward and reverse DNA sequences using Chromas v.2.6.6 (Treves 2010). Next, all DNA contigs were manually checked and aligned using MESQUITE v.3.51 (Madison and Madison 2018). Furthermore, the consensus sequence was then translated into amino acid to confirm no

possible gaps and stop codons. Finally, the consensus sequence of each specimen was converted into FASTA format and compared to the published sequence in GenBank using nucleotide BLASTN analysis (blast.ncbi.nlm.gov) to confirm species identity (McGinnis and Madden 2004; Li et al. 2019).

Then, the evaluation of the phylogenetic relationship of the newly obtained sequences from this study with other top 30 sequences of *B. binotatus* resulted from BLASTN analysis. Furthermore, we included the sequences from published articles to avoid incorrect taxonomic annotation of DNA sequences used in the phylogenetic analyses (e.g., erroneous taxonomic identification of the study specimens). Moreover, considering that sequences of *B. binotatus* collected from outside Indonesia could represent different species (e.g., Panprommin and Chanto 2014; Wu et al. (2016); Kottelat and Lim 2021), we selected sequences only from Indonesia. Finally, the nucleotide sequences from this study and GenBank were subsequently trimmed to create a COI dataset with an equal number of nucleotides (652 bp) for phylogenetic analyses.

Phylogenetic analyses were performed using Maximum Likelihood (ML) and Neighbor-Joining (NJ) methods in MEGA X (Kumar et al. 2018). The HKY+G was selected using Bayesian Information Criterion for the best-fit evolutionary model implemented in jModelTest v.2.1.10 (Posada 2008). Nodal support values in the ML and NJ trees were estimated by bootstrap analysis with 1,000 replications. *B. semifasciolatus* was selected for the outgroup. Details of sequences from BLASTN analysis with their accession numbers are presented in Table S1. Therefore, pairwise genetic distance was calculated in MEGA X to evaluate intra-or inter-specific genetic differences between sequenced individuals from the Salu Salibo and Lantora Rivers (Kumar et al. 2018). Kimura 2-Parameter (K2P) was used as a substitution model, as Hebert et al. (2003) suggested.

RESULTS AND DISCUSSION

Distribution extension record and notes on the habitat

Specimens of *B. binotatus* were collected from two rivers that have no direct connection and are separated ~3 km distance: Salu Salibo River and Lantora River (Figure 3). Individuals of *B. binotatus* were caught during the field surveys in both rivers with traditional fishing gear (nets, fishing rods, and fish traps). Salu Salibo River is characterized by the sandy and muddy substrate in most of the river bottom, while Lantora River has rocky bottom along the river flow. Salu Salibo and Lantora Rivers have heavy currents and exhibit swift current flow. Both rivers have vegetation cover on both riverbanks. These environments are typical habitats of several freshwater species, including *B. binotatus*. Species from the genus *Barbodes* occur in various habitat types, from rivers with strong currents with rocky bottoms in the highland to standing waters with sandy gravel to rocky substrates in the lowlands (Ng and Tan 2018; Ahmad et al. 2020). Species other than *B. binotatus* were also found in both rivers, e.g.,

Osteochilus vittatus (Valenciennes, 1842), *Barbonymus gonionotus* (Bleeker, 1849), *Nomorhamphus liemi* Vogt, 1978, *Marosatherina ladigesii* (Ahl, 1936), and *Oryzias celebensis* (Weber, 1894). Specimens collected varied in total length, ranging from 5 cm to 15 cm. Small specimens with a standard length of less than 8 cm were considered juvenile. The presence of juveniles in Salu Salibo River and Lantora River could indicate that this species can breed and constitute a self-sustaining population in both rivers.

Morphological characteristics

Our morphometric examinations suggested that all individuals collected from the Salu Salibo and Lantora Rivers can be identified as *B. binotatus* (Valenciennes 1842). All morphological characters observed in this study were in agreement with those described by Roberts (1989), Kottelat et al. (1993), and Froese and Pauly (2019). However, we noticed variation in the intensity of large spots at the edge caudal peduncle was observed among specimens. From 30 individuals collected in these two rivers, 18 appear to possess a large-prominent spot at the distal end of the caudal peduncle with high-intensity melanophore pigmentation. In contrast, the remaining individuals have faint and subtle intensity. According to Pethiyagoda (2012), the large black spots on *B. binotatus* may fade when the species is under stress. Fourteen morphometric characters of *B. binotatus* observed in this study are presented in Table 2.

Characterizations of meristic characteristics were done by counting eight items, followed by Hubbs and Lagler (1958) and Kottelat and Freyhof (2007) (Table 3). The results suggested that selected meristic characters are still within the range of previous studies (Haryono and Wahyudewantoro 2020; Azahar et al. 2021). Intra-and inter-specific morphological variations can be highly affected by genetic and environmental factors and their interaction with them (Waiho et al. 2021). Moreover, morphological variations among regions can be linked to adaptation to different environmental pressures (Ismen 2020). However, there are no significant differences in morphometric and meristic characteristics of *B. binotatus* from the Salu Salibo and Lantora Rivers. Both rivers have similar topographical, flow, ecosystem, and physicochemical characteristics (Tables 2 and 3).

BLASTN result and phylogenetic analysis

The four COI sequences of *B. binotatus* were identical regardless of sampling location. Pairwise genetic distance analysis confirmed no genetic differences between individuals from the two rivers (Table S2). A nucleotide BLASTN similarity sequence analysis of specimens from this study resulted in the top 30 sequences of *B. binotatus* from GenBank with a similarity value ranging from 99.25 to 100% (Table S3). Therefore, this result confirmed our morphological identification that specimens collected from the Salu Salibo and Lantora Rivers could be accurately identified as *B. binotatus*. According to Francis et al. (2018) and Yang et al. (2014), if a sequence of specimens has a similarity value between 98-100% with a sequence of

Table 1. Morphometric measurement of *B. binotatus* from Salu Salibo River and Lantora River. Morphometric characteristics are presented as a percentage of standard length except for eye diameter, interorbital width, and pre-orbital length

Characteristics	Code	Salu Salibo (n=15)		Lantora River (n=15)	
		Range	Mean ± std dev	Range	Mean ± std dev
Standard length		4.550-7.34 cmSL	5.521 ± 0.924	4.77-7.18 cmSL	5.76 ± 0.778
In percentage of standard length					
Total length	TL	1.220-1.294	1.264 ± 0.010	1.218-1.273	1.243 ± 0.025
Head length	HL	0.211-0.278	0.250 ± 0.012	0.217-0.270	0.241 ± 0.019
Pre dorsal length	PDL	0.490-0.544	0.510 ± 0.017	0.486-0.543	0.510 ± 0.017
Pre pectoral length	PPL	0.448-0.505	0.481 ± 0.023	0.453-0.503	0.468 ± 0.016
Pre anal length	PAL	0.687-0.728	0.705 ± 0.013	0.665-0.748	0.697 ± 0.014
Body depth	BD	0.277-0.320	0.292 ± 0.009	0.277-0.306	0.290 ± 0.008
Caudal peduncle	CPD	0.162-0.221	0.185 ± 0.018	0.136-0.208	0.180 ± 0.028
Pre pelvic length	PPvL	0.242-0.320	0.283 ± 0.028	0.233-0.350	0.139 ± 0.049
Anal length	AL	0.076-0.173	0.118 ± 0.036	0.064-0.227	0.155 ± 0.042
Pelvic fin length	LPF	0.242-0.320	0.283 ± 0.028	0.086-0.209	0.312 ± 0.028
In percentage of head length					
Eye diameter	ED	0.209-0.324	0.259 ± 0.030	0.225-1.761	0.261 ± 0.017
Interorbital width	IW	0.157-0.333	0.214 ± 0.054	0.112-0.303	0.171 ± 0.041
Pre orbital length	POL	0.276-0.592	0.374 ± 0.083	0.262-0.964	0.674 ± 0.253

Table 3. Meristic characters of *B. binotatus* from Salu Salibo River and Lantora River

Characteristic	Number in range	
	Salu Salibo (N=15)	Lantora River (N=15)
Number of dorsal fin rays	I, 7-8	I, 7-8
Number of pectoral fin rays	I, 9-11	I, 8-11
Number of anal fin rays	4-5	4-5
Number of pelvic fin rays	I, 6-8	I, 6-7
Number of caudal fin rays	14-16	14-16
Lateral line scales	9-10	9-10
Circumpeduncular scales	12	12
Transverse scales	4 ½ 4	4 ½ 4

Both nucleotide BLASTN and phylogenetic analyses revealed that *B. binotatus* from Sulawesi Island has an identical haplotype and therefore was grouped with sequences from Lumajang, East Java. This indicates the point source for introducing *B. binotatus* into Sulawesi Island. According to these results, the most plausible explanation for the occurrence of *B. binotatus* in the Salu Salibo River and Lantora River was due to human introduction, either unintentionally or deliberately. This hypothesis was supported by records from Nur et al. 2021 and Serdiati et al. 2021, that introduction around ninety thousand individuals of cyprinids was carried out for the restocking program in Sulawesi Island. Therefore, *B. binotatus* might have been accidentally transported to this island through this activity. Although we cannot rule out the possibility of the natural dispersal of *B. binotatus* from East Java to Sulawesi Island, this hypothesis is implausible as the Java Sea separates Sulawesi Island at a great geographical distance from East Java. Another additional hypothesis for *B. binotatus* in Sulawesi Island is that individuals of *B. binotatus* might have migrated from Borneo Island, the closest island to Sulawesi Island, where this species is naturally distributed.

Table S3. A total of 5 haplotypes with intra-species sequence from this study and the top 30 published sequence of *B. binotatus* from GeneBank

Haplotype	Individual's acc. no.	Locality
1	MW931716*	Salu Salibo, Sulawesi
	MW931717*	Salu Salibo, Sulawesi
	MW931719*	Lantora River, Sulawesi
	MW931720*	Lantora River, Sulawesi
	MG699684.1	Asem, Lumajang, East Java
	MG699680.1	Mujur, Lumajang, East Java
	MG699645.1	Asem, Lumajang, East Java
	MG699644.1	Asem, Lumajang, East Java
	MG699586.1	Asem, Lumajang, East Java
	2	MN640073.1
MN640074.1		Lebo Taliwang, Nusa Tenggara
MG699681.1		Buleleng, Bali
MG699678.1		Buleleng, Bali
MG699669.1		Buleleng, Bali
MG699650.1		Asem, Lumajang, East Java
MG699606.1		Kelungkung, Bali
3	MG699581.1	Kelungkung, Bali
	MG699648.1	Kelungkung, Bali
	MG699647.1	Jembrana, Bali
	MG699646.1	Jembrana, Bali
4	KT960754.1	Jembrana, Bali
	KT960753.1	Bendung Bekel, Bali
	MG699683.1	Mujur, Lumajang, East Java
	MG699682.1	Mujur, Lumajang, East Java
5	MG699641.1	Mujur, Lumajang, East Java
	MG699612.1	Mujur, Lumajang, East Java
	MG699677.1	Karang Anyar, Samin, Central Java
	MG699630.1	Purwokerto, Central Java
	MG699626.1	Camis, West Java
	MG699621.1	Camis, West Java
	MG699620.1	Camis, West Java
	MG699596.1	Mojokerto, East Java
MG699594.1	Karang Anyar, Central Java	
MG699593.1	Karang Anyar, Central Java	

Note: *) Sequences obtained from this study

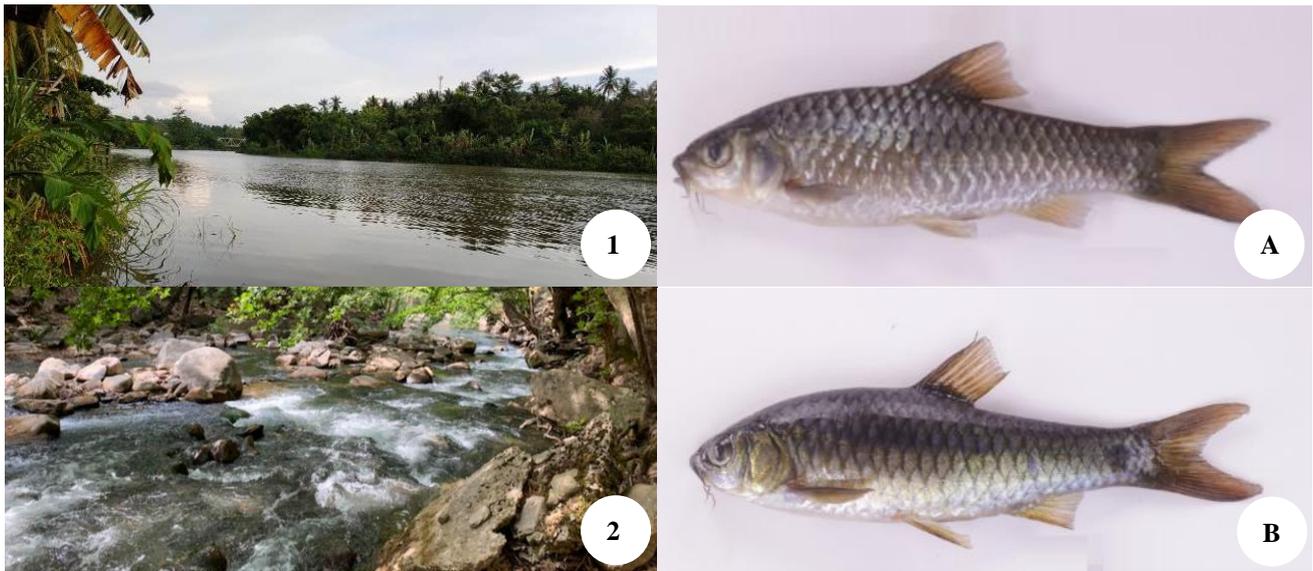


Figure 3. Habitats of *B. binotatus* at West Sulawesi: 1. Salu Salibo River, and 2. Lantora River. Lateral images of *B. binotatus* from Salu Salibo River (A: voucher number DIB.FPIKUB.022021.09; 10.5 cm standard length) and Lantora River (B: DIB.FPIKUB.022021.04; 8.7 cm)

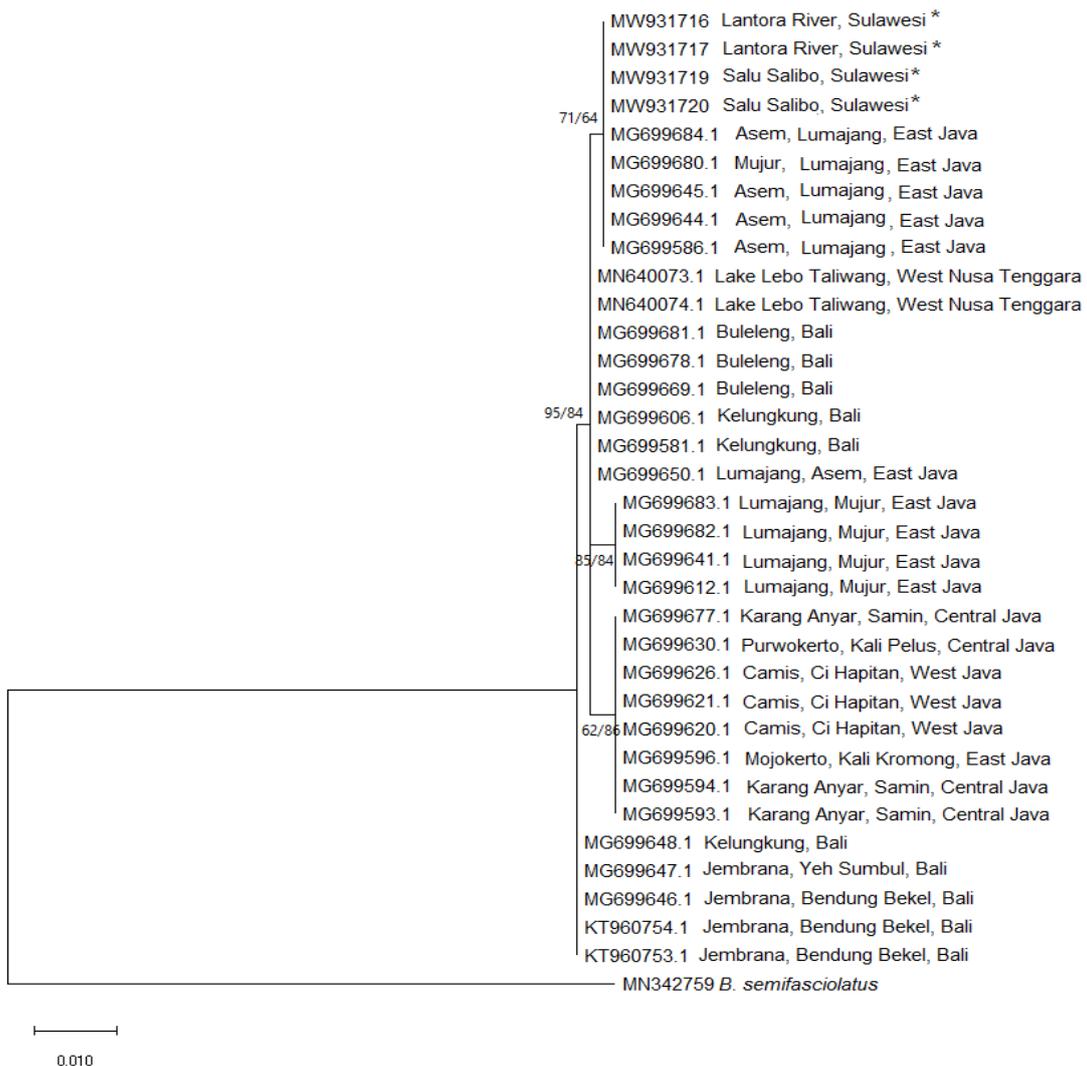


Figure 4. A maximum likelihood tree showing the phylogenetic relationships of *B. binotatus* from Indonesia. Sequences obtained from this study were denoted with an asterisk (*)

However, the latter explanation is unlikely as these two islands are separated by the deep and wide Makassar Strait (Gordon et al. 2019; Rossi et al. 2023). This deep and wide strait forms a physical barrier for species to inhibit migration, especially for primary freshwater fish like *B. binotatus*.

In this study, we recorded for the first time the occurrence of a cyprinid fish, *B. binotatus*, on Sulawesi Island, which is outside of its natural range. Our genetic data suggested that the existence of this species seems to be associated with the human introduction. Unfortunately, although Sulawesi Island is a region with enormous freshwater fish species with a high degree of endemism, its ichthyo-biodiversity is under severe threat due to the introduction of invasive species. These several invasive fish species, e.g., *O. niloticus*, *Cichlasoma nigrofasciatum* (Günther, 1867), *Poecilia reticulata* Peters, 1859, *Anabas testudineus* (Bloch, 1792), *Aplocheilichthys panchax* (Hamilton, 1822), have been reported to be introduced and caused negative impacts towards native species in this island (Herder et al. 2012; Umar et al. 2015; Nasution et al. 2019; Parenti 2019; Hediando and Sentosa 2020; Yanuarita et al. 2020). Introducing invasive fish species will cause negative consequences, e.g., predation and competition for native and endemic species, ecological imbalances, or even disruption in food webs, which will eventually cause a significant decline in the native biodiversity (Islami 2020; Saba et al. 2021). Therefore, further study and monitoring of *B. binotatus* in Sulawesi Island, e.g., distribution, abundance, ecological role, and feeding habits, are critical to evaluate this species' invasibility and mitigate its negative consequences on the native biota of Sulawesi Island.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for providing financial support under the grant *Program Magister Menuju Doktor untuk Sarjana Unggul* (PMDSU) No. 023 /E4.1/AK.04.PT/2019. The author also thanks Wahyu Widia Ningrum, Kiki Nur Azam Kholil, Muhammad Nurfaiz Hibatullah, Ahmad Muammar Kadafi (Universitas Palangka Raya), and Firda Rabbani Razaqi for their assistance in research activities. We acknowledged insightful comments from Dr. Tan Heok Hui and two other anonymous reviewers on the earlier version of the manuscript.

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