

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

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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*, *Systomus*, 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)

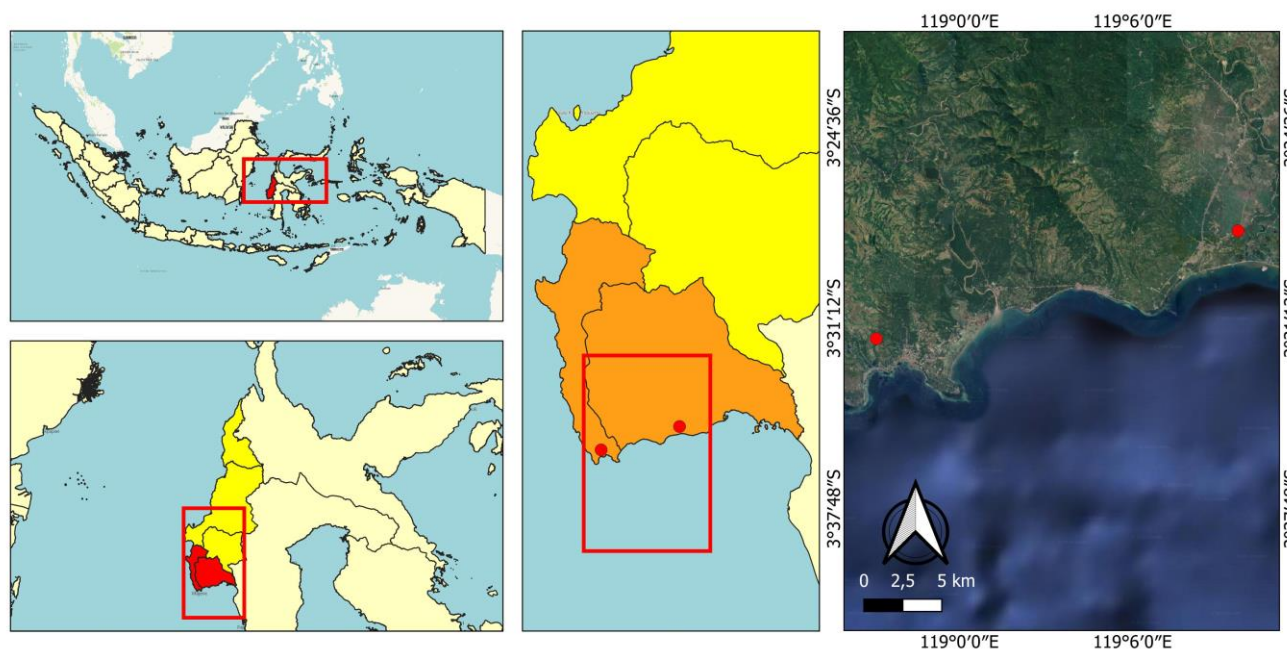


Figure 1. Maps showing research site: A. Indonesian islands with the position of Wallacea region are highlighted with light yellow color. The boundary of Wallacea region is adopted from Gill et al. (2015); B. Map of Sulawesi Island in which the locality of sampling is indicated with a red-dotted circle;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

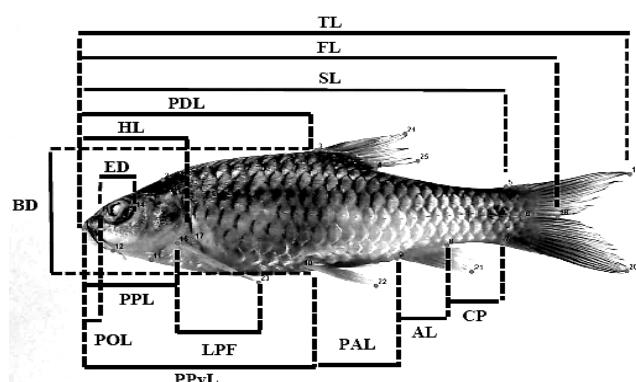


Figure 2. Fourteen morphometric characters of *B. binotatus* were measured in this study (Kottelat et al. 1999). BD: Body Depth; ED: Eye Diameter; TL: Total Length, SL: Standard Length; HL: Head Length; LPF: Pelvic Fin Length; PDL: Pre Dorsal Length; PAL: Pre Anal Length; CP: Caudal Peduncle; POL: Pre 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 µL which contained 25 µL PCR master mix, 5.0 µL forward primer (10 µM), 5.0 µL reverse primer (10 µM), 5 µL DNA template, 5 µL 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. semifasciatus* 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

a species deposited in GenBank, the obtained sequence can be assigned as the same species.

Therefore, to evaluate the phylogenetic relationships of the obtained sequences in this study with the top 30 sequences of *B. binotatus* resulting from nucleotide BLASTN similarity sequence search, we conducted a phylogenetic analysis using ML and NJ methods. Our ML and NJ trees corroborate the nucleotide BLASTN analysis

in which specimens from the Salu Salibo and Lantora Rivers were clustered together with those from Lumajang localities (Figure 4). Furthermore, the grouping of sequences from Sulawesi Island (Salu Salibo River and Lantora River) with Lumajang was further supported by moderate bootstrap value (71% and 64% for ML and NJ, respectively).

Table S1. The top 30 published sequences of *B. binotatus* from GenBank resulted from nucleotide BLASTN analysis

GenBank acc. no.	Locality	Length (bp)	Max Score	%Identity	% Query coverage	References
MN640073.1	Lake Lebo Taliwang, West Nusa Tenggara	652	1174	99.25	97	Arisuryanti, et al. (2017)
MN640074.1	Lake Lebo Taliwang, West Nusa Tenggara	652	1166	99.69	95	Arisuryanti, et al. (2017)
MG699684.1	Asem, Lumajang, East Java	652	1162	100	94	Hutama et al. (2017)
MG699680.1	Mujur, Lumajang, East Java	652	1162	100	94	Hutama et al. (2017)
MG699645.1	Asem, Lumajang, East Java	652	1162	100	94	Hutama et al. (2017)
MG699644.1	Asem, Lumajang, East Java	652	1162	100	94	Hutama et al. (2017)
MG699586.1	Asem, Lumajang, East Java	652	1162	100	94	Hutama et al. (2017)
MG699681.1	Buleleng, West Bali, Bali	652	1158	99.84	94	Hutama et al. (2017)
MG699678.1	Buleleng, West Bali, Bali	652	1158	99.84	94	Hutama et al. (2017)
MG699669.1	Buleleng, West Bali, Bali	652	1158	99.84	94	Hutama et al. (2017)
MG699606.1	Kelungkung, West Bali, Bali	652	1158	99.84	94	Hutama et al. (2017)
MG699581.1	Kelungkung, West Bali, Bali	652	1158	99.84	94	Hutama et al. (2017)
MG699648.1	Kelungkung, West Bali, Bali	652	1153	99.69	94	Hutama et al. (2017)
MG699647.1	Jembrana, Yeh Sumbul, Bali, West Bali,	652	1153	99.69	94	Hutama et al. (2017)
MG699646.1	Jembrana, Bendung Bekel, Bali, West Bali	652	1153	99.69	94	Hutama et al. (2017)
KT960754.1	Jembrana, Bendung Bekel, Bali, West Bali	652	1153	99.69	94	Dahrudin et al. (2016)
KT960753.1	Bendung Bekel, Bali, West Bali, Jembrana	652	1153	99.69	94	Dahrudin et al. (2016)
MG699650.1	Lumajang, Asem, East Java	652	1151	99.53	94	Hutama et al. (2017)
MG699683.1	Lumajang, Muju, East Java	652	1149	99.53	94	Hutama et al. (2017)
MG699682.1	Lumajang, Muju, East Java	652	1149	99.53	94	Hutama et al. (2017)
MG699677.1	Karang Anyar, Samin, Central Java	652	1149	99.53	94	Hutama et al. (2017)
MG699641.1	Lumajang, Muju, East Java	652	1149	99.53	94	Hutama et al. (2017)
MG699630.1	Purwokerto, Kali Pelus, Central Java	652	1149	99.53	94	Hutama et al. (2017)
MG699626.1	Camis, Ci Hapitan, West Java	652	1149	99.53	94	Hutama et al. (2017)
MG699621.1	Camis, Ci Hapitan, West Java	652	1149	99.53	94	Hutama et al. (2017)
MG699620.1	Camis, Ci Hapitan, West Java	652	1149	99.53	94	Hutama et al. (2017)
MG699612.1	Lumajang, Muju, East Java	652	1149	99.53	94	Hutama et al. (2017)
MG699596.1	Mojokerto, Kali Kromong, East Java	652	1149	99.53	94	Hutama et al. (2017)
MG699594.1	Karang Anyar, Samin, Central Java	652	1149	99.53	94	Hutama et al. (2017)
MG699593.1	Karang Anyar, Samin, Central Java	652	1149	99.53	94	Hutama et al. (2017)
MN342759	<i>Barbodes semifasciolatus</i> , Johor, Malaysia*	652	733	87.23	94	Sobri et al. 2021

Note: * Outgroup taxon used for phylogenetic analysis

Table S2. Pairwise genetic distance between individuals from Salu Salibo River and Lantora River. Sequences from this study were indicated by an asterisk (*)

[illegible]

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 \pm std dev	Range	Mean \pm std dev
Standard length		4.550-7.34 cmSL	5.521 \pm 0.924	4.77-7.18 cmSL	5.76 \pm 0.778
In percentage of standard length					
Total length	TL	1.220-1.294	1.264 \pm 0.010	1.218-1.273	1.243 \pm 0.025
Head length	HL	0.211-0.278	0.250 \pm 0.012	0.217-0.270	0.241 \pm 0.019
Pre dorsal length	PDL	0.490-0.544	0.510 \pm 0.017	0.486-0.543	0.510 \pm 0.017
Pre pectoral length	PPL	0.448-0.505	0.481 \pm 0.023	0.453-0.503	0.468 \pm 0.016
Pre anal length	PAL	0.687-0.728	0.705 \pm 0.013	0.665-0.748	0.697 \pm 0.014
Body depth	BD	0.277-0.320	0.292 \pm 0.009	0.277-0.306	0.290 \pm 0.008
Caudal peduncle	CPD	0.162-0.221	0.185 \pm 0.018	0.136-0.208	0.180 \pm 0.028
Pre pelvic length	PPvL	0.242-0.320	0.283 \pm 0.028	0.233-0.350	0.139 \pm 0.049
Anal length	AL	0.076-0.173	0.118 \pm 0.036	0.064-0.227	0.155 \pm 0.042
Pelvic fin length	LPF	0.242-0.320	0.283 \pm 0.028	0.086-0.209	0.312 \pm 0.028
In percentage of head length					
Eye diameter	ED	0.209-0.324	0.259 \pm 0.030	0.225-1.761	0.261 \pm 0.017
Interorbital width	IW	0.157-0.333	0.214 \pm 0.054	0.112-0.303	0.171 \pm 0.041
Pre orbital length	POL	0.276-0.592	0.374 \pm 0.083	0.262-0.964	0.674 \pm 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 $\frac{1}{2}$ 4	4 $\frac{1}{2}$ 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
2	MG699586.1	Asem, Lumajang, East Java
	MN640073.1	Lebo Taliwang, Nusa Tenggara
	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
	KT960754.1	Jembrana, Bali
4	KT960753.1	Bendung Bekel, Bali
	MG699683.1	Mujur, Lumajang, East Java
	MG699682.1	Mujur, Lumajang, East Java
	MG699641.1	Mujur, Lumajang, East Java
	MG699612.1	Mujur, Lumajang, East Java
5	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)

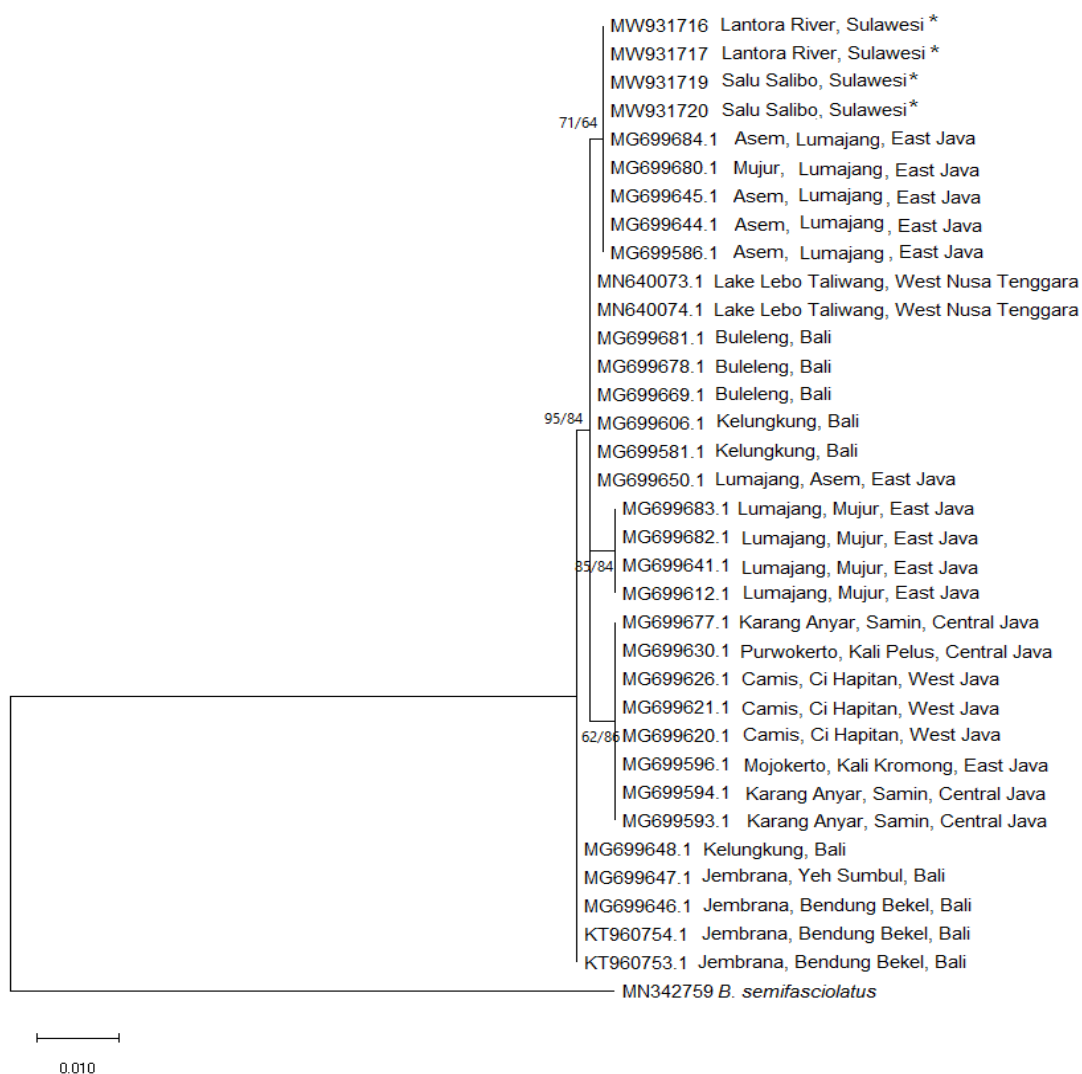


Figure 4. A maximum likelihood tree showing the phylogenetic relationships of *B. binotatus* from Indonesia. Sequences obtained from this study were denoted with an asterisk (*)

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 ichthyobiodiversity 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.

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REFERENCES

- Ahmad MF, Theng ECM, Nor SAM, Ahmad A. 2020. Deciphering species-group taxonomic complexity of common, *Barbodes binotatus* and saddle barbs, *B. banksi* in Peninsular Malaysia. *Mal J Fund Appl Sci* 16 (5): 536-547. DOI: 10.11113/mjfas.v16n5.1873.
- Ali JR, Heaney LR. 2021. Wallace's line, Wallacea, and associated divides and areas: History of a tortuous tangle of ideas and labels. *Biol Rev* 96 (3): 922-942. DOI: 10.1111/brv.12683.
- Andriyono S, Fitriani M. 2021. Non-native species existence and its potency to be invasive species on freshwater ecosystem in East Java Province, Indonesia. *Egypt J Aquat Biol Fish* 25 (2): 1013-1024. DOI: 10.21608/EJABF.2021.170621
- Arisuryanti T, Hasan RL, Ayu KL, Ratman N, Hakim L. 2019. Genetic identification of freshwater fish species through DNA barcoding from Lake Lebo Taliwang, West Nusa Tenggara. *J Trop Biodivers Biotechnol* 4 (3): 107-112. DOI: 10.22146/jtbb.46256.
- Azahar MA, Munian K, Shahfiz MA. 2021. Diversity of freshwater fish in Forest Research Institute Malaysia (FRIM): A comparison of diversity between man-made and natural forest in Selangor, Malaysia. *IOP Conf Ser: Earth Environ Sci* 842: 1-11. DOI: 10.1088/1755-1315/842/1/012027.
- Bacon CD, Michonneau F, Henderson AJ, McKenna MJ, Milroy AM, Simmons MP. 2013. Geographic and taxonomic disparities in species diversity: dispersal and diversification rates across Wallace's Line. *Evolution* 67 (7): 2058-2071. DOI: 10.1111/evo.12084.
- Batubara AS, Nur FM, Zulfahmi I, Rizal S, Efizon D, Elvyra R, Muchlisin ZA. 2019. Population dynamics of the groe fish *Barbodes binotatus* (Pisces: Cyprinidae) in the Nagan River, Aceh Province, Indonesia. *IOP Conf Ser: Earth Environ Sci* 348: 012038. DOI: 10.1088/1755-1315/348/1/012038.
- Berra TM. 2001. *Freshwater Fish Distribution*. Academic Press, California, USA.
- Buckley SJ, Brauer C, Unmack P, Hammer M, Beheregaray LB. 2021. The roles of acidification and sea level changes in the diversification and persistence of freshwater fish lineages. *Mol Ecol* 2 (1): 1-60. DOI: 10.1111/mec.16082.
- Chua KWJ, Tan HH, Yeo DCJ. 2019. Loss of endemic fish species drives impacts on functional richness, redundancy and vulnerability in freshwater ecoregions of Sundaland. *Biol Conserv* 234: 72-81. DOI: 10.1016/j.biocon.2019.03.019.
- Daban IB, Ismen A. 2020. Fish larvae assemblages of Gökçeada Island, North Aegean Sea: Effect of weekly sampling interval on their incidences. *Turk J Zool* 44: 165-172. DOI: 10.3906/zoo-1907-46.
- Dahrudin H, Hutama A, Busson F, Sauri S, Hanner R, Keith P, Hadiaty R, Hubert N. (2016). Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: Taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Mol Ecol Resour* 17 (2): 288-299. DOI: 10.1111/1755-0998.12528.
- Fisher MR, Verheijen B, Sahide MAK. 2020. Community and conservation in Wallacea: Making the case for the region, a methodological framework, and research trends. *For Soc* 4 (1): 1-19. DOI: 10.24259/fs.v4i1.9569.
- Francis CM, Borisenko AX, Ivanova NV, Eger JL, Lim BK, Servent AG, Mackie L, Hebert PDN. 2010. The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. *PLoS One* 5 (9): e12575. DOI: 10.1371/journal.pone.0012575.
- Fricke R, Eschmeyer WN, van der Laan R. 2022. Eschmeyer's catalog of fishes: Genera, species. (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>). Electronic version accessed 15 March 2022.
- Froese R, Pauly. 2019. Fishbase. World Wide Web Electronic Publication. www.fishbase.org. (December 2021).
- Gill A, Brown MN, Lubis KL. 2015. Wallacea corridor: A field guide of Wallacea biodiversity map. *Biogeograph J* 40 (3): 505-512.
- Gordon AL, Napitu A, Huber BA, Gruenbourg LK, Pujiana K, Agustadi T, Kuswardani A, Mbay N, Setiawan A. 2019. Makassar strait throughflow seasonal and interannual variability: An overview. *J Geophys Res Oceans* 124 (11): 3487-4364. DOI: 10.1029/2018JC014502.
- Hadiaty RK. 2018. Taxonomical status of endemic freshwater ichthyofauna of Sulawesi. *J Iktiologi Indones* 18 (2): 175-190. DOI: 10.32491/jii.v18i2.428.
- Hall R. 2009. Southeast Asia's changing palaeogeography. *Blumea* 54 (1-3): 148-161. DOI: 10.3767/000651909X475941.
- Haryono, Wahyudewantoro G. 2020. The alien freshwater fish of Mount Galunggung, West Java, Indonesia. *Biodiversitas* 21 (4): 1407-1414. DOI: 10.13057/biodiv/d210419.
- Hasan F, Jones DT, Syaekani S, Eggleton P. 2021. Termite transects from Buton Island, Sulawesi, have a low diversity compared with Sundaland sites. *J Trop Ecol* 37 (4): 161-164. DOI: 10.1017/S0266467421000146.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. *Proc Biol Sci* 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.
- Hediando DA, Sentosa AA. 2019. Trophic interactions of fish community in Lake Matano, South Sulawesi post-development of invasive alien

- fish species. Jurnal Penelitian Perikanan Indonesia 25 (2): 117-133. DOI: 10.15578/jppi.25.2.2019.117-133. [Indonesian].
- Herder F, Mohring J, Flury JM, Utama IV, Wantania L, Wowor D, Boneka FB, Stelbrink B, Hilgers L, Schwarzer J, Pfaender J. 2022. More non-native fish species than natives, and an invasion of Malawi cichlids, in ancient Lake Poso, Sulawesi, Indonesia. Aquat Invasions 17 (1): 72-91. DOI: 10.3391/ai.2022.17.1.05.
- Herder F, Schliwien UK, Geiger MF, Hadiaty RK, Gray SM, McKinnon JS, Walter RP, Pfaender J. 2012. Alien invasion in Wallace's dreamponds: Records of the hybridogenic "flowerhorn" cichlid in Lake Matano, with an annotated checklist of fish species introduced to the Malili Lakes System in Sulawesi. Aquat Invasions 7 (4): 521-535. DOI: 10.3391/ai.2012.7.4.009.
- Hubbs CL, Lagler KF. 1958. Fishes of the Great Lakes region. Univ. Mich. Press. Ann Arbor, Mich.
- Hubert N, Hanner R. 2015. DNA barcoding species delineation and taxonomy: A historical perspective. DNA barcodes 3: 44-58. DOI: 10.1515/dna-2015-0006.
- Hubert N, Lumbantobing D, Sholihah A, Dahruddin H, Delrieu-Trottin E, Busson F, Sauri S, Hadiaty R, Keith P. 2019. Revisiting species boundaries and distribution ranges of *Nemacheilus* spp. (Cypriniformes: Nemacheilidae) and *Rasbora* spp. (Cypriniformes: Cyprinidae) in Java, Bali and Lombok through DNA barcodes: Implications for conservation in a biodiversity hotspot. Conserv Genet 20 (3): 517-529. DOI: 10.1007/s10592-019-01152-w.
- Hutama AA, Hadiaty RK, Hubert N. (2016). Biogeography of Indonesian freshwater fishes: Current progress. Treubia 43: 17-30. DOI: 10.14203/treubia.v43i0.2969.
- Islami MM. 2020. Are they always bad? Assessing benefits of non-indigenous species in aquatic environment and their implications. Mar Res Indones 45 (2): 75-86. DOI: 10.14203/mri.v45i2.577.
- Kealy S, Louys J, O'Connor S. (2016). Islands under the sea: A review of early modern dispersal routes and migration hypotheses through Wallacea. J Island Coast Archaeol 11 (3): 364-384. DOI: 10.1080/15564894.2015.1119218.
- Kottelat M, Freyhof J. 2007. Handbook of European Freshwater Fishes. Maurice Kottelat (privately published), Berlin.
- Kottelat M, Lim KKP. 2021. Two new species of *Barbodes* from the Malay Peninsula and comments on 'cryptic species' in the *B. binotatus* group (Teleostei: Cyprinidae). Raffles Bull Zool 69 (1): 522-540. DOI: 10.26107/RBZ-2021-0069.
- Kottelat M. 1999. Nomenclature of the genera *Barbodes*, *Cyclocheilichthys*, *Rasbora* and *Chonerhinos* (Teleostei: Cyprinidae and Tetraodontidae), with comments on the definition of the first reviser. Raffles Bull Zool 47 (2): 591-600.
- Kottelat M. 2000. Diagnosis of a new genus and 64 new species of fishes from Laos (Teleostei: Cyprinidae, Balitoridae, Bagridae, Syngnathidae, Chaudhuriidae and Tetraodontidae). J South Asian Nat Hist 5 (1): 37-82.
- Kottelat M. 2013. The fishes of the inland waters of Southeast Asia : a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. Raffles Bull Zool 27: 1-663.
- Kumar S, Stetcher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.
- Lohman DJ, de Bruyn M, Page T, von Rintelen K, Hall R, Ng PKL, Shih HT, Carvalho GR, von Rintelen T. 2011. Biogeography of the Indo-Australian Archipelago. Annu Rev Ecol Evol Syst 42 (1): 205-226. DOI: 10.1146/annurev-ecolsys-102710-145001.
- Lourie SA, Vincent ACJ. 2004. Using biogeography to help set priorities in marine conservation. Conserv Biol 18: 1004-1020. DOI: 10.1111/j.1523-1739.2004.00137.x.
- Maddison WP, Maddison DR. 2018. Mesquite: A Modular System for Evolutionary Analysis. Version 3.61. <http://mesquiteproject.org>.
- McGinnis S, Madden TL. 2004. BLAST at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res 32: W20-25. DOI: 10.1093/nar/gkh435.
- Morley RJ. 2000. Origin and Evolution of Tropical Rain Forests. John Wiley & Sons, Chichester, UK.
- Nasution SH, Haryani GS, Dina R, Samir O. 2019. Threat of alien species to endemic fish in Lake Matano, South Sulawesi. Berita Biologi 18 (2): 233-245. DOI: 10.14203/beritabiologi.v18i2.2993. [Indonesian].
- Ng CKC, Tan J. 2021. Cryptic species and grey zone speciation of the *Barbodes binotatus* complex (Teleostei, Cyprinidae) in Sundaland. J Fish Biol 99 (4): 1256-1273. DOI: 10.1111/jfb.14829.
- Nur M, Fajriani, Tenriware, Simanjutak CPH, Nasyrah AFA, Kautsari N, Wahana S. 2021. Fish fauna of Batatangga River, West Sulawesi, Indonesia. E3S Web of Conf 322: 01026. DOI: 10.1051/e3sconf/202132201026.
- Ohee HL, Sujarta P, Surbakti SBR, Barclay H. 2018. Rapid expansion and biodiversity impacts of the red devil cichlid (*Amphilophus labiatus*, Günther 1864) in Lake Sentani, Papua, Indonesia. Biodiversitas 19 (6): 2096-2103. DOI: 10.13057/biodiv/d190615.
- Panprommin D, Chanto W. 2014. Fish species diversity and identification using DNA barcode in Than, Sawan Waterfall, Doi Phu Nang national park, Phayao province. Phayao University, Phayao, Thailand.
- Parenti LR, Hadiaty RK, Lumbantobing DN. 2015. Collection of freshwater and coastal fishes from Sulawesi Tenggara, Indonesia. J Iktiologi Indones 14 (1): 1-19.
- Parenti LR. 2019. Endemism and conservation of the native freshwater fish fauna of Sulawesi Indonesia. Prosiding Seminar Nasional Ikan 6: 1-10.
- Pethiyagoda R, Kottelat M. 2005. The identity of the south Indian barb *Puntius mahecola* (Teleostei: Cyprinidae). Raffles Bull Zool 12: 145-152.
- Pethiyagoda R, Meegaskumbura M, Maduwage K. 2012. A synopsis of the South Asian fishes referred to *Puntius* (Pisces: Cyprinidae). Ichthyol Explor Freshw 23 (1): 69-95.
- Posada D. 2008. jModelTest: Phylogenetic model averaging. Mol Biol Evol 25 (7): 1253-1256. DOI: 10.1093/molbev/msn083.
- Rainboth WJ. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. FAO, Rome.
- Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. Review: The detection of aquatic animal species using environmental DNA-a review of eDNA as a survey tool in ecology. J Appl Ecol 51 (5): 1450-1459. DOI: 10.1111/1365-2664.12306.
- Roberts TR. 1989. The freshwater fishes of Western Borneo (Kalimantan Barat, Indonesia). Mem Calif Acad Sci 14: 1-210.
- Roberts TR. 1993. The freshwater fishes of Java, as observed by Kuhl and van Hasselt in 1820-23. Zool Verh 285: 1-94.
- Rohlf FJ. 2015. The TPS Series of Software. Hystrix 26 (1): 9-12.
- Rossi VM, Longhitano SG, Olariu C, Chiocci FL. 2023. Straits and seaways: End members within the continuous spectrum of the dynamic connection between basins. Geol Soc 523 (1): 1-25. DOI: 10.1144/SP523-2022-159.
- Saba AO, Ismail A, Zulkifli SZ, Shohaimi S, Amal MNA. 2021. Public knowledge and perceptions of the impacts and importance of alien fish species in Malaysia: Implications for freshwater biodiversity and conservation. Manag Biol Invasion 12 (2): 441-456. DOI: 10.3391/mbi.2021.12.2.15.
- Serdiati N, Arfiati D, Widodo MS, Lelono TD, Ndobe S, Mansyur K, Moore AM. 2021. Perspectives on sustainable management of the Poso Lake (Indonesia) endemic ricefish, *Oryzias nigrimas* (Actinopterygii: Adrianichthyidae). Rev Biol Trop 69 (1): 139-152. DOI: 10.15517/rbt.v69i1.42404.
- Sobri NZA, Lavoué S, Aziz F, Nor SAM, Akib NAM, Khaironizam MZ. 2021. To lump, to split or to maintain? Molecular taxonomy of the spotted barb *Barbodes binotatus* (Cyprinidae) and closely related species in Peninsular Malaysia. J Fish Biol 99 (2): 656-668. DOI: 10.1111/jfb.14754.
- Stelbrink B, Albrecht C, Hall R, von Rintelen T. 2012. The biogeography of Sulawesi revisited: Is there evidence for a vicariant origin of taxa on Wallace's "anomalous island"? Evolution 66 (7): 2252-2271. DOI: 10.1111/j.1558-5646.2012.01588.x.
- Sultana M, Hashim ZH. 2015. Invasive alien fish species in freshwater of the continent. J Environ Sci Nat Resour 8 (2): 63-74. DOI: 10.3329/jesnr.v8i2.26868.
- Treves DS. 2010. Review of three DNA analysis applications for use in the microbiology or genetics classroom. J Microbiol Biol Educ 11 (2): 186-187. DOI: 10.1128/jmbe.v11i2.205.
- Tweedley JR, Bird DJ, Potter IC, Gill HS, Miller PJ, O'Donovan G, Tjakrawidjaja AH. 2013. Species compositions and ecology of the riverine ichthyofaunas in two Sulawesi islands in the biodiversity hotspot of Wallacea. J Fish Biol 82 (6): 1916-1950. DOI: 10.1111/jfb.12121.
- Umar C, Kartamihardja ES, Aisyah. 2015. Invasive impact of red devil fish (*Amphilophus citrinellus*) to fish diversity in inland water in Indonesia. J Kebijakan Perikan Ind 7 (1): 55-61. DOI: 10.15578/jkpi.7.1.2015.55-61. [Indonesian].

- Van der Geer A, Lyras G, de Vos J. 2021. Evolution of Island Mammals: Adaptation and Extinction of Placental Mammals on Islands, 2nd Edition. John Wiley and Sons Ltd, New Jersey.
- Van der Laan R, Eschmeyer WN, Fricke R. 2014. Family-group names of recent fishes. *Zootaxa* 3882 (2): 1-230. DOI: 10.11646/zootaxa.3882.1.1.
- Van der Laan R. 2018. Freshwater Fish List 25th Edition. Almere, Netherland.
- van Hasselt JC. 1823. Uittreksel uit een' brief van den Heer J.C. van Hasselt, aan den Heer C.J. Temminck, geschreven uit Tjecande, Residentie Bantam, den 29sten December 1822.-Op. cit., 2, 130-133.
- Waiho K, Ikhwanuddin M, Abualreesh MH, Shu-Chien AC, Ishak SD, Jalilah M, Azmie G, Fazhan H. 2021. Intra-and interspecific variation in sexual dimorphism patterns of mud crab genus *Scylla* along the equatorial region. *Front Mar Sci* 8: 1-9. DOI: 10.3389/fmars.2021.690836.
- Yang L, Tan Z, Wang D, Xue L, Guan MX, Huang T, Li R. 2014. Species identification through mitochondrial rRNA genetic analysis. *Sci Rep* 4: 4089. DOI: 10.1038/srep04089.
- Yanuarita D, Inaku DF, Nurdin N, Rahim SW, Kudsiah S, Parawansa BS, Rukminasari N, Irmawati, Moka W. 2020. Aquatic invasive species distribution within Wallace region: A preliminary review. *IOP Conf Ser: Earth Environ Sci* 564: 012038. DOI: 10.1088/1755-1315/564/1/012038.