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Sexual dimorphism in *Belobranchus segura* (Teleostei: Gobiiformes: Eleotridae), with notes on the distribution of *Belobranchus* species in the Philippines

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Abstract. Sexual dimorphism is a widely observed yet under–documented phenomenon in many fish species, particularly those that are less studied. This study provides the first detailed account of sexual dimorphism in *Belobranchus segura* based on specimens from the Philippines, Indonesia, and Taiwan. Our findings include new distribution records of *B. segura* in Palawan and Mindanao Island, and a new distribution record of *Belobranchus belobranchus* from Palawan in the Philippines. DNA sequencing also revealed the presence of two distinct subclades for both *B. segura* (Philippines/Taiwan and Indonesia) and *B. belobranchus* (Philippines/ Indonesia, Indonesia only), each separated by 2.0–2.8% K2P intraspecific genetic distance, suggesting potential species complexes among the samples.

Key words. bully/spiny cheek sleepers, cryptic diversity, freshwater fish diversity, ichthyology

INTRODUCTION

The members of the family Eleotridae Steindachner, 1870 are commonly known as gudgeons, spinycheek sleepers, or bully sleepers. It is an amphidromous fish group with a circumglobal distribution, predominantly inhabiting freshwater, brackish, and marine environments in tropical, subtropical, and occasionally temperate areas (Nelson et al., 2016). It is the third most speciose gobioid family, comprising 154 species distributed amongst 23 genera (Fricke et al., 2025).

The eleotrid genus *Belobranchus* Bleeker, 1856 is a group of gudgeons distributed throughout the Indo-West Pacific (Froese & Pauly, 2024). It was long considered monotypic following the synonymisation of *Belobranchus quoyi* Bleeker, 1856, and *Belobranchus taeniopterus* Bleeker, 1856 with *Belobranchus belobranchus* (Valenciennes, 1837) (Kottelat, 2013; Parenti, 2021). This perception changed with the description of a second species, *Belobranchus segura* Keith, Hadiaty & Lord, 2012 from Halmahera, Indonesia. *Belobranchus belobranchus* is widely distributed, occurring in Okinawa (Japan), Taiwan, the Philippines, Papua New Guinea, Timor Leste, the Solomon Islands, Vanuatu, Fiji, and the Andaman Islands (Herre, 1953b; Nakabo, 2002; Larson & Pidgeon, 2004; Boseto & Jenkins, 2006; Jenkins & Boseto, 2007; Keith et al., 2011; Rajan & Sreeraj, 2014; Chou et al., 2020). On the other hand, *B. segura* has been reported in Okinawa, Taiwan, the Philippines, Borneo, Indonesia, Papua New Guinea, Solomon Islands, Vanuatu, and the Andaman Islands (Keith et al., 2012; Kottelat, 2013; Rajan & Sreeraj, 2014; Manangkalangi et al., 2020; Amick & Toko, 2021; Parenti, 2021; Shao, 2024).

In the Philippines, *B. belobranchus* has been reported from various freshwater habitats across Luzon, Cebu, Negros, Leyte, and Mindanao Islands (Herre, 1927, 1953a, 1953b; Kottelat, 1993), as well as in Taal and Naujan lakes (Herre, 1953b; Mercene, 1997). Conversely, *B. segura* was first recorded from Panay Island (Larson, 2021), followed by a specimen collected from Tablas Island, Romblon (Gonzalez et al., 2023), and Aurora (Dimaquibo et al., 2025). A recent DNA barcoding study of common freshwater eleotrids in the Philippines included both *B. belobranchus* and an unidentified *Belobranchus* species (Abdulmalik-Labe & Quilang, 2024). Correspondingly, earlier studies, including the original description, of *B. segura* – did not provide detailed information on sexual dimorphism of the species, except for the differences in the shape of the urogenital papillae, and reported no evidence of sexual dichromatism between males and females (Keith et al., 2012). Similarly, a recent revision of diagnostic characteristics in the genus *Belobranchus* did not address sexual dimorphism in *B. segura* either (Afrisal et al., 2025).

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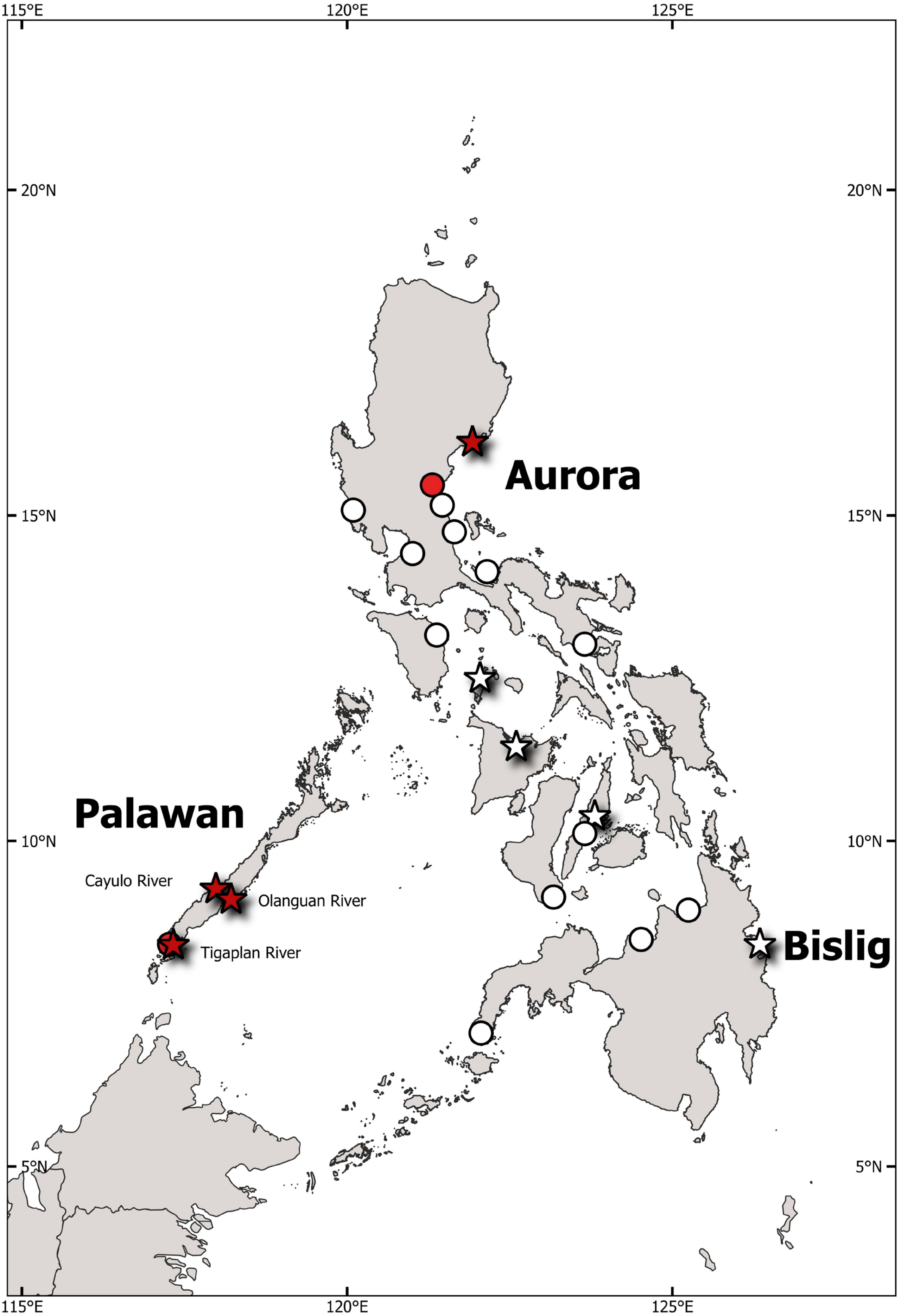

Fig. 1. Map showing the distributional records of *Belobranchus segura* (stars) and *Belobranchus belobranchus* (circles) in the Philippines. Red–filled stars and circles denote the sources of samples examined in this study.

Sexual dimorphism, characterised by morphological (e.g., body size, shape, colouration, etc.) and behavioural (e.g., aggression, territoriality, etc.) differences between male and female individuals within a species, is a widespread phenomenon observed in various animal taxa, including marine and freshwater fishes (Ritchie et al., 2007; Hulsey et al., 2015; Qiao et al., 2016; Maeda et al., 2021; Mori et al., 2022). This phenomenon has been observed for centuries and is believed to have been influenced by genetic, ecological, and evolutionary processes (Rypel, 2007; Odreitz & Sefc, 2015; Estlander et al., 2017). Understanding the extent of sexual dimorphism enhances our comprehension of species' reproductive strategies and identification, and also provides insights into ecological and evolutionary processes (Rypel, 2007; Estlander et al., 2017).

During our fieldwork in the rivers of Palawan, Aurora, and Bislig, we collected several *Belobranchus* specimens constituting new distribution records of *B. segura* in the Philippines and marking the first *Belobranchus* records from the mentioned localities. We also provided insights into the morphological differences between the sexes in *B. segura*, which were not addressed in the original description or previous occurrence records. Additionally, we supplied additional DNA sequences of *Belobranchus* species from the Philippines, contributing valuable genetic data to enhance our understanding of the diversity and distribution of these species.

MATERIAL AND METHODS

Collection and preservation of specimens. Specimens were collected from three river systems (i.e., Cayulo, Olanguan, and Tigaplan rivers) on Palawan Island (09°30′N, 118°30′E), Aurora (i.e., Dicapulao, 16°06′N, 121°44′E) on Luzon Island, and Bislig (i.e., Bonsadan Falls, 08°19′N, 126°16′E) on Mindanao Island, Philippines, using a hand net (Fig. 1). Additional specimens were also acquired from the Fangshan River, Pingtung, Taiwan, and in Java, Indonesia. A few specimens from each location were photographed before the fin clips were carefully taken. The specimens were identified as *Belobranchus segura* using the following characters: a red brown to mahogany coloured body, a greyish head with brown stripes originating from the eye to the snout and operculum, translucent first dorsal fin with black zebra stripes, and yellowish to orange second dorsal, anal, pectoral, pelvic, and caudal fins. A black spot with a bluish tinge and a solid, broken whitish margin at the base of the pectoral fin was also noted (Keith et al., 2012). Some *B. belobrachus* specimens from Aurora were also used in the analysis.

Fin clips were preserved in 95% ethanol and frozen at -20˚C before DNA extraction, while voucher specimens were fixed in 10% formalin and gradually transitioned to 70% ethanol for long-term preservation. Both the voucher specimens and fin clips were stored in the Department of Oceanography (DOS) collections at National Sun Yat-sen University, Kaohsiung. Wildlife gratuitous permits (GP No. 2023-21) and export certification (WEC No. 2023-013, ECC 0023-0002) were obtained before sample collection and exportation.

Morpho-meristic examination. Measurements and counts were taken from the left side of each specimen, using the methods of Watson (1995), Nakabo (2002), and Maeda et al. (2015), with the following modifications: the length of caudal peduncle was replaced by two measurements – length of caudal peduncle from anal fin base, and length of caudal peduncle from first dorsal fin. In addition, body depth at second dorsal fin origin and length of pelvic fin were also included as supplementary measurements. Twenty-three morphometric and nine meristic characters were taken on the left side of the sample using a dial calliper, with values expressed to the nearest tenth of a millimetre (0.1 mm). A stereo microscope was used to examine smaller individuals and characters (e.g., scale series). *Belobranchus belobranchus* specimens were also used for comparison alongside the *B. segura* specimens.

DNA extraction and amplification. DNA extraction using the fin clip was performed using the GeneMark Easy Tissue & Cell Genomic DNA Purification Kit (GMbiolab, Taichung, Taiwan) following the manufacturer's protocol. The mitochondrial cytochrome c oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) using the Fish F2 (5′ – TCG ACT AAT CAT AAA GAT ATC GGC AC – 3′) and Fish R2 (5′ – ACT TCA GGG TGA CCG AAG AAT CAG AA – 3′) primers (Ward et al., 2005). All PCR products were amplified in a 25 µL reaction volume containing 9.5 µL of ultrapure water, 12.5 µL of Zeju Master Mix, 1.0 µL of each primer (10µM, forward and reverse), and 1.0 µL of DNA template. Gel electrophoresis was used to verify the quality and quantity of PCR products using a 1.5% agarose gel. The PCR products obtained were purified using SAP-EXO Purification Kit (Jena Bioscience, Jena, Germany) following the manufacturer's instructions, and sent to Genomics Sci & Tech (Taipei, Taiwan) for sequencing.

Data analyses. All morphometric measurements were transformed into percentages against standard length (%SL) before comparison to mitigate any bias introduced by allometric growth. Independent sample *t*-tests were conducted using SPSS (version 28) (IBM Corp., 2021) to identify characters exhibiting differences between sexes. Subsequently, all morphometric characters were utilised for principal component analysis (PCA) for character clustering using Paleontological Statistics (PAST, version 4.06) (Hammer et al., 2001). DNA sequences obtained in this study were manually edited and trimmed using the Molecular Evolutionary Genetics Analysis software (MEGA version 11) (Tamura et al., 2021). To enhance the phylogenetic analysis, additional sequences of *B. segura* and *B. belobranchus* from other localities were retrieved from NCBI GenBank (Clark et al. 2016; https://www.ncbi.nlm.nih.gov/genbank) and BOLD Systems (Ratnasingham & Hebert, 2006; https://www.boldsystem.org). Additionally, a sequence of *Eleotris fusca* (Bloch & Snyder, 1801) (PV943659) was included as an outgroup. All sequences were aligned using the CLUSTALW function of MEGA 11 and trimmed to a

Fig. 2. *Belobranchus segura* from Palawan, Philippines. Top, DOS9372–3, male, 61.6 mm SL; Bottom, DOS9310–5, female, 53.1 mm SL. (Photos by: Wen-Chien Huang).

uniform length of 488 bp. Neighbour-Joining (NJ) method was used based on the Kimura 2-Parameter (K2P) model (Kimura, 1980) to reconstruct a phylogenetic tree with 1,000 bootstrap replications for node support (Felsenstein, 1985). The phylogenetic tree reconstruction was performed using MEGA 11. Graphs of morphometric relationships of male and female specimens were generated using Microsoft Excel.

TAXONOMY

Family Eleotridae Steindachner, 1870

Genus *Belobranchus* Bleeker, 1856

Belobranchus segura Keith, Hadiaty & Lord, 2012

Throatspine gudgeon
(Figs. 2–4; Tables 1–2)

Materials examined. Philippines: DOS9310-1 (male, 54.9 mm SL), DOS09310-2 (male, 51.0 mm SL), DOS9310-4 (female, 44.8 mm SL) and DOS9310-5 (female, 53.1 mm SL), all specimens collected from Tigaplan River (08°49.02′N, 117°41.07′E), Brooke's Point, Palawan Island, coll. W.C. Jhuang and R.A.T. Balisco, 25 April 2023; DOS9372-1 (female, 43.8 mm SL), DOS9372-2 (male, 52.9 mm SL), DOS9372-3 (male, 61.6 mm SL), DOS9372-4 (male, 56.5 mm SL), DOS9372-5 (male, 52.0 mm SL) and DOS9372-6 (female, 48.0 mm SL), all specimens collected from Cayulo River (10°02.37′N, 118°45.46′E), Puerto Princesa City, Palawan Island, coll. W.C. Jhuang and R.A.T. Balisco, 30 April 2023; **Indonesia:** DOS10068-4 (male, 52.7 mm SL), DOS10068-5 (male, 52.0 mm SL), DOS10068-1 (female, 54.8 mm SL), DOS10068-2 (female, 47.4 mm SL), and DOS10068-3 (female, 48.5 mm SL), all specimens collected from Java Island, acquired through aquarium trade, 2 February 2024; **Taiwan:** DOS10233-3 (male, 45.5 mm SL), DOS10233-4 (male, 41.1 mm SL), DOS10233-7 (female, 59.5 mm SL), DOS10233-8 (female, 59.2 mm SL) and DOS10233-9 (female, 38.2 mm SL), all specimens collected from Fangshan River (22°15.75′N, 120°40.21′E), Pingtung County, coll. C.C. Yu, 17 March 2024.

Diagnosis. Head greyish with five brown stripes on cheek originating from eyes to snout and operculum. A black spot with a bluish tinge and a solid, broken whitish margin

Table 1. Morphometric measurements and meristic counts of *Belobranchus segura* from Palawan (Philippines), Java (Indonesia), and Pingtung (Taiwan), and *Belobranchus belobranchus* from the Philippines. Values in bold highlight characters exhibiting differences between sexes.

Characters	***Belobranchus segura***					***Belobranchus belobranchus***				
	Male (n=10)		**Female (n=10)**		**Keith et al. (2012)**	**Male (n=3)**		**Female (n=3)**		**Keith et al. (2012)**
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
Standard length (SL, mm)	41.1–61.6	52.0 ± 5.6	37.6–54.8	46.3 ± 5.3	40.0–80.0	40.5–89.6	61.8± 25.2	43.3–46.8	45.5 ± 1.9	–
Morphometric (% SL)										
Head length	26.9–30.9	28.6 ± 1.5	24.4–30.0	27.6 ± 1.8	26.0–31.0	27.3–28.2	27.8 ± 0.5	26.7–27.6	27.1 ± 0.5	29.0–36.0
Snout length	**5.5–6.6**	**5.9 ± 0.4**	**4.1–6.1**	**4.9 ± 0.4**	–	5.2–6.9	6.0 ± 0.9	4.6–5.6	5.1 ± 0.5	–
Eye diameter	**5.7–7.0**	**6.3 ± 0.4**	**5.8–7.7**	**6.9 ± 0.6**	–	4.7–7.1	6.0 ± 1.2	5.5–7.1	6.2 ± 0.8	–
Post-orbital length of head	13.3–17.3	15.7 ± 1.3	12.1–17.6	15.8 ± 1.6	–	15.5–17.4	16.2 ± 1.1	14.8–16.4	15.7 ± 0.8	–
Upper jaw length	**7.5–12.0**	**10.0 ± 1.4**	**7.7–10.6**	**8.8 ± 0.8**	**12.0–17.0**	10.3–14.1	12.1 ± 1.9	9.3–10.4	10.0 ± 0.6	13.0–17.0
Body depth at D2 origin	**19.3–21.0**	**20.1 ± 0.6**	**14.0–20.5**	**18.8 ± 1.9**	**17.0–22.0**	19.6–20.4	20.0 ± 0.4	18.1–20.6	19.0 ± 1.4	16.0–21.0
Body depth at P2 origin	**17.8–20.4**	**19.4 ± 0.9**	**14.2–18.7**	**16.9 ± 1.5**	–	17.4–20.6	18.6 ± 1.7	16.6–18.3	17.3 ± 0.9	–
Body depth at A origin	**18.2–21.0**	**19.2 ± 0.7**	**16.0–18.8**	**17.9 ± 0.8**	–	17.8–22.1	19.3 ± 2.5	16.5–17.8	17.1 ± 0.7	–
Depth at caudal peduncle	**14.9–16.7**	**15.6 ± 0.6**	**13.9–15.5**	**14.5 ± 0.5**	**13.0–16.0**	14.1–16.7	15.2 ± 1.3	13.3–15.2	14.5 ± 1.0	11.0–15.0
Caudal length	**20.2–25.7**	**23.4 ± 1.8**	**15.5–25.0**	**20.3 ± 3.4**	**20.0–25.0**	20.0–23.5	22.0 ± 1.8	19.5–21.4	20.7 ± 1.0	18.0–21.0
Length of caudal peduncle from A base	**18.0–24.4**	**22.0 ± 1.7**	**20.9–26.6**	**23.6 ± 2.0**	**22.0–27.0**	22.7–26.1	23.9 ± 1.9	22.4–27.9	24.6 ± 2.9	23.0–28.0
Length of caudal peduncle from D2	**21.4–26.2**	**23.7 ± 1.8**	**22.5–31.1**	**25.8 ± 3.0**	–	22.4–26.6	24.8 ± 2.2	26.8–27.9	27.2 ± 0.6	–
Pre-dorsal length	38.3–44.4	40.9 ± 2.1	38.6–44.0	41.0 ± 1.6	38.0–42.0	36.9–42.9	40.4 ± 3.1	39.9–42.1	40.9 ± 1.1	40.0–46.0
D1 length	**16.9–20.2**	**18.2 ± 1.0**	**13.9–16.7**	**15.4 ± 1.0**	–	11.7–17.7	13.9 ± 3.3	12.6–16.1	14.3 ± 1.8	–
Length of D1 base	7.9–15.2	11.5 ± 2.6	9.5–14.3	12.0 ± 1.5	–	8.6–12.3	10.2 ± 1.9	9.6–11.8	10.8 ± 1.1	–
Length of longest spine of D1	9.0–15.9	11.7 ± 2.0	10.9–12.8	11.9 ± 0.8	–	8.1–13.3	10.1 ± 2.8	9.5–12.1	10.7 ± 1.3	–
Interval between D1 and D2 bases	5.4–10.2	7.7 ± 1.4	5.6–10.7	7.7 ± 1.5	–	7.6–11.9	10.1 ± 2.2	8.7–10.0	9.5 ± 0.7	–
D2 length	**21.1–24.9**	**23.0 ± 1.1**	**17.3–21.4**	**19.5 ± 1.7**	**21.0–27.0**	19.9–23.9	21.3 ± 2.2	19.6–21.8	20.4 ± 1.2	19.0–23.0
Length of D2 base	10.2–13.5	12.3 ± 1.1	11.0–14.2	12.5 ± 0.9	–	10.4–11.5	11.1 ± 0.6	10.0–10.3	10.2 ± 0.2	–
Length of longest ray of D2	**15.4–19.2**	**16.4 ± 1.1**	**12.9–15.8**	**14.5 ± 0.9**	–	14.5–18.0	15.8 ± 1.9	12.6–16.3	14.4 ± 1.9	–
Pre-A length	61.5–66.0	64.1 ± 1.4	59.5–67.7	63.8 ± 2.8	58.0–66.0	62.8–67.0	64.5 ± 2.2	61.3–65.2	63.5 ± 2.0	61.0–69.0

Characters	*Belobranchus segura*					*Belobranchus belobranchus*				
	Male (n=10)		Female (n=10)		Keith et al. (2012)	Male (n=3)		Female (n=3)		Keith et al. (2012)
	Range	Mean ± SD	Range	Mean ± SD	Range	Range	Mean ± SD	Range	Mean ± SD	Range
A length	**20.7–24.0**	**22.1 ± 1.1**	**18.1–21.9**	**19.9 ± 1.5**	**21.0–25.0**	22.2–23.0	22.7 ± 0.4	17.4–23.2	19.6 ± 3.1	20.0–23.0
Length of longest ray of A	12.7–15.6	14.4 ± 1.0	12.2–17.1	14.4 ± 1.6	–	14.9–16.1	15.5 ± 0.6	14.3–16.9	15.2 ± 1.5	–
Length of A base	**8.8–11.4**	**10.3 ± 0.9**	**10.2–12.7**	**11.0 ± 0.7**	**–**	8.3–9.9	9.2 ± 0.8	9.3–10.0	9.7 ± 0.4	–
Length between anus to A	5.0–8.0	6.5 ± 1.1	4.7–7.7	6.1 ± 0.9	–	5.7–6.1	5.9 ± 0.2	5.6–6.5	6.1 ± 0.5	–
Length of longest ray of P1	17.5–21.9	19.6 ± 1.3	17.1–20.8	18.7 ± 1.1	–	17.4–19.4	18.4 ± 1.0	17.4–20.8	19.4 ± 1.8	–
P2 length	16.1–21.0	18.6 ± 1.3	17.1–19.4	18.4 ± 0.7	–	17.0–20.0	18.7 ± 1.3	18.5–20.8	19.6 ± 1.2	–
Meristic counts										
Dorsal fin rays	VI–I, 6–7		VI–I, 7		VI–I, 7	VI–I, 7		VI–I, 7		VI–I, 7
Anal fin rays	I, 6–7		I, 6–7		I, 7	I, 7		I, 7		I, 7
Pectoral fin rays	18–19		18–20		21–24	19		16		–
Pelvic fin rays	5–6		5–6		–	6		6		–
Caudal fin rays	14–15		14–15		13–15	15		15		–
Lateral scales series	58–60		57–60		55–60	58		58		56–63
Transverse forward series	20–26		20–22		22–28	25		25		27–35
Transverse backward series	18–21		18–22		18–21	20		20		20–23
Zigzag series	18–19		17–19		15–19	19		18		14–18

D1, first dorsal fin; D2, second dorsal fin; A, anal fin; P1, pectoral fin; P2, pelvic fin.

Fig. 3. Aquarium photos of male (top) and female (bottom) *Belobranchus segura* from Dicapulao, Aurora, Philippines (photos: Jacky Che–Chi Yu).

found at base of pectoral fin. Males having a red-brown body with generally yellowish to orange fins, with upper part of first dorsal fin having orange colour, while females having a brownish-to-mahogany colouration with spotted fins (Figs. 2, 3).

Description. Refer to Table 1 for morphometric measurements and meristic counts of male and female *B. segura* samples from different locations. Body elongated, anteriorly cylindrical, and posteriorly slightly compressed. Head depressed, and snout convex. Dorsal fins VI–I, 6–7; anal fin I, 6–7 directly below second dorsal fin; pectoral fin 18–20 with a black spot and broken whitish margin in pectoral base and rounded posterior margin; pelvic fin 5–6; caudal fin 14–15 segmented rays with brown or orange margin at base and rounded posterior caudal margin. Lateral scales 57–60, transverse forward 21–26, transverse backward 20–21, and zigzag 18–19. Mouth large, lower jaw prominent projecting beyond upper jaw, and several rows of teeth in front. Eyes large with a bluish sheen, having an anterior nostril.

Table 2. Morphometric measurements of two subclades of *Belobranchus segura* identified in this study. Subclade I includes samples from the Philippines and Taiwan; Subclade I includes samples from Indonesia. M, male; F, female.

Character	Keith et al. (2012)	Subclade I (n=15)				Subclade II (n=5)	
		Philippines (5M, 5F)		Taiwan (2M, 3F)		Indonesia (2M, 3F)	
	Range	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Standard length (SL, mm)	40.0 – 80.0	43.8 – 61.6	44.74 ± 8.76	37.3 – 59.5	51.86 ± 5.36	47.4 – 54.8	51.08 ± 3.06
Morphometric (% SL)							
Head length	26.0 – 31.0	25.8 – 29.0	27.58 ± 2.58	24.4 – 30.9	27.57 ± 1.03	28.7 – 30.7	29.52 ± 0.82
Snout length		4.1 – 6.3	5.40 ± 0.34	5.1 – 5.9	5.28 ± 0.76	5.1 – 6.6	5.56 ± 0.61
Eye diameter		5.5 – 7.7	6.38 ± 1.02	5.1 – 7.9	6.34 ± 0.81	6.3 – 7.3	6.88 ± 0.42
Post-orbital length		12.1 – 16.7	16.46 ± 0.69	15.7 – 17.6	14.90 ± 1.45	15.6 – 17.3	16.80 ± 0.71
Upper jaw length	12.0 – 17.0	8.2 – 12.0	8.00 ± 1.24	6.7 – 9.6	9.85 ± 1.37	8.2 – 10.8	9.56 ± 1.11
Body depth at D2 origin	17.0 – 22.0	18.6 – 21.0	19.25 ± 1.77	18.0 – 20.5	19.72 ± 0.80	18.6 – 20.5	19.68 ± 0.79
Body depth at P2 origin	–	16.6 – 20.4	16.96 ± 1.05	15.6 – 18.2	19.15 ± 1.27	16.9 – 19.2	18.08 ± 0.95
Body depth at A origin	–	17.6 – 21.0	17.56 ± 0.96	16.5 – 18.6	18.93 ± 0.93	17.7 – 19.4	18.70 ± 0.63
Depth at caudal peduncle	13.0 – 16.0	13.9 – 16.3	14.84 ± 0.82	13.9 – 16.1	14.98 ± 0.69	14.4 – 16.7	15.44 ± 0.83
C length	20.0 – 25.0	19.8 – 25.7	21.60 ± 1.53	20.2 – 23.5	23.44 ± 2.02	21.0 – 24.1	22.16 ± 1.28
Length of caudal peduncle from A base	22.0 – 27.0	21.2 – 24.9	24.62 ± 1.99	22.1 – 26.6	22.63 ± 1.26	18.0 – 23.0	21.18 ± 1.93
Length of caudal peduncle from D2	–	21.4 – 25.6	27.98 ± 2.86	24.6 – 31.1	23.60 ± 1.53	22.4 – 26.2	23.68 ± 1.54
Pre-dorsal length	38.0 – 42.0	38.3 – 41.7	40.52 ± 2.39	38.6 – 44.4	39.79 ± 1.13	38.7 – 44.0	41.98 ± 1.98
D1 length	–	15.0 – 19.1	16.80 ± 2.13	14.9 – 20.2	17.26 ± 1.26	13.9 – 18.6	16.32 ± 1.69
Length of D1 base	–	9.6 – 15.2	10.72 ± 2.55	7.9 – 12.9	12.92 ± 1.72	9.5 – 11.8	10.46 ± 0.86
Length of longest spine of D1	–	10.4 – 12.8	10.80 ± 1.44	9.0 – 12.6	11.68 ± 0.86	11.0 – 15.9	13.04 ± 1.78
Interval between D1 and D2 bases	–	5.4 – 8.7	8.50 ± 1.95	6.2 – 10.7	7.12 ± 1.21	7.6 – 9.1	8.08 ± 0.63
D2 length	21.0 – 27.0	18.5 – 24.9	20.74 ± 2.17	17.4 – 23.1	22.03 ± 1.99	19.6 – 23.3	21.58 ± 1.37
Length of D2 base	–	11.7 – 14.2	11.38 ± 0.95	10.2 – 12.2	12.87 ± 0.78	11.0 – 12.8	12.32 ± 0.75
Length of longest ray of D2	–	13.6 – 16.7	15.34 ± 0.69	14.6 – 16.2	15.49 ± 1.13	14.2 – 19.2	16.00 ± 1.91
Pre- A length	58.0 – 66.0	61.8 – 66.0	61.36 ± 1.29	59.5 – 63.0	64.12 ± 1.37	65.2 – 67.7	66.30 ± 0.93

Character	Keith et al. (2012)	Subclade I (n=15)				Subclade II (n=5)	
		Philippines (5M, 5F)		Taiwan (2M, 3F)		Indonesia (2M, 3F)	
	Range	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
A length	21.0 – 25.0	20.2 – 24.0	20.94 ± 1.43	19.1 – 22.5	21.79 ± 1.26	18.1 – 22.1	20.44 ± 1.57
Length of longest ray of A	–	12.7 – 17.1	14.28 ± 0.78	13.6 – 15.6	14.93 ± 1.31	13.7 – 15.6	14.24 ± 0.81
Length of A base	–	9.5 – 12.7	10.36 ± 0.93	8.8 – 11.2	10.95 ± 0.93	9.7 – 10.8	10.36 ± 0.44
Length between anus to A base	–	5.0 – 7.7	5.74 ± 1.05	4.7 – 7.5	6.07 ± 0.88	6.3 – 8.0	7.08 ± 0.66
Length of longest ray of P1	–	18.5 – 21.9	18.68 ± 1.26	17.1 – 20.2	19.78 ± 1.23	17.5 – 19.2	18.34 ± 0.67
Length of P2	–	18.0 – 21.0	17.70 ± 0.90	16.1 – 18.2	19.12 ± 0.83	17.1 – 19.0	17.96 ± 0.77
Meristic counts							
Dorsal fin rays	VI–I, 7	VI–I, 6–7		VI–I, 7		VI–I, 7	
Anal fin rays	I, 7	I, 6–7		I, 6–7		I, 7	
Pectoral fin rays	21–24	18–20		18–19		–	
Pelvic fin rays	–	5–6		5		–	
Caudal fin rays	13–15	14–15		14–15		–	
Lateral scales series	55–60	57–60		58–60		–	
Transverse forward series	22–28	20–26		20–22		–	
Transverse backward series	18–21	18–21		18–22		–	
Zigzag series	15–19	18–19		17–19		–	

D1, first dorsal fin; D2, second dorsal fin; A, anal fin; P1, pectoral fin; P2, pelvic fin.

Fig. 4. Underwater photo of a *Belobranchus belobranchus* from Aurora, Philippines (photo: Wei-Cheng Jhuang).

Sexual dimorphism. Male. Body reddish-brown with alternating vertical white bands in posterior part; second dorsal, anal, pectoral, pelvic, and caudal fins yellowish to orange; upper part of first dorsal fin yellowish to orange, middle and lower parts mottled greyish to black; some individuals with stripes on dorsal fins. No obvious spots (Figs. 2, 3). Urogenital papilla rounded near anus and pointed or triangular near anal fin (Fig. 5).

Female. Body brownish-to-mahogany coloured with more visible white bands from middle to posterior part. Upper part of first and second dorsal fins slightly yellowish and spotted or with stripes in pectoral, pelvic, and caudal fins (Figs. 2, 3). Urogenital papilla bulbous, with cleavage on both ends (Fig. 5).

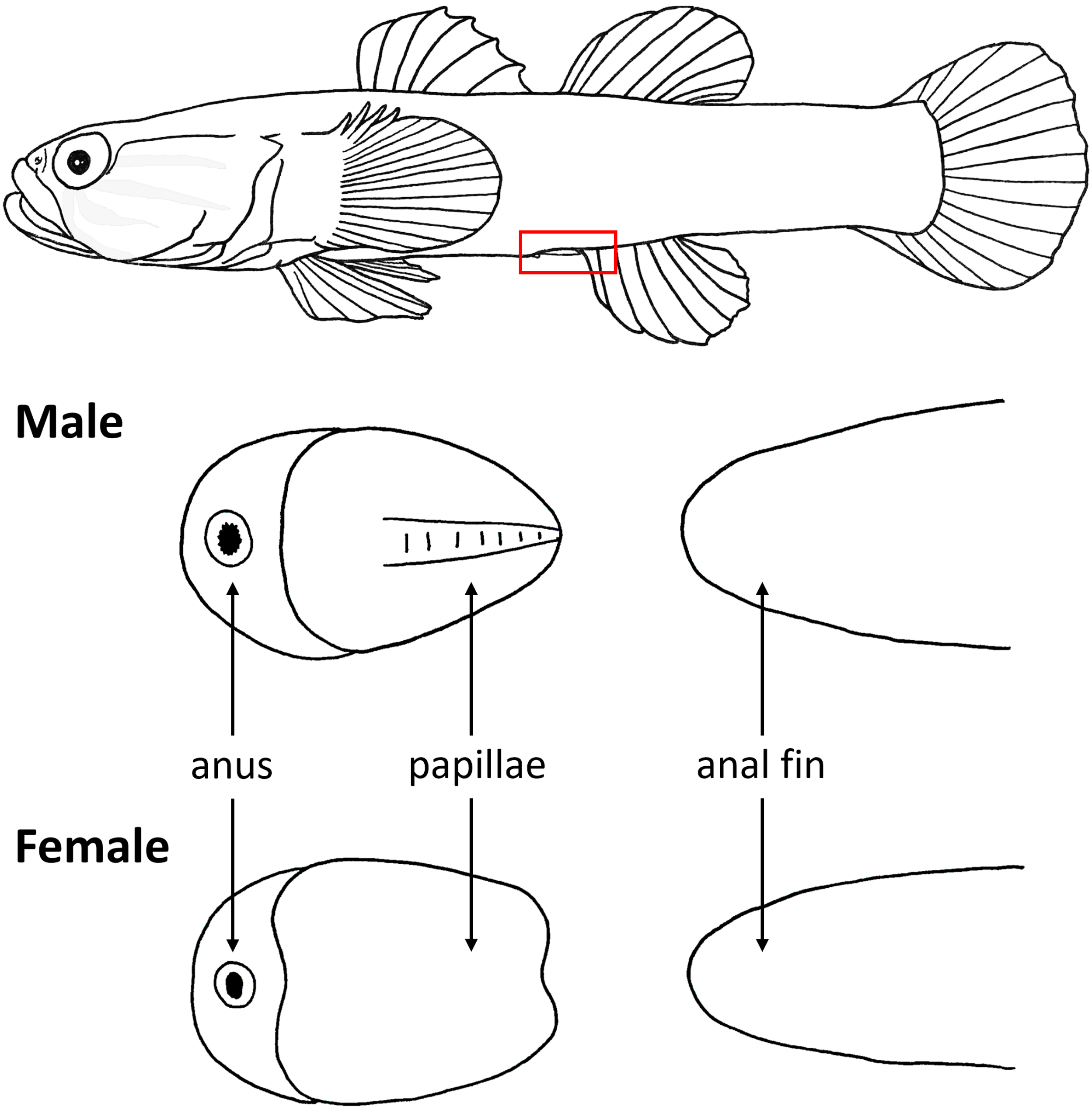

Fig. 5. Schematic diagram of urogenital papillae of male (middle) and female (bottom) *Belobranchus segura* from Palawan, Philippines. Ventral view of the red box.

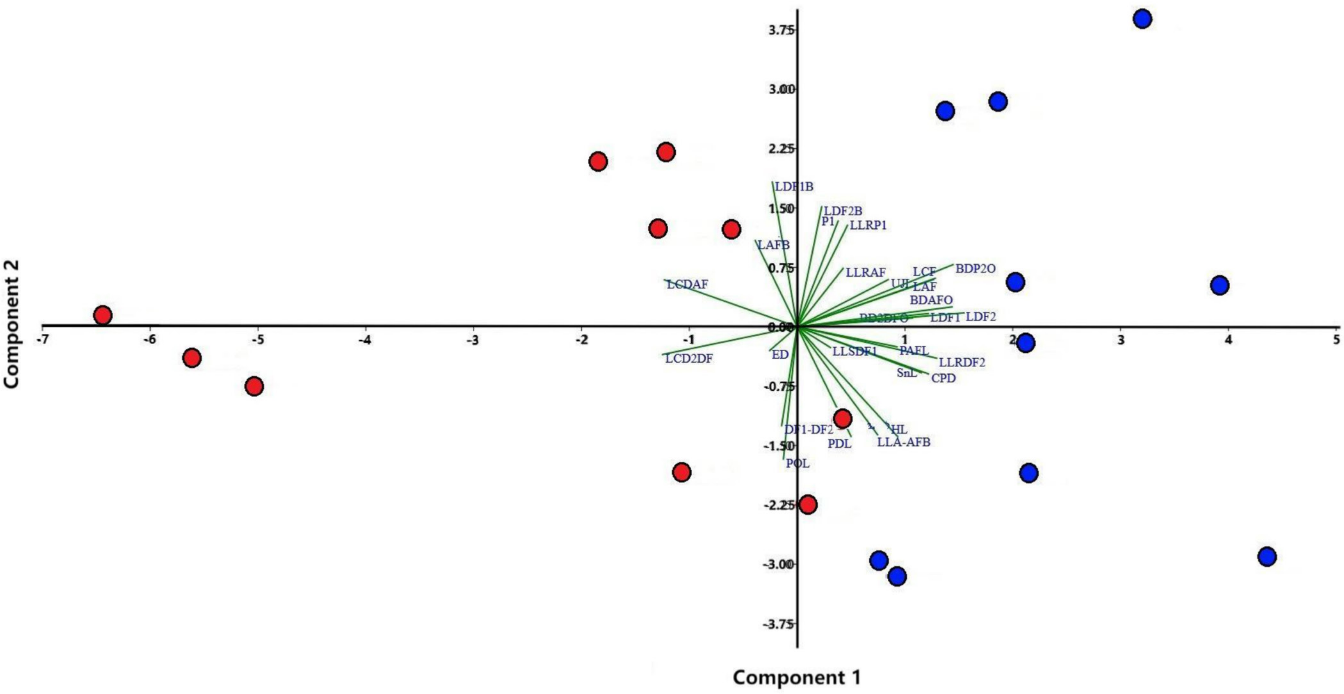

Fig. 6. Scatter plot of the first two components (PC1 and PC2) in the principal component analysis of different morphometric characters for male (blue) and female (red) *Belobranchus segura* from the Philippines, Indonesia, and Taiwan.

Morpho-meristic difference (% SL). Males with longer snout than females (5.5–6.6 vs. 4.1–5.3), along with greater body depths at pelvic fin origin (17.8–20.4 vs. 14.2–18.7), second dorsal fin origin (19.3–21.0 vs. 14.0–20.5), anal fin origin (18.2–21.0 vs. 16.0–18.8), and caudal peduncle (14.9–16.7 vs. 13.9–15.5). Additionally, first dorsal fin (16.9–20.2 vs. 13.9–16.7), second dorsal fin (21.1–24.9 vs. 17.3–21.4), longest ray of second dorsal fin (15.4–19.2 vs. 12.9–15.8), anal fins (20.7–24.0 vs. 18.1–21.9), caudal fins (20.2–25.7 vs. 15.5–25.0), and upper jaws (7.5–12.0 vs. 7.7–10.6) longer in males.

Eye diameter (5.8–7.7 vs. 5.7–7.0) larger in females; caudal peduncle from anal fin base (20.9–26.6 vs. 18.0–24.4), caudal peduncle from second dorsal fin base (22.5–31.1 vs. 21.4–26.2), and anal fin base (10.2–12.7 vs. 8.8–11.4) longer in females. No meristic difference observed between sexes (Table 1, Fig. 5).

The PCA conducted on 27 morphometric characters showed a cumulative variance of 49.8% across two PCs with eigenvalues >4.0. All morphometric characters exhibited a positive correlation in PC1; however, approximately half of these characters showed a negative correlation in PC2 (Table S3).

***Belobranchus belobranchus* (Valenciennes, 1837)**

Materials examined: DOS9310-3 (male, 89.6 mm SL), Tigaplan River (08°49.02′N, 117°41.07′E), Brooke's Point, Palawan Island, Philippines, coll. W.C. Jhuang and R.A.T. Balisco, 25 April 2023; DOS9257-1 (male, 55.5 mm SL), DOS9257-5 (male, 40.5 mm SL), DOS9257-2 (female, 46.5 mm SL), DOS9257-3 (female, 46.8 mm SL), and DOS9257-2 (female, 43.3 mm SL), Digno Tabon Falls (15°40.79′N, 121°30.53′E), Aurora, Luzon Island, Philippines, coll. W.C. Jhuang, 26 May 2024.

Diagnosis. Head greyish orange with striped pectoral fins and orangish pectoral fin base. Four alternating horizontal light and dark brown bands along body. Caudal fin always spotted with white (Fig. 4).

Description. Refer to Table 1 for morphometric measurements and meristic counts of *B. belobranchus*. Body elongated with four alternating brown bands. Dorsal fins, VI–I, 7; anal fin, I, 7; pectoral fin 15–16 with orange pectoral base and rounded posterior margin; pelvic fin 5–6; caudal fin 15 segmented rays. Lateral series 56–60, transverse forward 20–21, transverse backward 20–21, and zigzag 17–18 (Fig. 4).

RESULTS

Molecular phylogenetic analysis. The NJ phylogenetic tree based on COI sequences revealed two well-supported monophyletic subclades within *B. segura* with a 2.0% K2P genetic distance. Subclade I contained sequences from the Philippines and Taiwan, while Subclade II was comprised of Indonesian sequences. However, the subclades observed in *B. segura* are not supported by morphological characters, except that the length of the 1st dorsal fin base only slightly overlaps between subclades (Table 2). Similarly, two subclades were also detected within *B. belobranchus,* with a 2.8% K2P genetic distance. Subclade I consisted of sequences from the Philippines (Palawan and Aurora) and Indonesia (Jawa Barat, Bali, and Sulawesi), while Subclade II was exclusively composed of sequences from Jawa Barat, Indonesia. The interspecific K2P genetic distance between *B. segura* and *B. belobranchus* is 19.0% (Fig. 7). Details of the

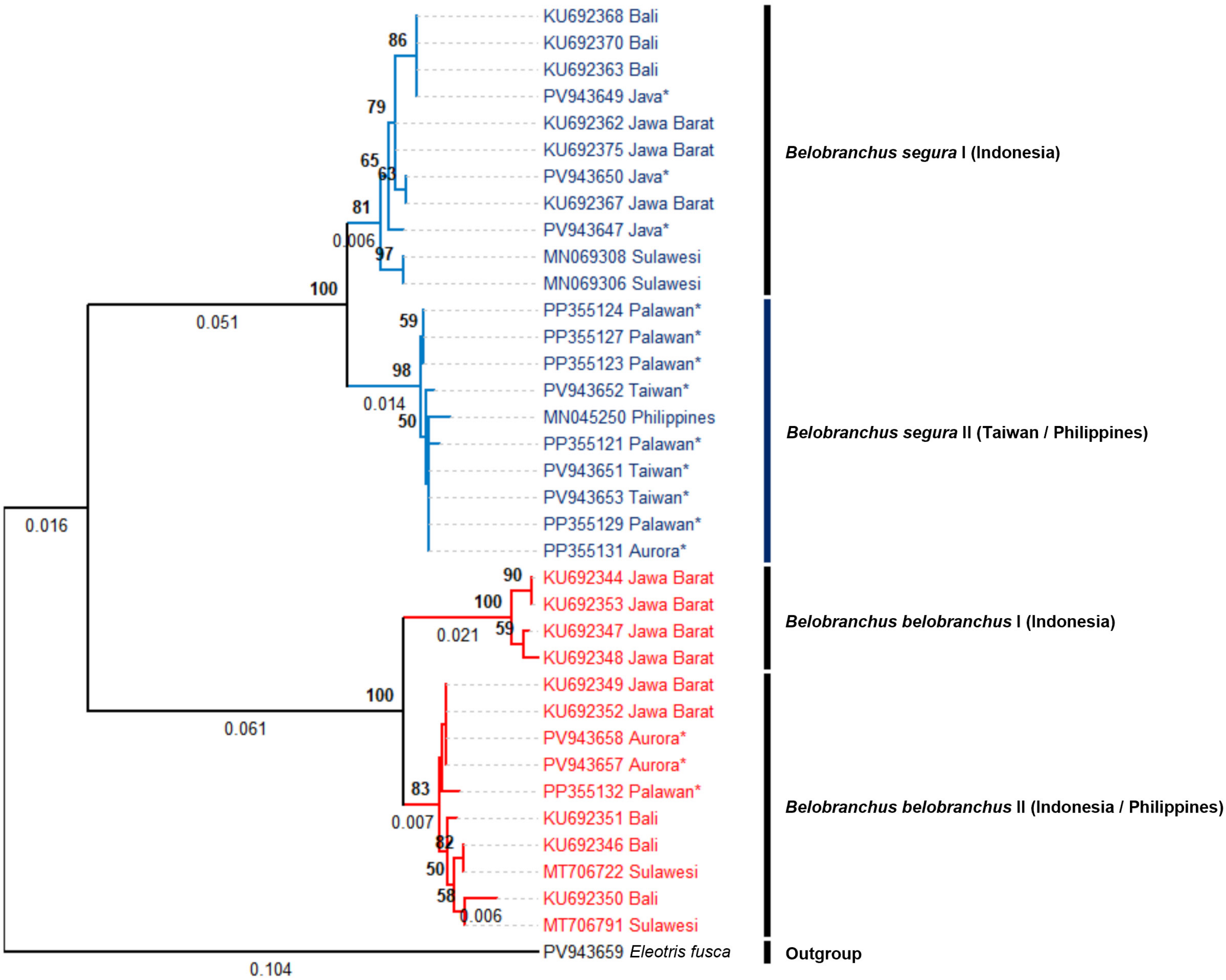

Fig. 7. Neighbour-Joining (NJ) tree based on the partial COI gene sequences (488 bp) among the *Belobranchus* species from the Philippines, Indonesia, and Taiwan based on the Kimura 2-Parameter (K2P) model. Numerals beside the internal branch are the bootstrap support, with values below 50% not shown. *Eleotris fusca* was used as the outgroup. Newly obtained sequences in this study are indicated by an asterisk (*) next to their GenBank accession numbers and locality.

COI sequences used in this study, including their geographic origins from the Philippines, Taiwan, and Indonesia, are provided in Table S1.

Principal Component Analysis. A Principal Component Analysis (PCA) was performed on the standardised morphometric data (%SL) to examine variation between male and female *B. segura* samples from different locations. The PCA biplot (Figure 6) shows individual fish distributed along the first two principal components (PC1 and PC2). PC1 and PC2 accounted for 49.79% of the total variation in the dataset (Table S3), with PC1 explaining 33.30% and clearly separating males from females. This indicates that PC1 reflects the primary morphometric differences between the sexes.

Traits with strong positive loadings on PC1—such as length of second dorsal fin base (LDF2B), length of longest ray of pectoral fin (LLRP1), longest ray of anal fin (LLRAF), and length of caudal fin (LCF), body depth at pectoral fin origin (BDP2O), body depth at anal fin origin (BDAFO), length of first dorsal fin (LDF1), length of second dorsal fin (LDF2), and length of anal fin (LAF)—were associated with males, suggesting these contribute most to observed sexual dimorphism. In contrast, traits associated with females were located on the negative side of PC1, though with weaker loadings.

PC2 explained 16.49% of the variance and did not differentiate sexes, instead capturing individual variation with groups. The clear spatial separation of males and females in the PCA biplot highlights a pronounced morphometric divergence between the sexes and supports sexual dimorphism in *B. segura*. Additional scatter plots of 10 morphometric traits against the standard length (mm) for male and female *Belobranchus segura* from the Philippines, Taiwan, and Indonesia are presented in Figure S1.

Distribution of *Belobranchus* species. *Belobranchus segura* is currently reported across various locations in the Indo-West Pacific, such as Okinawa (Japan), Taiwan, the Philippines, Borneo, Indonesia, Papua New Guinea, the

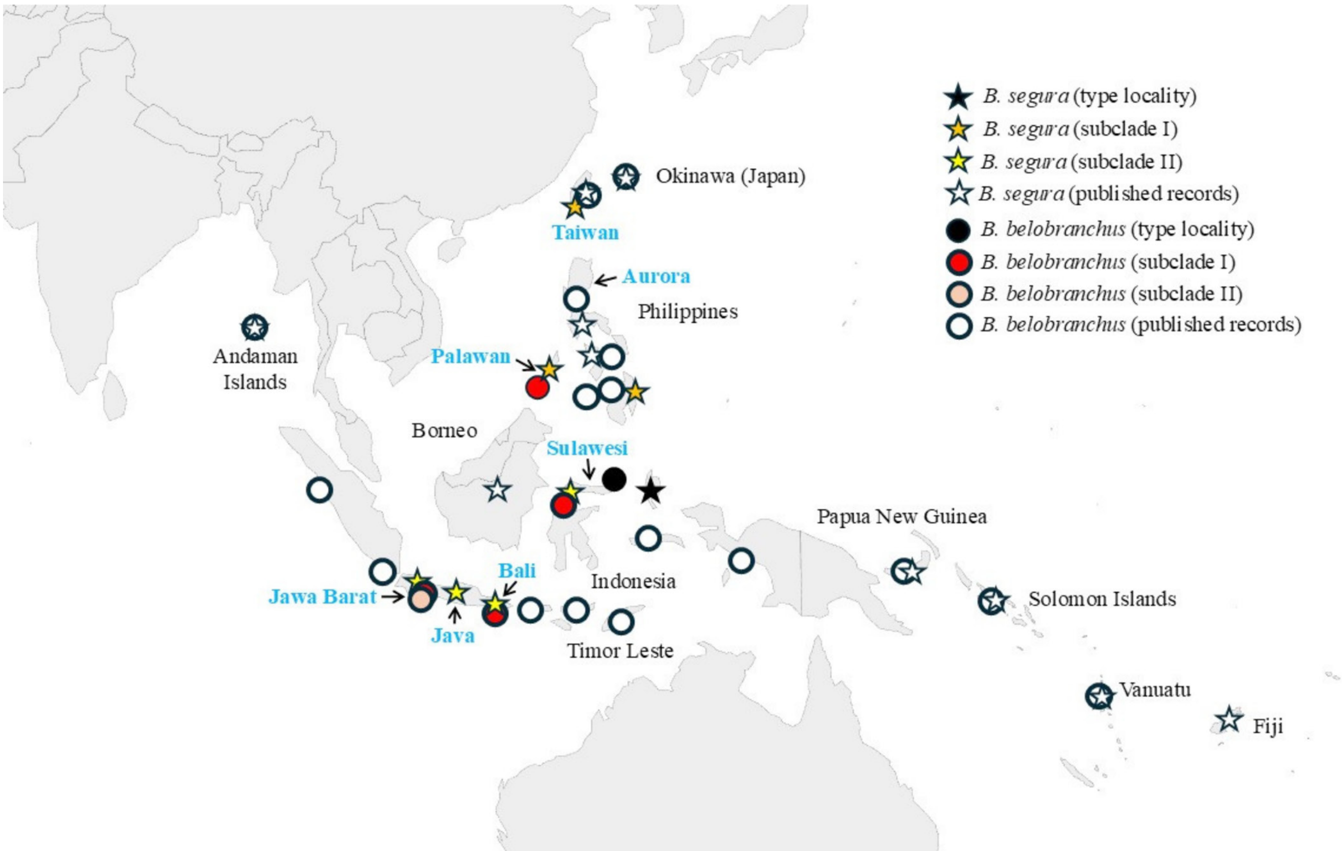

Fig. 8. Current known distribution of *Belobranchus* species in the Indo-West Pacific. Locations labelled in blue indicate the sources of molecular sequences used in this study.

Solomon Islands, Vanuatu, and Fiji, as well as the Andaman Islands in the Eastern Indian Ocean (Fig. 8). Previous reports in the Philippines indicated its presence in Aurora, Panay, and Romblon islands, and more records from Palawan and Bislig (Mindanao) in the present study. The new record of *B. belobranchus* from Palawan Island has extended its known distribution to the westernmost Philippines. It was previously reported from Luzon, Mindoro, Cebu, Negros, Leyte, and Mindanao Islands (Fig. 1).

DISCUSSION

Sexual dimorphism has been documented in various freshwater fish taxa, including guppies (Houde, 1997), gobies (Amick & Toko, 2021; Pasisingi et al., 2024), blennies (Laporte et al., 2018), and barbs (Ibrahim et al., 2013), among others. Despite its prevalence in freshwater fishes, studies on sexual dimorphism in eleotrids are relatively scarce, with only a few species having been investigated, such as *Dormitator maculatus* (Dávila-Camacho et al., 2023), *Hypseleotris* (Thacker & Unmack, 2005), and *B. belobranchus* (Pasisingi et al., 2024). Sexual dimorphism is believed to provide advantages in reproductive success, resource acquisition, parental investment, territorial defence, and environmental adaptation (Stamps, 1983; Bisazza, 1993; Magurran & Garcia, 2000; Székely et al., 2004). By elucidating the extent and underlying mechanisms of sexual dimorphism, we can gain a deeper understanding of the adaptive significance of these differences and their implications for species fitness and survival.

Similar to other freshwater fish exhibiting sexual dimorphism, male *B. segura* also displays distinct morphological characters, including a longer snout and greater body depths at various points, such as in the pelvic fin origin, anal fin origin, and caudal peduncle. Males also possess larger and yellowish fins (e.g., first dorsal fin, second dorsal fin, and anal fin), and longer upper jaws compared to females which typically have smaller and striped fins. These traits are believed to confer ecological advantages, particularly in courtship and mating, where males often invest more effort in securing mates and guarding offspring (Bisazza, 1993; Amundsen & Forsgren, 2003; Méndez-Janovitz & Macías Garcia, 2017). Likewise, striking colour patterns and a more slender body shape have also been observed in male *B. belobranchus* from Sulawesi (Gorontalo Bay), Indonesia, in contrast to their female counterparts (Pasisingi et al., 2024).

The original description of *B. segura* included a photo identified as a juvenile *B. belobranchus* (40.0 mm SL; Fig. 3B, p. 481). However, upon closer examination of its morphological characteristics, it bears a resemblance to the female *B. segura* specimens we examined in this study, particularly in body colouration and the presence of stripes in the fins. Moreover, the original description noted some features, such as yellowish to orange fins, an orange distal part of the first dorsal fin, and a translucent caudal fin with yellow or reddish zones on the caudal bases, which align with the male *B. segura* specimens we analysed. This suggests that the original description of *B. segura* may have been based solely on the morphological characteristics of male specimens, with no female specimens used for the description. Obtaining COI sequences from the type specimens or topotypes of both sexes could help validate our hypothesis. Nevertheless, we are certain that the specimens (both males and females) we examined in this study are *B. segura,* as their morpho-meristic characters largely correspond to the range reported in the original description (Table 1).

Our findings also reveal that *B. segura* sequences from the Philippines, Indonesia, and Taiwan form two monophyletic subclades, separated by a 2.0% K2P genetic distance, suggesting that *B. segura* may represent a species complex. Subclade I comprised specimens from Taiwan and the Philippines, while subclade II includes specimens exclusively from Indonesia. These subclades were also observed by Afrisal et al. (2025), although their study included only a single COI sequence from the Philippines and none from Taiwan. Similarly, two unidentified *Belobranchus* specimens analyzed by Abdulmalik-Labe & Quilang (2024) in their DNA barcoding of Philippine freshwater eleotrids are likely *B. segura,* as their sequences clustered with *Belobranchus* sp. (MN045250), which also appears in our NJ phylogenetic tree. The presence of stripes in the dorsal and caudal fins in these specimens further corroborated this identification, as these markings are characteristic of female *B. segura* examined in this study.

This study also confirms the presence of two genetic lineages within *B. belobranchus* from the Philippines and Indonesia, as previously reported by Abdulmalik-Labe & Quilang (2024) and Afrisal et al. (2025). Afrisal et al. (2025) identified two *B. belobranchus* subclades in Indonesia: one including samples from Bali, Gorontalo (=Sulawesi), and West Java (=Jawa Barat), and another confined to West Java (=Jawa Barat) and Banten. However, the limited access to other Indonesian specimens restricts our ability to determine whether these genetic differences are accompanied by morphological divergence. Moreover, our COI sequences from Indonesia are limited to populations west of the Wallace Line, leaving uncertainty on the genetic status of *Belobranchus* lineages from the eastern side, including the Halmahera and Manado populations, the type localities of *B. segura* and *B. belobranchus*, respectively. Further sampling and the inclusion of additional genetic markers are necessary to clarify the potential existence of divergent lineages across the species' full geographic range.

Despite its recent report in the Philippines, *B. segura* was previously misidentified as "*B. belobranchus*" by some fish enthusiasts over 10 years ago. For instance, a blogger named Odyssey (2012) provided underwater photos of two specimens initially identified as *B. belobranchus*, later recognised to match the morphological descriptions of male and female *B. segura* in this study. Similarly, Filipino Cave Diver (2013) documented an eleotrid specimen from Balamban, Cebu, displaying characteristics of a male *B. segura*, including cheek stripes and a yellowish distal part of the first dorsal fin. Furthermore, the morpho-meristic characters recorded in this study represent the first comprehensive documentation of *Belobranchus* species in the Philippines. The report of sexual dimorphism in *B. segura* is particularly important, as many records of *B. belobranchus* in other parts of the West Pacific could have been misidentifications of female *B. segura*, which closely resemble *B. belobranchus*.

The new record of *B. belobranchus* in Palawan significantly contributes to our understanding of its distribution and range expansion in comparison to *B. segura* in the Philippines. On the other hand, *B. segura* has so far been officially reported only from Luzon, Visayas, and Palawan islands, with its occurrence now also confirmed in Mindanao. Further ichthyofaunal surveys in the freshwater habitats of southern Philippines could provide valuable information on its distribution in the Philippines considering that Mindanao is near the type locality (Halmahera, Indonesia) compared to the other major islands in the country where they were first reported. A comprehensive collection of *Belobranchus* samples across its known distribution range is crucial in elucidating population genetic structures and identifying potential cryptic species within the genus.

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APPENDICES

Table S1. Information on the *Belobranchus* COI sequences from the Philippines, Indonesia, and Taiwan used in reconstructing the Neighbour-Joining (NJ) tree.

Identifier	Species	Sex	Country	Location	Site	GenBank Accession No.	Reference
DOS09877	*Belobranchus segura*	–	Philippines	Aurora	Dicapulao	PP355131	Dimaquibo et al. (2025)
DOS09310-2	*Belobranchus segura*	Male	Philippines	Palawan	Tigaplan River	PP355121	This study
DOS09310-5	*Belobranchus segura*	Female	Philippines	Palawan	Tigaplan River	PP355123	This study
DOS09372-1	*Belobranchus segura*	Female	Philippines	Palawan	Cayulo River	PP355124	This study
DOS09372-4	*Belobranchus segura*	Male	Philippines	Palawan	Cayulo River	PP355127	This study
DOS09372-6	*Belobranchus segura*	Female	Philippines	Palawan	Cayulo River	PP355129	This study
DOS10068-1	*Belobranchus segura*	Female	Indonesia	Java	–	PV943647	This study
DOS10068-4	*Belobranchus segura*	Male	Indonesia	Java	–	PV943649	This study
DOS10068-5	*Belobranchus segura*	Male	Indonesia	Java	–	PV943650	This study
DOS10233-7	*Belobranchus segura*	–	Taiwan	Pingtung	Fangshan River	PV943651	This study
DOS10233-8	*Belobranchus segura*	–	Taiwan	Pingtung	Fangshan River	PV943652	This study
DOS10233-9	*Belobranchus segura*	–	Taiwan	Pingtung	Fangshan River	PV943653	This study
ANGBF57288-19	*Belobranchus segura*	–	Indonesia	Gorontalo	Gorontalo Bay	MN069306	Sahami et al. (2019)
ANGBF57290-19	*Belobranchus segura*	–	Indonesia	Gorontalo	Gorontalo Bay	MN069308	Sahami et al. (2019)
BIFD1933-14	*Belobranchus segura*	–	Indonesia	Bali	–	KU692363	Dahruddin et al. (2017)
BIFD2019-14	*Belobranchus segura*	–	Indonesia	Bali	–	KU692368	Dahruddin et al. (2017)
BIFD2019-14	*Belobranchus segura*	–	Indonesia	Bali	–	KU692370	Dahruddin et al. (2017)
BIFD1043-14	*Belobranchus segura*	–	Indonesia	Jawa Barat	Cibeber	KU692362	Dahruddin et al. (2017)
BIFB397-13	*Belobranchus segura*	–	Indonesia	Jawa Barat	Ci Haur	KU692367	Dahruddin et al. (2017)
GBMNB8442-20	*Belobranchus* sp.	–	Philippines	-	–	MN045250	Mennesson et al. (2019)
DOS10257-1	*Belobranchus belobranchus*	–	Philippines	Aurora	DignoTabon Falls	PV943657	This study
DOS10257-2	*Belobranchus belobranchus*	–	Philippines	Aurora	DignoTabon Falls	PV943658	This study
DOS09310-3	*Belobranchus belobranchus*	Male	Philippines	Palawan	Tigaplan River	PP355132	This study
BIF0255	*Belobranchus belobranchus*	–	Indonesia	Jawa Barat	Ci Tiis	KU692344	Dahruddin et al. (2017)
BIF0256	*Belobranchus belobranchus*	–	Indonesia	Jawa Barat	Ci Tiis	KU692347	Dahruddin et al. (2017)
BIF0361	*Belobranchus belobranchus*	-	Indonesia	Jawa Barat	Ci Pawenang	KU692348	Dahruddin et al. (2017)
	Belobranchus belobranchus	–	Indonesia	Jawa Barat	Ci Pawenang	KU692349	Dahruddin et al. (2017)
BIF0423	*Belobranchus belobranchus*	–	Indonesia	Jawa Barat	Ci Haur	KU692352	Dahruddin et al. (2017)
BIF1696	Belobranchus belobranchus		Indonesia	Jawa Barat	Citiis	KU692353	Dahruddin et al. (2017)
BIF2383	*Belobranchus belobranchus*	–	Indonesia	Bali	Nbang	KU692346	Dahruddin et al. (2017)
	Belobranchus belobranchus	–	Indonesia	Bali	Nbang	KU692350	Dahruddin et al. (2017)
BIF2385	*Belobranchus belobranchus*	–	Indonesia	Bali	Nbang	KU692351	Dahruddin et al. (2017)
GBMNC15216-20	*Belobranchus belobranchus*	–	Indonesia	Gorontalo	Gorontalo Bay	MT706791	Sahami et al. (2019)
GBMNC15218-20	*Belobranchus belobranchus*	–	Indonesia	Gorontalo	Gorontalo Bay	MT706722	Sahami et al. (2019)
DOS09306-1	*Eleotris fusca*	–	Philippines	Palawan	–	PV943659	This study

Table S2. Pairwise K2P distance between clades on *Belobranchus belobranchus* and *Belobranchus segura* from the Philippines, Taiwan, and Indonesia.

Clade	***B. segura* I**	***B. segura* II**	***B. belobranchus* I**	***B. belobranchus* II**
***B. segura* I**		0.028	0.019	0.017
***B. segura* II**	0.007		0.018	0.017
***B. belobranchus* I**	0.157	0.148		0.037
***B. belobranchus* II**	0.140	0.137	0.008	

Table S3**.** Eigenvalues and percent variance of different principal components of morphometric characters in *Belobranchus segura.*

PC	**Eigenvalue**	**% variance**
1	8.991	33.300
2	4.454	16.497
3	2.586	9.576
4	2.305	8.539
5	1.631	6.041
6	1.339	4.069
7	1.125	4.167
8	1.082	4.009
9	0.867	3.213
10	0.591	2.188
11	0.533	1.974
12	0.481	1.781
13	0.352	1.305
14	0.246	0.912
15	0.182	0.674
16	0.104	0.385
17	0.072	0.266
18	0.037	0.137
19	0.021	0.079

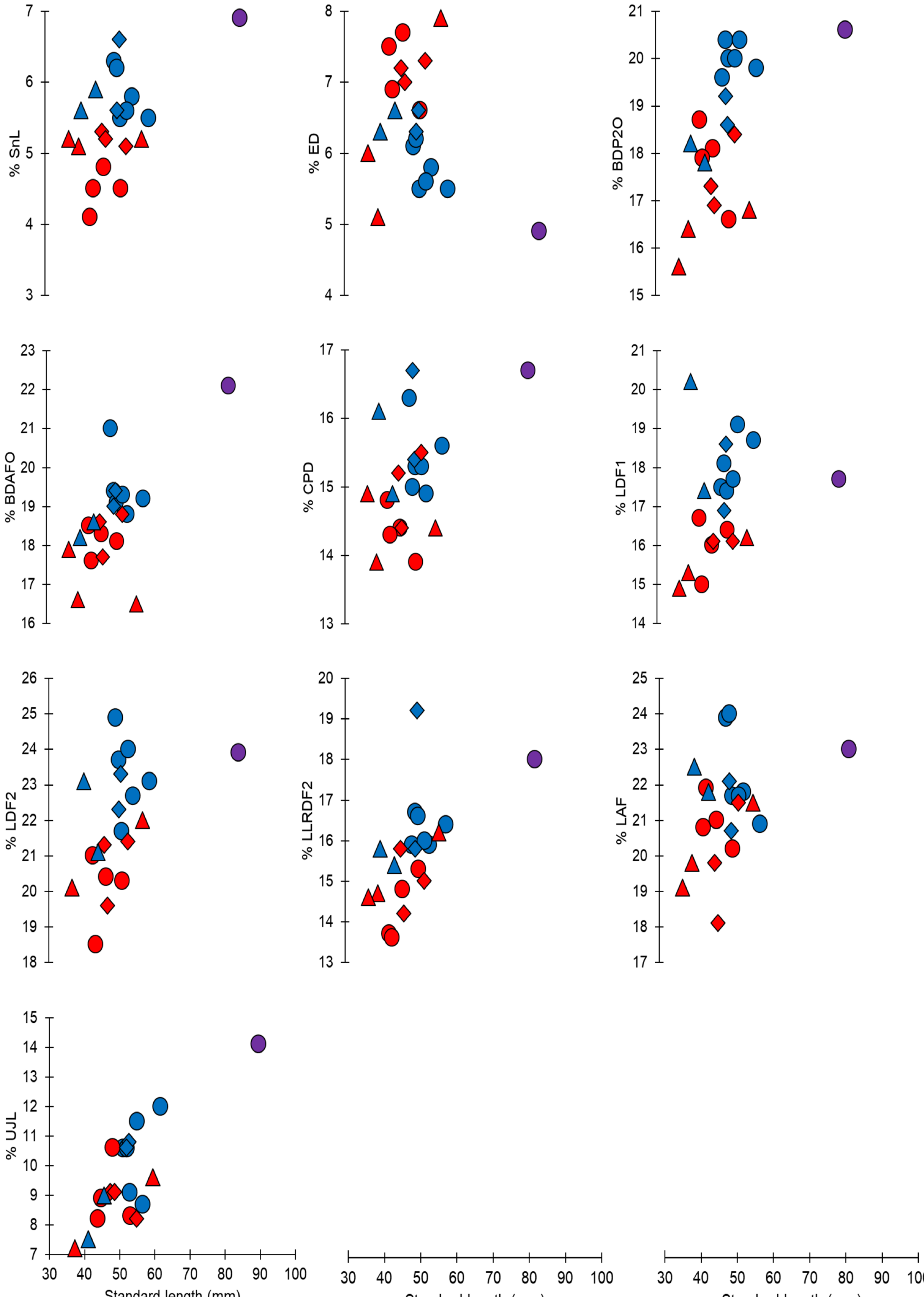

Fig. S1. Scatter plots of 10 selected morphometric measurements against the standard length (mm) of male (blue) and female (red) *Belobranchus segura* from Palawan, Philippines (circle), Java, Indonesia (diamond), Pintung, Taiwan (triangle), and *Belobranchus belobranchus* (violet circle).