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Molecular and morphological evidence reveals two fish species of *Giuris* (Eleotridae: Gobiiformes) in Sulawesi, with a range extension of *G. aporocephalus*

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ABSTRACT

Introduction: Gobies of the freshwater or amphidromous eleotrid genus *Giuris* (Sauvage 1880) are widespread in tropical rivers and lakes. Eight species are recognized to date through extensive taxonomic revision of the species complex *G. margaritaceus* and remain challenging to identify based solely on morphological characteristics.
Objective: The aim of this study was to clarify the *Giuris* species occurring in three lakes along the Northern arm of Sulawesi Island in the Wallacea bioregion (Limboto, Tondano and Bolano Sau) using an integrated molecular (DNA barcoding) and morphological approach.
Methods: *Giuris* specimens were collected from the three lakes, and their morphological characters were recorded. Before voucher specimen preservation, fin clippings were preserved in 96 % ethanol for genetic analysis. DNA barcodes were produced through DNA extraction, PCR (primers Fish F1, Fish R1) and Sanger sequencing of Cytochrome C Oxidase Subunit I Mitochondrial DNA (COI mtDNA) nucleotide sequences.
Results: The DNA barcodes (615 bp) nested in two clades: *G. laglaizei* (Bolano Sau Lake) and *G. aporocephalus* (all three lakes). Morphometric and meristic data were consonant with DNA barcode species assignments.
Conclusions: Our study confirms that the fish known locally as payangka in Limboto and Tondano Lakes belong to *G. aporocephalus*, while those from Bolano Sau Lake correspond to *G. aporocephalus* and *G. laglaizei*. This study also expands the known range of *G. aporocephalus*, with five *Giuris* species now confirmed from Tomini Bay watersheds.

Key words: Wallacea; DNA barcoding; morphology; amphidromy; Tomini Bay.

RESUMEN

Evidencia molecular y morfológica revela dos especies de peces *Giuris* (Eleotridae: Gobiiformes) en Sulawesi, con extensión del rango de *G. aporocephalus*

Introducción: Los peces gobios del género eleótrido dulceacuícola o anfidromo *Giuris* (Sauvage 1880) están ampliamente distribuidos en ríos y lagos tropicales. Las ocho especies identificadas hasta la fecha mediante

una extensa revisión taxonómica del complejo de especies *G. margaritaceus* siguen siendo difíciles de identificar morfológicamente.

Objetivo: El propósito de este estudio fue identificar las especies de *Giuris* presentes en tres lagos situados a lo largo del brazo norte de la isla de Sulawesi en la bioregión de Wallacea (Limboto, Tondano y Bolano Sau), utilizando un enfoque integrado de códigos de barras de ADN y caracteres morfomerísticos.

Métodos: Se recolectaron especímenes de *Giuris* en los tres lagos y se registraron los caracteres morfomerísticos. Antes de preservar los especímenes como ejemplares de referencia, se tomaron muestras de aletas, las cuales fueron preservadas en etanol al 96 % para análisis genéticos. Los códigos de barras de ADN se obtuvieron mediante extracción de ADN, PCR (usando los cebadores Fish F1 y Fish R1) y secuenciación Sanger de las secuencias de nucleótidos del ADN mitocondrial de la subunidad I del citocromo C oxidasa (COI mtDNA).

Resultados: Los códigos de barras de ADN (615 bp) formaron dos clados: *G. laglaizei* (lago Bolano Sau) y *G. aporocephalus* (en los tres lagos). Los caracteres morfomerísticos fueron congruentes con las asignaciones de especies basadas en los códigos de barras de ADN.

Conclusiones: Nuestro estudio confirma que los peces conocidos localmente como payangka en los lagos Limboto y Tondano pertenecen a la misma especie, mientras que en el lago Bolano Sau el payangka incluye dos especies de *Giuris*. Este estudio también amplía el rango conocido de *G. aporocephalus*, con cinco especies de *Giuris* ahora confirmadas en las cuencas hidrográficas de la Bahía de Tomini.

Palabras clave: Wallacea; código de barras de ADN; *Giuris*; morfomerística; anfidromía; Bahía de Tomini.

INTRODUCTION

The gobies (Gobiiformes) are a highly diverse taxonomic group of marine, freshwater and diadromous fishes found throughout the Indo-Pacific region and the Indo-Malay islands (Keith & Lord, 2012; Larson et al., 2014). The phylum Gobiiformes comprises 13 currently recognized families, including the Gobiidae (> 167 genera and > 1 432 species) and Eleotridae (> 36 genera, > 215 species) (Fricke et al., 2025; Froese & Pauly, 2025). Typically, amphidromous gobies contribute most to the diversity of fish communities in the Indo-Pacific and the Caribbean insular systems, and have the highest levels of endemism (Keith, 2002; Keith, 2003; Lim et al., 2002; Marquet et al., 1999), although many detailed aspects of the biological cycle and the factors driving evolution in amphidromous gobies remain poorly understood (Keith & Lord, 2012).

Typically, amphidromous like most eleotrids, gobies of the genus *Giuris* Sauvage 1880 are found in rivers (mostly estuarine and lower reaches) and lakes, often associated with aquatic vegetation and rocky or gravel bottoms (Keith et al., 2020). The adults of some of the larger diadromous gobies are also locally important as food fish, particularly the gudgeons (Eleotridae) generally known as payangka (or payangga in some regions) in Sulawesi, that can reach at least 16 cm (Makmur et al., 2019; Putra et al., 2020). These payangka populations are considered at risk, primarily from the introduction of alien fish but also from environmental degradation and, due to their organoleptic qualities, from fishing pressure (Paramata et al., 2025; Syafei, 2017).

The genus *Giuris* has a complicated taxonomic history; all previously described taxa assigned to the genus *Giuris* were synonymized as *G. margaritaceus* Valenciennes 1837 around 50 years ago (Akihito & Meguro, 1974; Keith et al., 2020). *Giuris* has been assigned the masculine gender (Kottelat, 2013); however, the grammatical gender of this genus has been inconsistent, with both *G. margaritaceus* and *G. margaritacea* appearing in the literature, along with at least 12 other synonyms (Froese & Pauly, 2025). Despite external morphological similarities, Kottelat (2013) argued that *Giuris* likely comprised multiple cryptic species, based on the extensive distribution and variability of this taxon. Recent taxonomic research integrating classical morphological and molecular data have confirmed the species complex hypothesis

and described or re-described eight *Giuris* species to date (Keith & Mennesson, 2020; Keith et al., 2020; Ndobe et al., 2023).

The largest semi-enclosed bay in the world, Tomini Bay (also known as the Gulf of Tomini) is bounded by the Southern coast of the Northern arm of Sulawesi, the Northern coast of Central Sulawesi, and to the West by the "neck" joining them together (Fig. 1). Payangka (*Giuris* spp.) are present in at least three lakes: Bolano Sau in Central Sulawesi Province, Limboto Lake in Gorontalo Province, and Tondano Lake in North Sulawesi Province. Three species *Giuris* were identified to the South of Tomini Bay by a major Indonesia-wide study (Keith et al., 2020), and a DNA barcoding study revealed that the payangka in Bolano Sau lake, identified as *G. laglaizei* (Ndobe et al., 2023), is not the same species as the payangka in Limboto and Tondano.

There is anecdotal historical evidence that the *Giuris* in Tondano Lake were introduced over 100 years ago from Limboto Lake (Soeroto, 1988), so it is likely that the same species will be present in these two lakes. It is also possible that more than one species may be present in each lake. The aim of our study was therefore to elucidate the *Giuris* species present in the three main lakes on the Northern arm of Sulawesi (Limboto, Tondano and Bolano Sau) using an integrated morphological and molecular (DNA barcoding) approach. These data will enrich DNA barcoding databases, inform management at the local level in three Indonesian provinces, and provide input for conservation assessments at local to global scales.

MATERIAL AND METHODS

Specimen collection and preservation: Specimens of the genus *Giuris* were collected on 13-18 August 2023 from three lakes on the Northern arm of Sulawesi Island, Indonesia: Limboto Lake, Gorontalo Province; Tondano Lake, North Sulawesi Province; and Bolano Sau Lake, Central Sulawesi Province (Fig. 1). The

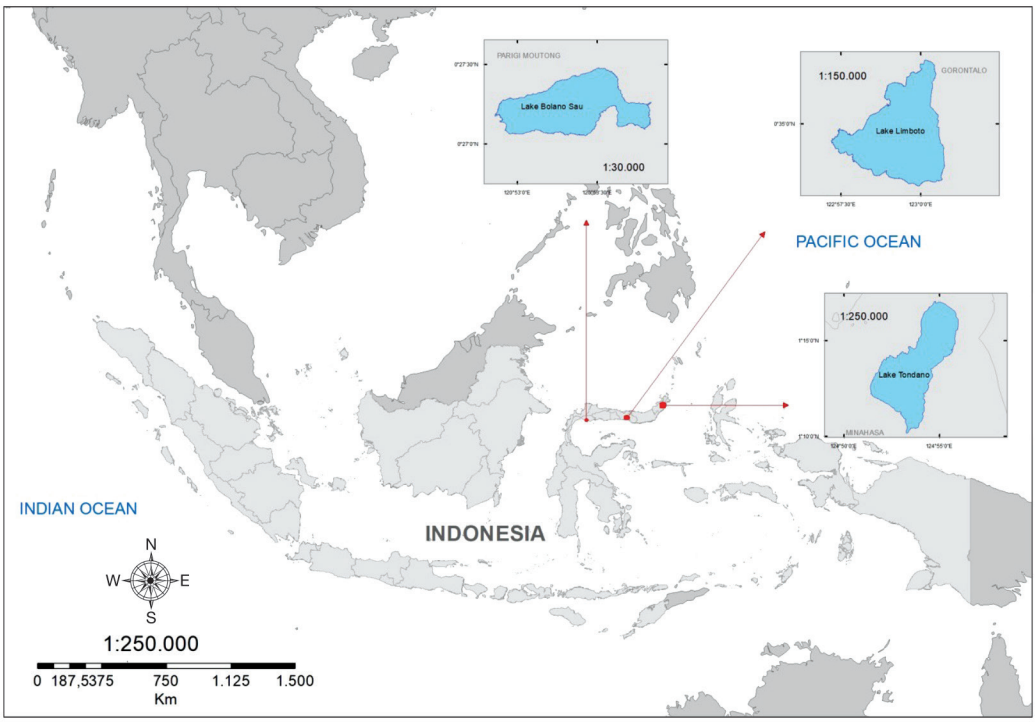

Fig. 1. Research sites in the Northern arm of Sulawesi, Wallacea, Indonesia.

Fig. 2. Freshly caught *Giuris* sp. specimen P1LL collected on 13 August 2023 from Limboto Lake, Gorontalo Province, Indonesia.

collection was authorized under research permit No. 18057/UN4.15/PT.01.04/2024 issued by Hasanuddin University and was approved by the relevant provincial government agencies. The specimens were obtained from local fishermen using a throw net with mesh size 3.5". Each specimen collected was photographed using an iPhone digital camera (Fig. 2) and euthanized using clove oil following standard protocols (Fernandes et al., 2017; Neiffer & Stamper, 2009).

Each specimen was weighed (digital scales, precision 0.1 g), labelled, and the total length (TL) and standard length (SL) were measured (digital calipers, precision 0.1 mm). A tissue sample for genetic (DNA barcoding) analysis was taken from the right ventral fin of each specimen, cleaned with distilled water and placed in a 2 ml cryotube containing 96 % absolute ethanol. The tube was then labelled with the same field sample code as the specimen: P1LL, P2LL, P3LL for samples from Limboto Lake; P1TL, P2TL, P3TL for samples from Tondano Lake; P1BL, P2BL for samples from Bolano Sau Lake.

The specimens were then preserved following the protocol developed for the Hasanuddin University scientific collection (Omar et al., 2021) with catalog numbers UNH24-NAS001-UNH24-NAS008. After soaking in 4 % formalin for 2-3 days, each specimen was rinsed in clean water to remove formalin before conducting the ethanol dehydration series (Suzuki et al., 2012). The specimens were placed in 96 % ethanol to remove excess water for 3-7 days, then transferred to glass jars filled with 70 % alcohol which was changed periodically until discoloration was minimal.

Morphometric and meristic data: Where relevant, counts and measurements were taken from the left side of each specimen. Morphometric measurements (Fig. 3A, Fig. 3B, Table 1) were made with digital calipers in mm (precision 0.1 mm). Meristic counts included scale counts (Fig. 3C) and fin spine/ray counts. Character codes used followed references (Keith & Mennesson, 2020; Keith et al., 2020). All morphometric traits except standard length (SL) were expressed as dimensionless ratios (% SL).

DNA barcoding: Genomic DNA was extracted from each sample using Geneaid Gsync kits following the manufacturer's protocols. DNA presence and quality was visualized through electrophoresis on 2 % agar gel soaked in the fluorescent dye ethidium bromide and viewed under ultraviolet (UV) radiation. The target cytochrome oxidase I mitochondrial DNA (COI mtDNA) gene fragment (DNA barcode region) was amplified through polymerase chain reaction (PCR) using the forward primer Fish F1 (5'TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and reverse primer Fish R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA3') (Ward et al., 2005).

PCR amplification was conducted (BiosystemsTM VeritiTM 96-Well Thermal Cycler,

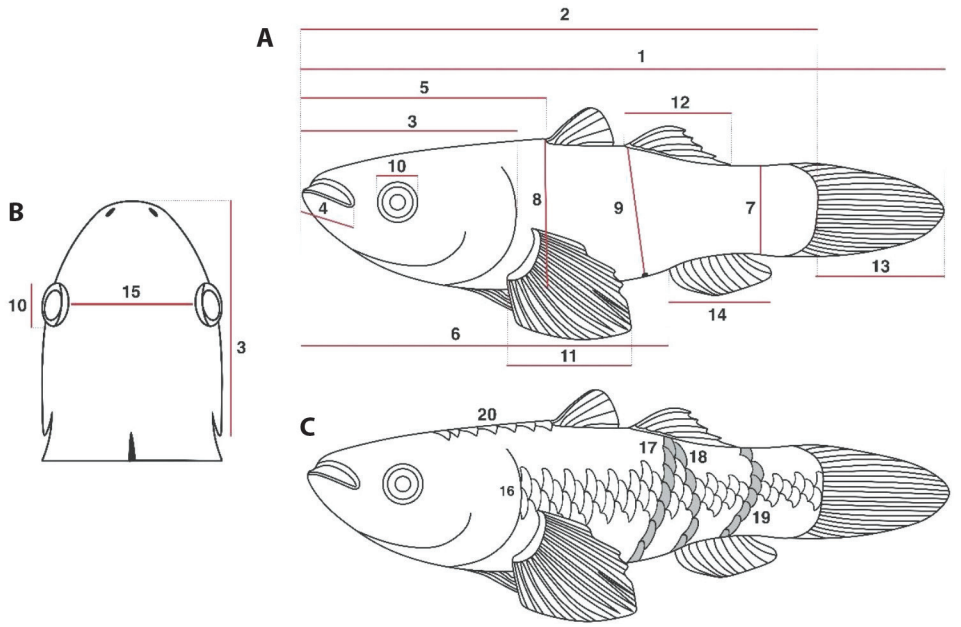

Fig. 3. *Giuris* sp. **A.** and **B.** morphometric and **C.** meristic characters used in this study.

Thermo Fisher Scientific) using the following PCR profile: predenaturation at 94 °C for 2 minutes; 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 45 °C for 45 seconds, and extension at 72 °C for 1.5 minutes; and final extension at 72 °C for 10 minutes. The PCR product (was visualized through electrophoresis (110 V for 20 minutes) on 2 % agar gel with TAE buffer and GelRed™ stain; a 4 µl aliquot of product was placed in each well. Sanger sequencing (ABI 3500 Genetic Analyzer, Thermo Fisher Scientific) was then performed on all PCR products with clear electrophoresis bands.

The chromatogram (.abi) files were imported into MEGA 11 (Tamura et al., 2021) for quality control and analysis. The forward/reverse sequences from each specimen were cleaned, aligned using ClustalW performed in MEGA 11 (Tamura et al., 2021), merged and trimmed to produce a single nucleotide sequence (DNA barcode). These vouchered DNA barcode sequences were then deposited in the NCBI GenBank repository with accession numbers PQ409574-PQ409581 (Table 2).

Phylogenetic analysis: Sequence alignment, trimming and phylogenetic analyses were performed in MEGA 11 (Tamura et al., 2021). Homologous sequences from the genus *Giuris* obtained using the NCBI Basic Local Alignment Search Tool BLAST-n online function were downloaded in FASTA format and aligned (ClustalW) with the *Giuris* DNA barcodes obtained from this study and a homologous vouchered eleotrid sequence from a different genus (*Oxyeleotris marmorata*, GenBank accession PQ637337, catalog number UNH24-NAS009, collected from Limboto Lake). Sequences with 90 % or greater overlap with the sequences from our specimens (Table 2) were included in evolutionary analyses using the Kimura-2- Parameter (K2P) model (Kimura, 1980) in MEGA 11 (default settings) with 1 000 bootstrap test replicates (Felsenstein, 1985). The Neighbor-Joining (NJ) method was applied to the full data set in Table 2 (83 sequences, 643 nucleotide positions) and the Maximum Likelihood method was applied to a reduced and trimmed data set (77 sequences, 604 nucleotide

Table 1
Morphometric characters measured on *Giuris* specimens from lakes in the Northern arm of Sulawesi.

No.	Code	Description
1	TL	Total length
2	SL	Standard length
3	HL	Head length
4	JL	jaw length
5	PDL	Predorsal length
6	PAL	Preanal length
7	CPM	Caudal peduncle height (minimum distance)
8	BDD1	Body depth at first dorsal fin
9	BDa	Body depth at anus
10	O	Eye diameter
11	Pect-L	Pectoral fin length
12	SDFL	Second dorsal fin length
13	CFL	Caudal fin length
14	AFL	Anal fin length
15	IO	Interorbital length
16	LS	Lateral line scale series (counted from upper pectoral base, or anteriormost scale along lateral midline, to central hypural base)
17	TRF	Transverse forward series (counted from the first scale anterior to the second dorsal fin, diagonally, anterior and ventral to the middle of the abdomen or most ventral scale)
18	TRB	Transverse backward series (counted from the first scale on the front to the second dorsal fin, diagonally, on the back and ventral side to the base of the anal fin or most ventral scale)
19	ZZ	Zig-zag series (scales on the narrowest part of the caudal peduncle counted from the most dorsal scale to the most ventral scale in an alternating manner)
20	PD	Pre-dorsal series (counted from scale directly anterior to first dorsal fin insertion to the anteriormost midline scale)
21	D1	Anterior dorsal fin spines (Latin numbers) and rays (Arabic numbers)
22	D2	Posterior dorsal fin spines (Latin numbers) and rays (Arabic numbers)
23	P	Pectoral fin spines (Latin numbers) and rays (Arabic numbers)
24	V	Ventral fin spines (Latin numbers) and rays (Arabic numbers)
25	A	Anal fin spines (Latin numbers) and rays (Arabic numbers)
26	C	Caudal fin rays (Arabic numbers)

positions). The resulting phylogenetic trees were exported as Newick files and edited in the Interactive tree of life (iTOL) v5 (Letunic & Bork, 2021).

The *Giuris* clades formed were identified with assistance from Philippe Keith of the French National Museum of Natural History.

RESULTS

Morphometric and meristic data: the specimens showed considerable variation in appearance (Fig. 4). The variability in the morphological traits (Table 3) and meristic counts (Table 4) of these specimens indicates the presence of more than one species. Based on a comparison with the most recent descriptions or redescriptions of the eight currently recognized species within the genus *Giuris* (Keith & Mennesson, 2020; Keith et al., 2020), the most likely species candidates were *G. aporocephalus* Macleay, 1884, originally described from Australia, and *G. laglaizei* Sauvage, 1880, originally described from the Philippines.

Table 2
Nucleotide sequences used in the phylogenetic analyses.

No	GenBank Accession		Taxon given in reference	Taxon based on phylogenetic analysis	Country/ Region[b]	Reference
	Number(s)	Verbatim taxon[a]				
1	AF391368	*O. aporos*	*O. aporos*	*G. aporocephalus*	Australia	(Thacker, 2003)
2	AY722159, AY722160	*O. aporos*	*O. aporos*	*G. aporocephalus*	Australia	(Thacker & Hardman, 2005)
3	AY722161	*O. aporos*	*O. aporos*	*G. aporocephalus*	Sulawesi *	(Thacker & Hardman, 2005)
4	HQ654732-HQ654738, HQ654740	*O. aporos*	*O. aporos*	*G. laglaizei*	Philippines	(Aquino et al., 2011)
5	HQ682711, HQ682712	*G. margaritacea*	*G. margaritacea*	*G. laglaizei*	Philippines	(Aquino et al., 2011)
6	JN021218, JN021219	*G. tolsoni*	*G. tolsoni*	*G. tolsoni*	Philippines	(Abdulmalik-Labe & Quilang, 2024)
7	KU692503, KU692508, KU692513	*G. margaritaceus*	*G. margaritacea*	*G. viator*	Java *	(Dahruddin et al., 2017)
8	KU692504-KU692506, KU692514	*G. margaritaceus*	*G. margaritacea*	*G. tolsoni*	Bali *	(Dahruddin et al., 2017)
9	KU944837	*G. margaritaceus*	N/A	*G. tolsoni*	Taiwan	(Chang et al., 2017)
10	MG407388-MG407392	*G. margaritaceus*	*G. margaritaceus*	*G. laglaizei*	Philippines	(Abdulmalik-Labe & Quilang, 2019)
11	MK572389	*O. porocephalum*	*O. porocephala*	*G. viator*	Bangladesh	(Rahman et al., 2019)
12	MW497105, MW497106, MW497109, MW497111, MW497112, MW497116, MW497125, MW497133, MW497145, MW497146	*Giuris* sp.	*G. tolsoni*	*G. tolsoni*	Lombok *	(Keith et al., 2020)
13	MW497123	*Giuris* sp.	*G. tolsoni*	*G. tolsoni*	Ampana *	(Keith et al., 2020)
14	MW497113, MW497137, MW497139, MW497147	*Giuris* sp.	*G. margaritaceus*	*G. margaritaceus*	Ambon *	(Keith et al., 2020)
15	MW497110, MW497117, MW497122	*Giuris* sp.	*G. margaritaceus*	*G. margaritaceus*	Ampana *	(Keith et al., 2020)
16	MW497141, MW497143	*Giuris* sp.	*G. margaritaceus*	*G. margaritaceus*	Luwuk *	(Keith et al., 2020)
17	MW497121	*Giuris* sp.	*G. margaritaceus*	*G. margaritaceus*	Seram *	(Keith et al., 2020)
18	MW497144[c]	*Giuris* sp.	*G. viator*	*G. viator*	Ambon *	(Keith et al., 2020)
19	MW497108, MW497120	*Giuris* sp.	*G. viator*	*G. viator*	Lombok *	(Keith et al., 2020)
20	MW497135, MW497150[c]	*Giuris* sp.	*G. viator*	*G. viator*	Luwuk *	(Keith et al., 2020)
21	MW497107, MW497114, MW497118, MW497132, MW497136[c], MW497149, MW497151, MW497152, MW497153[c]	*Giuris* sp.	*G. viator*	*G. viator*	Seram *	(Keith et al., 2020)
22	OM674613	*G. laglaizei*	*G. laglaizei*	*G. laglaizei*	Bolano Sau Lake *	(Ndobe et al., 2023)
23	ON604188	*G. margaritaceus*	N/A	*2020-0156 14545 G. yahayai*	Madagascar	Unpublished 2022

No	GenBank Accession		Taxon given in reference	Taxon based on phylogenetic analysis	Country/ Region[b]	Reference
	Number(s)	Verbatim taxon[a]				
24	OQ386785	*G. tolsoni*	*G. tolsoni*	*G. tolsoni*	Philippines	(Bemis et al., 2023)
25	OQ386907	*G. tolsoni*	*G. tolsoni*	*G. tolsoni*	Philippines	(Bemis et al., 2023)
26	OQ788245-OQ788247, OQ788250	*G. margaritaceus*	*G. margaritaceus*	*Giuris aporocephalus*	Limboto Lake*	(Lamadi et al., 2023)
27	LC864454	*G. tolsoni*	N/A	*G. tolsoni*	Japan	Unpublished 2025

Giuris sequences obtained from this study and outgroup sequence

No.	Specimen Code	GenBank Accession		UNHAS catalog number	Collection site	
		Number	Taxon		Lake	Coordinates[d]
1	P1LL	PQ409574	*G. aporocephalus*	UNH24-NAS001	Limboto	0°35’02” N, 122°58’48” E
2	P2LL	PQ409575	*G. aporocephalus*	UNH24-NAS002	Limboto	0°35’02” N, 122°58’48” E
3	P3LL	PQ409576	G. aporocephalus	UNH24-NAS003	Limboto	0°35’02” N, 122°58’48” E
4	P1BL	PQ409577	*G. laglaizei*	UNH24-NAS004	Bolano Sau	0°27’05” N, 120°53’36” E
5	P2BL	PQ409578	*G. aporocephalus*	UNH24-NAS005	Bolano Sau	0°27’05” N, 120°53’36” E
6	P1TL	PQ409579	*G. aporocephalus*	UNH24-NAS006	Tondano	1°13’38” N, 124°53’49” E
7	P2TL	PQ409580	*G. aporocephalus*	UNH24-NAS007	Tondano	1°13’38” N, 124°53’49” E
8	P3TL	PQ409581	*G. aporocephalus*	UNH24-NAS008	Tondano	1°13’38” N, 124°53’49” E
9	Outgroup	PQ637337	*Oxyeleotris marmorata*	UNH24-NAS009	Limboto	0°35’02” N, 122°58’48” E

[a] The taxon name given in the GenBank accession metadata. / [b] The sub-national region is given for accessions from Indonesian specimens (marked with *). / [c] Included in Neighbor Join analysis but not in Maximum Likelihood analysis (insufficient overlap with sequences from this study). / [d] Approximate coordinates, given to the nearest second.

Fig. 4. *Giuris* specimens collected from lakes in the Northern arm of Sulawesi, Indonesia: **A.** *G. aporocephalus* (P1TL, SL = mm; TL = mm); **B.** *G. aporocephalus* (P2TL, SL = mm; TL = mm); **C.** *G. aporocephalus* (P3TL, SL = mm; TL = mm); **D.** *G. aporocephalus* (P1LL, SL = mm; TL = mm); **E.** *G. aporocephalus* (P2LL, SL = mm; TL = mm); **F.** *G. aporocephalus* (P3LL, SL = mm; TL = mm); **G.** *G. laglaizei* (P1BL, SL = mm; TL = mm); **H.** *G. aporocephalus* (P2BL, SL = mm; TL = mm); codes: TL = Tondano Lake; LL= Limboto Lake; BL = Bolano Sau Lake.

Table 3

Comparison of ten morphometric traits of eight *Giuris* sp. specimens from lakes in the Northern arm of Sulawesi and reported ranges for eight *Giuris* species.

Specimen code/ species	Morphometric trait (ratio to standard length SL, in %, all measurements in mm) [a]									
	BDa	BDD1	CPM	PDL	PAL	CFL	HL	JL	O	IO
P1LL	22	28	17	50	67	12	30	10	8	11
P2LL	22	25	14	43	58	12	32	9	6	12
P3LL	20	23	13	44	58	14	30	9	6	13
P1TL	24	26	13	46	66	12	33	10	7	11
P2TL	26	28	13	43	64	12	32	10	4	12
P3TL	22	24	13	44	67	13	33	10	6	12
P1BL	31	27	18	47	64	13	37	13	5	0
P2BL	26	28	18	47	69	12	34	8	6	0
G. aporocephalus[b]	21-26	20-25	13-16	44-51	62-67	22	31-36	9-11	5-7	12-16
G. laglaizei [b]	23-27	22-27	14-17	43-47	64-70	18	30-35	8-11.	5-6.	10-12.
G. laglaizei [d]		15-27	10-16	27-40		9-15	20-31	4-8	3-6	
G. viator [bc]	21-24	20-24	14-15	42-47	59-66	7-12	31-35	10-12	6-8	10-14
G. *margaritaceus* [bc]	20 -24	20-25	13-16	43-39	59-66	18	30-35	10-11	6-8	12-14
G. *tolsoni* [bc]	16 -22	20-24	13-15	39-47	59-68	11	31-37	9-12	6-7	9-12
G. yahayai [b]	26 - 36	26-39	16-20	44-50	60-68	11-17	31-36	9-12	4-6	14-16
G. charpini [b]	20 -24	19-25	13-16	43-44	59-65	10-12	31-33	10-11	5-7	12-14
G. caussei [b]	16 -21	22-25	14-16	50	63-64	6	37	10	4-6	13;-5

[a] BDa = Body depth at anus; BDD1 = Body depth at first dorsal fin; CPM = Caudal peduncle height; PDL = Predorsal length; PAL = Preanal length; CFL= Caudal fin length; HL = Head length; JL= Jaw length; O = Eye diameter; IO = Interorbital length. See Table 1 for definitions. The full data set is available on reasonable requests from the corresponding author. / [b] (Keith & Mennesson, 2020) / [c] (Keith et al., 2020). / [d] (Ndobe et al., 2023).

The specimens in this study displayed three color morphs. The first group (Fig. 4B, Fig. 4D, Fig. 4E, Fig. 4F) have a greyish background along the dorsal ridge, with poorly-defined and variable pale reddish and brownish patterns on the flanks, becoming paler on the belly. Some scales in lines running from the pectoral fin base to the caudal peduncle along and mostly close to the lateral line have a bluish or greenish tinge, with orange to reddish spots

Table 4
Meristic count of eight *Giuris* sp. specimens from lakes in the Northern arm of Sulawesi and ranges for eight *Giuris* species.

	Meristic fin spine/ray and scale counts[a]									
	D1-D2	A	P	V	C	LS	PD	TRB	TRF	ZZ
P1LL	VI-I.8	I.7	10	I.5	14	27	15	10	12	8
P2LL	VI-I.10	I.9	13	I.5	15	29	16	11	13	9
P3LL	VI-I.9	I.9	15	I.5	15	30	18	12	14	9
P1TL	VI-I.8	I.7	12	I.5	15	27	15	8	11	8
P2TL	VI-I.8	I.9	13	I.5	15	29	14	10	12	8
P3TL	VI-I.8	I.9	14	I.5	15	30	18	11	13	9
P1BL	VI-I.8	I.9	12	I.5	14	31	13	12	12	9
P2BL	VI-I.8	I.9	15	I.5	15	32	13	12	11	9
G. aporocephalus [b]	VI-I.8	I.9	14-15	I.5	13-14	28-31	15-18	8-10	11-13	8-10
G. laglaizei [b]	VI-I.8	I.9	15	I.5	14-15	29-31	15-17	9-11	12-14	8-9
G. laglaizei [d]	VI-I.8	I.8	13	I.5	15					
G. viator [c]	VI-I.8	I.9	14	I.5	13-14	28-32	14-16	10-12	14-15	9-10
G. margaritaceus [c]	VI-I.8	I.9	14-15	I.5	13-15	28-31	15-17	9-10	13-14	9-10
G. tolsoni [c]	VI-I.8	I.9	14	I.5	13-15	29-31	14-16	10-11	13-16	8-10
G. yahayai [b]	VI-I.8-9	I.9	14	I.5	15	29-32	15-18	10-12	17-20	7-9
G. charpini [b]	VI-I.8	I.8-9	13-14	I.5	13-14	27-29	13-15	9-11	13-14	8-9
G. caussei [b]	VI-I.8-9	I.9	14-15	I.5	13	29	15-16	9	11-12	9

[a] Fin spines /rays: Roman numerals represent spines; Arabic numerals represent rays; D = dorsal fin; A = anal fin; P = pectoral fin; V = ventral fin; C = caudal fin. Scale counts: LS = lateral line series; PD = pre-dorsal series; TRB = transverse backward series; TRF = transvers forward series; ZZ = zig-zag series. See Table 1 for definitions. / [b] (Keith & Mennesson, 2020). / [c] (Keith et al., 2020). / [d] (Ndobe et al., 2023).

on some scales on the flanks from the pectoral fin base to the hypural base. These colors can cause the fish to shine in some light conditions. Top of head greyish, lateral part yellowish with 3 dark brown to black stripes radiating from the eye to the cheeks and operculum, the highest stripe continuing to the pectoral base which is yellowish. Both dorsal fins and the anal fin are greyish with a reddish stripe at the distal margin. Pectoral fins greyish to hyaline and pelvic fins greyish to hyaline with a red distal margin. Caudal fin brownish with a reddish base and paler outer margin.

The second group (Fig. 4A, Fig. 4C, Fig. 4H) have a brownish to orange dorsal color, orange to dark red or brown on the flanks with bluish coloration along and parallel to the lateral line from the pectoral fin base to the caudal peduncle. The pinkish orang to reddish tinged scales on the flanks have a dark red dot. Belly bright yellow or orange at the isthmus, head brownish on top, sides orange to bright red with 3 dark red stripes radiating from the eye to the cheeks and operculum, the highest stripe continuing to the bright yellow pectoral base, and lips orange. The specimen P2BL (Fig. 4G) had more similarities to the first group but displayed several differences from both groups.

DNA Barcoding and phylogenetic analysis: The DNA barcodes obtained from the eight *Giuris* sp. specimens collected from three lakes in the Northern arm of Sulawesi were 587-604 bp in length and submitted to GenBank as accessions PQ409574-PQ409581. The BLAST results identified AF391368 (Thacker, 2003) as the closest accession for seven of our *Giuris* sequences (PILL, P2LL, P3LL, PITL, P2TL, P3TL, and PIBL), with around 99 % identity. This sequence was deposited as *Ophieleotris aporos* and is currently listed in GenBank as *Giuris margaritaceus*. The closest accession for

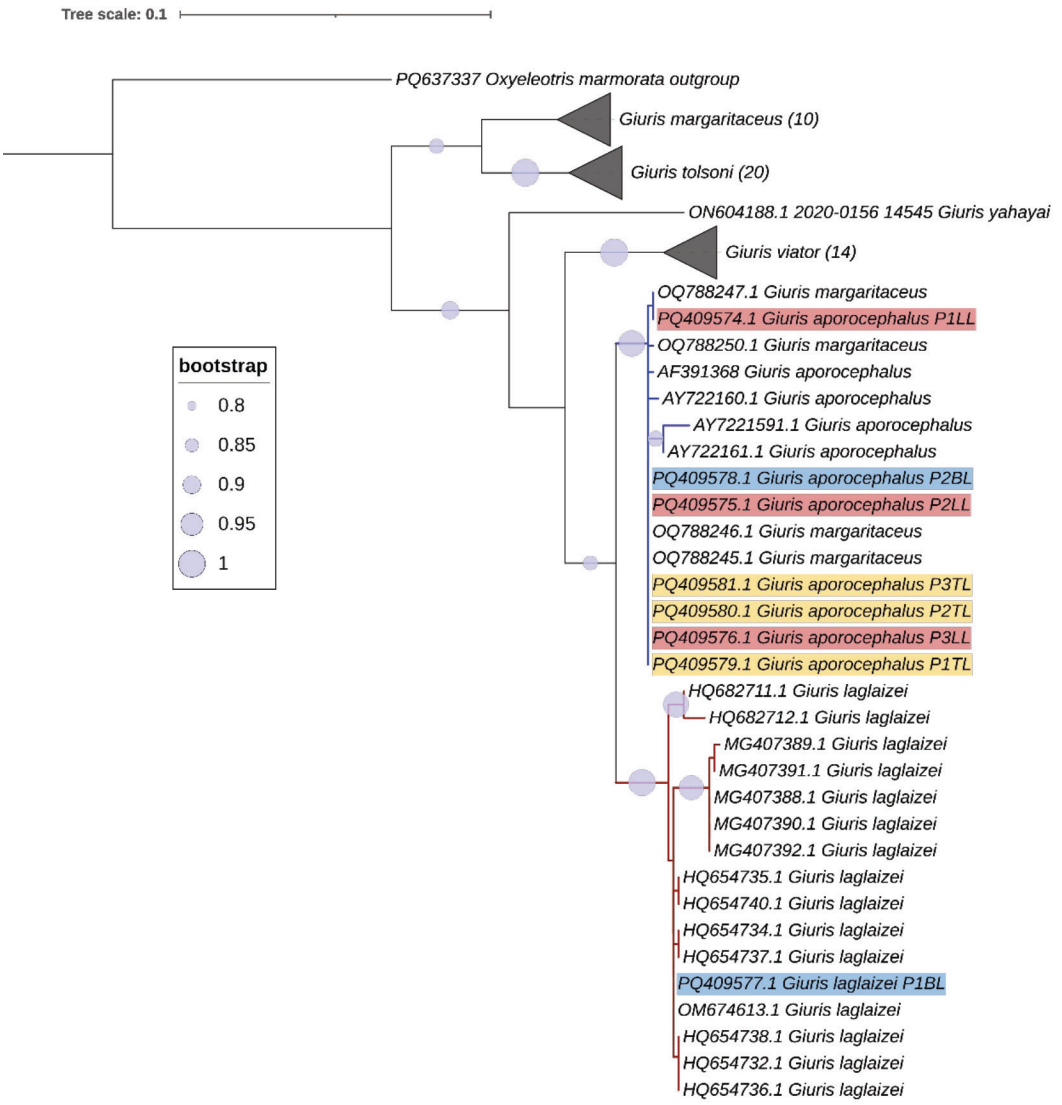

Fig. 5. Maximum Likelihood phylogenetic tree of the genus *Giuris* based on 77 COI mtDNA barcode sequences (604 bp, x 1000 bootstrap test replicates) including 8 specimens from the Northern arm of Sulawesi, 68 *Giuris* GenBank accessions, and the outgroup *Oxyeleotris marmorata* Bleeker, 1852. Highlight color: red = Limboto Lake; blue = Bolano Sau Lake; yellow = Tondano Lake. Line color: red = *G. laglaizei* clade; blue = *G. aporocephalus* clade. The number of sequences in each clade is given in parentheses.

one sequence (PIBL) with 99 % identity was OM674613 (Ndobe et al. 2023), submitted as *G. laglaizei*. The clustering patterns of the phylogenetic analyses using the Neighbor Join (not shown) and Maximum Likelihood (Fig. 5) methods were consistent, with six major clades and all sequences included in both analyses resolving within the same clade.

The eight DNA barcode sequences from our study were resolved within two of the six clades in the phylogenetic tree incorporating *Giuris* GenBank accessions. The DNA barcodes

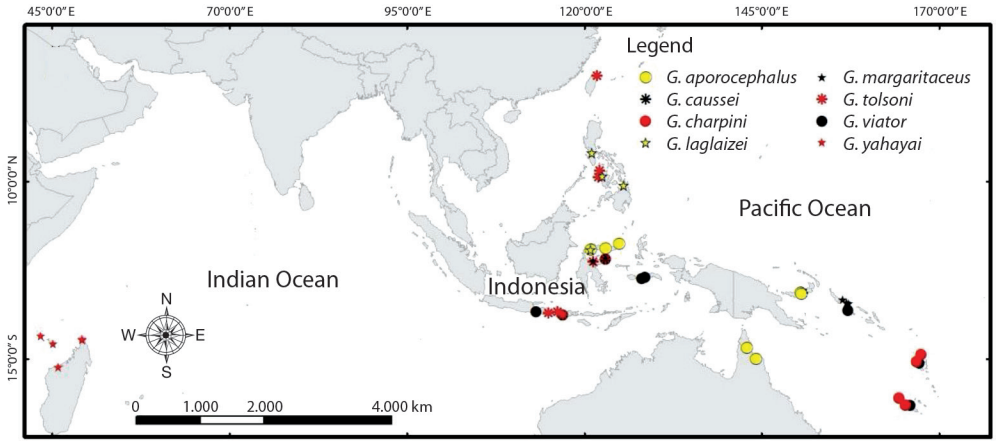

Fig. 6. Georeferenced records for eight *Giuris* species, indicating a range extension of around 3 000 km Northwards for *G. aporocephalus*.

from seven specimens (P1LL, P2LL, P3LL from Limboto Lake; P1TL, P2TL, P3TL from Tondano Lake; P2BL from Bolano Sau Lake) resolved in the same clade as accessions of *Ophieleotris aporos* from Australia (Thacker, 2003; Thacker & Hardman, 2005). This clade (comprising *Giuris* accessions PQ409574-PQ409580 from this study) was identified as *Giuris aporocephalus* (Philippe Keith, French National Museum of Natural History, personal communication 2024). The Bolano Sau Lake specimen (code P1BL, GenBank accession PQ409581) resolved within the *G. laglaizei* clade.

The map in Fig. 6 shows the distribution of records for the eight currently accepted *Giuris* species. The closest georeferenced record for *G. aporocephalus* is around 3 000 km from the Northern arm of Sulawesi.

DISCUSSION

Molecular identification of *Giuris* spp.: Until recently, all *Giuris* populations were classified as a single species, *G. margaritaceus*, originally described as *Eleotris margaritacea* Valenciennes 1837. This collective taxonomy is still used in FishBase (Froese & Pauly, 2025) and the IUCN Red List (Larson, 2019), although 17 invalid synonyms (including 4 misspellings) are listed in FishBase, and 14 are listed in Eschmeyer's Catalog of Fishes (Fricke et al., 2025). Of these, two (originally ascribed to the genus *Eleotris*) have now been resurrected as extant species-level taxa in the genus *Giuris*: *G. laglaizei* and *G. aporocephalus*. Current revisions recognize eight species within the morphologically cryptic *Giuris* species complex (Keith & Mennesson, 2020; Keith et al., 2020). Our study generated eight COI barcodes (PQ409574-PQ40981; one specimen failed to amplify), complementing existing data on the distribution of *Giuris* species. These barcodes reveal the presence of at least two *Giuris* species in the Northern arm of Sulawesi: (1) *G. laglaizei* (confirming the record by Ndobe et al. (2023) from Lake Bolano Sau) and (2) *G. aporocephalus*, a new record for Sulawesi, found in all three lakes (Bolano Sau, Limboto, and Tondano). This dual presence makes Bolano Sau a new multi-species site (sympatric zone), while conclusively resolving the taxonomic identity of payangka in lakes Limboto and Tondano as *G. aporocephalus*. Thereby, we resolve the taxonomic question raised by Ndobe et al. (2023) regarding these populations.

The genetic distances (measured in the number of substitutions per site and represented visually by the branch lengths in Fig. 5) between

the six major (congeneric species) clades were in the order of 0.01, while the inter-generic distance with the outgroup was in excess of 0.1, and the within-clade (intra-species) distances were of the order of 0.001 or less. The exception was the group within the *G. laglaizei* clade comprising sequences from Lanao Lake in the Philippines (GenBank accessions MG407388-MG407392) originally labelled as *G. margaritaceus* by Abdulmalik-Labe & Quilang (2019). The genetic distance between this sub-clade and the *G. laglaizei* sequences from other sites was around 0.01, indicating considerable genetic diversity between populations within this putative species. Nonetheless, in general the inter-and intra-specific genetic distances in the COI mtDNA barcode region are consonant with other taxonomic groups, including marine fishes (Wang et al., 2024), while within the Gobiiformes, the interspecies distances are within the range reported for the subfamily Sicydiinae (Taillebois et al., 2014).

Notably, our study did not detect *G. margaritaceus sensu stricto* (the taxon currently recognized under this name after taxonomic revisions). This supports the hypothesis proposed by Ndobe et al. (2023) and Lamadi et al. (2024) that previous reports of *G. margaritaceus* from this region (including misapplied synonyms *G. margaritacea* and *O. aporos*: Auliyah, 2019; Hasim et al., 2021; Lamadi et al., 2023; Makmur et al., 2015; Makmur et al., 2019; Nuha et al., 2020; Putra et al., 2020) likely represent misidentifications of other *Giuris* species, stemming from historical taxonomic conflations. However, absence of detection is not proof of absence. Both *G. margaritaceus* and *G. tolsoni* have been detected at other Sulawesi sites, including Ampana on the South coast of Tomini Bay (Keith et al., 2020). This distribution pattern suggests potential recruitment via riverine connections between Tomini Bay and our study lakes, implying that additional *Giuris* species may occur in the region despite only two being found here.

Integrative taxonomic identification and range extension of *G. aporocephalus*: The present study provides new records for *Giuris aporocephalus* based on molecular and morphological data. This species was previously recorded at georeferenced sites in Australia, Papua New Guinea and the Solomon Islands (Keith & Mennesson, 2020). Our records of the species represent an approximate range extension of around 1 100 km to the North (from the Soloman Islands) and 2 000 km to the West (from Queensland, Australia). This amphidromous fish exhibits a patchy distribution across Sulawesi, with potential occurrence in areas between our study sites and its confirmed range. However, these distributional patterns require verification through molecular, morphological and geographical evidence.

Overall, the morphological characteristics of *G. aporocephalus* (morphometric ratios based on standard length and meristic counts) aligned with diagnostic ranges established in the redescription by Keith & Mennesson (2020) which validated its distinction from *G. margaritaceus*. The key diagnostic characters (15 pectorals rays, mid-body depth at anus 20-26 % SL, and 11-13 scales in transverse forward series) were consistently observed. Table 3 and Table 4 show several morphometric deviations from Keith & Mennesson (2020) redescription. For instance, in specimen P1LL, the body depth at anus was (20 % SL vs 21-26 %); the body depth at the first dorsal fin (28 % vs 20-25 %), caudal peduncle height (17 % vs 13-16 %), head length (30 % vs 31-36 %), eye diameter (8 % vs 5-7 %), and interorbital distance (11 % vs 12-16 %). Similarly, in P2TL the specimen body depth at the first dorsal fin exceeded the expected range (28 % vs 20-25 %).

Meristic data (Table 4) largely align with reported ranges for *G. aporocephalus*, though some deviations occur (compared with ranges by Keith & Mennesson, 2020): D2 rays (10 in P2LL, 9 in P3LL vs. 8); A rays (7 in PILL/PITL vs. 9); pectoral rays (10-13 vs. 14-15); C rays (15 in five specimens vs. 13-14); lateral line scales (27 in PILL/PITL vs. 28-31); PD scales (14 in P2TL vs. 15-18); and TRB rows (12 in PILL vs. 8-10). Longitudinal (11-13) and zigzag (8-10) scale series matched expected ranges.

These minor differences likely represent natural intraspecific variation, contributing to our understanding of morphological diversity in *G. aporocephalus*. As genetically verified specimens accumulate, character ranges may expand beyond current reports (Keith & Mennesson, 2020; Keith et al., 2020). However, increasing overlaps between species' morphological ranges underscore the necessity of integrative taxonomic approaches for reliable identification.

The *G. aporocephalus* specimens studied here exhibited two color morphs differing primarily in flank coloration. Like other *Giuris* gobies (Keith et al., 2020), they are considered omnivorous, feeding mainly on filamentous algae, small crustaceans and aquatic insects, and inhabiting vegetated areas such as ponds, lakes, swamps, and the lower reaches of rivers. The observed variation in coloration and morphometric/meristic data (including deviations from published ranges) likely reflect phenotypic plasticity in response to environmental factors (Hossain et al., 2010; Mittelbach et al., 2014), a common source of intraspecific diversity.

Sympatric *Giuris* species in Bolano Sau Lake: DNA barcoding identified two *Giuris* species in Northern Sulawesi lakes: *G. aporocephalus* (all three lakes) and *G. laglaizei* (in Lake Bolano Sau). The confirmation of *G. laglaizei* (PDB1 specimen, Fig. 4G) is consistent with a prior record (OM674613) from this lake by Ndobe et al. (2023), while PDB2 specimen from Bolano Sau Lake represent *G. aporocephalus*, demonstrating sympatry. Such multi-species occurrences mirror patterns observed elsewhere, including the Philippines (Abdulmalik-Labe & Quilang, 2024), Australia (Thacker, 2003; Thacker & Hardman, 2005), and Indonesia (Dahruddin et al., 2017), including Central Sulawesi (*G. margaritaceus* and *G. tolsoni* in Luwuk/Ampana and *G. viator* in Luwuk (Keith et al., 2020).

The P1BL specimen shows similar morphological characters to specimens from Lake Mainit (Philippines, Mindanao) identified as *G. laglaizei*, as redescribed by Keith & Mennesson (2020). The *G. laglaizei* specimen found in Bolano Sau Lake during this study (Fig. 4G) has a dark brown dorsal region; brown flanks; brownish-dark grey head; grayish belly; brownish first dorsal fin; greyish pelvic fins; brownish anal fin with 2-3 rows of grayish dots; and brownish-dark grey caudal fin with two rows of grayish dots.

The morphometric and meristic characters of *G. laglaizei* (Table 3, Table 4) generally fall within the ranges reported in Keith & Mennesson (2020), with slight differences found in PDB1 specimen: body depth at anus (31 % vs 21-26 %), head length (37 % vs 31-36 %), body depth at the first dorsal fin (28 % vs 22-27 %), caudal peduncle depth (18 % vs 14-17 %), pectoral fin ray count (12 vs 13), caudal fin ray count (14 vs 15), and predorsal scales (13 vs 15-17).

The similarity between the two sympatric *Giuris* species from Lake Bolano Sau (*G. aporocephalus* and *G. laglaizei*) (Fig. 4G, Fig. 4H) explains both the lack of historical reports recognizing multiple species and the single local name "payangka". A diagnostic difference observed was the presence of reddish-yellow flank spots in *G. aporocephalus*, absent in *G. laglaizei*.

Implications for conservation and management: Taxonomy is often perceived as an academic pursuit with limited practical application, yet it can have extremely important implications for conservation and resource management, including fisheries (Agnarsson & Kuntner, 2007). For the genus *Giuris*, the previous classification as a single widespread species (*G. margaritaceus*) led to its "Least Concern" IUCN assessment (Larson, 2019), as population declines of this amphidromous species could theoretically be offset by natural recruitment across watersheds, or eventually through assisted recruitment. However, the recognition of at least eight distinct species, each with potentially restricted ranges, demands revised conservation assessments, as some may now face extinction risks. Accurate management thus requires precise species identification using integrative taxonomy (DNA barcoding and morphology),

as advocated for cryptic taxa (Packer et al., 2009; Yang et al., 2022).

Given the apparently patchy distribution, widely separated populations (e.g., Sulawesi and Philippine populations of *G. laglaizei* and the Australian and Sulawesi populations of *G. aporocephalus*) may represent separate stocks or even evolutionarily significant units (ESUs) *sensu* Moritz (1994), thus requiring separate assessment at a sub-species level. Where connectivity between populations is lacking or extremely limited, several management units may be needed within a single ESU (Hohenlohe et al., 2021) or at fine geographical scales (Moore et al., 2021).

This research highlights the value of integrative taxonomy approaches for exploring biodiversity. We confirmed that the fish known locally as payangka in Limboto and Tondano Lakes belong to the same species (*G. aporocephalus*), while Bolano Sau hosts two *Giuris* species (*G. aporocephalus* and *G. laglaizei*). Our findings expand the known range of *G. aporocephalus* and reveal five *Giuris* species now confirmed in Tomini Bay watersheds, necessitating updates to IUCN Red List and FishBase classification to reflect this hidden diversity.

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REFERENCES

Abdulmalik-Labe, O. P., & Quilang, J. P. (2019). DNA barcoding of fishes from Lake Lanao, Philippines. *Mitochondrial DNA Part B*, *4*(1), 1890–1894. http://dx.doi.org/10.1080/23802359.2019.1614890

Abdulmalik-Labe, O. P., & Quilang, J. P. (2024). DNA barcoding of common freshwater eleotrids (Gobiiformes: Eleotridae) in the Philippines, with a global comparison and notes on their conservation and management. *Global Ecology and Conservation*, *53*(3), e03014. http://dx.doi.org/10.1016/j.gecco.2024.e03014

Agnarsson, I., & Kuntner, M. (2007). Taxonomy in a changing world: seeking solutions for a science in crisis. *Systematic Biology*, *56*(3), 531–539. http://dx.doi.org/10.1080/10635150701424546

Akihito, P., & Meguro, K. (1974). On Gobiid fishes *Ophiocara porocephala* and *Ophieleotris aporos*. *Japanese Journal of Ichthyology*, *21*, 72–84.

Aquino, L. M. G., Tango, J. M., Canoy, R. J. C., Fontanilla, I. K. C., Basiao, Z. U., Ong, P. S., & Quilang, J. P. (2011). DNA barcoding of fishes of Laguna de Bay, Philippines. *Mitochondrial DNA*, *22*(4), 143–153. http://dx.doi.org/10.3109/19401736.2011.624613

Auliyah, N. (2019). Length-weight relationship and condition factor of huluu fish (*Giuris margaritacea*) in Limboto Lake. *IOP Conference Series: Materials Science and Engineering*, *567*(1), 012027. http://dx.doi.org/10.1088/1757-899X/567/1/012027

Bemis, K. E., Girard, M. G., Santos, M. D., Carpenter, K. E., Deeds, J. R., Pitassy, D. E., Flores, N. A. L., Hunter, E. S., Driskell, A. C., Macdonald, K. S., Weigt, L. A., & Williams, J. T. (2023). Biodiversity of Philippine marine fishes: A DNA barcode reference library based on voucher specimens. *Scientific Data*, *10*(1), 411. https://doi.org/10.1038/s41597-023-02306-9

Chang, C. H., Shao, K. T., Lin, H. Y., Chiu, Y. C., Lee, M. Y., Liu, S. H., & Lin, P. L. (2017). DNA barcodes of the native ray-finned fishes in Taiwan. *Molecular Ecology Resources*, *17*(4), 796–805. http://dx.doi.org/10.1111/1755-0998.12601

Dahruddin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R., & Hubert, N. (2017). Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: Taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Molecular Ecology Resources*, *17*(2), 288–299. http://dx.doi.org/10.1111/1755-0998.12528

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*(4),

783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fernandes, I. M., Bastos, Y. F., Barreto, D. S., Lourenço, L. S., & Penha, J. M. (2017). The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. *Brazilian Journal of Biology*, *77*(3), 444–450. https://doi.org/10.1590/1519-6984.15015

Fricke, R., Eschmeyer, W. N., & Van der Laan, R (Eds.). (2025). *Eschmeyer's catalog of fishes: genera, species, reference*. California Academy of Science.

Froese, R., & Pauly, D. (2025). *The Global Database of Fishes*. FishBase. http://www.fishbase.org

Hasim, H., Tuheteru, J., & Fazrin, D. N. (2021). Comparison of growth pattern, condition factor, gonadosomatic index of Manggabai (*Glosogobius giuris*) and Payangka (*Ophieleotris aporos*) in Limboto Lake, Gorontalo, Indonesia. *Biodiversitas Journal of Biological Diversity*, *22*(6), 3388–3393. http://dx.doi.org/10.13057/biodiv/d220646

Hohenlohe, P. A., Funk, W. C., & Rajora, O. P. (2021). Population genomics for wildlife conservation and management. *Molecular Ecology*, *30*(1), 62–82. https://doi.org/10.1111/mec.15720

Hossain, M. A. R., Nahiduzzaman, M. D., Saha, D., Khanam, M. U. H., & Alam, M. S. (2010). Landmark-based morphometric and meristic variations of the endangered carp, kalibaus *Labeo calbasu*, from stocks of two isolated rivers, the Jamuna and Halda, and a hatchery. *Zoological Studies*, *49*(4), 556–563.

Keith, P. (2002). Threatened fishes of the world: *Rhyacichthys guilberti* Dingerkus Séret, 1992 (Rhyacichthyidae). *Environmental Biology of Fishes*, *63*(1), 40. http://dx.doi.org/10.1023/A:1013827820971

Keith, P. (2003). Biology and ecology of amphidromous Gobiidae of the Indo- Pacific and the Caribbean regions. *Journal of Fish Biology*, *63*(4), 831–847. http://dx.doi.org/10.1046/j.1095-8649.2003.00197.x

Keith, P., & Lord, C. (2012). Tropical freshwater gobies: Amphidromy as a life cycle. In R. A. Patzner, J. L. Van Tassell, M. Kovačić, & B. G. Kapoor (Eds.), *The biology of gobies* (pp. 243–277). CRC Press.

Keith, P., & Mennesson, M. I. (2020). Review of Giuris (Teleostei: Eleotridae) from Indo-Pacific islands, with description of three new species. *Cybium: International Journal of Ichthyology*, *44*(4), 331–349. http://dx.doi.org/10.26028/cybium/2020-444-004

Keith, P., Mennesson, M. I., Sauri, S., Busson, F., Delrieu-Trottin, E., Limmon, G., Nurjirana, Dahruddin, H., & Hubert, N. (2020). *Giuris* (Teleostei: Eleotridae) from Indonesia, with description of a new species. *Cybium: International Journal of Ichthyology*, *44*(4), 317–329. http://dx.doi.org/10.26028/cybium/2020-444-003

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111–120. https://doi.org/10.1007/BF01731581

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. *The Raffles Bulletin of Zoology* (Supplement 27), S1–S663.

Lamadi, A., Paricahya, A., Iranawati, F., Widodo, M. S., Wiadyna, D., & Wiadyna, R. (2024). Sexual dimorphism of ambigous Hulu'u fish (*Giuris* sp. Limboto) from Limboto Lake, North Sulawesi. *Egyptian Journal of Aquatic Biology and Fisheries*, *28*(2), 429–440. http://dx.doi.org/10.21608/EJABF.2024.348615

Lamadi, A., Paricahya, A. F., Iranawati, F., Widodo, M. S., & Wiadnya, R. (2023). Genetic and morphological analysis of complex *Giuris* group species from Limboto Lake, Gorontalo Province, Indonesia. *Biodiversitas Journal of Biological Diversity*, *24*(10), 5223–5232. http://dx.doi.org/10.13057/biodiv/d241002

Larson, H. (2019). *Giuris margaritaceus*. The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/274979439/246550801

Larson, H. K., Geiger, M. F., Hadiaty, R. K., & Herder, F. (2014). *Mugilogobius hitam*, a new species of freshwater goby (Teleostei: Gobioidei: Gobiidae) from Lake Towuti, central Sulawesi, Indonesia. *Raffles Bulletin of Zoology*, *62*, 718–725.

Letunic, I., & Bork, P. (2021). Interactive tree of life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, *49*(W1), W293–W296. https://doi.org/10.1093/nar/gkab301

Lim, P., Meunier, F. J., Keith, P., & Noël, P. Y. (2002). *Atlas des poissons et des crustacés d'eau douce de la Martinique*. Patrimoines naturels.

Makmur, S., Muthmainnah, D., & Subagdja, S. (2019). Biological characters of snakehead gudgeon (*Giuris margaritacea* Valenciennes, 1837) in Tondano Lake, Minahasa, North Sulawesi, Indonesia. *BIOVALENTIA: Biological Research Journal*, *5*(2), 1–9. http://dx.doi.org/10.24233/BIOV.5.2.2019.125

Makmur, S., Subagdja, S., Makri, Sudrajat, A., Irawan, B., & Bataragoa, N. E. (2015). *Karakteristik lingkungan, keanekaragaman jenis ikan dan aktivitas penangkapan sumberdaya ikan Danau Tondano, Sulawesi Utara. Jakarta* [Thechnical Report]. Balai Penelitian Perikanan Perairan Umum.

Marquet, G., Keith, P., & Vigneux, E. (1999). *Atlas des poissons et des crustacés d'eau douce de Nouvelle-Calédonie*. Service du patrimoine naturel.

Mittelbach, G. G., Ballew, N. G., & Kjelvik, M. K. (2014). Fish behavioral types and their ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences*, *71*(6), 927–944. http://dx.doi.org/10.1139/cjfas-2013-0558

Moore, A. M., Ndobe, S., & Yasir, I. (2021). Importance of monitoring an endangered endemic species-intraspecies biodiversity perspectives on the Banggai cardinalfish conservation and trade. *IOP Conference Series: Earth and Environmental Science*, *681*, 012120. http://dx.doi.org/ 10.1088/1755-1315/681/1/012120

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, *9*(10), 373–375. https://doi.org/10.1016/0169-5347(94)90057-4

Ndobe, S., Nurdin, M. S., Hasanah, N., Putra, A. E., Mansyur, K., Nasir, M., Rabuna, M. L., & Moore, A. M. (2023). DNA barcoding detects resurrected taxon *Giuris laglaizei* (Sauvage 1880) in Sulawesi, Indonesia: Bolano Sau Lake payangka phylogeny, phenotypic characters and implications for *Giuris* spp. conservation. *F1000Research*, *11*, 295. http://dx.doi.org/10.12688/f1000research.108970.2

Neiffer, D. L., & Stamper, M. A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. *ILAR Journal*, *50*(4), 343–360. http://dx.doi.org/10.1093/ilar.50.4.343

Nuha, U., Amin, M., & Lestari, U. (2020). The molecular phylogenetic of payangga (*G. margaritacea*), manggabai (*G. giuris*) and hulu'u from Limboto Lake based on cytochrome B sequences. *Journal of Physics: Conference Series*, *1465*, 012006. http://dx.doi.org/10.1088/1742-6596/1465/1/012006

Omar, S. B. A., Hidayani, A. A., Yanuarita, D., Umar, M. T., & Andriyono, S. (2021). Phylogenetic analysis of endemic fish from the Maros Karst Region, South Sulawesi, Indonesia. *International Journal of Agriculture and Biology*, *26*(6), 661–666. http://dx.doi.org/10.17957/IJAB/15.1880

Packer, L., Gibbs, J., Sheffield, C., & Hanner, R. (2009). DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources*, *9*(Supplement 1), S42–S50. https://doi.org/10.1111/j.1755-0998.2009.02631.x

Paramata, A. R., Tresnati, J., Kadir, N. N., & Moore, A. M. (2025). Native and introduced fish caught with gillnets in Limboto Lake, Gorontalo Province. *Indonesia. Fisheries and Aquatic Sciences*, *28*(4), 236–247. http://dx.doi.org/10.47853/FAS.2025.e21

Putra, A. E., Nurdin, M. S., Hasanah, N., Ndobe, S., & Mansyur, K. (2020). Sex ratio and size at first maturity of snakehead gudgeon (*Giuris margaritacea*) caught with gillnets at Bolano Sau Lake, Parigi Moutong District. *Jurnal AgriSains*, *21*(3), 111–118.

Rahman, M. M., Norén, M., Mollah, A. R., & Kullander, S. O. (2019). Building a DNA barcode library for the freshwater fishes of Bangladesh. *Scientific Reports*, *9*(1), 9382. https://doi.org/10.1038/s41598-019-45379-6

Soeroto, B. (1988). *Makanan dan reproduksi ikan payangka* (Ophieleotris aporos (Bleeker)) *di Danau Tondano*. Institut Pertanian Bogor.

Suzuki, Y., Imada, T., Yamaguchi, I., Yoshitake, H., Sanada, H., Kashiwagi, T., & Takaba, K. (2012). Effects of prolonged water washing of tissue samples fixed in formalin on histological staining. *Biotechnic and Histochemistry*, *87*(4), 241–248. https://doi.org/10.3109/10520295.2011.613410

Syafei, L. S. (2017). Keanekaragaman hayati dan konservasi ikan air tawar. *Jurnal Penyuluhan Perikanan dan Kelautan*, *11*(1), 48–62. https://doi.org/10.33378/jppik.v11i1.85

Taillebois, L., Castelin, M., Lord, C., Chabarria, R., Dettaï, A., & Keith, P. (2014). New Sicydiinae phylogeny (Teleostei: Gobioidei) inferred from mitochondrial and nuclear genes: Insights on systematics and ancestral areas. *Molecular Phylogenetics and Evolution*, *70*(1), 260–271. https://doi.org/10.1016/j.ympev.2013.09.026

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Thacker, C. E. (2003). Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). *Molecular Phylogenetics and Evolution*, *26*(3), 354–368. https://doi.org/10.1016/S1055-7903(02)00361-5

Thacker, C. E., & Hardman, M. A. (2005). Molecular phylogeny of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidei). *Molecular Phylogenetics and Evolution*, *37*(3), 858–871. https://doi.org/10.1016/j.ympev.2005.05.004

Wang, Y., Ma, C., Song, X., Li, M., & Zhang, H. (2024). Assessment of fish diversity in the East China Sea hairtail national aquatic germplasm resources conservation zone using DNA barcoding. *Global Ecology and Conservation*, *53*, e03013. https://doi.org/10.1016/j.gecco.2024.e03013

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B*, *360*(1462), 1847–1857. http://dx.doi.org/10.1098/rstb.2005.1716

Yang, B., Zhang, Z., Yang, C. Q., Wang, Y., Orr, M. C., Wang, H., & Zhang, A. B. (2022). Identification of species by combining molecular and morphological data using convolutional neural networks. *Systematic Biology*, *71*(3), 690–705. http://dx.doi.org/10.1093/sysbio/syab076