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Insights from DNA barcoding endangered endemic *Caridina* shrimps (Decapoda: Atyidae) in Lindu Lake, Indonesia

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ABSTRACT

Introduction: Humanity faces a multidimensional crisis with severe threats to often poorly known freshwater biodiversity. Molecular tools, like DNA barcoding, can aid in biodiversity exploration, monitoring, and conservation. Sulawesian *Caridina* shrimps are both ecologically significant and endangered yet remain understudied.
Objective: To contribute well-documented DNA barcode (COX1 mtDNA) sequences for three *Caridina* species endemic to Lindu Lake.
Methods: We collected 224 *Caridina* shrimps from three sites around Lindu Lake in August (n = 73), and October 2023 (n = 117), and July 2024 (n = 34). We measured and analyzed morphological traits as dimensionless ratios of total length. We extracted DNA from six specimens (two per species) and COX1 mtDNA barcodes obtained through PCR (primers jgLCO and jgHCO) and Sanger sequencing. We obtained homologous sequences from GenBank (BLAST routine) and BOLD repositories for phylogenetic analyses. All molecular analyses were performed in MEGA 11.
Results: One species was identified at each site. Morphological traits differ between species. Three haplotypes with low divergence: one in *Caridina kaili*, one in *Caridina linduensis*, and one in both *Caridina dali* and *C. linduensis*. Homologous sequences in GenBank and BOLD included very few Sulawesi endemic species; these formed the closest sister clades to Lindu Lake *Caridina*.
Conclusions: We submitted to the GenBank repository the first reference DNA barcodes for each of the Lindu Lake *Caridina* species. The poor resolution of COX1 mtDNA for Lindu Lake *Caridina* may be due to recent evolutionary processes. Our study highlights the ongoing need for barcoding freshwater invertebrates, particularly atyid shrimps from Sulawesi, in the Wallacea bioregion.

Keywords: atyid shrimps; Crustacea; cryptic species; lacustrine; mitochondrial DNA; Wallacea.

RESUMEN

Información obtenida del código de barras de ADN en camarones *Caridina* (Decapoda: Atyidae) endémicos en peligro de extinción en el Lago Lindu, Indonesia

Introducción: La humanidad enfrenta una crisis multidimensional con graves amenazas a la biodiversidad de agua dulce, a menudo poco conocida. Las herramientas moleculares, como el código de barras de ADN, pueden ayudar en la exploración, monitoreo y conservación de la biodiversidad. Los camarones *Caridina* de Sulawesi son ecológicamente significativos y están en peligro, pero siguen siendo poco estudiados.

Objetivo: Contribuir con secuencias bien documentadas de código de barras de ADN (COX1 mtDNA) para tres especies de *Caridina* endémicas del Lago Lindu.

Métodos: Recolectamos 224 camarones *Caridina* de tres sitios alrededor del Lago Lindu en agosto (n = 73), octubre 2023 (n = 117) y en julio 2024 (n = 34). Medimos y analizamos rasgos morfológicos como proporciones adimensionales de la longitud total. Extrajimos ADN de seis especímenes (dos por especie) y obtuvimos códigos de barras COX1 mtDNA mediante PCR (primers jgLCO y jgHCO) y secuenciación Sanger. Obtuvimos secuencias homólogas de GenBank (rutina BLAST) y repositorios BOLD para análisis filogenéticos. Todos los análisis moleculares se realizaron en MEGA 11.

Resultados: Identificamos una especie en cada sitio. Los rasgos morfológicos diferían entre las especies. Tres haplotipos con baja divergencia: uno en *C. kaili*, uno en *C. linduensis*, y uno en *C. dali* y *C. linduensis*. Las secuencias homólogas en GenBank y BOLD incluyeron muy pocas especies endémicas de Sulawesi; estas formaron los clados hermanos más cercanos a *Caridina* del Lago Lindu.

Conclusiones: Enviamos al repositorio GenBank los primeros códigos de barras de ADN de referencia para cada una de las especies de *Caridina* del Lago Lindu. La baja resolución del COX1 mtDNA para *Caridina* del Lago Lindu puede deberse a procesos evolutivos recientes. Nuestro estudio destaca la necesidad de codificar genéticamente los invertebrados de agua dulce, en particular los camarones átidos de Sulawesi, en la región biogeográfica de Wallacea.

Palabras clave: camarones átidos; crustáceos; especies crípticas; lacustres; ADN mitocondrial; Wallacea.

INTRODUCTION

Humanity is facing a triple crisis with synergistic challenges in biodiversity loss, climate change, and threats to human wellbeing (Baldwin-Cantello et al., 2023). The current rate of biodiversity loss has been described as a 6th mass extinction (Ceballos et al., 2015). Freshwater ecosystems cover less than 1 % of the earth's surface, but account for over 6 % of described species, and present complex management challenges (Dudgeon et al., 2006). Freshwater biodiversity is increasingly at risk due to direct and indirect anthropogenic threats, calling for urgent action from local to global scales to "bend the curve of freshwater biodiversity loss" (Albert et al., 2021; Reid et al., 2019; Tickner et al., 2020). Data are crucial in addressing this crisis and stemming or reversing biodiversity losses, in particular to support planning and action at appropriate spatial and temporal scales; however, for many aquatic taxa, appropriate data are often still lacking or incomplete, especially for invertebrates (Ahmed et al., 2022; Dudgeon et al., 2006; Reid et al., 2019; Strayer & Dudgeon, 2010; Tickner et al., 2020).

Sulawesi, formerly known as the Celebes, is the largest island in Wallacea, formed through the meeting of the Eurasian, Pacific and Australasian geotectonic plates, and a hotspot for terrestrial and aquatic endemism (Michaux, 2010; Stelbrink et al., 2012; Struebig et al., 2022). The unique Sulawesian freshwater fauna includes radiations of closely related species in both vertebrate and invertebrate taxa (Mokodongan & Yamahira, 2015; Schubart et al., 2008; Stelbrink et al., 2014; Vaillant et al., 2013; von Rintelen, Bouchet & Glaubrecht, 2007), including atyid shrimps of the genus *Caridina* (von Rintelen, von Rintelen, Meixner et al., 2007; von Rintelen & Cai, 2009). The genus *Caridina* comprises at least 763 species (De Grave et al., 2015) with a complex biogeography (de Mazancourt

et al., 2019). By 2021 at least 39 Sulawesian *Caridina* species were known (Dwiyanto et al., 2021), with 26 species endemic to ancient lakes (Poso and the Malili complex), including their tributary rivers (Klotz et al., 2021; von Rintelen, 2011; von Rintelen & Cai, 2009). Although data on their biology and ecology are limited, these freshwater shrimps likely play important ecological roles in their typically oligotrophic habitats (von Rintelen & Cai, 2009).

Molecular (DNA-based) tools are increasingly used for biodiversity exploration and monitoring, supporting conservation planning and action (Deiner et al., 2017; Gostel & Kress, 2022; Ruppert et al., 2019), including in freshwater ecosystems (Reid et al., 2019; Takahashi et al., 2023). The use of molecular markers (short nucleotide sequences) called DNA barcodes theoretically enable the identification of organisms based on small samples taken from the target organism (Gostel & Kress, 2022). One widely used DNA barcode is a fragment of the cytochrome oxidase I mitochondrial DNA (COI or COX1 mtDNA) (Hebert & Gregory, 2005). Environmental DNA (eDNA) methods can detect the recent presence of organisms from samples (e.g. water, soil, air) of the environment where they live and have shed their DNA (Ruppert et al., 2019). Molecular methods can, *inter alia*, help detect cryptic diversity within an accepted taxon (Keith et al., 2020; Keith & Mennesson, 2020; Siriwut et al., 2021). The results can help determine community composition (Hernawati et al., 2020), change known species distributions (Moore et al., 2019) and require revisions in their conservation status (Ndobe et al., 2023). However, the accuracy and usefulness of barcoding and metabarcoding in key biodiversity-related tasks such as the identification and monitoring of specific taxa or biotic communities, are heavily dependent on the taxonomic coverage and quality of data deposited in nucleotide sequence databases (Hestetun et al., 2020) such as the NCBI GenBank (Sayers et al., 2022) and Barcode of Life Database (Bold) (Ratnasingham & Hebert, 2007).

Research on Sulawesian *Caridina* shrimps has contributed to the study of evolution, in particular speciation and species radiation (de Mazancourt et al., 2019; von Rintelen et al., 2010; von Rintelen & Cai, 2009). Their attractive appearance and their behavior have also made them popular in the global aquarium trade (Calado et al., 2003; Herjayanto, Ndobe, et al., 2019; Kiruba-Sankar et al., 2018). In addition to fishing pressure for the ornamental trade, many are threatened habitat degradation or loss (De Grave et al., 2015; Klotz et al., 2021), and at least one *Caridina dennerli* (von Rintelen & Cai, 2009) may be extinct (von Rintelen, 2018). Despite their popularity and awareness of their vulnerability, Sulawesian atyid shrimps remain understudied. Specific gaps include data on species distributions, incomplete exploration of species complexes, and biodiversity exploration, as evidenced by the ongoing discovery and description of new species (Annawaty et al., 2022; Klotz et al., 2021; Klotz et al., 2023). Given the number of species and the complexity of identification based on external morphology alone, the use of molecular methods has the potential to support the study of atyid shrimp diversity and distribution patterns. However, many *Caridina* species (including most Sulawesian *Caridina*) lack reference sequences in the NCBI and BOLD databases.

Lindu Lake has a surface area of around 34.5 km^2, a maximum depth of around 72.6 m, and lies within an enclave in the Lore Lindu National Park in Central Sulawesi, Indonesia (Annawaty & Wowor, 2015). The lake and its watershed are within the UNESCO-recognized Lore Lindu Biosphere Reserve (Coordination and Communication Management of Lore Lindu Biosphere Reserve, 2020). Three *Caridina* species have been described from the lake (Annawaty & Wowor, 2015). Of these, *C. linduensis* (Roux, 1904) has been assessed as Critically Endangered (CR) under IUCN Red List criteria (De Grave & Wowor, 2020), while *C. dali* (Annawaty & Wowor, 2015) and *C. kaili* (Annawaty & Wowor, 2015) have not been assessed. The purpose of this study was to contribute well-documented DNA barcode (COX1

mtDNA) sequences obtained from *Caridina* spp. specimens collected from Lindu Lake.

MATERIAL AND METHODS

Material-shrimp specimens: The material examined in this study comprised 224 atyid shrimps of the genus *Caridina* collected around Lindu Lake in Central Sulawesi, Indonesia (01°16'-01°23' S, 120°1-120°11' E) on 21 August (n = 73) and 14 October 2023 (n = 117), and on 6 July 2024 (n = 34). The specimens were captured using dip nets collected from three sites (Fig. 1) and euthanized using clove oil at a concentration of 200 mg/L until fully unresponsive (Coyle et al., 2007).

Material-DNA: In each year (2023 and 2024), body tissue from a specimen of each of the three morphospecies collected was preserved in a 1.5 ml Eppendorf tube filled with 96 % absolute ethanol for genetic analysis. These six samples were sent to the Bionesia Laboratory in Denpasar, Indonesia for further analysis.

Morphological analysis: Each specimen was measured (digital calipers, precision 0.1 mm), weighed (digital scales, precision 0.01 g) and identified based on morphological characters with reference to species descriptions (Annawaty & Wowor, 2015; Roux, 1904) and other information on the Lindu shrimps (Annawaty et al., 2016).

Fig. 1. Samples of *Caridina* shrimps from Lindu Lake and their collection sites. **A.** *C. kaili* (ID: UNTAD_2024_CS01). **B.** *C. dali* (ID: UNTAD_2024_CS02). **C.** *C. linduensis* (ID: UNTAD_2024_CS03).

Morphometric traits measured in mm (digital calipers, precision 0.1 mm) were: total length (TL), head length (HL), rostrum length (RL), body length (BL) and telson length (TS). Dimensionless ratios to total length (TL) were calculated (%) and given as range and mean ± standard deviation (SD). Statistical tests were conducted in Microsoft Excel 365 at the 95 % confidence level. Mean values of each trait were compared through analysis of variance (ANOVA) followed by post-hoc Student's t-test if significant differences in mean values were detected ($p < 0.05$).

DNA Barcoding and Phylogenetic Analysis: Genomic DNA was extracted from 10 g sub-samples of muscle tissue taken from each of the preserved (96 % absolute ethanol) shrimp samples using DNeasy Tissue Kits (Qiagen) following the manufacturer's protocol. The extracted DNA samples were stored in a freezer for further analysis (-20 °C).

A fragment of the cytochrome oxidase I (COX1) mitochondrial DNA (mtDNA) gene was amplified through polymerase chain reaction (PCR) using the forward primer jgLCO (5'-TIT CIA AYC AYA ARG AYA TTG G-3') and reverse primer jgHCO (5'-TAI ACY TCI GGR TGI CCR AAR AAY CA-3') (Geller et al., 2013) on an Applied Biosystems™ 2720 Thermal Cycler machine. Each PCR reaction tube contained 25 µl comprising: 2 µl extracted DNA template; 1.25 µl of each primer (10 mM), 9 µl of ddH2O, and 12.5 µl Ready Mix. The PCR profile comprised initial denaturation at 94 °C for 3 min; 38 cycles of denaturation at 94 °C for 30 s, annealing at 50°C for 30 s, extension at 72 °C for 60 s; and final extension at 72 °C for 2 min. PCR product presence was verified through electrophoresis on 1 % agarose gel stained with Nucleic Acid Gel Stain (GelRed®). Verified PCR product was sent for Sanger sequencing at PT. Genetika Science, Jakarta, Indonesia.

The Sanger sequencing trace files (.abi files) were imported into MEGA 11 (Tamura et al., 2021). The forward and reverse sequences produced from each sample were cleaned, aligned, trimmed, and combined to produce a consensus sequence (DNA barcode). The six barcodes obtained were submitted to the NCBI GenBank nucleotide repository (submission SUB14743114) and allocated GenBank accession numbers PQ361195-PQ361200. The genetic distance between the DNA barcodes (Kimura, 1980), their protein and nucleotide composition were calculated in MEGA 11.

The on-line NCBI BLAST routine (Altschul et al., 1990) was used with the Organism option set to *Caridina* (taxid: 96236). All 65 resulting homologous sequences obtained were downloaded using the FASTA (aligned sequences) option. COX1 DNA barcodes were downloaded from the Barcode of Life Database (BOLD). The BOLD Identification routine did not find any records similar to the Lindu Lake *Caridina* barcodes. The BOLD database had 118 publicly available clusters or Barcode Index Numbers (BINS) containing 2 554 records from 20 countries. Of these, 2 452 had species names, representing 197 species. BOLD BINS typically represent low-level taxa (species or sub-species/populations within a species). BINS with Indonesia as a country of origin were downloaded as FASTA files.

Phylogenetic analysis was conducted in MEGA 11 (Tamura et al., 2021). Sequences were aligned using the ClustalW (Thompson et al., 1994) option. Phylogenetic trees representing inferred evolutionary history within the genus *Caridina* were constructed using the Neighbor-Join (NJ) algorithm (Saitou & Nei, 1987) with 1 000 bootstrap test replicates (Felsenstein, 1985). Evolutionary distances (as number of base substitutions per site) were computed using the Kimura 2-parameter method (Kimura, 1980). The first analysis included 65 GenBank accession nucleotide sequences from 16 nominal species and covered a total of 673 positions. The second analysis included 141 selected BOLD accessions from 19 BINS (SMT 1) representing 12 nominal species and one BIN labelled *Caridina* sp. and covered a total of 654 nucleotide positions. Representative trees were exported as a Newick files and edited using the interactive tree of life (iTOL) version 5 (Letunic & Bork, 2021).

RESULTS

Morphological traits: At each site, only one species was identified (Fig. 1). The main diagnostic character for distinguishing the three Lindu Lake *Caridina* species is the relative length of the rostrum (Annawaty & Wowor, 2015). This is longest in *C. linduensis*, extending well beyond the basal antennular peduncle segment; in *C. dali*, the rostrum barely overreaches this segment, while in *C. kaili* the rostrum barely reaches or falls short of the end of this segment. A further diagnostic trait for *C. kaili* is a stouter second pereiopod compared to *C. dali*, while *C. kaili* also has the largest eggs, visible in the ovigerous female specimen in Fig. 1A.

The typology of the collection sites corresponded with previous studies on the three species (Annawaty et al., 2016; Annawaty & Wowor, 2015), which describe *C. kaili* as an obligate stream species and *C. linduensis* as a truly lacustrine shrimp, while *C. dali* was found in both stream and lacustrine habitat. They also note that they never found two species at the same site. In this study, all specimens from the tributary river site (Fig. 1A) were identified as *C. kaili*, consonant with its designation as an obligate stream species. All specimens from the first site along the shore of the lake were identified as *C. dali* (Fig. 1B), and those from the second lakeshore site as *C. linduensis* (Fig. 1C).

Morphometric data on the specimens caught show high variability between individuals within each species and considerable overlap between species (Table 1). Total length (TL) of the 224 specimens collected ranged from 5.1 to 21.8 mm, with a weight range of 0.01-0.12 g.

The data in Table 1 indicates considerable morphological plasticity within each of the Lindu Lake *Caridina* species; *C. kaili*, considered an obligate stream-dwelling rather than lacustrine shrimp, had the lowest intra-species variation in each character. Each species differed significantly from the other two in at least one character. The mean value of the diagnostic character rostrum length (RL) relative to total length (RL/TL) followed the order given in the species descriptions (Annawaty & Wowor, 2015) for the length relative to the basal antennular peduncle segment. However, there was considerable overlap and intra-species variation in this ratio (Fig. 2).

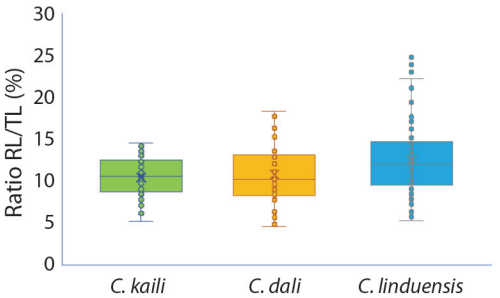

Fig. 2. Boxplot of the ratio rostrum length to total length (RL/TL, in %) for *Caridina* spp. (n = 224) from Lindu Lake.

DNA barcodes: The DNA barcode sequences obtained from Lindu Lake *Caridina* shrimps belonging to three morphotypes were 673 bp long. These sequences were submitted to the NCBI GenBank database (Submission SUB14743114, accession numbers PQ361195-PQ361200). The GC-AT ratio was 69 %

Table 1
Morphometric characters of *Caridina* spp. (n = 224) from Lindu Lake*.

N.°	Species	n	TL (mm)		HL/TL (%)	RL/TL (%)	BL/TL (%)	TS/TL (%)	W (g)
			Range	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Range
1	*C. kaili*	92	12.0-21.8	15.9 ± 1.9	26.02 ± 5.30^{a}	10.46 ± 2.22^{a}	60.22 ± 5.35^{a}	13.26 ± 3.12^{a}	0.01-0.12
2	*C. dali*	49	5.1-20.7	11.66 ± 3.72	29.49 ± 8.06^{b}	10.78 ± 3.49^{a}	55.25 ± 10.90^{b}	14.22 ± 3.52^{ab}	0.01-0.10
3	*C. linduensis*	83	8.9-20.0	13.5 ± 2.5	29.91 ± 6.28^{b}	12.50 ± 4.22^{b}	61.55 ± 11.37^{a}	14.81 ± 2.90^{b}	0.01-0.10

* For each character (column), mean values with the same superscript letter do not differ significantly from each other (95 % confidence level).

(59.138:40.862). The genetic distances between sequences ranged from zero (0.0) to nearly 0.003 (Table 2).

There were three sites with single nucleotide polymorphisms and 3 alleles within this data set (Table 3). The nucleotide and protein composition were similar for all six sequences from the three putative species (Table 4). The mutations were all silent, meaning that the protein translation was the same for all six barcodes.

LAST and phylogenetic reconstruction: The BLAST-n routine output included very few

Table 2
Genetic distance (base substitutions per site) between COX1 mtDNA barcodes of *Caridina* shrimp from Lindu Lake.

N.°	Sample ID	GenBank Accession	Sequence number				
			1	2	3	4	5
1	UNTAD_2023_CS01	PQ361195					
2	UNTAD_2024_CS01	PQ361196	0.0000000				
3	UNTAD_2023_CS02	PQ361197	0.0044726	0.0044726			
4	UNTAD_2024_CS02	PQ361199	0.0029806	0.0029806	0.0014875		
5	UNTAD_2023_CS03	PQ361198	0.0029806	0.0029806	0.0014875	0.0000000	
6	UNTAD_2024_CS03	PQ361200	0.0029806	0.0029806	0.0014875	0.0000000	0.0000000

Table 3
Single nucleotide polymorphisms (SNPs) and alleles in 673 bp COX1 mtDNA barcodes of *Caridina* shrimp from Lindu Lake.

N.°	Sample ID	Sequence Lab ID	Morphospecies	Nucleotide site number			Allele
				21	245	270	
1	UNTAD_2023_CS01	BIOSUB280.001	*Caridina kaili*	A	G	T	1
2	UNTAD_2023_CS02	BIOSUB280.002	*C. dali*	G	A	A	2
3	UNTAD_2023_CS03	BIOSUB280.003	*C. linduensis*	G	A	T	3
4	UNTAD_2024_CS01	BIOSUB237.004	*C. kaili*	A	G	T	1
5	UNTAD_2024_CS02	BIOSUB237.003	*C. dali*	G	A	T	3
6	UNTAD_2024_CS03	BIOSUB237.005	*C. linduensis*	G	A	T	3

Table 4
Nucleotide and translated protein composition of Lindu Lake *Caridina* shrimp COX1 mtDNA barcodes.

No	GenBank Accession Number	Base composition (% of 673 positions)			
		T	C	A	G
1	PQ361195-PQ361197, PQ361199, PQ361200	34.324	21.545	24.814	19.316
3	PQ361198	34.175	21.545	24.963	19.316
	Mean	34.299	21.545	24.839	19.316

Translated proteins (identical for all six sequences)					
Protein	%	Protein	%	Protein	%
Alanine	8.929	Isoleucine	6.250	Arginine	2.232
Cysteine	0.000	Lysine	0.000	Serine	7.589
Aspartic acid	3.125	Leucine	15.625	Threonine	5.357
Glutamic acid	0.893	Methionine	7.143	Valine	6.696
Phenylalanine	7.143	Asparagine	4.018	Tryptophan	2.679
Glycine	11.161	Proline	5.804	Tyrosine	1.786
Histidine	2.679	Glutamine	0.893	Total:	224 codons

homologous *Caridina* sequences. All sequences with a high cover % (90-100 %) had a low identity (% ID range 77.81-86.03 %), well below thresholds for species identity. Of these, the closest match in the GenBank database (88.63 % ID) was deposited as *Caridina boehmei* (accession OR765944) obtained from a domesticated population of this Indonesian native shrimp (Romadhona et al., 2024). Conversely, all overlapping sequences with the highest high ID scores (> 95 %) had low cover % (29 %). All phylogenetic trees produced using this data set had similar topography. The Lindu Lake barcode sequences were consistently nested in the genus *Caridina* within a clade containing other species from Sulawesi, while the next nearest neighbors were mostly species known to occur in Indonesia (Fig. 3).

The BOLD BINs with geolocation data in Indonesia comprised many more *Caridina* COX1 sequences compared to GenBank. Most of these had a high cover % with the Lindu Lake *Caridina* DNA barcode sequences. Most of the species in the Indonesian BINs have geolocated reports from Sulawesi in the GBIF database (Global Biodiversity Information Facility, 2024); however, there were no BOLD Bins representing named Sulawesi endemic species. The neighbor-join phylogenetic tree shows three major clades, one comprising the Lindu Lake sequences (Fig. 4).

DISCUSSION

The sequences obtained are likely the first reference barcode sequences for the Lindu

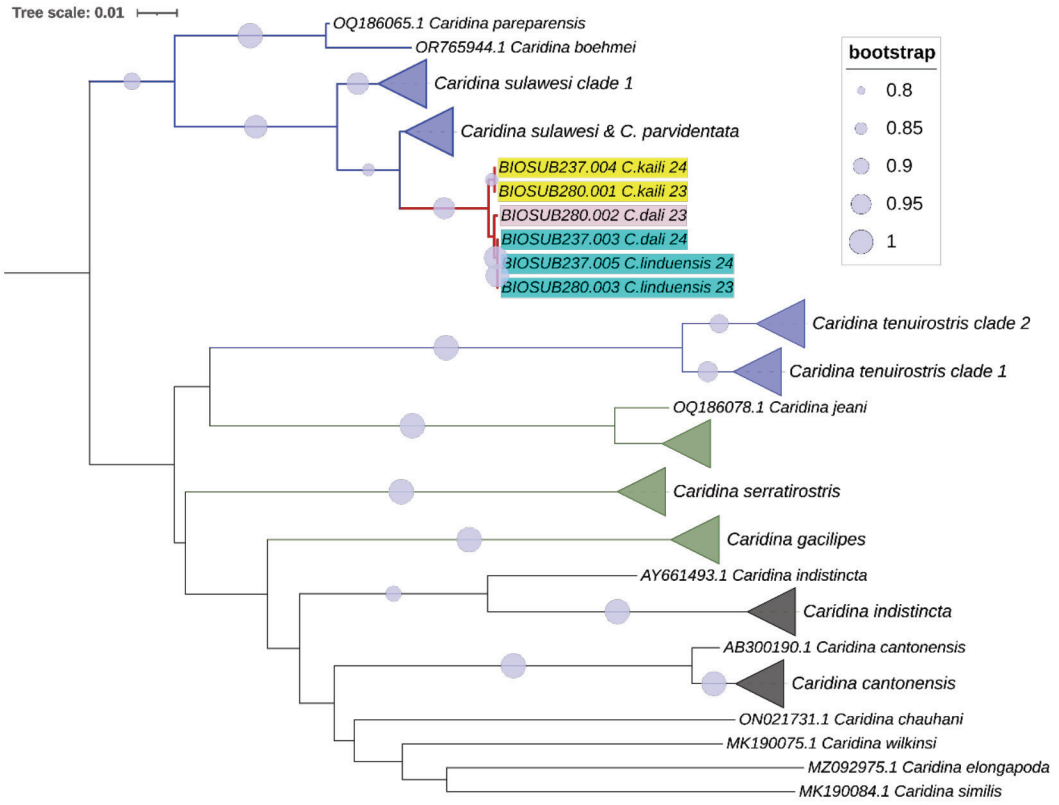

Fig. 3. Phylogenetic tree (Neighbor-Join algorithm, 1 000 bootstrap replicates) inferred from six Lindu Lake *Caridina* DNA barcodes (COI mtDNA) and 65 homologous NCBI GenBank accessions (673 nucleotide positions). Branch color indicates nominal species distribution: Red = Lindu Lake (this study); Blue = Sulawesi endemic; Green = Indonesian distribution; Black = not reported from Indonesia.

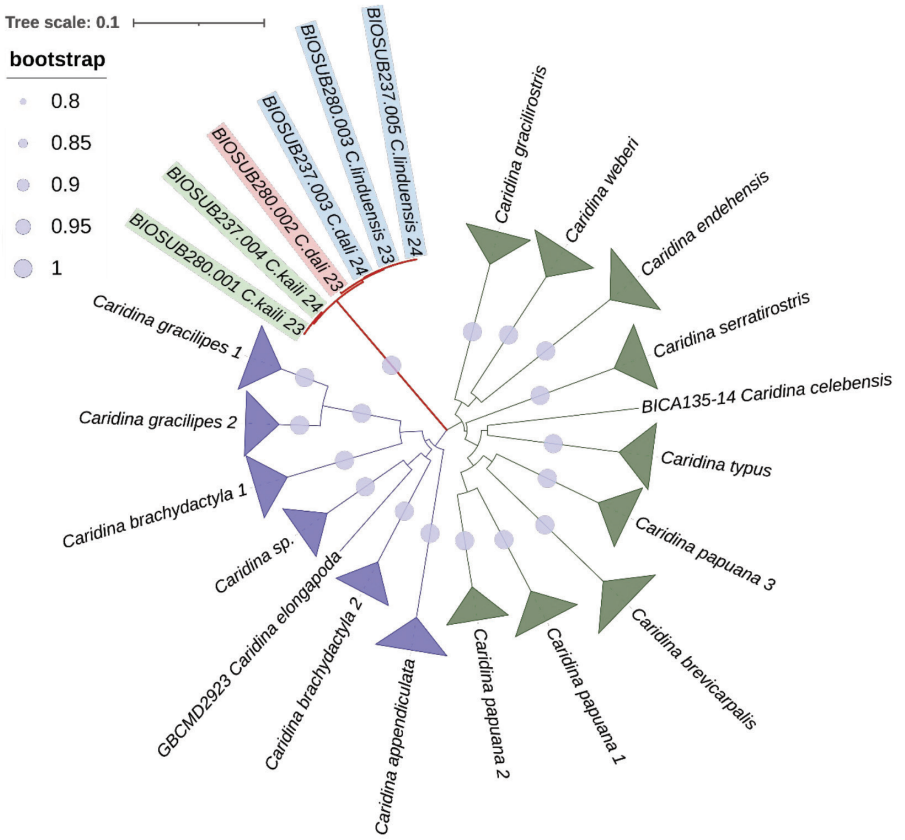

Fig. 4. Phylogenetic tree (Neighbor-Join algorithm, 1 000 bootstrap replicates) inferred from six Lindu Lake *Caridina* DNA barcodes (COX1 mtDNA) and 141 homologous COX1 sequences from 19 Indonesian BOLD BINS (654 nucleotide positions). Different highlight colors represent the terminal sub-clades of the Lindu Lake clade (red branch).

Lake *Caridina* species *Caridina linduensis*, *C. dali* and *C. kaili*, and are the first to be made publicly available. The identity with the closest homologous atyid shrimp barcodes was well below 90 %, whereas reported inter-specific variation (p-distance in %) in the COI sequences ranged from 0.8 to 4.9 with 7.7 % divergence over the entire Poso watershed clade for *Caridina* shrimps in Poso Lake (von Rintelen, von Rintelen, & Glaubrecht, 2007) and from 0.5 to 9.7 with 11.5 % divergence over the entire Malili lake complex clade (von Rintelen, 2011).

Fig. 2 and Fig. 3 show multiple well-separated clades for several nominal species, in particular *C. brachydactyla*, *C. cantonensis*, *C. gracilipes*, *C. indistincta*, *C. papuana*, *C. sulawesi* and *C. tenuirostris*. This indicates a need for further research integrating classic morphomeristic and molecular approaches to clarify the taxonomy and distribution of species within the genus *Caridina*. Conversely, in both trees the COX1 sequences for the three putative species present in Lindu Lake are grouped in a clade with shorter internal (sub-clade) branches than most single species clades. Furthermore, one sub-clade contains identical sequences from two morphospecies (*C. dali* and *C. linduensis*).

We conclude that the COX1 mtDNA fragment used as a DNA barcode in this study could not be used to reliably distinguish between the three putative *Caridina* species present in Lindu Lake. However, this marker can distinguish

Lindu Lake *Caridina* from all other congeners for which homologous DNA barcodes are currently available in the BOLD and GenBank sequence repositories, even with overlaps as low as 29 % in terms of the gene region covered.

The usefulness of the COI/COX1 mtDNA barcode (and mitochondrial DNA more generally) for successful species delineation is limited in cases where recent speciation events have occurred (Raupach & Radulovici, 2015). The low interspecific genetic distances between the three putative species in this study may be due to recent evolutionary radiation. However, incongruence between taxonomic units based on mtDNA genotype and phenotype can result from high phenotypic plasticity (Fritz et al., 2007; González-Castellano et al., 2020), and patterns of COX1 variability can vary considerably between decapod taxa (Matzen-da Silva et al., 2011). These results call for further research with a range of molecular markers to determine whether the Lindu Lake morphotypes do indeed correspond to species at a genetic level and, if so, identify a suitable molecular marker (alternative "barcode") capable of reliably distinguishing them from one another. Ambiguity in DNA barcodes for crustacean identification are also reported for the genus *Macrobrachium* (Rosyida et al., 2023). These cases highlight the need to combine molecular and classical taxonomic methods rather than relying fully on one or the other, in particular to delineate taxa and identify individuals.

With respect to other Sulawesi endemic *Caridina*, homologous sequences were only accessible for four out of around 40 species. Therefore, no conclusions can be drawn regarding the power of the COX1 mtDNA molecular marker used in distinguishing the majority of Sulawesian *Caridina* species from the Lindu Lake *Caridina* or from each other. This underlines the ongoing need for DNA barcoding of freshwater and diadromous invertebrates, as well as fish (Hubert et al., 2015).

Threats to native Sulawesi freshwater fish and invertebrates, especially the many endemic species, include habitat degradation or loss; unsustainable exploitation, most often for human consumption or the ornamental aquarium trade; and the introduction of alien species (Annawaty et al., 2016; Herder et al., 2012; Herder et al., 2022; Klotz & von Rintelen, 2013; Rahmawati et al., in press; Serdiati et al., 2021; von Rintelen et al., 2012; von Rintelen, von Rintelen, Meixner et al., 2007; von Rintelen & Cai, 2009). *Caridina* shrimps are considered particularly sensitive to pollution (von Rintelen & Cai, 2009), vulnerable to predation by alien invasive species (Herder et al., 2012; Herder et al., 2022), and popular in the poorly documented aquarium hobby (Calado et al., 2003; Udin, 2013; von Rintelen & Cai, 2009). While posing a threat to wild populations, this popularity has also raised public awareness of their plight (von Rintelen et al., 2012). Although management measures are clearly vital to conserve native/endemic species in Sulawesi lakes and their watersheds, no *in situ* conservation measures are reported in studies on *Caridina* shrimps in Lindu Lake and its tributaries (Annawaty et al., 2016; De Grave & Wowor, 2020).

Lindu Lake lies within the Lore Lindu UNESCO Biosphere Reserve (LLBR) established in 1978; the lake and riparian villages form an enclave in the Lore Lindu National Park, gazette in 1999 (Nontji, 2016). Tourism and fisheries have been promoted as livelihood options for Lindu Lake villagers (Muhamad et al., 2023), and alien fish have been introduced for this purpose (De Grave & Wowor, 2020; Herjayanto, Gani, et al., 2019). Conservation management of the Lindu Lake watershed has been complicated by complex socio-economic and cultural dynamics, with considerable in-migration and potential conflicts between ethnic groups, illegal logging, and other land-use issues (Anugrahsari et al., 2020; Mappatoba et al., 2017; Rahman et al., 2021). Initiatives to conserve the biodiversity of Lindu Lake and its watershed, in particular the *Caridina* shrimps in this study, include a project funded in 2022 by the Mohamed bin Zayed Species Conservation Fund for the conservation of *Caridina linduensis* (Mohamed Bin Zayed-Species Conservation Fund [MBZ-SCF], 2022). The overall project goal was to develop a multi-pronged

conservation strategy, with proposed activities including studies on the reproductive biology and ecology of *C. linduensis* as well as developing and deploying artificial spawning grounds in the lake and educational/awareness-building programs for local communities, and the release of larvae. However, the source of these larvae was not specified in (MBZ-SCF, 2022), and it is not clear how far the targets were accomplished. The project report does not mention the complexities raised by the presence of more than one *Caridina* taxon, possibly as only *C. linduensis* (De Grave & Wowor, 2020) has been assessed under IUCN Red List criteria and, as an endangered species, is therefore eligible for funding from the Mohamed Bin Zayed-Species Conservation Fund . The only information found on output or outcome from this project is a blog page (Dwiyanto, 2022) which makes general recommendations similar to those proposed for endemic species conservation in other Sulawesi lakes such as Poso Lake (Serdiati et al., 2021). In addition to identifying and protecting or restoring critical habitat, the threat from alien species could be reduced through promoting the use of low-value or unused (invasive) alien species for human consumption or as fish/animal feed (Rahmawati et al., in press). There is still a need to develop awareness regarding endemic species, seek and leverage local traditions that could support conservation, and involve communities and other stakeholders in developing workable conservation strategies, while monitoring of conservation targets (e.g., *Caridina* populations and habitat condition) is crucial to evaluate effectiveness.

In addition to *in-situ* measures, *ex-situ* conservation can support the conservation of endangered aquatic species (Calado et al., 2003; Stoeckle et al., 2022), including those found in Sulawesi lakes (Herjayanto et al., 2023; Herjayanto, Gani, et al., 2019; Wicaksono et al., 2022). *Ex-situ* husbandry and breeding of decapods has been developed for supplying the ornamental trade (Calado et al., 2003), and could help alleviate one of the greatest threats to Sulawesi aquatic organisms, including *Caridina* shrimps. Captive breeding could also enable re-stocking of native/endemic species populations (Theissinger et al., 2021). Such interventions should follow national (Sadili et al., 2015) and international (e.g. IUCN) guidelines, in particular with respect to biosecurity and genetic diversity considerations (Bouwmeester et al., 2021; Theissinger et al., 2021; Velle et al., 2025). In the case of cryptic/morphologically similar species, DNA barcoding can assist in ensuring prospective broodstock belong to the same species, and molecular markers can also be used to evaluate intra-species genetic diversity in captive populations and wild populations enhanced through restocking (Fox et al., 2018; Roques et al., 2018; Stoeckle et al., 2022), as well as supporting traceability (Ng et al., 2016). With respect to the Lindu *Caridina* species, *ex-situ* conservation efforts include research on the husbandry as a first step towards captive breeding of *C. kaili* (Herjayanto, Ndobe, et al., 2019).

In conclusion, this study provides the first reference DNA barcodes (COI/COX1 mtDNA) for atyid shrimps from Lindu Lake in Central Sulawesi, Indonesia with lengths of 673 bp. The similarity between the sequences, obtained from specimens identified as *Caridina linduensis*, *C. dali*, and *C. kaili* based on external morphomeristic traits, and the mismatch between morphotypes and genotypes (alleles based on three single nucleotide polymorphisms), may indicate poor resolution of this commonly used molecular marker for the genus *Caridina*, and call for further research with a range of molecular markers. More broadly, efforts are needed to fill the gap in standard COI/COX1 DNA barcodes for Sulawesi endemic aquatic invertebrates, in particular atyid shrimps of the genus *Caridina*. Such research can contribute to urgently needed conservation measures, in particular the monitoring of wild populations as well as *ex-situ* initiatives and traceability in the international aquarium trade.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we

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See supplementary material
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REFERENCES

Ahmed, S. F., Kumar, P. S., Kabir, M., Zuhara, F. T., Mehjabin, A., Tasannum, N., Hoang, A. T., Kabir, Z., & Mofijur, M. (2022). Threats, challenges and sustainable conservation strategies for freshwater biodiversity. *Environmental Research*, *214*(P1), 113808. https://doi.org/10.1016/j.envres.2022.113808

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller, K. O., & Ripple, W. J. (2021). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*, *50*(1), 85–94. https://doi.org/10.1007/s13280-020-01318-8

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Annawaty, A., & Wowor, D. (2015). The atyid shrimps from Lake Lindu, Central Sulawesi, Indonesia with description of two new species (Crustacea: Decapoda: Caridea). *Zootaxa*, *3957*(5), 501–519. https://doi.org/10.11646/zootaxa.3957.5.1

Annawaty, A., Lapasang, N. H. E., Rahayu, P., Hairul, H., Tadeko, F. R. I., & Dwiyanto, D. (2022). Checklist of the freshwater shrimps (Crustacea, Decapoda, Caridea) from the Banggai Archipelago, Central Sulawesi, Indonesia. *Check List*, *18*(2), 341–355. https://doi.org/10.15560/18.2.341

Annawaty, A., Wowor, D., Farajallah, A., Setiadi, D., & Suryobroto, B. (2016). Habitat preferences and distribution of the freshwater shrimps of the genus *Caridina* (Crustacea: Decapoda: Atyidae) in Lake Lindu, Sulawesi, Indonesia. *HAYATI Journal of Biosciences*, *23*(2), 45–50. https://doi.org/10.1016/j.hjb.2016.04.001

Anugrahsari, I., Sardjono, M. A., Fitriyah, N., & Golar, G. (2020). Social contracts: Pillars of community conservation partnerships in Lore Lindu National Park, Indonesia. *Forest and Society*, *4*(1), 115. https://doi.org/10.24259/fs.v4i1.8682

Baldwin-Cantello, W., Tickner, D., Wright, M., Clark, M., Cornelius, S., Ellis, K., Francis, A., Ghazoul, J., Gordon, J. E., Matthews, N., Milner-Gulland, E. J., Smith, P., Walmsley, S., & Young, L. (2023). The triple challenge: synergies, trade-offs and integrated responses for climate, biodiversity, and human wellbeing goals. *Climate Policy*, *23*(6), 782–799. https://doi.org/10.1080/14693062.2023.2175637

Bouwmeester, M. M., Goedknegt, M. A., Poulin, R., & Thieltges, D. W. (2021). Collateral diseases: Aquaculture impacts on wildlife infections. *Journal of Applied Ecology*, *58*(3), 453–464. https://doi.org/10.1111/1365-2664.13775

Calado, R., Lin, J., Rhyne, A. L., Araújo, R., & Narciso, L. (2003). Marine ornamental decapods-popular, pricey, and poorly studied. *Journal of Crustacean Biology*, *23*(4), 963–973. https://doi.org/10.1651/C-2409

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., & Palmer, T. M. (2015). Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances*, *1*(5), e1400253. https://doi.org/10.1126/sciadv.1400253

Coordination and Communication Management of Lore Lindu Biosphere Reserve. (2020). *History: The Lore Lindu Biosphere Reserves*. Lore Lindu Biosphere Reserve. https://www.lorelindu-br.org/about

Coyle, S. D., Dasgupta, S., Tidwell, J. H., Beavers, T., Bright, L. A., & Yasharian, D. K. (2007). Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society*, *36*(3), 282–290. https://doi.org/10.1111/j.1749-7345.2005.tb00332.x

De Grave, S., & Wowor, D. (2020). Caridina linduensis *(amended version of 2013 assessment) [e.T198228A176123703]*. The IUCN Red List of Threatened Species. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T198228A176123703.en

De Grave, S., Smith, K. G., Adeler, N. A., Allen, D. J., Alvarez, F., Anker, A., Cai, Y., Carrizo, S. F., Klotz, W., Mantelatto, F. L., Page, T. J., Shy, J. Y., Villalobos, J. L., & Wowor, D. (2015). Dead shrimp blues: A global assessment of extinction risk in freshwater shrimps (Crustacea: Decapoda: Caridea). *PLoS ONE*, *10*(3), e0120198. https://doi.org/10.1371/journal.pone.0120198

de Mazancourt, V., Klotz, W., Marquet, G., Mos, B., Rogers, D. C., & Keith, P. (2019). The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution,

biogeography, and habitat. *Molecular Phylogenetics and Evolution*, *131*, 164–180. https://doi.org/10.1016/j.ympev.2018.11.002

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. https://doi.org/10.1111/mec.14350

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, *81*(2), 163–182. https://doi.org/10.1017/S1464793105006950

Dwiyanto, D. (2022). *Fauna Endemik Danau Lindu: Rekam Jejak Kehidupan Danau Lindu dari Masa ke Masa [Lindu Lake endemic species: a record of life in Lindu Lake over time*]. Zoological Community of Celebes. https://www.zccindonesia.org/post/fauna-endemik-danau-lindu-rekam-jejak-kehidupan-danau-lindu-dari-masa-ke-masa

Dwiyanto, D., Annawaty, A., Farajallah, A., & Wowor, D. (2021). A preliminary survey of the freshwater shrimp genus *Caridina* from Eastern Sulawesi, Indonesia. *Tropical Natural History*, *21*(2), 337–342. https://doi.org/10.58837/tnh.21.2.251715

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

Fox, G., Darolti, I., Hibbitt, J.-D, Preziosi, R. F., Fitzpatrick, J. L., & Rowntree, J. K. (2018). Bespoke markers for ex-situ conservation: application, analysis and challenges in the assessment of a population of endangered undulate rays. *Journal of Zoo and Aquarium Research*, *6*(2), 50–56.

Fritz, U., Hundsdörfer, A., Široký, P., Auer, M., Kami, H., Lehmann, J., Mazanaeva, L., Türkozan, O., & Wink, M. (2007). Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western Palaearctic tortoises (*Testudo graeca* complex; Testudines, Testudinidae). *Amphibia-Reptilia*, *28*(1), 97–121. https://doi.org/10.1163/156853807779799135

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, *13*(5), 851–861. https://doi.org/10.1111/1755-0998.12138

Global Biodiversity Information Facility. (2024). *GBIF Home page*. https://www.gbif.org

González-Castellano, I., Pons, J., González-Ortegón, E., & Martínez-Lage, A. (2020). Mitogenome phylogenetics in the genus *Palaemon* (Crustacea: Decapoda) sheds light on species crypticism in the rockpool shrimp *P. elegans*. *PLoS ONE*, 15(8), e0237037. https://doi.org/10.1371/journal.pone.0237037

Gostel, M. R., & Kress, W. J. (2022). The expanding role of DNA barcodes: Indispensable tools for ecology, evolution, and conservation. *Diversity*, *14*(3), 213. https://doi.org/10.3390/d14030213

Hebert, P. D. N., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology*, *54*(5), 852–859. https://doi.org/10.1080/10635150500354886

Herder, F., Möhring, J., Flury, J. M., Utama, I. V., Wantania, L., Wowor, D., Boneka, F. B., Stelbrink, B., Hilgers, L., Schwarzer, J., & Pfaender, J. (2022). More non-native fish species than natives, and an invasion of Malawi cichlids, in ancient Lake Poso, Sulawesi, Indonesia. *Aquatic Invasions*, *17*(1), 72–91. https://www.reabic.net/aquaticinvasions/2022/AI_2022_Herder_etal.pdf

Herder, F., Schliewen, U. K., Geiger, M. F., Hadiaty, R. K., Gray, S. M., McKinnon, J. S., Walter, R. P., & Pfaender, J. (2012). Alien invasion in Wallace's Dreamponds: records of the hybridogenic "flowerhorn" cichlid in Lake Matano, with an annotated checklist of fish species introduced to the Malili Lakes system in Sulawesi. *Aquatic Invasions*, *7*(4), 521–535. https://doi.org/10.3391/ai.2012.7.4.009

Herjayanto, M., Carman, O., Tri Soelistyowati, D., Alimuddin, Wicaksono, A. W., & Arfah, H. (2023). The ontogenic study of early life stages of culture-bred *Nomorhamphus* sp. (Zenarchopteridae) from Lindu, Central Sulawesi. *Jurnal Akuakultur Indonesia*, *22*(2), 179–186. https://doi.org/10.19027/jai.22.2.179-186

Herjayanto, M., Gani, A., Adel, Y. S., & Suhendra, N. (2019). Frehswater fish of lakes and it's inlet rivers in Sulawesi Tengah Province, Indonesia. *Journal of Aquatropica Asia*, *4*(1), 1–9. https://doi.org/10.33019/aquatropica.v4i1.1679

Herjayanto, M., Ndobe, S., Abdillah, A., Muamar, M., Melaty, P., Gani, A., Fadli, M., Suhendra, N., Waris, A., & Musdalifa, M. (2019). Preliminary study of *Caridina kaili* domestication, endemic shrimp to Lake Lindu, Central Sulawesi, Indonesia. *Jurnal Perikanan Dan Kelautan*, *9*(2), 165–173. https://doi.org/10.33512/jpk.v9i2.8629

Hernawati, R., Nurhaman, U., Busson, F., Suryobroto, B., Hanner, R., Keith, P., Wowor, D., & Hubert, N. (2020). Exploring community assembly among Javanese and Balinese freshwater shrimps (Atyidae, Palaemonidae) through DNA barcodes. *Hydrobiologia*, *847*(2), 647–663. https://doi.org/10.1007/s10750-019-04127-7

Hestetun, J. T., Bye-Ingebrigtsen, E., Nilsson, R. H., Glover, A. G., Johansen, P.-O., & Dahlgren, T. G. (2020).

Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. *Marine Biodiversity*, *50*(5), 70. https://doi.org/10.1007/s12526-020-01093-5

Hubert, N., Kadarusman, Wibowo, A., Busson, F., Caruso, D., Sulandari, S., Nafiqoh, N., Pouyaud, L., Rüber, L., Avarre, J. C., Herder, F., Hanner, R., Keith, P., & Hadiaty, R. K. (2015). DNA barcoding Indonesian freshwater fishes: challenges and prospects. *DNA Barcodes*, *3*(1), 144–169. https://dx.doi.org/10.1515/dna-2015-0018

Keith, P., & Mennesson, M. I. (2020). Review of *Giuris* (Teleostei: Eleotridae) from Indo-Pacific islands, with description of three new species. *Cybium*, *44*(4), 331–349. https://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*, *44*(4), 317–329. https://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*(2), 111–120. https://doi.org/10.1007/BF01731581

Kiruba-Sankar, R., Raj, J. P., Saravanan, K., Kumar, K. L., Angel, J. R. J., Velmurugan, A., & Roy, S. D. (2018). Invasive species in freshwater ecosystems-threats to ecosystem services. In C. Sivaperuman, A. Velmurugan, A. K. Singh, & I. Jaisankar (Eds.), *Biodiversity and climate change adaptation in tropical islands* (pp. 257–296). Academic Press.

Klotz, W., & von Rintelen, K. (2013). Three new species of *Caridina* (Decapoda: Atyidae) from Central Sulawesi and Buton Island, Indonesia, and a checklist of the islands' endemic species. *Zootaxa*, *3664*(4), 554. https://doi.org/10.11646/zootaxa.3664.4.8

Klotz, W., von Rintelen, T., Annawaty, A., Wowor, D., & von Rintelen, K. (2023). *Caridina clandestina*, new species, an unusual new freshwater shrimp (Crustacea: Decapoda: Atyidae) from the remote high elevation Napu Valley of Sulawesi, Indonesia. *Raffles Bulletin of Zoology*, *71*, 12–25. https://doi.org/10.26107/RBZ-2023-0002

Klotz, W., von Rintelen, T., Wowor, D., Lukhaup, C., & von Rintelen, K. (2021). Lake Poso's shrimp fauna revisited: the description of five new species of the genus *Caridina* (Crustacea, Decapoda, Atyidae) more than doubles the number of endemic lacustrine species. *ZooKeys*, *1009*, 81–122. https://doi.org/10.3897/zookeys.1009.54303

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

Mappatoba, M., Kassa, S., Palebori, H., Anwar, C., Tanamal, C. E., & Ilyas, S. (2017). Exploring nature conservation problems at Lore Lindu National Park Central Sulawesi, Indonesia. *International Journal of Agriculture Innovations and Research*, *6*(2), 279–284.

Matzen-da Silva, J., Creer, S., dos Santos, A., Costa, A. C., Cunha, M. R., Costa, F. O., & Carvalho, G. R. (2011). Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE*, *6*(5), e19449. https://doi.org/10.1371/journal.pone.0019449

Michaux, B. (2010). Biogeology of Wallacea: geotectonic models, areas of endemism, and natural biogeographical units. *Biological Journal of the Linnean Society*, *101*(1), 193–212. https://doi.org/10.1111/j.1095-8312.2010.01473.x

Mohamed Bin Zayed-Species Conservation Fund. (2022). *Mohamed bin Zayed Species project number 220528372 Conservation of the critically endangered and endemic shrimp Caridina linduensis in Lake Lindu, Central Sulawesi, Indonesia*. Mohamed Bin Zayed Species Conservation Fund. https://www.speciesconservation.org/case-studies-projects/lindu-shrimp/28372

Mokodongan, D. F., & Yamahira, K. (2015). Origin and intra-island diversification of Sulawesi endemic Adrianichthyidae. *Molecular Phylogenetics and Evolution*, *93*, 150–160. https://doi.org/10.1016/j.ympev.2015.07.024

Moore, A. M., Tassakka, A. C. M., Ambo-Rappe, R., Yasir, I., Smith, D. J., & Jompa, J. (2019). Unexpected discovery of *Diadema clarki* in the Coral Triangle. *Marine Biodiversity*, *49*(5), 2381–2399. https://doi.org/10.1007/s12526-019-00978-4

Muhamad, H., Saleh, M., & Mascunra, A. (2023). Patron-client and Lindu lake tourism development. *Jurnal Penelitian Pendidikan Indonesia*, *9*(3), 1585. https://doi.org/10.29210/020232202

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*(2), 295. https://doi.org/10.12688/f1000research.108970.2

Ng, T. H., Tan, S. K., Wong, W. H., Meier, R., Chan, S. Y., Tan, H. H., & Yeo, D. C. J. (2016). Molluscs for sale: Assessment of freshwater gastropods and bivalves in the ornamental pet trade. *PLoS ONE*, *11*(8), e0161130. https://doi.org/10.1371/journal.pone.0161130

Nontji, A. (2016). *Danau-danau alami Nusantara*. Masyarakat Limnologi Indonesia.

Rahman, A., Cyio, M. B., Darman, S., & Hasanah, U. (2021). Outlooks for developing land degradation mitigation policy plans in the Lindu watershed hope for the present and the future. *International Journal of Research and Innovation in Social Science*, *5*(5), 501–509. https://doi.org/10.47772/IJRISS.2021.5529

Rahmawati, Budimawan, Arief, A. A., Moore, A. M., & Jompa, J. (in press). Fisheries in the Malili ancient lake complex: from endemic to introduced species. *Cybium*.

Ratnasingham, S., & Hebert, P. D. N. (2007). Bold: The barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Raupach, M. J., & Radulovici, A. E. (2015). Looking back on a decade of barcoding crustaceans. *ZooKeys*, *539*, 53–81. https://doi.org/10.3897/zookeys.539.6530

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Romadhona, E. I., Megawati, N., Widantara, H., Sutanti, S., Rahman Ardiansyah, A., Mariya Dewi, K., Fitri Larassagita, A., Firman, A., Risky, M., Rahmat, R., & Ayub, A. (2024). Characterization of CO1 and 18S rDNA genes from indonesian native ornamental shrimp *Caridina boehmei*. *Jurnal Akuakultur Indonesia*, *23*(2), 147–156. https://doi.org/10.19027/jai.23.2.147-156

Roques, S., Berrebi, P., Rochard, E., & Acolas, M. L. (2018). Genetic monitoring for the successful re-stocking of a critically endangered diadromous fish with low diversity. *Biological Conservation*, *221*, 91–102. https://doi.org/10.1016/j.biocon.2018.02.032

Rosyida, E., Moore, A. M., Serdiati, N., Ndobe, S., & Siddik, J. (2023). DNA barcoding of an amphidromous palaemonid shrimp recruiting to the Palu River, Central Sulawesi, Indonesia. In N. Edy, A. Alam, S. Rijal, R. Pratama, A. Wibowo, & A. A. Kasim (Eds.), *Proceedings of the 2nd International Interdisciplinary Conference on Environmental Sciences and Sustainable Developments 2022 Environment and Sustainable Development (IICESSD-ESD 2022)* (vol. 36, pp. 153–158). Atlantis Press.

Roux, J. (1904). Décapodes d'eau douce de Cèlébes (Genres *Caridina* et *Potamon*). *Revue Suisse de Zoologie*, *12*, 539–571. https://doi.org/10.5962/bhl.part.10150

Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, *17*, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sadili, D., Haryono, P., Kamal, M. M., Sarmintohadi, D., & Ramli, I. (2015). *Pedoman Umum Restoking Jenis Ikan Terancam Punah*. Direktorat Jenderal Kelautan Pesisir dan Pulau-Pulau Kecil.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sayers, E. W., Cavanaugh, M., Clark, K., Pruitt, K. D., Schoch, C. L., Sherry, S. T., & Karsch-Mizrachi, I. (2022). GenBank. *Nucleic Acids Research*, *50*(D1), D161–D164. https://doi.org/10.1093/nar/gkab1135

Schubart, C. D., Santl, T., & Koller, P. (2008). Mitochondrial patterns of intra- and interspecific differentiation among endemic freshwater crabs of ancient lakes in Sulawesi. *Contributions to Zoology*, *77*(2), 83–90. http://dx.doi.org/10.1163/18759866-07702004

Serdiati, N., Arfiati, D., Sri Widodo, M., Lelono, T. D., Ndobe, S., Mansyur, K., & Moore, A. M. (2021). Perspectives on sustainable management of the Poso Lake (Indonesia) endemic ricefish, *Oryzias nigrimas* (Actinopterygii: Adrianichthyidae). *Revista de Biología Tropical*, *69*(1), 139–152. https://doi.org/10.15517/rbt.v69i1.42404

Siriwut, W., Jeratthitikul, E., Panha, S., Chanabun, R., Ngor, P. B., & Sutcharit, C. (2021). Evidence of cryptic diversity in freshwater *Macrobrachium* prawns from Indochinese riverine systems revealed by DNA barcode, species delimitation and phylogenetic approaches. *PLoS ONE*, *16*(6), e0252546. https://doi.org/10.1371/journal.pone.0252546

Stelbrink, B., Albrecht, C., Hall, R., & von Rintelen, T. (2012). The biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on Wallace's "anomalous island"? *Evolution*, *66*(7), 2252–2271. https://doi.org/10.1111/j.1558-5646.2012.01588.x

Stelbrink, B., Stöger, I., Hadiaty, R. K., Schliewen, U. K., & Herder, F. (2014). Age estimates for an adaptive lake fish radiation, its mitochondrial introgression, and an unexpected sister group: Sailfin silversides of the Malili Lakes system in Sulawesi. *BMC Evolutionary Biology*, *14*(1), 94. https://doi.org/10.1186/1471-2148-14-94

Stoeckle, B. C., Mueller, M., Nagel, C., Kuehn, R., & Geist, J. (2022). A conservation genetics perspective on supportive breeding: A case study of the common nase (*Chondrostoma nasus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *32*(10), 1596–1605. https://doi.org/10.1002/aqc.3863

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society*, *29*(1), 344–358. https://doi.org/10.1899/08-171.1

Struebig, M., Aninta, S. G., Beger, M., Bani, A., Barus, H., Brace, S., Davies, Z. G., De Brauwer, M., Diele, K., Djakiman, C., Djamaluddin, R., Drinkwater, R.,

Dumbrell, A., Evans, D., Fusi, M., Herrera-Alsina, L. L., Iskandar, D. T., Jompa, J., Juliandi, B., … Supriatna, J. (2022). Safeguarding imperiled biodiversity and evolutionary processes in the Wallacea center of endemism. *BioScience*, *72*(11), 1118–1130. https://doi.org/10.1093/biosci/biac085

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., van der Heyde, M., Heydenrych, M. J., Juszkiewicz, D. J., Nevill, P., Dawkins, K. L., Bessey, C., Fernandes, K., Miller, H., Power, M., Mousavi-Derazmahalleh, M., Newton, J. P., White, N. E., Richards, Z. T., & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. *Science of The Total Environment*, *873*, 162322. https://doi.org/10.1016/j.scitotenv.2023.162322

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

Theissinger, K., Edsman, L., Maguire, I., Diéguez-Uribeondo, J., & Jussila, J. (2021). Editorial: conservation of European freshwater crayfish. *Frontiers in Ecology and Evolution*, *9*, 1–4. https://doi.org/10.3389/fevo.2021.804629

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC308517/

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., McClain, M. E., Muruven, D., Olden, J. D., … Young, L. (2020). Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. *BioScience*, *70*(4), 330–342. https://doi.org/10.1093/biosci/biaa002

Udin, S. (2013, September 14). *Eight new shrimps described from Sulawesi*. Sulawesi Shrimp Udang Hias Sulawesi. http://sulawesishrimp.blogspot.com/2013/09/eight-new-shrimps-described-from.html

Vaillant, J. J., Bock, D. G., Haffner, G. D., & Cristescu, M. E. (2013). Speciation patterns and processes in the zooplankton of the ancient lakes of Sulawesi Island, Indonesia. *Ecology and Evolution*, *3*(9), 3083–3094. https://doi.org/10.1002/ece3.697

Velle, G., Thorstad, E. B., Garseth, Å. H., Gjøen, T., Gulla, S., Lo, H., Mo, T. A., & Malmstrøm, M. (2025). Balancing the restoration of a native fish and the risks of hitch-hiking invasive species. *Management of Biological Invasions*, *16*(1), 247–276. https://doi.org/10.3391/mbi.2025.16.1.15

von Rintelen, K. (2011). Intraspecific geographic differentiation and patterns of endemism in freshwater shrimp species flocks in ancient lakes of Sulawesi. In C. Held, S. Koenemann, & C. D. Schubart (Eds.), *Phylogeography and Population Genetics in Crustacea* (1st ed., pp. 257–271). CRC Press.

von Rintelen, K. (2018). Caridina dennerli *[e.T198055A109683594]*. The IUCN Red List of Threatened Species 2018. https://doi.org/10.2305/IUCN.UK.2018-2.RLTS.T198055A109683594.en

von Rintelen, K., & Cai, Y. (2009). Radiation of endemic species flocks in ancient lakes: Systematic revision of the freshwater shrimp *Caridina* H. Milne Edwards, 1837 (Crustacea: Decapoda: Atyidae) from the ancient lakes of Sulawesi, Indonesia, with the description of eight new species. *The Raffles Bulletin of Zoology*, *57*(2), 343–452.

von Rintelen, K., Glaubrecht, M., Schubart, C. D., Wessel, A., & von Rintelen, T. (2010). Adaptive radiation and ecological diversification of Sulawesi's ancient lake shrimps. *Evolution*, *64*(11), 3287–3299. https://doi.org/10.1111/j.1558-5646.2010.01043.x

von Rintelen, K., von Rintelen, T., & Glaubrecht, M. (2007). Molecular phylogeny and diversification of freshwater shrimps (Decapoda, Atyidae, *Caridina*) from ancient Lake Poso (Sulawesi, Indonesia)-The importance of being colourful. *Molecular Phylogenetics and Evolution*, *45*(3), 1033–1041. https://doi.org/10.1016/j.ympev.2007.07.002

von Rintelen, K., von, Rintelen, T., Meixner, M., Lüter, C., Cai, Y., & Glaubrecht, M. (2007). Freshwater shrimp-sponge association from an ancient lake. *Biology Letters*, *3*(3), 262–264. https://doi.org/10.1098/rsbl.2006.0613

von Rintelen, T., Bouchet, P., & Glaubrecht, M. (2007). Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of *Tylomelania* (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi, Indonesia. *Hydrobiologia*, *592*(1), 11–94. https://doi.org/10.1007/s10750-007-0765-8

von Rintelen, T., von Rintelen, K., Glaubrecht, M., Schubart, C. D., & Herder, F. (2012). Aquatic biodiversity hotspots in Wallacea: the species flocks in the ancient lakes of Sulawesi, Indonesia. In D. J. Gower, K. Johnson, J. Richardson, B. Rosen, L. Rüber, & S. Williams (Eds.), *Biotic evolution and environmental change in Southeast Asia* (pp. 290–315). Cambridge University Press.

Wicaksono, A. W., Carman, O., Soelistyowati, D. T., Arfah, H., Alimuddin, Herjayanto, M., Solahudin, E. A., & Ahmadi, K. (2022). Biometric characterization and morphogenesis of Anasa ornamental fish (*Nomorhamphus* sp.) endemic to Lake Lindu, Central Sulawesi, Indonesia. *AACL Bioflux*, *15*(5), 2479–2487. http://www.bioflux.com.ro/home/volume-15-5-2022/