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**MORPHOLOGICAL CHARACTERISTICS AND PHYLOGENETIC ANALYSIS OF *MACROBRACHIUM* SP. IN PATTUNUANG RIVER, MAROS REGENCY, SOUTH SULAWESI**Afifah Nur Fadhilah<sup>1</sup>, Nadiarti Nurdin Kadir<sup>2,5,6\*</sup>, Andi Aliah Hidayani<sup>3</sup>, Abigail Mary Moore<sup>4,5</sup><sup>1</sup>Master Program in Fisheries Science, Hasanuddin University. Jl. Perintis Kemerdekaan km 10, Makassar 90245, Indonesia<sup>2</sup>Aquatic Resource Management Study Program, Hasanuddin University. Jl. Perintis Kemerdekaan km 10, Makassar 90245, Indonesia<sup>3</sup>Aquaculture Study Program, Hasanuddin University. Jl. Perintis Kemerdekaan km 10, Makassar 90245, Indonesia<sup>4</sup>Graduate School, Hasanuddin University. Jl. Perintis Kemerdekaan km 10, Makassar 90245, South Sulawesi, Indonesia<sup>5</sup>Learning Centre for Ecosystems Approach to Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan km 10, Makassar 90245, South Sulawesi, Indonesia<sup>6</sup>Laboratory of Fisheries Resource Management and Conservation, Hasanuddin University, Jl. Perintis Kemerdekaan km 10, Makassar 90245, Indonesia\*Corresponding Author: [nadiarti@unhas.ac.id](mailto:nadiarti@unhas.ac.id)

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[Doi :10.48047/AFJBS.7.7.2025.241-260](https://doi.org/10.48047/AFJBS.7.7.2025.241-260)**ABSTRACT**

This study evaluated the effectiveness of using combined morphological and molecular (integrative) taxonomic approaches in the identification of freshwater shrimp specimens of the genus *Macrobrachium* collected from the Pattunuang River, Maros, South Sulawesi. Morphological identification referred to identification keys. Cytochrome oxidase I mitochondrial DNA (COI mtDNA) barcodes (634 bp) were produced through DNA extraction, PCR (primer pair jgLCO/jgHCO) and Sanger sequencing. Key morphological traits included a slightly upward-curved rostrum and straight post-antennular carapace. Both approaches classified the specimens within the *Macrobrachium* genus, but neither produced a definitive identification. Morphologically, the specimens most closely resembled *Macrobrachium latidactylus* described from North Morowali, Sulawesi. The closest reference sequence was a *Macrobrachium latidactylus* (MN526229) accession from East Java. Phylogenetic analysis showed the Pattunuang River barcodes forming a well-supported (100% bootstrap) clade with no close (< 98% identity) sequences in the NCBI GenBank. The results strongly indicate that *Macrobrachium latidactylus* as currently recognized is likely a species complex. The barcodes are the first reference sequences for the *Macrobrachium* sp. from Pattunuang River in this database. However, further research is needed to determine whether this taxon is a new species or a described species lacking prior reference sequences in GenBank.

**Keywords:** COI mtDNA, DNA barcoding, integrative taxonomy, freshwater crustacea, species complex, Sulawesi

## INTRODUCTION

The genus *Macrobrachium* Spence Bate 1868 is a group of freshwater shrimp (Decapoda: Palaemonidae) found in tropical and subtropical regions (Cai & Ng, 2001). Several species (e.g., *M. rosenbergii*, *M. lanchesteri*, *M. lar*) have become popular aquaculture commodities (Annawaty & Wowor, 2015). However, many other species of *Macrobrachium* remain undescribed in detail, particularly in Eastern Indonesia, including Sulawesi (Laewa *et al.*, 2018). Sulawesi is known for its high biodiversity, including freshwater shrimp (Wowor *et al.*, 2009). One common species found in Sulawesi is *M. latidactylus*, which is widely distributed across Southeast Asia and several major islands in Indonesia (Jayachandran *et al.*, 2019). This species has previously been reported in the Halmahera River (Cai & Ng, 2001) and the Gililana River in North Morowali, Sulawesi (Laewa *et al.*, 2018). Studies covering the many rivers in Sulawesi are needed to enhance the understanding of the *Macrobrachium* genus.

Morphological identification is essential for classifying freshwater shrimp species. Key morphological traits, such as rostrum shape, spine patterns and counts on the carapace, and the structure of segments on swimming and walking legs, are commonly used for species differentiation (Jayachandran *et al.*, 2019). However, Fransen *et al.* (2010) highlight that this process can be challenging due to shared characteristics among species and significant morphological variation within a single species, especially those with broad geographic ranges. Therefore, complementary methods, such as molecular identification through DNA barcoding, can be necessary (Abdullah *et al.*, 2011). DNA barcoding is a molecular technique used to identify and distinguish species based on genetic information from DNA sequences. This method involves extracting short sequences from a designated “barcode” gene and comparing them to references in a DNA database to determine the species (Ward *et al.*, 2005). The most commonly used gene for DNA barcoding in animals is cytochrome oxidase I (COI) from mitochondrial DNA (Kress *et al.*, 2015).

Nugroho *et al.* (2009) noted that the genetic diversity of freshwater shrimp in Sulawesi differs significantly from that found in Kalimantan, West Java, and Sumatra. However, there are few reports of this genus in South Sulawesi. This study aimed to identify the morphological characteristics and conduct DNA barcoding of freshwater shrimp in the Pattunuang River, Maros Regency, South Sulawesi using an integrative taxonomic approach. This included analysis of the morphometric and meristic traits of *Macrobrachium* sp. collected from the Pattunuang River as

well as obtaining DNA barcodes (COI partial sequences) and constructing a phylogenetic tree to elucidate the relationship of this species with its close relatives based on homologous DNA barcode sequence data from the NCBI GenBank. This research represents a crucial initial step in the morphological identification and DNA barcoding of freshwater crustacea in the Pattunuang River, Maros Regency.

## MATERIALS AND METHODS

Shrimp samples were collected from the Pattunuang River, Maros Regency, South Sulawesi, Indonesia (Fig.1) from March to September 2023. Morphological identification was conducted at the Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar. Prior to DNA analysis, each sample was photographed using a digital camera. The pereopods of each sample were placed in 2 ml cryotubes filled with 96% absolute alcohol. DNA extraction and polymerase chain reaction (PCR) amplification of the target gene fragment were conducted at the Biotechnology Laboratory of the Brackishwater Aquaculture Research Institute (BPPBAP), Maros Regency. Sanger sequencing was performed at PT Genetika Science, Jakarta.

A



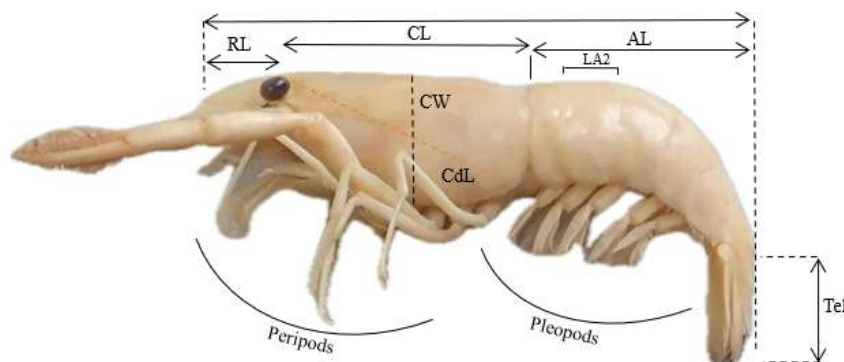
B



**Figure 1.** A. Map of the sample collection site. B. Condition of the Pattunuang River, Maros Regency, South Sulawesi, Indonesia (left: looking downstream, right looking upstream).

### Morphological characterization

Morphological data were collected following Wowor *et al.* (2009). The morphometric measurements followed Munasinghe & Thushari (2010) and were taken using a ruler (precision 1 mm). Meristic characters focused on the percentage distribution of spines above and below the rostrum of *Macrobrachium* specimens. The morphometric parameters were: total length (TL), abdomen length (AL), telson length (Tel), carapace length (CL), carapace width (CW), carapace diagonal length (CdL), length of abdominal segment 1 (LA1), rostrum length (RL), length of abdominal segment 2 (LA2) (Fig. 2). Meristic counts recorded were the numbers of dorsal and ventral rostral spines. Morphological identification was conducted according to the guidelines in Wowor *et al.* (2004). The observed characters were compared with previous studies on species within the genus *Macrobrachium* found in Sulawesi.



**Figure 2.** Morphometric parameters of *Macrobrachium* sp. from Pattunuang River.

### DNA barcoding

DNA was extracted from the preserved shrimp pereopods using the genomic DNA mini kit (Geneaid) following the manufacturers protocol. The target region of the COI mtDNA gene region

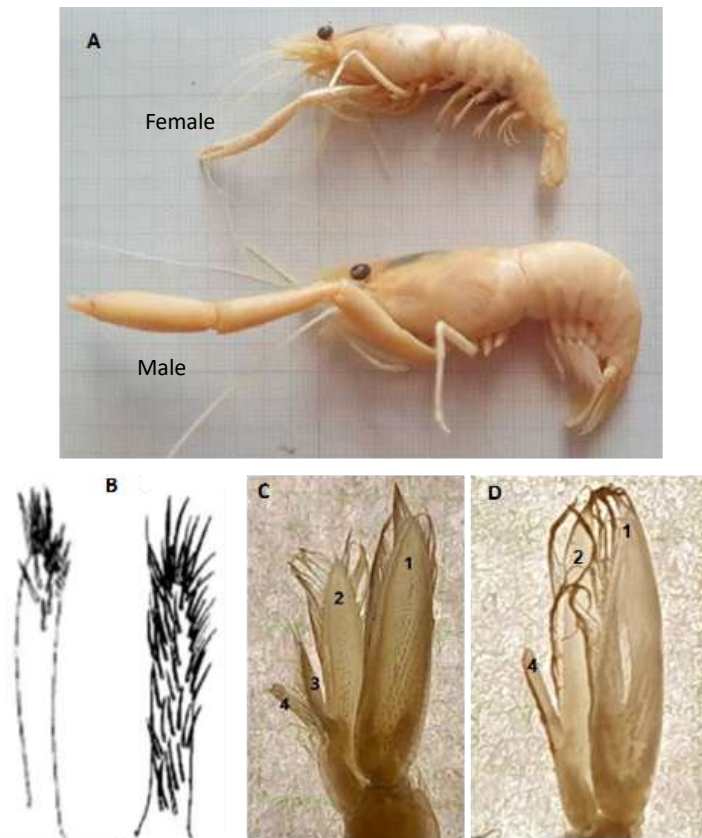
was amplified through PCR using the primers jgLCO1490 (forward) and jgHCO2198 (reverse) (Geller *et al.*, 2013). The PCR reaction mixtures included 4  $\mu$ L of 10x PCR buffer, 2.5  $\mu$ L of 10 mM dNTPs, 1.2  $\mu$ L of each 10 mM primer, 2  $\mu$ L of 25 mM MgCl<sub>2</sub>, 0.125  $\mu$ L of AmplyTaq Gold™, and 14.5  $\mu$ L of ddH<sub>2</sub>O, and 1  $\mu$ L template DNA, resulting in a total volume of 25  $\mu$ L. The PCR profile included an initial denaturation at high temperature (95 °C) for 5 min to separate the double-stranded DNA template. This was followed by 35 cycles of high-temperature denaturation (30 s at 95 °C), annealing (30 s at 50 °C), and extension (45 s at 72°C). A final extension at 72°C for 5 minutes ensured complete elongation of each DNA strand (Veneza *et al.*, 2018). PCR product was verified using electrophoresis on 1% agarose gel mixed with a DNA stain. The amplified PCR products producing clear, clean bands were sent for Sanger sequencing to PT Genetics Science Indonesia in Jakarta.

The sequence (chromatogram) files obtained were imported into Mega 11 (Tamura *et al.*, 2021). Sequences meeting the quality criteria were cleaned, trimmed, aligned, and the forward and reverse sequences for each specimen were combined to produce a DNA barcode. Homologous DNA barcode sequences were obtained from the NCBI GenBank using the Basic Local Alignment Search Tool (BLAST), accessible through the NCBI website (<https://blast.ncbi.nlm.nih.gov>) (Madden, 2013). Genetic distances were computed and a phylogenetic tree was constructed (neighbor-joining, Kimura 2 parameter model, 1000 bootstrap replicates) in Mega 11 (Tamura *et al.*, 2021). Haplotype analysis included geo-referenced sequences from the closest clades in the phylogenetic analysis and was conducted using DnaSP, Network and Notepad++ software.

## RESULTS

The morphological characterization of freshwater prawn samples from the Pattunuang River, Maros Regency, confirmed that all three samples (S1, S2 and S3) belong to the genus *Macrobrachium*. This classification was based on characteristics such as the presence of a hepatic spine on the carapace, simple (non-bifid) dactyls on the last three pereopods, and long, robust second pereopods. Identifying the specimens to the species level was challenging, one reason being the limitation arising from the fact that the identification keys for the genus *Macrobrachium* are only fully applicable to adult male individuals, as otherwise morphologically similar species are characterized by distinctive features such as a fully developed appendix masculina and the

second pereiopod which is significantly larger in males than in females (Short, 2004). These features are illustrated in Fig. 3.



**Figure 3.** A. Difference between the pereiopods of male and female *Macrobrachium* sp. from Pattunuang River. B. undeveloped (left) and developed (right) *appendix masculina* on the pleopod of male *Macrobrachium* (Short, 2004). C. Second pleopod of male *Macrobrachium* (S2). 1: exopodite, 2: endopodite, 3: *appendix masculina* , 4: *appendix interna*. D. Second pleopod of female *Macrobrachium* (S1).

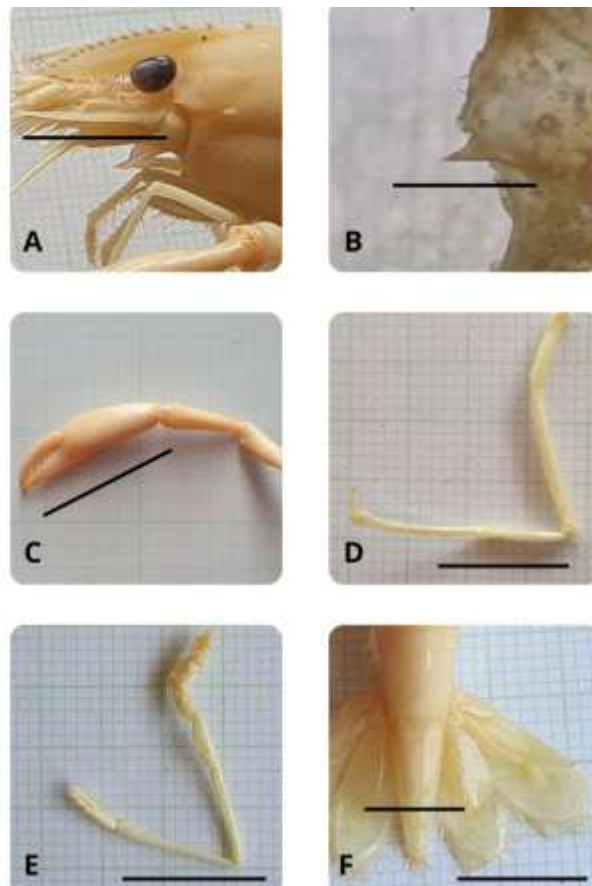
The three prawn samples identified, two (S1 and S3) were female, as indicated by the absence of the *appendix masculina* on the second pleopod and further supported by the presence of eggs in the abdominal region (Table 1).

**Table 1.** Morphological characterization of three *Macrobrachium* sp. specimens from the Pattunuang River

Specimen	<i>Spina hepatica</i>	<i>Appendix masculina</i>	Information
S1	Present	None	Adult female, eggs present
S2	Present	Developed	Adult male
S3	Present	None	Adult female, eggs present

The male specimen S2 (Fig. 4) had similar traits to *Macrobrachium latidactylus* from the Gililana River, North Morowali, Sulawesi (Laewa *et al.*, 2018) as indicated by similar morphometric and meristic characteristics (Table 2). The identification steps based on the key compiled by Wowor *et al.* (2004) is shown below:

1. The rostrum is slightly curved upward or knife-shaped, with the ventral margin having fewer than 7 teeth.....Fig. 4A
2. The second pereopod is not covered by attached scales, and the third to fifth pereopods are smooth.....Fig. 4(C&D)
3. The second pereopod has a carpus longer than the merus, with the carpus being sub-cylindrical.....Fig. 4C
4. The post-antennular carapace margin is straight.....Fig. 4B
5. The second pereopod has a chela longer than the carpus, with the surface of the carapace, telson, and uropod being spiny in adult males.....Fig. 4(A, C, F)
6. The second pereopod is asymmetrical, with the major pereopod having a broad chela and smooth fingers, while the minor pereopod has a slender chela with numerous long, stiff setae on the fingers; the fingers of both chelae are open (adult males).....*Macrobrachium latidactylus*.



**Figure 4.** *Macrobrachium sp.* from the Pattunuang River (male specimen S2). A. Cephalothorax and its parts, a: *spina hepatica*. B. Post-antennular carapace. C. Pereiopod 2. D. Pereiopod 5. E. Pereiopod 1. F. Telson and Uropod

**Table 2.** Morphometric and meristic characters of three specimens from the Pattunuang River and *Macrobrachium* spp. from other studies

No	Character	This study	Laewa <i>et al.</i> (2018)	Dwiyanto <i>et al.</i> (2017)	Aswandi & Annawaty (2019)	Rahayu & AnnCawaty (2019)	
			(Giliana River, North Morowali)	(Batuyusa River, Donggala)	(Maraja River, Toli-Toli)	(Labolo Island, Central Sulawesi)	
			<i>M. latidactylus</i>	<i>M. scabriculum</i>	<i>M. rosenbergi</i>	<i>M. equidens</i>	<i>M. latidactylus</i>
1	Total length	60-80	-	-	-	-	-
2	Abdomen length	28-30	-	-	-	-	-
3	Telson length	6-10	-	-	-	-	-
4	Carapace length	18-25	26	17	45	-	-
5	Carapace width	14-18	-	-	-	-	-
6	Carapace diagonal length	18-27	-	-	-	-	-
7	Length of abdominal segment 1	2-3	-	-	-	-	-
8	Rostrum length	15-18	-	-	-	-	-
9	Length of abdominal segment 2	5-6	-	-	-	-	-
10	Rostrum formula	$(3-4)+(10-11)/3$	15/3	$(4-5)+10/2$	3+9/11	$(1-3)+(5+10)/(3-7)$	$(3-5)+(10-11)/(2-5)$
11	Number of teeth on chela	11-15	15	15-18	-	-	-
12	Post antenular carapace	Straight	Straight	Rounded	Straight	Straight	Straight

Based on the comparison between the specimens and the literature, in particular the key provided by Wowor *et al.* (2004), all three samples were identified as members of the genus *Macrobrachium*. Only sample S2 could be tentatively identified to the species level and, based on the key, appeared most likely to be *M. latidactylus*.

### Molecular characters (DNA barcoding)

The COI mtDNA barcodes obtained from two of the three *Macrobrachium* sp. specimens collected from the Pattunuang River (S1 and S2) had a length of 634 bp and were submitted to the NCBI GenBank (submission number SUB14896909, accessions PQ658041 and PQ658042). The chromatograms obtained from the third specimen (S3) did not meet quality standards. The BLAST results (Table 3) show that the homologous sequence in GenBank most closely related to the *Macrobrachium* sp. from the Pattunuang River was accession MN526229, submitted as *M. latidactylus* from East Java (Hernawati *et al.*, 2020), with a query cover of 99% and an identity of 93.68%. According to Pangastuti (2006), a similarity percentage of less than 97% between COI DNA barcodes generally indicates that the sequences come from individuals belonging to different species.

**Table 3.** The similarity of the specimen with samples from GenBank

Pattunuang river sequence			Closest GenBank BLAST Result			
Lab ID	Species	Bp	Accession Number	Species	Query cover (%)	Identity (%)
4907280	<i>Macrobrachium</i> sp.	634	MN526229	<i>M. latidactylus</i>	99%	93.68%
4907282	<i>Macrobrachium</i> sp.	634	MN526229	<i>M. latidactylus</i>	100%	93.60%

\*MN526229 (Hernawati *et al.*, 2020)

### Phylogenetic and Network Analysis

The genetic distances calculated in MEGA 11 are shown in Table 4. The smallest genetic distance between samples S1 and S2 and homologous GenBank accessions was 0.062, with accession MN526229 (*M. latidactylus* from East Java, Indonesia). According to Nei (1972), genetic distance ranges from 0 to infinity, where a value of 0 indicates no genetic difference.

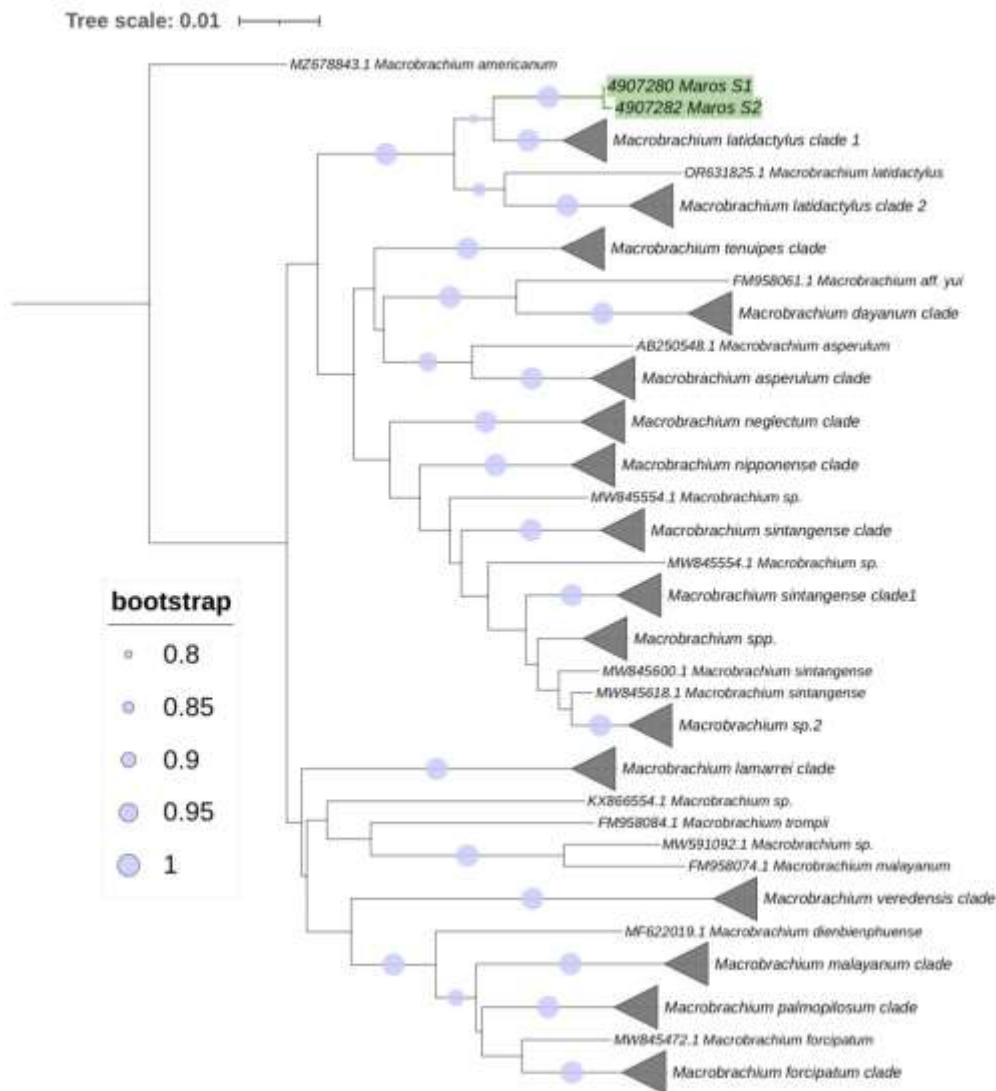
**Tabel 4.** Genetic distance *Macrobrachium* sp. from Pattunuang River and sample from GenBank

No.	Sampel	1	2	3	4	5	6	7	8	9	10	11
1	4907280 S1											
2	4907282 S2	0,000										
3	MW84557	0,074	0,074									
4	MW84558	0,078	0,078	0,004								
5	MN526229	0,062	0,062	0,008	0,013							
6	OR631825	0,138	0,138	0,135	0,139	0,126						
7	OR631826	0,130	0,130	0,107	0,111	0,095	0,123					
8	OR631828	0,130	0,130	0,107	0,111	0,095	0,122	0,003				
9	OR631831	0,134	0,134	0,111	0,115	0,100	0,127	0,003	0,007			
10	OR631832	0,126	0,126	0,111	0,115	0,100	0,126	0,007	0,003	0,010		
11	OR631833	0,134	0,134	0,111	0,115	0,100	0,126	0,007	0,003	0,003	0,007	

S1 & S2 : Sample of this study

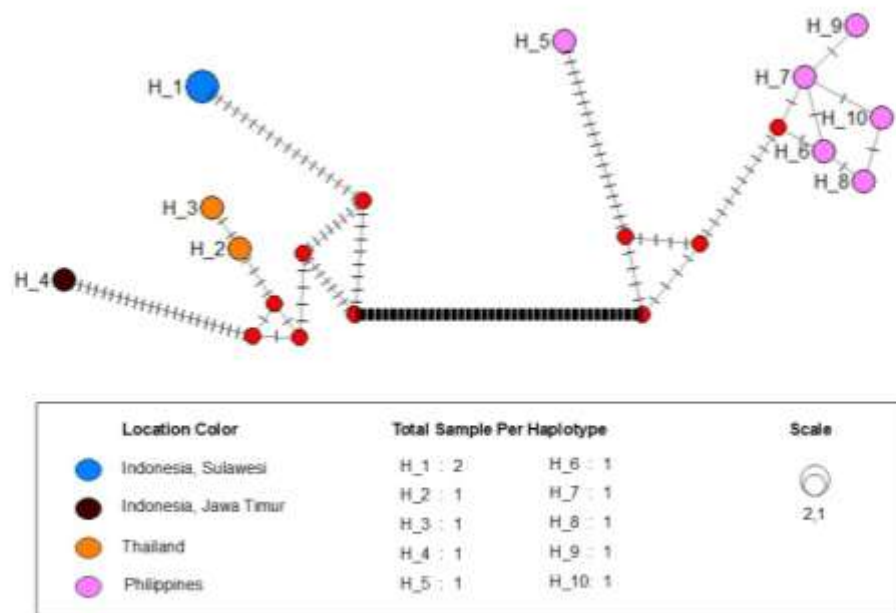
Accessions number from 3-11 as *M. latidactylus*

The phylogenetic tree shows four main clades within the genus *Macrobrachium*. Of these, the uppermost clade in Fig. 5 comprises three clades containing accessions submitted as *M. latidactylus* and a fourth well-separated clade comprising the two *Macrobrachium* sp. from the Pattunuang River. The closest (sister) clade to the Pattunuang Rivers specimens contained the previously mentioned *M. latidactylus* from East Java (Hernawati *et al.*, 2020). This clear (100% bootstrap) separation and the high genetic distance indicate that the *Macrobrachium* sp. specimens from the Pattunuang River belong to a species that does not yet have a reference sequence in the NCBI GenBank database.



**Figure 5.** Phylogenetic tree showing inferred evolutionary relationships within the genus *Macrobrachium* based on the COI DNA barcodes from this study (green highlight) and homologous GenBank accessions

The Network analysis included 10 geo-referenced haplotypes, one from the samples in this study, and nine from *M. latidactylus* GenBank accessions from East Java, Thailand, and the Philippines (Fig. 6). This figure also shows the Pattunuang River *Macrobrachium* sp. specimens (H1) forming a separate group, more closely related to haplotypes from Java and Thailand than to those from the Philippines.



**Figure 6.** Haplotype network of *Macrobrachium* sp. from Pattunuang River, Sulawesi and the closest geo-referenced homologous sequences from GenBank (all submitted as *M. latidactylus*)

## DISCUSSION

Morphological characterization involves the analysis of the shape, size, and physical structure of an organism or its parts, including observable external characteristics such as color, patterns, and organ shapes. In biology, the study of morphology is essential for identifying and classifying organisms based on their characteristics (Wowor & Ng, 2007). Morphological analysis was performed on all samples collected during sampling, and included morphometric and meristic traits. The key morphological characteristics included the shape of the rostrum, the structure of the pereopods, the presence of a hepatic spine on the carapace, and the size of the chela in adult males (Short, 2004). Based on comparisons between the characters observed and measured (Table 2, Fig. 2-4) and identification keys or current literature, all samples were identified as belonging to the genus *Macrobrachium*. Only one specimen (S2) was identified as an adult male *Macrobrachium*, based on the presence of an appendix masculina (Wowor & Choy, 2001), allowing for further analysis to determine the species, as some morphological features in female prawns are less developed, making their identification more challenging (Short, 2004). Specimens S1 and S3 were identified as females due to the absence of the appendix masculina on the endopod of the second pleopod, and the presence of eggs in the abdominal region.

The adult male *Macrobrachium* (S2) was tentatively identified as *M. latidactylus* based on morphological traits visible in Fig 4. The morphological characteristics of *M. latidactylus* include a slightly upward-curving or knife-shaped rostrum, a straight post-antennular carapace, a rostrum that does not extend beyond the scaphocerite, the presence of four post-orbital spines, the absence of a preanal carina, and dense pubescence on the chela of the second pereopod (Wowor *et al.*, 2004). The meristic features of *M. latidactylus* as described by Wowor *et al.* (2004) and Chace & Bruce (1993) include the number of dorsal rostrum teeth ranging from 9 to 15, and the ventral rostrum teeth being fewer than 7, with a rostrum formula of (3–5)+(10–11)/(2–5). Meanwhile, morphometric characteristics include the second pereopod having a chela longer than the carpus, with the carpus being sub-cylindrical. In this study, specimen S2 conformed to these guidelines and showed similarities with *M. latidactylus* found in the Giliana River, North Morowali, Sulawesi (Laewa *et al.*, 2018). The carapace lengths (25 and 26 mm) were similar, as were the meristic counts. The morphological characteristics of the specimens also match the descriptions of *M. latidactylus* previously reported in Halmahera (Cai & Ng, 2001).

The Pattunuang River seems to provide suitable habitat for *Macrobrachium* shrimps, as the observed specimens had total body lengths of 60–80 mm and carapace lengths of 16–25 mm, which fall within the standard size range for this genus (Brown, 2022). The morphological characterization results indicate that specimen S2 might be *M. latidactylus*. If this is confirmed, the presence of this species at various locations in Sulawesi with differing habitat characteristics demonstrates its adaptability to diverse freshwater habitats (Jayachandran *et al.*, 2019).

Phylogenetic analysis seeks to understand the evolutionary relationships between species and can be based on genetic, morphological, and other characteristics of organisms. Phylogenetic analysis based on genetic characters can assess genetic distances and typically includes the construction of phylogenetic trees to depict the kinship between species and provide insights into evolutionary history (Omar *et al.*, 2021). According to Hernawati *et al.* (2020), genetic variation in *Macrobrachium* species across different regions can be quite high. Genetic distance refers to the level of genomic difference between species or between populations within a species, measured as the proportion of nucleotide sites that differ between two compared sequences (Nei, 1972). Two congeneric groups are generally considered to have undergone speciation if the genetic distance between them exceeds 3% (Jose *et al.*, 2016). The 634 bp DNA barcode for the *Macrobrachium* sp. found in the Pattunuang River through using the mitochondrial COI gene was most similar to

with *M. latidactylus* from East Java, but the low percentage identity (93.68%) strongly indicates that these specimens belong to a different species. The genetic distances between samples S1 and S2 and all homologous GenBank sequences were greater than 0.06 (Table 4). This reflects differences of over 6%, typical of the distance between congeneric species (Jose *et al.*, 2016). The phylogenetic tree (Fig. 5) shows that the *Macrobrachium* sp. specimens from the Pattunuang River form a separate clade from the closest species in the GenBank database. The closest clade is *M. latidactylus* clade 1, but the clear separation (100% of 1000 bootstrap replicates) suggests that these specimens belong to an unrecognized species or possibly a new species, despite the morphological resemblance to *M. latidactylus*.

Haplotype diversity refers to the number of haplotype variations within a population, reflecting the likelihood of two random samples having different alleles (Nei & Li, 1979). Haplotype variation within a population can be influenced by various interconnected complex factors such as habitat fragmentation, changes in water quality, and microhabitat geographic isolation create barriers that limit gene flow, enabling local genetic differentiation and the formation of distinct haplotypes (Nehemia & Kochzius, 2017). Specific environmental conditions, such as water temperature, oxygen availability, and substrate characteristics, play an essential role in driving local adaptations that can be reflected in haplotype variations (Griffiths & Brinkman, 2020; Hurwood *et al.*, 2014). The haplotype analysis (Fig. 6) shows that, with the exception of the haplotypes from this study (H1), each of the other haplotypes was only represented by one sample, and resolve into three main groups representing three distinct biogeographical regions: H1 alone (from Pattunuang River, Sulawesi, in Wallacea); H2-H4 (from Java and Thailand, in Sundaland); and H5-H10 (from the Philippines), each of which likely represents a species-level taxon. The haplotype network analysis (Fig. 6) supports the hypothesis that the Pattunuang *Macrobrachium* belongs to a species which is not represented in the NCBI GenBank database.

Species radiations and cryptic species occur in several taxonomic groups in Sulawesi (Jayadi *et al.*, 2019; Siriwut *et al.*, 2021), and new amphidromous species or species distributions are also being discovered in Sulawesi rivers (Wibowo *et al.*, 2024). Therefore, it is not unlikely that there could be as yet undescribed *Macrobrachium* species in this region, while the NCBI GenBank coverage of Sulawesi freshwater taxa is known to be incomplete (Hubert *et al.*, 2016).

Overall, the results indicate that the observed differences between the *Macrobrachium* sp. in the Pattunuang River and other taxa within this genus may be due to genetic variation but most

likely represent a cryptic species within the *M. latidactylus* group. South Sulawesi is an area known for its high biodiversity but still underexplored (Vari & Hadiaty, 2012). This study demonstrates the need for in-depth research to further improve our understanding of the genetic diversity of *Macrobrachium* in the study region, across Sulawesi, and more widely across the distribution of this genus.

## CONCLUSION

Although they share many morphological similarities, the high genetic distance between *Macrobrachium* sp. from the Pattunuang River in Maros, South Sulawesi and other *M. latidactylus* with GenBank accessions from different regions indicates that the specimens collected in this study most likely represent a distinct species. The results strongly indicate that the taxon currently referred to as *Macrobrachium latidactylus* is likely a species complex comprising at least three (possibly four or more) cryptic species-level taxa, with the *Macrobrachium* sp. from the Pattunuang River belonging to one of these congeneric species, demonstrating the importance of integrative morphological and molecular approaches. The Pattunuang River *Macrobrachium* sp. may be a species as yet undescribed or a species previously described but lacking COI DNA barcodes in the NCBI GenBank. Therefore, this research provides the first DNA barcodes for a previously unrepresented taxon and opens up an exciting avenue for further studies on this genus and its diversity in the Pattunuang River and other watersheds in Sulawesi and the Indo-Pacific region.

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