RESEARCH ARTICLE

Morphological and Genetic Diversity Assessment of Freshwater Prawns (*Macrobrachium* spp.) in the Cairawan River, Antique Province, Panay Island, Philippines

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ABSTRACT

Freshwater prawns of the genus *Macrobrachium* are among the most valuable cultured aquatic species in the world. The systematic study of *Macrobrachium* species in the Philippines has often been based on morphological trait assessment. Here, we combined morphology and molecular approaches to study the presence and actual distribution of *Macrobrachium* species along the Cairawan River in Laua-an, Antique Province, Panay Island. Using morphological characteristics, we identified 11 *Macrobrachium* species, the highest number of species reported from a single river. Molecular results based on the cytochrome oxidase subunit I (COI) mitochondrial DNA (mtDNA) sequences confirmed the morphological identification. The analysis revealed eight major clades with 19 distinct haplotypes within the species. Our results provide information on the identification of *Macrobrachium* species inhabiting the Cairawan River. The baseline information can be helpful for future genetic studies involving freshwater prawns with potential use in aquaculture. It can provide scientific data to facilitate the formulation of selective breeding plans and direct governance policies in freshwater prawn resource conservation and management in the Philippines.

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1. INTRODUCTION

Macrobrachium is one of the most abundant and widespread crustacean genera, with 309 described species and a number of cryptic species (WoRMS Editorial Board 2023). The Oriental region has the most species, i.e., 123 species (De Grave et al. 2008), of which more than 100 are found in East and Southeast Asia (Wowor et al. 2004).

Because of their size and wide distribution, *Macrobrachium* species are the most valuable cultured freshwater prawns in the world and have gained a reputation as a high-value food resource from inland waters (Cavallo et al. 2001). The giant freshwater prawn (*M. rosenbergii*), in particular, comprises an important fishery in natural freshwater bodies in several countries worldwide, and culture is a crucial activity mainly in Asia, United States, and Brazil (David et al. 2018). Moreover, freshwater prawn

aquaculture is considered a rapidly growing sector with the expansion of prawn hatcheries and grow-out farms in many countries. Marketable forms of prawns command a good price in local and foreign markets in both developing and developed countries. It has also been identified as an alternative to black tiger shrimp and white leg shrimp because it is moderately more disease-resistant (Ravi et al. 2009; Jang et al. 2022). World aquaculture production of *M. rosenbergii* from 2000 to 2020 increased by 44%, from 130,700 tons to 294,000 tons (FAO 2022). However, many natural populations are being heavily depleted, and the species have become widely translocated for aquaculture (New and Valenti 2000). There have been studies of exploited *Macrobrachium* stocks in Brazil (Lima et al. 2014), Nigeria (Okogwu et al. 2010), Indonesia (Aprila et al. 2020), and the Philippines (Cuvin-Aralar 2014). Currently, four *Macrobrachium* species are considered critically endangered or possibly extinct (De Grave et al. 2015).

In the Philippines, fisheries for the giant freshwater prawn used to occur in most of the major rivers in the country, such as the Cagayan, Agno, Pampanga, Bicol, Jalaud, Agusan, and Mindanao rivers. Prawn fisheries also existed in lake/outlet river complexes such as the Laguna Lake/Pasig River, Naujan Lake/Butas River, Bato Lake/Bicol River, and Mainit Lake/Agusan River. Due to human population pressure, urbanization, overfishing, and the establishment of industries, the global natural fishery has been declining and has reached deficient levels (FAO 2020). Aquaculture production declined dramatically, from 29 tons in 2009 to only 0.48 tons in 2021 (BFAR 2022).

The earliest survey in the Philippines that enabled the identification of various *Macrobrachium* species was the 1907–1910 Albatross Philippine Expedition (Chace and Bruce 1993). From then, few studies were conducted, reporting new records collected from all over the Philippines (Cai and Anker 2004; Freitag 2005; Cai and Shokita 2006; Cai et al. 2009). In 2007, Eguia et al. validated the natural distribution of local *Macrobrachium* species through morphometric characterization. Among the 27 sampling sites around the Philippines, the Cairawan River in Laua-an, Antique Province, had the greatest number of species, suggesting its rich biological diversity (Eguia et al. 2009). To date, about 39 species have been reported in the Philippines (Chace and Bruce 1993; Cai and Shokita 2006; Chen et al. 2009; Eguia et al. 2009).

Here, we study the presence and distribution of Macrobrachium species along the Cairawan River in Laua-an, Antique, using integrative taxonomy based on morphological and molecular markers. Macrobrachium is a difficult genus, especially when identifying young (both sexes) and female specimens based on morphology (Suzuki and Kusamura 1997).

The morphological variations in a species based on sex and age are wide, and the available identification keys are suitable for fully developed adult males only. Therefore, a molecular approach was also used. We used cytochrome c oxidase I (COI) mitochondrial marker as its sequence divergence differentiates even closely related species (Jose and Harikrishnan 2016). COI has been used for phylogenetic inferences (Liu et al. 2007; Wowor et al. 2009), phylogeographic analysis, and research in population genetics (Murphy and Austin 2004; De Bruyn and Mather 2007; Liu et al. 2011; Vergamini et al. 2011; Hurwood et al. 2014; Bernays et al. 2015; Cui et al. 2018; Aliah et al. 2022). Based on the COI gene, the work of Liu et al. (2007) and Pileggi and Mantelatto (2010) led to the molecular systematics of Australian, Indo-Pacific, and some American species of *Macrobrachium*, respectively.

In this study, COI was used to analyze the phylogenetic relationships of wild stocks of *Macrobrachium* species sampled along the Cairawan River to infer its presence and distribution. The data on the distribution of wild stocks can help develop appropriate management plans and identify species with potential for aquaculture.

2. MATERIALS AND METHODS

2.1 Sample collection

Samples were collected from the Cairawan River in Laua-an, Antique Province, for six consecutive months (March to August 2009). Local catching gears were used to gather samples from four sampling points: upstream (Barangays Guiamon and San Ramon), midstream (Barangay Lupaan), and downstream (Barangay Liya-liya) of the river (**Figure 1**). A barangay is a sub-division within a municipality or a city in the Philippines. A global positioning system (GPS) navigation device was used to mark the sampling areas. The sampling site and relative information per site are presented in **Table 1**. The specimens were transported live using oxygenated plastic bags and styrofoam boxes to the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC/AQD) in Tigbauan, Iloilo, Philippines.

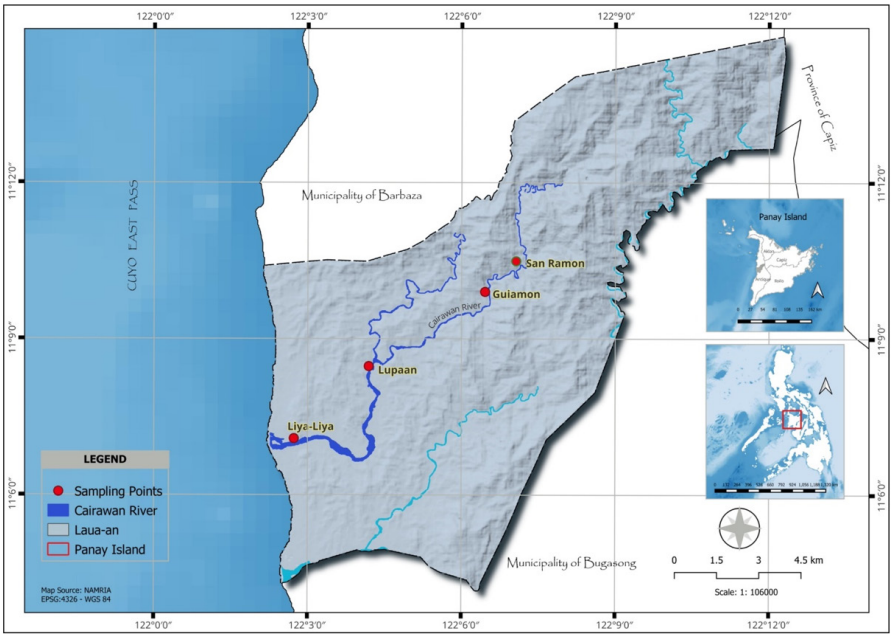

Figure 1. Map showing the location of the study area and sampling sites. The map was generated using Quantum Geographic Information System (QGIS version 3.28.0) based on the Philippines' National Mapping and Resource Information Authority (NAMRIA), Philippine GIS Data Clearinghouse (PhilGIS), Bureau of Soils and Water Management (BSWM), Mines and Geosciences Bureau (MGB) Geoportal, Department of Environment and Natural Resources (DENR), and United States Geological Survey.

Table 1. Sampling sites, distance from the sea, and geographical location of *Macrobrachium* sp. samples collected from Cairawan River, Antique, Philippines.

Site	Distance from the sea (km)	Barangay / Village	Elevation, above sea level (m)
Downstream	0.1	Liya-liya	13
Midstream	9	Lupaan	48
	18	Guiamon	98
Upstream	24	San Ramon	146

2.2 Morphological identification

A total of 131 specimens were identified up to the species level using taxonomic keys by Chace and Bruce (1993) and Eguia et al. (2009). Ten morphometric characters were measured, namely: total length (distance between rostrum tip and distal tip of telson), body weight (BW), carapace length (distance between the posterior margin of the right orbit and midpoint of the posterior margin of the carapace), rostral length (distance of epigastric tooth basis to rostrum tip), dactylus length (distance between proximal and distal margin of dactyl), palm length (distance between proximal and distal margin of palm), carpus length (distance from the proximal to the distal end of carpus), merus length (distance between lateral line of merus), ischium length (distance between lateral line of ischium) and palm width (distance between lateral line of palm). The number of rostral teeth (dorsal and ventral), length of the rostrum in relation to the antennal scale, pereopod morph (e.g., type and presence or absence of spines, spinules, long-stiff and short-velvety seta on the second pereopod, presence or absence of longitudinal groove), and size and shape of chelipeds were also examined. Morphological characters were recorded, including shape traits that are considered necessary in *Macrobrachium* systematics (e.g., rostrum and second pereopod).

Representative samples were sent to the Research Center for Biosystematics and Evolution (formerly Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences), National Research and Innovation Agency in Cibinong, Indonesia, for identification. Additional morphological characters such as the fourth thoracic sternite or T4 (located between the bases) of the first pair of pereopod, teeth, and tubercles at the cutting edges of the fingers of the second pereopod, were used to distinguish three morphologically similar species, i.e. *M. jaroense*, *M. placidulum,* and *M. horstii*. The T4, armed with a well-developed median process, has various shapes from rounded to sharp tips in some of the same species, but the presence or absence is very

consistent between sexes and individuals at different stages of development. The presence of a median process is a useful key character in identifying a species (Short 2004). Representative *Macrobrachium* specimens were deposited at the Museum Zoologicum Bogoriense (MZB) or Bogor Zoological Museum in Cibinong, Indonesia, with reference numbers from MZB Cru 3128 up to MZB Cru 3150 (Appendix 1).

2.3 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from pleopods of at least 10 individuals per species of the prawn samples using DNAzol (Invitrogen). The DNA extracts were purified using Fujifilm QuickGene DNA tissue kit S (DT-S) and Fujifilm QuickGene-Mini80. Fragments of mitochondrial DNA cytochrome c oxidase subunit I gene (COI or *cox1*) were amplified for sequence analysis. The primers used to amplify the mitochondrial COI gene were C1-J-1718 5' GGAGGATTTGGAAATTGATTAGTTCC 3' and C1-N-2191 5' CCCGGTAAAATTAAAATATAACCTTC 3' (Simon et al. 1994). Blend Taq Plus (Tokobo) was used according to the manufacturer's specifications, and the Polymerase Chain Reactions (PCR) were run in 27 µL reactions. The cycling profile was one step at 95°C for 5 min, followed by 15 cycles at 94°C for 30 s, 40°C for 30 s, and 72°C for 1 min, 30 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, and a final 5 min extension step at 72°C adapted from Cook et al. (2006).

PCR products were purified with the Agencourt Ampure XP PCR purification kit (Beckman Coulter) following the supplier's instructions and directly sequenced using the BigDyeTM terminator cycle sequencing chemistry, following the manufacturer's protocol (Applied Biosystems). Cycle sequencing conditions were 30 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. The sequences were recorded with an ABI 3130 automated sequencer (Perkin-Elmer). At least ten individuals per species were sequenced. DNA sequencing was done at the Laboratory of Integrative Aquatic Biology, Graduate School of Agricultural Science of the Tohoku University in Onagawa, Miyagi Prefecture, Japan.

2.4 Data analysis

The sequence chromatograms were checked using 4Peaks software (Mekentosj, Amsterdam) and FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA). A total of 48 sequences, nine from GenBank, including one outgroup, were aligned using MUSCLE v3.8.31 (Edgar 2004) in MEGA version X (Kumar et al. 2018). The alignment was used as input for phylogenetic inference, obtained using the Maximum-Likelihood (ML) and the Bayesian inference (BI) methods. Prior to phylogenetic analyses, the General Time Reversible (GTR) model +G+I (Tavaré 1986) was selected using jModelTest 2, according to AIC criteria (Darriba et al. 2012) and used for both ML and BI analyses.

The ML phylogenetic trees were reconstructed using MEGA X version 10.1.7 (Kumar et al. 2018). Statistical support for each node was calculated based on 1,000 bootstrap replications. The phylogenetic tree was visualized using FigTree.v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). The BI phylogenetic tree was reconstructed using MrBayes v3.2.6 (Ronquist et al. 2012). Four MCMC chains run for 50,000,000 generations, sampling every 40,000 generations. Eighty percent of the resulting Bayesian trees were discarded as 'burn-in.' The posterior probabilities for each node were visualized using FigTree.v1.4.4 and reported beside bootstrap values obtained in ML analyses for the same nodes.

The p-distances among all sequences were computed using MEGA version X (Kumar et al. 2018). The number of haplotypes (as unique sequences) for each species was determined with a custom Python script, while the haplotype diversity (h) and nucleotide diversity (π) were calculated with the R package pegas (Paradis 2010).

3. RESULTS

3.1 Morphological identification

The morphological assessment enabled the identification of eleven species (Supplementary Figure 1) of *Macrobrachium*, namely *M. australe* (Guérin-Méneville, 1838) (Koua river prawn) (MA), *M. esculentum* (Thallwitz, 1891) (Sweet river prawn) (ME), *M. horstii* (De Man, 1892) (MH), *M. jaroense* (Cowles, 1914) (Jaro river prawn) (MJ), *M. lar* (Fabricius, 1798) (Monkey river prawn) (LAR), *M. latidactylus* (Thallwitz, 1891) (Scissor river prawn) (ML), *M. latimanus* (von Martens, 1868) (Mountain river prawn) (LM), *M. lepidactyloides* (De Man, 1892) (Malayan scale prawn) (LEP), *M. placidulum* (De Man, 1892) (PLA), *M. bariense* (De Man, 1892) (MB) and *M. mammillodactylus* (Thallwitz, 1892) (Knobtooth prawn) (MM). Three morphologically similar species, i.e., *M. jaroense*, *M. placidulum*, and *M. horstii*, were identified by using the number of rostral teeth behind

the eyes and the presence of a median process on the fourth thoracic sternite (T4) as a defining character. *M. jaroense* and *M. horstii* have a triangular median process, while in *M. placidulum* this process is absent. Moreover, the ischium and basal segments of the first pereopod of *M jaroense* are covered with squamiform (scale form) seta, while the same segments were glabrous in both *M placidulum* and *M horstii*. The distal part of the fingers of the large second pereopod of *M horstii* and *M placidulum* were observed to have a double row of tubercles, 3–6 and 4–6 on each row, respectively.

The morphometric and meristic characteristics of *Macrobrachium* species collected from the Cairawan River were measured (Supplementary Table 1). Generally, female samples have smaller body sizes, the second pereopod is shorter and more slender, the teeth on the cutting edges of the fingers are smaller, no double row of tubercles, and the pereopod is more heterogeneous compared to the male.

3.2 Sequencing analysis

The partial COI gene sequence alignment from the 39 generated *Macrobrachium* showed 77–100% identity. The percentage identity within species ranges from 80% to 100%. The new sequence data were submitted to Genbank with Accession Nos. OR631813-OR631851 and additional mtDNA COI sequences were used from the Genbank for molecular analysis (Supplementary Table 2). The genetic divergence was analyzed using the ML method based on partial COI sequences (393 bp consensus sequences), as shown in Figure 2.

There were eight clades corresponding to eight described species. While each of the eight clades

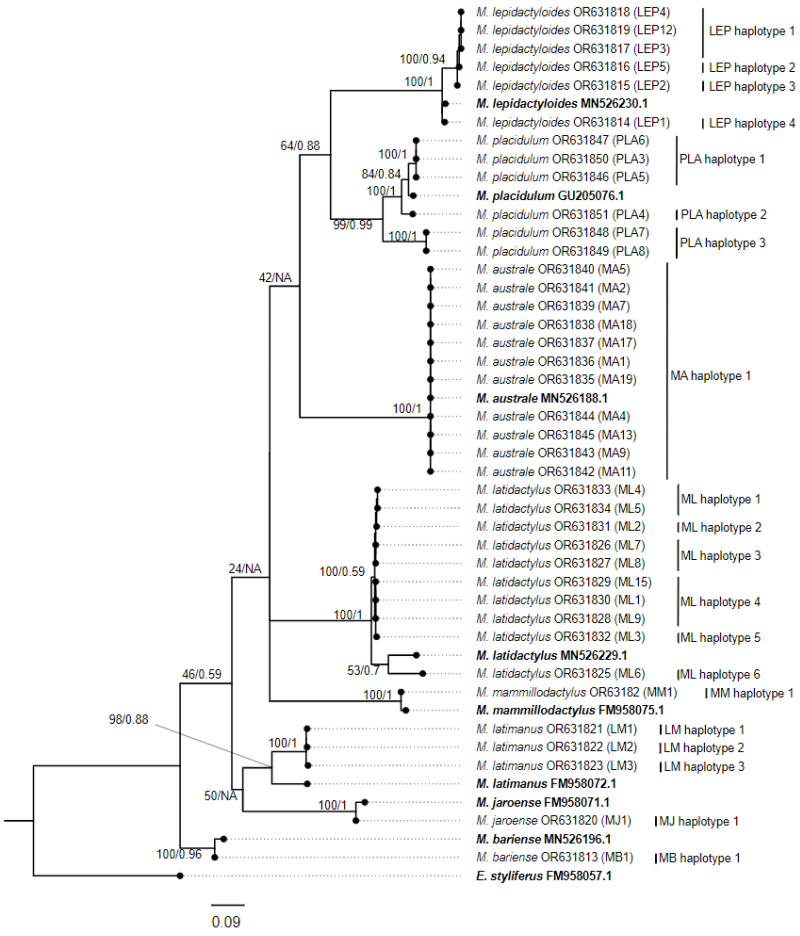

Figure 2. Phylogenetic tree of *Macrobrachium* populations in Cairawan River, based on 393 nt cox1 sequences, inferred from Maximum Likelihood (ML) analysis of partial mtDNA COI gene sequences showing bootstrap from ML and posterior probabilities from Bayesian Inference (BI) above the nodes. Reference sequences from Genbank are in bold font, and the accession number of sequences produced in this study is indicated above the nodes. Only percentages ≥ 50% and posterior probabilities ≥ 0.5 are shown.

was well defined (bootstrap ≥ 99% and posterior probability ≥ 0.99), the phylogenetic relationships among clades were less clear, having lower nodal support. *M. lepidactyloides* is a sister species to *M. placidulum* (bootstrap = 64%, and posterior probability = 0.88), while *M. latimanus* is possibly a sister species to *M. jaroense* (bootstrap = 50%, and posterior probability < 0.5). *M. bariense* seems to be a more distant species, possibly a basal clade (bootstrap < 50%, and posterior probability = 0.59). Other relationships among clades cannot be established with reasonable certainty due to low nodal support (bootstrap < 50% and posterior probability < 0.5). We do not have sequences for *M. lar, M. horstii*, and *M. esculentum*, and some species only have a few representative sequences in the analysis since the sequencing generated low-quality reads for these samples. All the species clustered with reference samples. The outgroup – *Exopalaemon styliferus* aligned with the sample sequences.

The genetic distance within species ranges from 0 (0%) (*M. australe*) to 0.092 (9.2%) (*M. placidulum*), while the distance between species ranges from 0.210 (21%) (*M. latimanus* vs *M. bariense*) to 0.301 (30.1%) (*M. mammillodactylus* vs. *M. jaroense*) (Table 2). The p-distance value among the other eight populations is about 0.20.

Based on the COI fragment, 19 distinct haplotypes were recognized (Figure 2, Table 3). *M. latidactylus* showed the highest haplotype diversity (h=6), followed by *M. lepidactyloides* (h = 4), *M. placidulum* (h = 3), *M. latimanus* (h = 3), and one haplotype each for *M. bariense*, *M. mammillodactylus* and *M. jaroense*. The haplotype for *M. australe* samples was shared with the reference sequence. In *M. latidacylus*, there was one haplotype each for ML6, ML2, ML3; the fourth haplotype was shared by three individuals (ML9/ ML15/ ML1); the fifth and sixth haplotypes were shared among two individuals, ML7/ML8 and ML4/ML5, respectively. For *M. lepidactyloides*, one haplotype for each individual (LEP1, LEP2, LEP5) and the fourth haplotype is shared between LEP3/LEP4/LEP12. For *M. placidulum*, the first haplotype is shared by PLA5/PLA6/PLA3, the second haplotype by PLA7/ PLA8, and one individual (PLA4) for haplotype three. For *M. latimanus*, the three samples have three distinct haplotypes.

Table 2. The mean uncorrected pairwise genetic distance (p-distance) within and between species based on the 393 nt *cox1* sequences. Within species distance are in gray shading. The number of analyzed sequences is in parenthesis after the species name.

	M. bariense	*M. lepidactyloides*	*M. jaroense*	*M. latimanus*	*M. mammillodactylus*	*M. latidactylus*	*M. australe*	*M. placidulum*
M. bariense (n=2)	0.023							
M. lepidactyloide (n=7)	0.297	0.032						
M. jaroense (n=2)	0.240	0.298	0.023					
M. latimanus (n=4)	0.210	0.281	0.229	0.064				
M. mammillodactylus (n=2)	0.238	0.279	0.301	0.221	0.013			
M. latidactylus (n=11)	0.215	0.255	0.249	0.249	0.257	0.059		
M. australe (n=12)	0.250	0.271	0.293	0.265	0.262	0.248	0.000	
M. placidulum (n=7)	0.264	0.228	0.248	0.247	0.260	0.241	0.249	0.092

Table 3. Number of haplotypes, haplotype diversity, and nucleotide diversity in mitochondrial DNA sequences of *Macrobrachium* species collected from Cairawan River, Antique, Philippines.

Species	Sample size (n)	Number of haplotypes (Nh)	Haplotype diversity (h)	Nucleotidediversity (π)
Macrobrachium bariense	1	1	NA	NA
Macrobrachium lepidactyloides	6	4	0.8	0.01917
Macrobrachium jaroense	1	1	NA	NA
Macrobrachium latimanus	3	3	1	0.00679
Macrobrachium mammillodactylus	1	1	NA	NA
Macrobrachium latidactylus	10	6	0.889	0.02404
Macrobrachium australe	11	1	NA	0
Macrobrachium placidulum	6	3	0.733	0.07803

3.3 Distribution in the river

Table 4 shows the distribution of *Macrobrachium* spp along the Cairawan River, Antique Province, Philippines. Almost all species were found downstream, midstream, and upstream of the river. *Macrobrachium mammillodactylus* was only collected downstream of the river, while *M. bariense* was only collected from midstream. *M. latimanus*, *M. horstii*, and *M. jaroense* were collected only upstream.

4. DISCUSSION

4.1 Morphological identification

Eguia et al. (2007) collected *Macrobrachium* spp from the Cairawan River and identified seven species using morphometric characterization, including *M. esculentum*, *M. latidactylus*, *M. jaroense*, *M. horstii*, *M. lar*, *M. australe,* and *M. lepidactyloides*. In the present study, we identified 11 *Macrobrachium* spp using a morphological approach. Four additional species were identified, namely *M. bariense*, *M. mammilodactylus*, *M. latimanus*, and *M. placidulum*. Among the identified samples, five are commercially important in the Philippines, i.e., *M. lar*, *M. esculentum*, *M. jaroense*, *M. lepidactyloides*, and *M. mamillodactylus*. In the 1950s, *M. esculentum* was listed as one of the most commercialized species in the Philippines (Jurniati et al. 2021).

The data showed high variability of morphological characters within species, where some characters displayed a high degree of developmental variation, particularly among males of *M. australe*. This is common in adult males, especially in the second walking legs (Barki et al. 1991). Different morphotypes in male *Macrobrachium*, particularly, can have similar body sizes but different cheliped sizes and forms, distribution and arrangement of spines, and presence of setae type (Rios et al. 2021). High phenotypic variation suggests that taxonomic traits may not be under strict genetic control in this species. The observation by Dimmock et al. (2004) showed that individuals within a single river have a high degree of morphological variation, which was evident in the current study. The variation in dactylus length, rostrum length, carapace length, and body length are highly significant, while carpus length, merus length, and total chelae are significant in wild *M. australe*. In *M. rosenbergii*, Ibrahim et al. (2023) observed a significant variation in rostrum length.

Obvious morphological differences within species were only observed in *M. latidactylus*, particularly in the structure of the second pereopod. The second pereopods are either isochelous (when the left and right second pereopods are equal in size or shape, mainly in young adult males and females) or heterochelous (when pereopods significantly differ in size or shape particularly in fully adult males) (Mossolin and Bueno 2003). The fingers of the large second pereopod are shorter or longer than the palm (per. observation). Adult *M. olfersi* are heterochelous, and the development of the second pair of pereopods coincides with its sexual maturity (Mossolin Bueno 2002). Like *M. olfersi*, the physiological dependence of *M. latidactylus* on brackishwater for development may have contributed to heterochely.

Table 4. Distribution of *Macrobrachium* spp along Cairawan River, Antique Province, Philippines.

Species	Barangay Liya-liya	Barangay Lupaan	Barangay Guiamon	Barangay San Ramon
Macrobrachium australe	x	x	x	
Macrobrachium bariense		x		
Macrobrachium esculentum	x			x
Macrobrachium horstii				x
Macrobrachium jaroense				x
Macrobrachium lar		x	x	x
Macrobrachium latidactylus	x	x		
Macrobrachium latimanus				x
Macrobrachium lepidactyloides	x	x		x
Macrobrachium mammillodactylus	x			
Macrobrachium placidulum	x	x	x	x

4.2 Sequencing analysis

The identities of eight species of *Macrobrachium* were confirmed using genetic analysis based on the mtDNA sequences. The clades in the dendrogram are consistent with those based on the analysis of morphological characters. One clade consisted of *M. lepidactyloides* and *M. placidulum*. This is congruent with the results of Liu et al. (2007), Cai and Shokita (2006), and Jose and Harikrishnan (2018), wherein *M. lepidactyloides* formed a clade with the morphologically similar species of *M. horstii* and *M. placidulum*. Along with other studies, the result contradicts a suggestion by Chace and Bruce (1993) that there is a possibility that these species may eventually prove to be synonymous. Another clade is composed of amphidromous species: *M. latidactylus* and *M. mammillodactylus*. This is concordant with the phylogenetic tree constructed by Jose and Harikrishnan (2018) and Chen et al. (2009) using COI, 16S rRNA, and nuclear 28S rDNA sequences. The third clade was formed by congeners of *Macrobrachium* species *M. jaroense* and *M. latimanus*. Other congeners within this clade included *M. bariense*.

The genetic distance between species ranging from 21% (*M. latimanus* and *M. bariense*) to 30.1% (*M. mammillodactylus* and *M. jaroense*) suggested that these species were valid species as they are greater than the 15% threshold to delineate species. The *M. mammillodactylus* and *M. jaroense* showed a higher genetic differentiation due to geographical isolation, the former being found only downstream of the river. The same is true with the two morphologically similar *M. jaroense* and *M. placidulum*, wherein *M. jaroense* is only found upstream of the river. This result suggests that the COI is useful to distinguish between species with similar morphological traits. On the other hand, the genetic distance within species ranges from 0% (*M. australe*) to 9.2% (*M. placidulum*). Although the results of the study were limited by the small number of individuals used for some species, such as *M. bariense*, *M. jaroense*, and *M. mammillodactylus*, the comparison with reference strains showed that their genetic distance is within the <15% threshold at the species level (Schütte et al. 2023).

Maximum Likelihood (ML) has been considered a good reconstruction method in decapod phylogenetic studies (Pileggi and Mantelatto 2010). The resolution and probabilities of ML and Bayesian Inference (BI) were similar; thus, the species are very well resolved. Patterns of mtDNA diversity provided valuable information about the demographics of populations. Our results showed that wild populations of *M. latidactylus*, *M. lepidactyloides*, *M. placidulum*, and *M. latimanus* had high levels of genetic diversity, suggesting that these species have good chance of surviving and good reproductive potential (Avise et al. 1987). Management efforts should be implemented to preserve the processes allowing such diversity to exist. These species also can have good aquaculture potential. Populations with high intra-population variation are more preferred for stock improvement programs as the traits may respond positively to genetic selection and can be more effectively used as baseline stocks for selective breeding (Nguyen et al. 2006). The wider the genetic diversity of wild aquatic species, the more opportunities for developing farmed stocks adapted for desired characteristics. Shokita et al. (1984) identified three *Macrobrachium* species (*M. lar*, *M. australe*, and *M. equidens*) with aquaculture potential. Of the three species, *M. lar* was the leading candidate because it attains a larger size than the other two species. In addition, it has a relatively fast growth rate. Williams (2018) evaluated the aquaculture potential of *M. lar* and found that *M. lar* had a better growth rate than the domesticated *M. rosenbergii*. There were several efforts to domesticate *M. lar*, including its complete larval development (Lal et al. 2014; Amos et al. 2014). Another potential candidate for aquaculture is *M. australe*. Apart from being the most abundant and widespread in the river, its good recruitment data implies that it can provide the advantage of constant broodstock sources from natural populations.

The mtDNA COI information in freshwater prawns generated from this study distinguished the species and revealed the pattern and extent of phylogenetic structuring within the genera. The cladogram gives us an idea of genetic structuring of *Macrobrachium* species in the Cairawan River. Other marker genes (mitochondrial and nuclear) and more variable molecular markers, such as microsatellites, can be used for future studies. DNA sequence data from ATP8, ND2, and ND6 protein-coding genes may also have the potential to provide valuable information for the elucidation of stock structure within these species, given that these genes demonstrate high nucleotide substitution rates in crustacean mitochondrial genomes (Machida et al. 2004).

4.3 Species distribution along the river

As expected, all species were found in all sampling points, as they are amphidromous species. Morphologically similar *M. jaroense* and *M.*

horstii were only found in the upstream part of the river, supporting Dimmock et al. (2004) that the environment can strongly influence morphological traits but may not be indicative of genetic diversity. In contrast, *M. jaroense* (upstream only) and *M. placidulum* are morphologically similar but not found in the same habitat. *M. mammillodactylus* is a lowland coastal species commonly found in relatively shallow depths (0.5–3.0 m) and euryhaline habitats (Short 2004; Jose and Harikrishnan 2018), corroborating with our collection in the downstream of the river. *M. mammillodactylus* is essential to local fishing communities and can be used for resource enhancement to replenish dwindling stocks in the wild and aquaculture. Importantly, it has been successfully bred in captivity (Cuvin-Aralar 2014). *M. esculentum* is relatively large and found upstream of rivers with sand or mud substrate but also in downstream areas in brackish water (Jurniati et al. 2021; De Grave et al. 2013), corroborating our study. It is a migratory freshwater prawn. No *M. lar* was collected downstream of the river, as it mainly inhabits freshwater, but the larvae have to develop in the marine environment (Lal et al. 2014). *Macrobrachium latidactylus* were only found downstream and midstream of the river. This species depends on brackishwater to successfully complete its development, restricting the distribution to river systems that drain to the sea (Mossolin and Bueno 2003).

5. CONCLUSION

This study recorded 11 *Macrobrachium* species in Cairawan River, Antique Province, Philippines, using morphological and morphometric approaches. Genetic characterization using mtDNA confirmed the identity of eight species. The partial COI gene sequences have a sufficient variation for species discrimination. Notably, the COI gene was useful to distinguish between species with similar morphological characters. However, additional molecular markers such as nuclear and microsatellite markers and better taxon sampling will be necessary to resolve some species' sequence quality reads and low nodal support. The species distribution along the river corresponds to their ecological character. It may be necessary to validate the existing species composition in the sampling sites. The data generated from this study can be used for biological conservation and management, as areas of high diversity are important for future conservation efforts. Scientific data was provided to the Local Government Unit to support governance policies in aquatic resource management and conservation. This undertaking could also direct conservation efforts in similar areas supporting high biodiversity. It also provides baseline reference information for genetic studies involving freshwater prawns in aquaculture.

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SUPPLEMENTARY MATERIAL

Below is the link to the electronic supplementary material.
Supplementary file

AUTHOR CONTRIBUTIONS

Romana-Eguia MRR: Conceptualization, Funding Acquisition, Methodology, Project administration, Supervision, Writing – Reviewing and Editing. **Santos MNM:** Conceptualization, Methodology, Analysis, Investigation, Data curation, Writing – Original draft preparation, Writing – Review and Editing, Visualization. **Wowor D:** Methodology, Specimen identification, Analysis, Investigation, Data curation, Writing – Reviewing and Editing. **Ikeda M:** Methodology, Resources, Writing – Reviewing and Editing, Supervision. **Padilla PI:** Writing – Reviewing and Editing

CONFLICTS OF INTEREST

The authors report no conflict of interest with respect to the research, authorship, and publication of this article.

ETHICS STATEMENT

Cairawan River is used for artisanal fisheries, and freshwater prawn fishing is an authorized activity. The collection of samples was coordinated and conducted with the Municipal Agriculturist Office in the Municipality of Laua-an, Antique Province. All of the experimental procedures were conducted in accordance with the institutional guidelines of the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC/AQD) under Study Code Br-01-C2006B. There are no human subjects in this study.

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