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Diversity of freshwater shrimp species of the genus *Macrobrachium* (Decapoda: Caridea) in the big river of East Kalimantan, Indonesia

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Abstract. *Annisa MN, Widayati KA, Jusmaldi, Farajallah A. 2025. Diversity of freshwater shrimp species of the genus* Macrobrachium *(Decapoda: Caridea) in the big river of East Kalimantan, Indonesia. Biodiversitas 26: 1474-1484.* Freshwater prawns are widely distributed in Indonesia, belonging to the subphylum Crustacea and order Decapoda. Freshwater shrimp commonly found in Indonesia belong to the families Atyidae and Palaemonidae. The Palaemonidae family is usually dominated by species of the genus *Macrobrachium.* Information on freshwater shrimp diversity in East Kalimantan, Indonesia, has never been reported, while water quality in East Kalimantan is declining due to increased exploitation of natural resources. This study was conducted to provide baseline information on freshwater shrimp species diversity for the future, before further changes occur in the East Kalimantan region and we provide Cytochrome Oxidase subunit I (COI) sequence data for molecular identification. Methods used for species identification based on morphological characters and species confirmation through molecular analysis using Cytochrome Oxidase subunit I (COI) gene fragments for molecular analysis. This study reports the diversity of freshwater shrimp in East Kalimantan, the results of morphological analysis showed confirmation of 7 species of freshwater shrimp. The family Palaemonidae consists of 7 species: *Macrobrachium rosenbergii, M. lanchesteri, M. scabriculum, M. mammillodactylus, M. sintangense, M. equidens*, and *M. villosimanus*. Molecular confirmation of species of the genus *Macrobrachium* using the COI gene showed genetic distance values ranging from 0 to 0.024. The species in this study have been confirmed based on genetic distance values and phylogenetic trees.

Keywords: COI, DNA barcode, genetic distance, morphology**,** Palaemonidae

INTRODUCTION

Crustacea is a subphylum within the kingdom Animalia, characterized by a high level of species diversity. This subphylum consists of 6 classes, 13 subclasses, 38 orders, and 849 families (Martin and Davis 2001; Krishanthan et al. 2015). The term "Crustacea" originates from the Latin word *crusta*, meaning a hard shell, which refers to the hard-shell crustaceans have due to calcium carbonate deposition in their cuticle. Freshwater crustacean species are divided into two distinct categories: species found exclusively in freshwater and species that can migrate between freshwater, brackish water, and even saltwater environments (Keith 2002; Rahmi et al. 2016). Freshwater crustaceans commonly encountered include freshwater crabs and shrimp. Crustaceans of the order Decapoda are predominantly found in shallow waters and can also be found in freshwater regions (Annawaty et al. 2022).

The Caridea superfamily consists of three families: Palaemonidae, Atyidae, and Alpheidae (Martin and Davis 2001). Among these, the Palaemonidae family, particularly the genus *Macrobrachium* is the most commonly found, and economically important because widely cultivated worldwide (Tantri 2016; Siriwut et al. 2021) with a total of 417 recorded species (WORMS 2024). Freshwater shrimp inhabit various aquatic environments, such as rivers, swamps, and lakes (Wowor et al. 2004). The general *Macrobrachium* contain a relatively large number of species, found in environments ranging from freshwater to brackish water (Susilo et al. 2020). Freshwater shrimp play multiple roles in aquatic ecosystems, including as a component of food webs, scavengers of organic material, and detritus feeders (Fahlevi 2021). The presence of shrimp in a body of water can serve as a bioindicator of the water ecosystem's quality, as these organisms are typically found only in healthy habitats and are sensitive to disturbances from human activities and pollution (Wowor et al. 2004; Taufik 2011).

The life cycle of genus *Macrobrachium* can be divided into two types: amphidromous and land-locked (Irving et al. 2017). The amphidromous group consists of shrimp that migrate to estuarine waters to spawn and amphidromous group has a wide distribution, in contrast the land-locked group spends its entire life cycle in freshwater (Wowor et al. 2004). Generally, shrimp that migrate to brackish waters to spawn have relatively small eggs, and the life cycle of this group is more complex. In comparison, shrimp that spend their entire life cycle in freshwater tend to have larger eggs. These eggs often hatch into more developed larvae, resulting in a shorter life cycle with fewer stages (Rasch and Bauer 2016). An example of an amphidromous species is *M. rosenbergii* (Wowor et al. 2004), while a land-locked species is *M. sintangense* (Said et al. 2014).

The distribution of genus *Macrobrachium* species is wide and varied, depending on the type of shrimp. Several factors influencing the distribution of freshwater shrimp include environmental conditions, food availability, ecological adaptation, and life cycle (Wowor et al. 2004). Some species have been introduced to new areas through the aquarium trade or aquaculture, which can sometimes cause ecological issues in their new habitats (New et al. 2010). For instance, the giant freshwater shrimp *M. rosenbergii* is widely distributed in Southeast Asia, India, and the Indo-Pacific region. This species is often found in rivers, lakes, and freshwater ponds (New et al. 2010).

East Kalimantan, Indonesia, known for its tropical rainforest, is rich in natural resources and has many rivers and lakes. Patang (2019) stated that water quality in East Kalimantan has been declining due to increased exploitation of natural resources, such as mining, deforestation, and infrastructure development. In connection with this, the planned development of the Ibu Kota Nusantara (IKN) in East Kalimantan is suspected to pose a threat to the habitat of several organisms. This is suspected of threatening the habitats of several organisms, potentially disrupting aquatic ecosystems and leading to a decrease in the diversity of various species, including those of the genus *Macrobrachium* (Tchakonte et al. 2014). Given the lack of information on freshwater shrimp in East Kalimantan and the rapid changes occurring in the region, aims to study the diversity of freshwater shrimp species in East Kalimantan. This species list is useful as a database of the current condition of the species composition of the freshwater shrimp in this region also it serves as a resource for conservation efforts.

MATERIALS AND METHODS

Study area

Research was conducted from December 2022 to July 2023 in East Kalimantan, Indonesia. Sampling was carried out in the big river of East Kalimantan, specifically Danau Harapan Baru, Lempake, Pinang Bahari, Karang Mumus, Perjiwa, Bongkok, Tenggarong, Panji, Handil, Babulu, Dondang, and Sesumpun (Figure 1).

Procedures

Samples collection

This study is exploratory used a purposive sampling method, considering information about freshwater shrimp from local communities and the safety of sample collection. We collected samples was conducted in the morning between 7-9 AM at one location, with a once collected. The samples were collected using a hand net with a mesh size of 0.5 cm or by directly catching shrimp by hand. The sampling points were selected based on the researcher convenience in obtaining samples while considering locations inhabited by freshwater shrimp such as between aquatic plants, behind rocks or among leaf litter deposited in rivers. The samples were preserved in 70% alcohol in the field, and then in 96% alcohol in the laboratory (Ng 2016). The specimens were stored in the Animal Physiology and Behavior Laboratory, Department of Biology, Institut Pertanian Bogor, Bogor, Indonesia.

The environmental parameters measured include physical parameters of temperature, and chemical parameters of pH (degree of acidity) were measured using a Multifunction 5in1 Digital Water Quality Tester and Dissolved Oxygen (DO) using a DO meter. The type of substrate and type current freshwater shrimp were found were also observed visually.

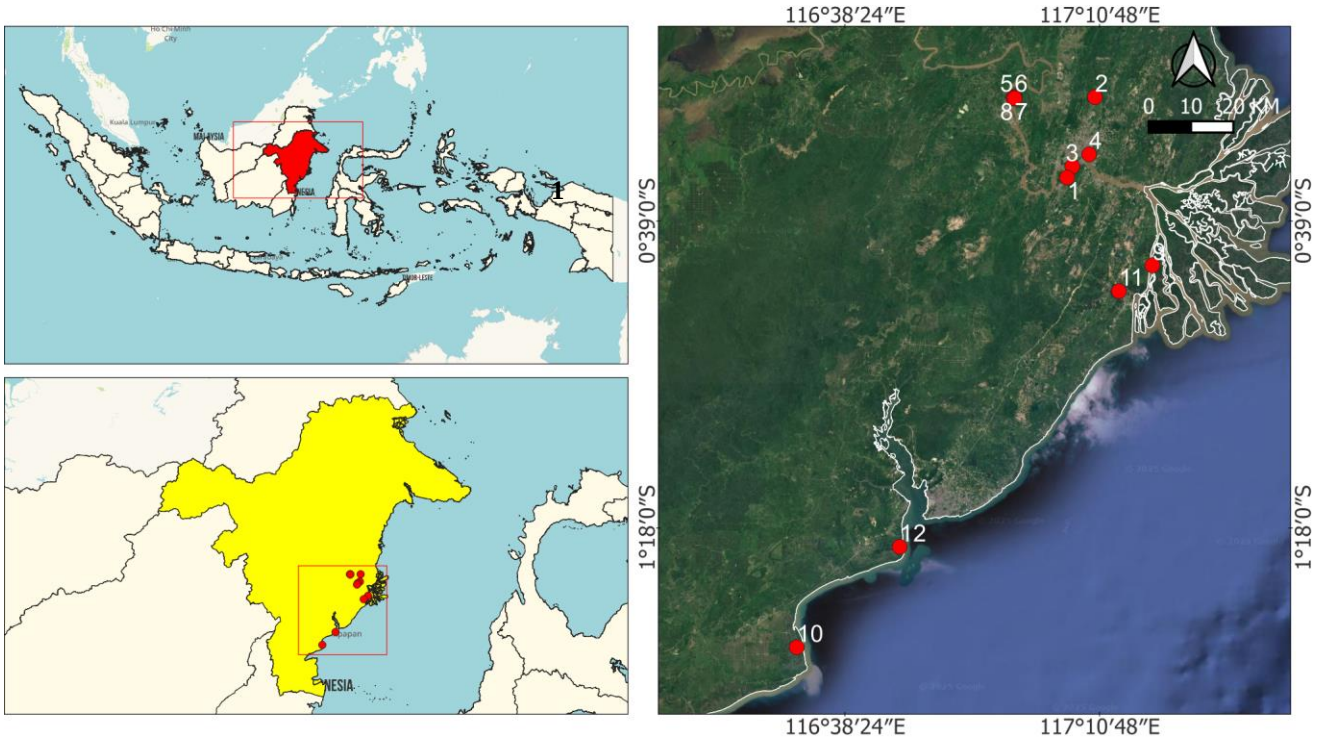

Figure 1. Freshwater shrimp sampling location in East Kalimantan, Indonesia. 1. Danau Harapan Baru, 2. Lempake, 3. Pinang Bahari, 4. Karang Mumus, 5. Perjiwa, 6. Bongkok, 7. Tenggarong, 8. Panji, 9. Handil, 10. Babulu, 11. Dondang, and 12. Sesumpun

Morphological identification

Sample identification was conducted using identification keys from several references: Chace and Bruce (1993), and Wowor et al. (2004). For specimens that were difficult to identify morphologically, molecular analysis was performed. The taxonomic status and distribution of the samples were confirmed Marine Species (http://www.marinespecies.org), and GBIF (http://www.gbif.org).

Molecular identification

DNA barcodes were analyzed using the genomic DNA Mini Kit provided by Geneaid (https://www.geneaid.com) with samples collected from muscle tissue. The Cytochrome Oxidase Subunit I (COI) gene segment from the mitochondrial genome was amplified using forward primer AF286 (5'-TCTACAAAYCATAAAGAYATYGG 3') and reverse primer AF287 (3'- GTGGCRGANGTRAARTARGTCCG 5'). Polymerase Chain Reaction (PCR) was conducted for a total of 30 cycles with an annealing temperature of 54°C. The obtained amplicons were then electrophoresis in a 1% agarose gel at 70V for 25 minutes. The agarose gel stained with FloroSafe dye was then observed under UV light. Amplicons with good results were sent to 1st Base service company for sequencing. We Supply sequence data available in the Data Bank of Japan (DDBJ), for public access. This sequence can be utilized for molecular identification by comparing nucleotide sequences with existing sequence databases and performing statistical significance analysis to confirm species.

Data analysis

The freshwater shrimp sequence data used for molecular analysis are listed in Table 1. The sequences from this study have been submitted to the Data Bank of Japan (DDBJ). The sequences were then edited and analyzed using Molecular Evolutionary Genetics Analysis (MEGA) software version 11 (Tamura et al. 2021). The statistical method used for phylogenetic tree construction was the Maximum Likelihood (ML) method with the Kimura 2-Parameter (K2P) nucleotide substitution model (Kimura 1980). Genetic distance analysis was conducted using the Kimura 2-Parameter model.

RESULTS AND DISCUSSION

Based on morphological identification, freshwater shrimp found in East Kalimantan consist of the family Palaemonidae, which totals seven species (Table 2).

***Macrobrachium rosenbergii* (De Man, 1879)**

Material examined

Indonesia-East Kalimantan: Karang Mumus, Samarinda, East Kalimantan: 20 males (12.9-20.8 mm CL), 16 females (13.4-19.7 mm CL), collected by Maulida on December 28, 2022. Tenggarong, Kutai Kartanegara, East Kalimantan: 2 males (17.4-25.8 mm CL), 2 females (15.6-24.2 mm CL). Handil, Kutai Kartanegara, East Kalimantan: 1 male (17.8 mm CL).

Description

This species has a long rostrum that exceeds the length of the scaphocerite. The rostrum is curved, with the anterior part higher than the posterior. It is equipped with 12-13 dorsal teeth and 11-13 ventral teeth. The first tooth is located approximately one-third or one-fourth proximally. The second pereiopod has fingers with varying sizes in each individual; the fingers are shorter in size. The carpus is longer than the palm and merus and is covered with spinules and large spines, except for the dactylus, which is covered with pubescence. The tips of the fingers have pubescence. In the third pereiopod, the dactylus and carpus vary based on the age and size of the specimen. The telson is medium to large in size and is equipped with 2 pairs of dorsal spines. It has a pre-anal carina.

Table 1. Freshwater shrimp sequence data used for molecular analysis

Spesies	Accession number	Location	References
Macrobrachium rosenbergii	LC843385	Karang Mumus	This study
	LC843384	Tenggarong	This study
	MZ781227	China	Zhong et al. (2021)
	KY865098	China	Li et al. (2019)
Macrobrachium lanchesteri	LC843389	Pinang Bahari	This study
	LC843388	Lempake	This study
	NC012217	Thailand	Ngernsiri dan Sangthong (2009)
	MW845503	Thailand	Siriwut et al. (2021)
Macrobrachium scabriculum	LC843390	Lempake	This study
	LC843391	Bongkok	This study
	MN526237	Indonesia	Hernawati et al. (2020)
Macrobrachium mammillodactylus	LC843392	Tenggarong	This study
	LC843393	Tenggarong	This study
	FM958075	Indonesia	Wowor et al. (2009)
Macrobrachium sintangense	LC843386	Karang Mumus	This study
	LC843387	Karang Mumus	This study
	MW845607	Thailand	Siriwut et al. (2021)
Macrobrachium equidens	LC843394	Babulu	This study
	LC843395	Handil	This study
	ON753700	Hong Kong	Chow et al. (2022)
Macrobrachium villosimanus	LC843396	Babulu	This study
	MW845638	Thailand	Siriwut et al. (2021)
Macrobrachium saigonense	MN200394	Bangladesh	Ahmed et al. (2019)

Table 2. List of freshwater shrimp species genus *Macrobrachium* found in East Indonesia, Indonesia

Species	Locations											
	DHS	L	PB	KM	P	B	T	Pa	H	Ba	D	S
Macrobrachium rosenbergii				●		●	●		●			
Macrobrachium lanchesteri	●	●	●	●			●	●				
Macrobrachium scabriculum		●		●	●	●	●					
Macrobrachium mammillodactylus				●			●					
Macrobrachium sintangense				●								
Macrobrachium equidens							●		●	●	●	●
Macrobrachium villosimanus										●		

Note: DHS: Danau Harapan Baru; L: Lempake; PB: Pinang Bahari; KM: Karang Mumus; P: Perjiwa; B: Bongkok; T: Tenggarong; Pa: Panji; H: Handil; Ba: Babulu; D: Dondang; S: Sesumpun. ●: Present

Remarks

Macrobrachium rosenbergii has been separated into two subspecies: the western *M. rosenbergii schenkeli* and the eastern *M. rosenbergii rosenbergii* (Johnson 1973), supported by Hedgecock et al. (1979), Lindenfelser (1984), and de Bruyn et al. (2004). Holthuis (1950) provided the correct nomenclature for these two subspecies, noting that they should be recognized as *M. rosenbergii* and *M. dacqueti* (Sunier 1925). According to Wowor and Ng (2007), their study (Figure 2.A) identified differences in the carpus of the second pereiopod as distinguishing features between the two. In this species (Figure 2.A), the carpus of the second pereiopod is covered with abundant spinules interspersed with large, widely spaced spines, indicating it as *M. rosenbergii*.

Habitat and ecological information

This large freshwater shrimp, known as the 'Giant River Shrimp' is caught in most major rivers and tributaries and is also locally cultivated for local consumption (Choy 1984). Although *M. rosenbergii* is considered a freshwater species, living in rivers and lakes, it is also commonly found in brackish waters at elevations ranging from 24.2 to 93.9 km above sea level. Large females are rarely collected from freshwater habitats because breeding occurs in estuaries and the sea; after breeding, females return to freshwater, and the cycle repeats several times (Johnson 1973). The larvae migrate upstream, so juvenile shrimps inhabit freshwater until they reach maturity, at which point they migrate downstream to breed.

Distribution

Found along the Huxley Line, in the Philippines, the Lesser Sunda Islands, Papua New Guinea, and northern Australia (Wowor and Ng 2007). Also found in Malaysia, Singapore, and Brunei (Wowor et al. 2004). This study found them in the waters of East Kalimantan.

***Macrobrachium lanchesteri* (De Man, 1911)**

Material examined

Indonesia-East Kalimantan: Danau Harapan Baru, Loa Janan Ilir, East Kalimantan: 65 males (5.3-10.6 mm CL), 22 females (8.4-11.3 mm CL), collected by Maulida on December 29, 2022. Pinang Bahari, Samarinda, East Kalimantan: 42 males (7.2-8.9 mm CL), 19 females (9.5 mm CL), collected by Maulida on December 28, 2022. Karang Mumus, Samarinda, East Kalimantan: 7 males (10.5-14.3 mm CL), 3 females (7.5-8.8 mm CL), collected by Maulida on December 28, 2022. Lempake, Samarinda, East Kalimantan: 2 males (8.9-12.5 mm CL), collected by Maulida on January 12, 2023. Panji, Kutai Kartanegara, East Kalimantan: 4 males (7.6-10.8 mm CL), 4 females (9.3-12.6 mm CL), collected by Maulida on July 11, 2023.

Description

The rostrum is straight and does not exceed the length of the scaphocerite. It is equipped with 6-7 dorsal teeth and 4-5 ventral teeth, with 1-2 apical teeth at the tip of the rostrum. The inferior orbit is moderate and blunt. The second pereiopod has a pair of limbs that are identical in size and shape, with the chela being shorter than the carpus. The palm and fingers are nearly the same length. In the third pereiopod, the merus is longer than the propodus, and the propodus is longer than both the dactylus and carpus. The telson has a pre-anal carina.

Remarks

Macrobrachium lanchesteri was first discovered and described in southern Thailand (De Man 1911) and later reported from central Thailand (Suvatti 1937), Peninsular Malaysia (Chong and Khoo 1988), Singapore, and Sabah (Chong and Khoo 1988; Ng and Choy 1990). This species (Figure 2.B) is characterized by having one or two apical teeth at the tip of the rostrum, separated from the other teeth by a clearly visible gap.

Habitat and ecological information

Macrobrachium lanchesteri is found in a variety of water bodies, from stagnant water to calm and fast-flowing streams, such as lakes, around dams, and along the edges of large rivers at elevations ranging from 62.1 to 100 km above sea level. *Macrobrachium lanchesteri* has the ability to adapt to almost all types of freshwater environments, showing tolerance to low temperatures and low oxygen levels (Aliya et al. 2017).

Distribution

Macrobrachium lanchesteri is known to be distributed in Thailand, Peninsular Malaysia, Singapore, and southern Thailand. It was first recorded in Malaysia by Ng (1995). It was first discovered in Brunei and later in Kalimantan by Wowor et al. (2009). In this study, it was found in the East Kalimantan region.

***Macrobrachium scabriculum* (HelIer, 1862)**

Material examined

Indonesia-East Kalimantan: Karang Mumus, Samarinda, East Kalimantan: 6 males (4.2-8.3 mm CL), 4 females (5.85-12.4 mm CL), collected by Maulida on December 28, 2022. Lempake, Samarinda, East Kalimantan: 2 males (5.85-11.9 mm CL), collected by Maulida on January 12, 2023. Bongkok, Kutai Kartanegara, East Kalimantan: 1 male (5.9 mm CL), collected by Maulida on July 11, 2023. Tenggarong, Kutai Kartanegara, East Kalimantan: 1 male (6.4 mm CL), 2 females (5.8-8.7 mm CL), collected by Maulida on July 11, 2023.

Description

The rostrum is moderately convex, with 13-14 dorsal teeth and 2-3 ventral teeth. Its length is equal to and does not exceed the scaphocerite. This species has a carapace equipped with spinules on the dorsal and branchiostegal regions. The second pereiopod has carpus and merus of equal length, tubular in shape. The palm and fingers are densely covered with spinules, with the palm being longer than the fingers and carpus. The telson lacks a pre-anal carina.

Remarks

Macrobrachium scabriculum was described from Sri Lanka. The current specimens closely match the description provided by Heller (1862). This species has many spinules on the surface of the carapace (Figure 2.C). The second pereiopod has robust carpus and merus, with the palm and the proximal two-thirds of the fingers covered with dense spinules. The fingers are slightly shorter than the palm, and the carpus is shorter than the palm (Figure 2.C).

Habitat and Ecological Information

This species is found in shallow, fast-flowing river areas with large rocks and small rocky substrates at elevations ranging from 62.1 to 96.4 km above sea level. It is also found in river streams with aquatic vegetation (Yeo et al. 1999; Wowor and Choy 2001).

Distribution

Macrobrachium scabriculum is distributed in eastern Africa and Madagascar (Chace and Bruce 1993), as well as the Indo-West Pacific region, including Sri Lanka, southern India, Peninsular Malaysia, Tioman Island, Brunei, Singapore, and the coastal regions of the Indian Ocean from Sumatra to Sarawak on the border of Kalimantan (Wowor and Choy 2001) and Sulawesi (Dwiyanto et al. 2017). In this study, it was found in the waters of East Kalimantan.

***Macrobrachium mammillodactylus* (Thallwitz, 1892)**

Material examined

Indonesia-East Kalimantan: Karang Mumus, Samarinda, East Kalimantan: 2 males (20.2-24.4 mm CL), 3 females (17.6-21.8 mm CL), collected by Maulida on December 28, 2022. Tenggarong, Kutai Kartanegara, East Kalimantan: 2 males (18.2-20.2 mm CL), collected by Maulida on July 11, 2023.

Description

This species exhibits variable rostrum morphology; the rostrum does not exceed the length of the scaphocerite and is equipped with 10-12 dorsal teeth and 4-6 ventral teeth. Antennal scale with straight or concave lateral margins. The first pereiopod has a carpus longer than the chela. The second pereiopod has a pair of limbs of equal length and similar shape; the carpus is longer than the palm, which is subcylindrical in shape. The palm is longer than the fingers, which are covered with spinules and pubescence. In the third pereiopod, the propodus is longer than both the carpus and dactylus; the merus and propodus are of equal length, with the propodus adorned with setae. The telson is equipped with a pair of dorsal spines and has a pre-anal carina.

Remarks

The rostrum (Figure 2.D) has a maximum depth exceeding the diameter of the eye cornea, and the dorsal rostrum is slightly convex above the eyes in *M. mammillodactylus*.

Habitat and ecological information

This species is found in large, fast-flowing rivers at elevations ranging from 62.1 to 93.9 km above sea level, with tolerance to salinity. Mature females require brackish water to hatch their eggs (Jurniati et al. 2021). Specimens (Figure 2.D) are often found hiding among rocks along the banks of large rivers.

Distribution

This species is found in the Philippines, northern Sulawesi, and East Java (Wowor and Choy 2001), and in this study, it was found in East Kalimantan.

***Macrobrachium sintangense* (De Man, 1898)**

Material examined

Indonesia-East Kalimantan: Karang Mumus, Samarinda, East Kalimantan: 4 males (11.3-24.5 mm CL), 4 females (9.35-20.25 mm CL), collected by Maulida on December 28, 2022.

Description

The rostrum is moderately convex and tapering at the tip, longer than the scaphocerite, with 12-13 dorsal teeth and 4-5 ventral teeth. Post-antennular margin on the carapace is rounded. The first pereiopod has a carpus longer than the palm and merus; the palm is longer than the fingers, which have pubescence. The second pereiopod is covered with setae, with limbs of similar shape but different lengths; the merus is shorter than the carpus, the carpus is longer than the palm, and the palm is longer than the fingers, which have pubescence and 2 small teeth at the tips of the fingers. Tubercles are present on each side of the dactylus. In mature shrimp, the cheliped has spinules. The telson has a distolateral tooth and lacks a pre-anal carina.

Remarks

Macrobrachium sintangense was first described from Sintang in the Kapuas Basin, Kalimantan. This species has also been reported in Peninsular Malaysia and Thailand. It has

a short, moderately convex rostrum that does not exceed the tip of the antenna peduncle and prominent pubescence on the second pereiopod fingers, with the proximal half covered with short setae in adults. The specimens found in this study (Figure 2.E) closely match the description (De Man 1898). The fingers (Figure 2.E) exhibit clear pubescence in large males (Holthuis 1950; Aprila et al. 2020).

Habitat and ecological information

Macrobrachium sintangense is found in large rivers and relatively calm flowing rivers at elevations around 62.1 km above sea level. Specimens are often found hiding among tall stacked rocks. Johnson (1966) stated that this species cannot be found in waters with very low oxygen content.

Distribution

Macrobrachium sintangense is distributed first described from Sintang in the Kapuas Basin, and has been reported from many Southeast Asian regions (Holthuis 1950; Ng and Choy 1990), including Borneo (De Man 1898), Thailand (Suvatti 1937; Holthuis 1950; Naiyanetr 1980), Malaysia (Wowor and Choy 2001), Nepal (Kamita 1974), and in this study, it was found in East Kalimantan.

***Macrobrachium equidens* (Dana, 1852)**

Material examined

Indonesia-East Kalimantan: Dondang, Muara Jawa, East Kalimantan: 5 males (12.4-14.7 mm CL), 2 females (13.6-15.9 mm CL), collected by Maulida on December 29, 2022. Handil, Samarinda, East Kalimantan: 3 males (12.6-17.9 mm CL), 1 female (14.7 mm CL), collected by Maulida on December 28, 2022. Babulu, North Penajam Paser, East Kalimantan: 2 males (17.7-24.5 mm CL), 3 females (17.5-22.8 mm CL), collected by Maulida on December 28, 2022.Sesumpun, North Penajam Paser, East Kalimantan: 2 males (18.8-22.7 mm CL), collected by Maulida on January 12, 2023. Tenggarong, Kutai Kartanegara, East Kalimantan: 4 males (14.3-20.7 mm CL), 4 females (13.8-17.5 mm CL), collected by Maulida on July 11, 2023.

Description

This species has a rostrum that curves upward anteriorly; the rostrum has 9-11 dorsal teeth and 5-6 ventral teeth. The carapace has a moderately produced and blunt inferior orbital margin. The second pereiopod has identical shape and length; the carpus width is larger than the palm; the carpus is longer than the palm; the palm is longer than the fingers, which have setae; both palm and carpus have spinules. The telson lacks a pre-anal carina; the uropod is uniformly large.

Remarks

Macrobrachium equidens from Brunei exhibits two forms of rostrum, one curving upward distally and the other straight. According to Holthuis (1950), based on specimens by De Man (1902) from Baram River, both forms are common in samples from Baram River near Sarawak, indicating significant variation in rostral shape. Rostral form is not related to age. A characteristic feature of *M. equidens* (Figure 2.F) includes a moderately produced inferior orbital margin, softly angled, lacking a pre-anal carina, and equally large uropods (Wowor and Choy 2001).

Habitat and ecological information

Macrobrachium equidens is widely distributed in estuaries including mangrove forests. This species prefers brackish water but sometimes spends its remaining life in freshwater at elevations ranging from 10 to 93.9 km above sea level. Mature females require brackish or seawater to hatch their eggs (Riyanto et al. 2015)

Distribution

This species has a broad distribution in the Western Indo-Pacific, from India to southern China, Southeast Asia, Papua New Guinea, New Britain, Solomon Islands, New Caledonia to Fiji (Choy 1984). In this study, it was found in East Kalimantan.

***Macrobrachium villosimanus* (Tiwari, 1949)**

Material examined

Indonesia-East Kalimantan: Babulu, Loa Janan Ilir, East Kalimantan: 5 males (14.9-20.3 mm CL), 2 females (13.9-15.6 mm CL), collected by Maulida on December 29, 2022.

Description

This species has a long and pointed rostrum; it has 7-10 dorsal teeth and 5-6 ventral teeth; the length of the rostrum exceeds the scaphocerite. The first pereiopod has a dactylus shorter than the carpus. The second pereiopod is elongated and slender; it is equipped with a carpus longer than the palm and merus; the palm is longer than the fingers covered with pubescence. The third pereiopod has a propodus longer than the dactylus and carpus. The telson is small and slender, equipped with 1 pair of lateral spines, and has a pre-anal carina.

Remarks

This species resembles *M. rosenbergii* (Cai and Ng 2002), distinguishable by its rostrum structure with fewer dorsal teeth (7-10 vs 11-14) (Figure 2.G). The fourth and fifth pereiopods have spines and pubescence.

Habitat and ecological information

Macrobrachium villosimanus is found widely in estuaries at elevations ranging around 22 km above sea level (Cai and Ng 2002). This species also requires freshwater during its life cycle. Mature females need brackish or seawater to hatch their eggs (Cai and Ng 2002).

Distribution

Kolkata, India; Chittagong, Bangladesh; Yangon, Myanmar (Cai and Ng 2002), and in this study, East Kalimantan.

Discussion

Life cycle and habitat preferences

The distribution of each species collected from aquatic systems in East Kalimantan, including 12 sample sites was analyzed. Among these, *M. rosenbergii* was the most

abundant species, recorded at four sampling sites: Karang Mumus, Bongkok, Tenggarong, and Handil. This species is known to have an amphidromous life cycle (Wowor et al. 2009). *Macrobrachium lanchesteri* was found widely spread in 6 sampling sites namely Danau Harapan Baru, Lempake, Pinang Bahari, Karang Mumus, Tenggarong and Panji. *Macrobrachium lanchesteri* has a landlocked life cycle. However, its increasing tolerance adaptations have led to its classification as an invasive species (Wowor et al. 2004). Species with broad distribution and high tolerance for diverse environmental conditions are often difficult to determine their habitat preferences (Sandriliana 2018). *Macrobrachium scabriculum* was recorded at four sampling sites: Lempake, Karang Mumus, Bongkok, and Tenggarong. These sites were characterized by riverine areas with high vegetation cover. This species exhibits an amphidromous life cycle (Simeon et al. 2014). *Macrobrachium mammillodactylus* was recorded at two sites, Karang Mumus and Tenggarong, while *M. equidens* was recorded at five sites: Tenggarong, Handil, Babulu, Dondang, and Sesumpun. *Macrobrachium villosimanus* was recorded at two sites, Babulu and Sesumpun. All three species *M. mammillodactylus*, *M. equidens*, and *M. villosimanus* are amphidromous and were primarily found in river areas close to seawater (Wowor et al. 2009). In contrast, *M. sintangense* which has a landlocked life cycle, was only found at a single sampling site, Karang Mumus. Its restricted distribution is likely due to its limited dispersal ability (Wowor et al. 2009).

Macrobrachium lanchesteri and *M. rosenbergii* were identified as the dominant species in the rivers and streams of East Kalimantan during this study. Although these two species have different life cycles, they share similar habitat preferences. *Macrobrachium lanchester*i has a landlocked life cycle, occupying freshwater environments with calm currents, distant from sea level. In contrast, *M. rosenbergii* has an amphidromus life cycle, occupying both freshwater and brackish waters with varying currents speeds, from calm to swift. These two species were found to coexist at certain locations, namely at Karang Mumus and Tenggarong. *Macrobrachium sintangense*, *M. equidens*, and *M. villosimanus* are typically found in riverine waters with moderate currents. Both *M. equidens* and *M. villosimanus* are distributed across freshwater and brackish environments. Species that are found to occupy the same location are capable of coexisting, this is supported by environmental conditions that have sufficient food and resource availability to support the survival of shrimp.

Species inhabiting similar currents tend to exhibit comparable morphological characteristics. For instance, *M. sintangense*, *M. equidens*, and *M. villosimanus*, found in locations with moderate flow and sandy substrates, all possess long and slender second pereiopods, an adaptation that aids in their survival (Rahayu and Annawaty 2019). Similarly, *M. scabriculum*, which inhabits areas with swift currents and rocky substrates, has evolved a larger and stronger second pereiopod, facilitating its adaptation to the more turbulent environment. In contrast, *M. lanchesteri*, found in locations with calm flows, exhibits a smaller and more slender second pereiopod, reflecting its adaptation to its tranquil habitat.

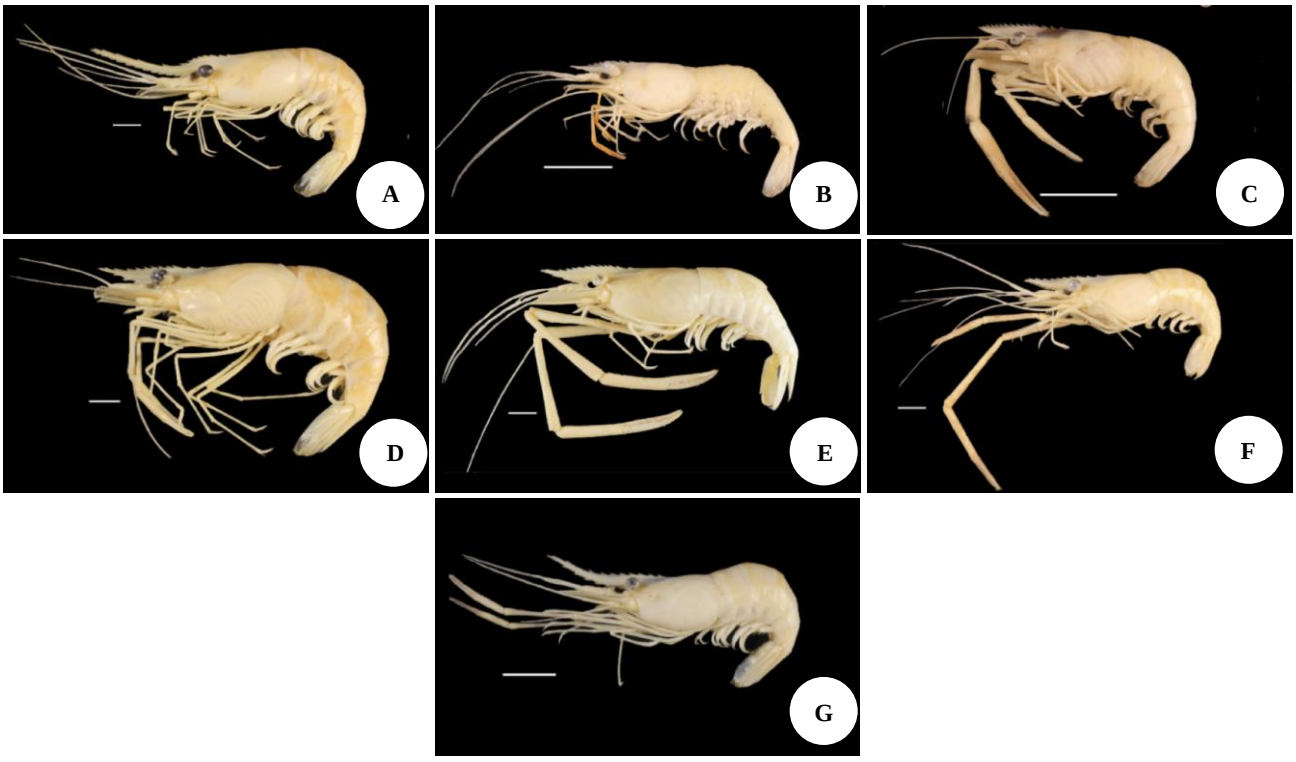

Figure 2. *Macrobrachium* species from East Kalimantan, Indonesia. A. *M. rosenbergii*; B. *M. lanchesteri*; C. *M. scabriculum*; D. *M. mammillodactylus*; E. *M. sintangense*; F. *M. equidens*; G. *M. villosimanus* (Scales: 1 cm)

Environmental conditions of sampling sites

The observed water temperature ranged from 27.4°C to 31°C; water pH ranged from 5 to 8; and DO levels ranged from 6.6 to 8.8 mg/L. Based on the result of this study, the river and lake systems in East Kalimantan display diverse environmental conditions, including variations in substrate type, the presence of aquatic plants, water temperature, pH, and Dissolved Oxygen (DO) levels. Samples were collected from 12 locations, including rivers, reservoirs, and lakes, with surrounding environments ranging from traditional villages, urban areas, gardens, and rice fields at the time of observation. According to Agustina (2016), the presence of freshwater shrimp species is influenced by the surrounding environment. The highest number of freshwater shrimp individuals was found near rice fields and areas without settlements, while the lowest numbers were found around residential areas.

Other observed parameters included substrate types at the sampling locations, which varied rocky, sandy, and muddy soils, with one sampling locations had a combination of these substrate types. The size of sediment particles is related to the organic matter content, with water containing soft sediment having a high percentage of organic matter due to sediment deposition accompanied by organic matter accumulation (Kate et al. 2016). Coarse sediment, on the other hand, has lower organic content but provides a good substrate for freshwater shrimp to shelter.

The presence of aquatic plants at the sampling locations supports the existence of freshwater shrimp. Freshwater shrimp prefer locations with aquatic plants (Wowor et al. 2004; Purnamasari et al. 2024). Dominant aquatic plants include water algae (*Spirogyra* sp.) and water hyacinth (*Eichornia crassipes*). The presence of aquatic plants in the water is very beneficial for freshwater shrimp, providing good habitat and hiding places.

The observed water temperature ranged from 27.4°C to 31°C; water pH ranged from 5 to 8; and DO levels ranged from 6.6 to 8.8 mg/L (Table 3). Freshwater shrimp from the families Palaemonidae and Atyidae can inhabit various aquatic habitats, such as lakes, rivers, swamps, ponds, and underground rivers with varying water flow speeds (Wowor et al. 2004). Freshwater shrimp in this study were found at temperatures between 27.4°C and 31°C, which is consistent with Nofrita et al. (2015), who reported that the optimal temperature for shrimp survival is between 28 and 31°C, and the ideal pH for freshwater environments ranges from 6.50 to 8.50. DO levels that support freshwater shrimp range from 3 to 7 mg/L (New 2002). In our study, all sampling locations had DO levels between 6.6 and 8.8 mg/L, which can support the survival of shrimp and freshwater shrimp.

Molecular confirmation of genetic distance and phylogenetic

Representatives from each species were analyzed molecularly by comparing genetic distance values with reference sequences from GenBank (Table 4) and using outgroup sequences (*M. saigonense*). Molecular confirmation using the COI gene validate the results of morphological identification. The COI gene fragment used for analysis was 485 bp. The sequences were compared with freshwater shrimp sequences from GenBank to analyze the genetic distance and the phylogenetic tree.

Molecular confirmation of species showed the genetic distance values ranging from 0 to 0.024 (Table 4). The highest genetic distance value was observed in the *M. rosenbergii* sequences from Karang Mumus and Tenggarong compared to *M. rosenbergii* references MZ781227 and KY865098, with genetic distances ranging from 0.0 to 0.024. The lowest genetic distance value was found in the *M. equidens* sequences from Babulu and Handil compared to *M. equidens* references ON753700, and in the *M. lanchesteri* references from Pinang Bahari and Bendungan Lempake compared to *M. lanchesteri* sequences NC012217 and MW845503, with genetic distances ranging from 0.0 to 0.002. The genetic distance among freshwater shrimp species in the subphylum Crustacea using the K2P method is 0-0.05 (Lefébure et al. 2006; Syuhada et al. 2024). The phylogenetic tree of the analyzed species, constructed using the Maximum Likelihood (ML) method (Figure 3), formed clades based on species and were separate from *M. saigonense* as outgroups. *Macrobrachium* species in this study were confirmed based on genetic distance values and the phylogenetic tree (Figure 3).

Table 3. Conditions of 12 sampling sites *Macrobrachium* species from East Kalimantan, Indonesia

Location	Water temperature (°C)	pH	Dissolved oxygen levels (DO) (mg/L)	Substrate type	Current type
Danau Harapan Baru	30.8	6.8	6.7	Mud	Calm
Lempake	27.4	6.9	6.9	Cobble	Strong
Pinang Bahari	30.2	6.5	6.5	Mud	Calm
Karang Mumus	28.6	5.5	6.6	Sand	Moderate
Perjiwa	28	7	8	Cobble	Strong
Bongkok	29.5	6.5	8.6	Sand	Calm
Tenggarong	30	6.8	8	Sand	Strong
Panji	28	6.5	8.6	Mud	Calm
Handil	29	5.5	6.6	Mud	Moderate
Babulu	31	5.9	7.5	Mud	Calm
Dondang	31	6	7.5	Mud	Moderate
Sesumpun	29	6.5	7.2	Mud	Moderate

Table 4. The genetic distance between different species of freshwater shrimp using K2P

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	23
*M. rosenbergii*_MZ781227_China																						
*M. rosenbergii*_KY865098_China	0.000																					
*M. rosenbergii*_LC843384_Tenggarong	0.004	0.004																				
*M. rosenbergii*_LC843385_Karang Mumus	0.026	0.026	0.024																			
*M. sintangense*_MW845607_Thailand	0.182	0.182	0.185	0.185																		
*M. sintangense*_LC843386_Karang Mumus_10_1	0.180	0.180	0.182	0.182	0.002																	
*M. sintangense*_LC843387_Karang Mumus_13	0.185	0.185	0.188	0.188	0.002	0.004																
*M. lanchesteri*_NC012217_ Thailand	0.184	0.184	0.181	0.181	0.202	0.199	0.199															
*M. lanchesteri*_MW845503_Thailand	0.184	0.184	0.181	0.181	0.202	0.199	0.199	0.000														
*M. lanchesteri*_LC843388_Lempake	0.184	0.184	0.181	0.181	0.202	0.199	0.199	0.002	0.002													
*M. lanchesteri*_LC843389_Pinang_Bahari	0.178	0.178	0.175	0.175	0.196	0.193	0.193	0.004	0.004	0.006												
*M. scabriculum*_MN526237_East Java	0.199	0.199	0.202	0.204	0.164	0.162	0.164	0.243	0.243	0.243	0.237											
*M. scabriculum*_LC843390_Lempake	0.187	0.187	0.190	0.193	0.164	0.162	0.164	0.237	0.237	0.237	0.231	0.009										
*M. scabriculum*_LC843391_Bongkok	0.193	0.193	0.196	0.199	0.162	0.159	0.162	0.243	0.243	0.243	0.237	0.006	0.011									
*M. mammillodactylus*_FM958075_East Kalimantan	0.220	0.220	0.223	0.226	0.183	0.186	0.183	0.234	0.234	0.237	0.228	0.207	0.201	0.204								
*M. mammillodactylus*_LC843392_Tenggarong 1	0.220	0.220	0.223	0.226	0.180	0.183	0.180	0.237	0.237	0.240	0.231	0.201	0.195	0.198	0.009							
*M. mammillodactylus*_LC843393_Tenggarong 2	0.220	0.220	0.223	0.226	0.180	0.183	0.180	0.237	0.237	0.240	0.231	0.201	0.195	0.198	0.009	0.000						
*M. equidens*_ON753700_Hong kong	0.210	0.210	0.210	0.213	0.190	0.187	0.187	0.190	0.190	0.190	0.187	0.191	0.182	0.191	0.196	0.190	0.190					
*M. equidens*_LC843394_Babulu	0.213	0.213	0.213	0.216	0.190	0.187	0.187	0.193	0.193	0.193	0.190	0.191	0.182	0.191	0.199	0.193	0.193	0.002				
*M. equidens*_LC843395_Handil	0.213	0.213	0.213	0.216	0.190	0.187	0.187	0.193	0.193	0.193	0.190	0.191	0.182	0.191	0.199	0.193	0.193	0.002	0.000			
*M. villosimanus*_MW845638_Thailand	0.224	0.224	0.227	0.227	0.210	0.207	0.210	0.218	0.218	0.218	0.218	0.214	0.214	0.211	0.262	0.266	0.266	0.257	0.257	0.257		
*M. villosimanus*_LC843396_Babulu	0.228	0.228	0.231	0.231	0.210	0.207	0.210	0.210	0.210	0.210	0.210	0.220	0.214	0.217	0.238	0.244	0.244	0.242	0.242	0.242	0.053	
*M. saigonense*_MN200394_Bangladesh	0.198	0.198	0.195	0.204	0.171	0.169	0.171	0.210	0.210	0.210	0.207	0.179	0.174	0.182	0.224	0.218	0.218	0.212	0.212	0.212	0.201	0.190

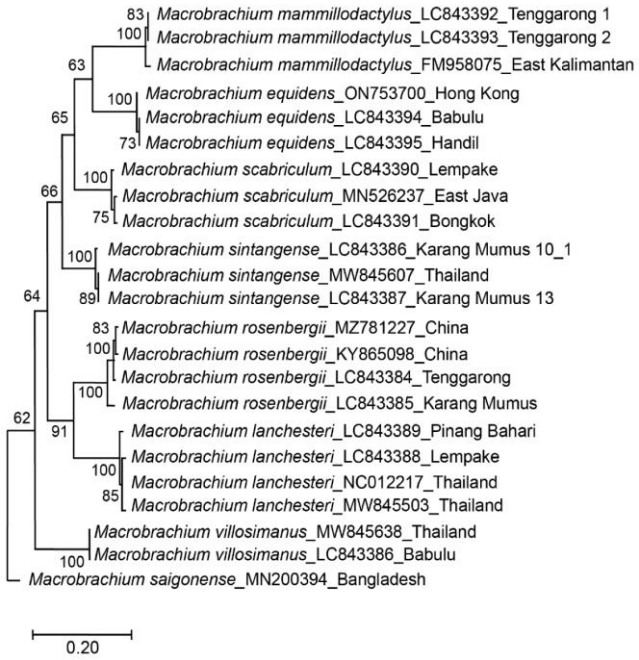

Figure 3. Species differentiation was analyzed using Maximum Likelihood (ML) based on COI gene fragments from seven *Macrobrachium* species found in East Kalimantan, Indonesia, with *Macrobrachium saigonense* used as the outgroup

In conclusion, the results seven species of freshwater shrimp distributed in East Kalimantan have been confirmed family Palaemonidae: *Macrobrachium rosenbergii*, *M. lanchesteri*, *M. scabriculum*, *M. mammillodactylus*, *M. sintangense*, *M. equidens*, and *M. villosimanus.* In the future, research on freshwater shrimp in East Kalimantan should focus on areas with stagnant water far from the coast and in highland regions to discover different species.

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