

NEW RECORD SPECIES OF GIANT FRESH WATER SHRIMP FROM THE DOWNSTREAM OF SERAYU RIVER

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ABSTRACT

Indonesia is characterized by great diversity of freshwater shrimp, consisting of several islands due to the geographical location on the equator. A recent investigation conducted in Central Java has identified Serayu River as the major habitat of freshwater shrimp genus *Macrobrachium*. Therefore, this research aimed to determine the type of giant freshwater shrimp found downstream of Serayu River using explanatory method. The experiment was conducted for one year (January - December 2023) and the results found two freshwater giant prawn species, namely *Macrobrachium rosenbergii* and *Macrobrachium spinipes*. Moreover, this research is the first record of *M. spinipes* based on the Cytochrome C oxidase Subunit I (CO1) gene. The mtDNA segment of *M. rosenbergii* was 631 bp (base pairs) (GenBank accession: LC813231.1), while *M. spinipes* was 616 bp (GenBank accession: PP735157.1). *M. spinipes* and *M. rosenbergii* downstream of Serayu River showed similar locations and characteristics. Genetic distance conducted on 15 species of the genus *Macrobrachium* showed that giant freshwater prawn serayu had a matrix value of 0.09 - 0.18. Genetic analysis conducted with GenBank indicated that *M. spinipes* showed significant differences from other species, suggesting the natural dispersal of juvenile through currents across Wallace Line. This research also found that *M. rosenbergii* was similar to *M. dacqueti* and related to *M. malcolmsonii*, while *M. spinipes* was close to *M. suphanense*. To support these results, recommendation was made to explore further the rivers in the western part of Java, Indonesia.

Key words: Bio geography, DNA Barcoding, *M. rosenbergii*, *M. spinipes*, Serayu River, life below water

بارونا وأخرون

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تسجيل جديد لروبيان المياه العذبة العملاق من مصب نهر سيرايو

⁵ سابوترا	⁴ سوديبيا	³ سابدانينغسيه	² س برايتنو	¹ بارونا ك
أستاذ مساعد	أستاذ مساعد	أستاذ مساعد	أستاذ	باحث
إدارة الموارد بجامعة دييونيفور ^{3,5}			استزراع الأحياء المائية بجامعة سوديرمان ¹	
استزراع الأحياء المائية بجامعة دييونيفور ^{2,4}				

المستخلص

يوجد في إندونيسيا تنوع كبير لروبيان المياه العذبة. ويرجع ذلك إلى موقعها الجغرافي على خط الاستواء وتكوينها من عديد الجزر. نهر سيرايو هو أحد الأنهار الكبيرة في جاوة الوسطى وهو شيمة لجنس الروبيان *Macrobrachium*. الهدف من هذا البحث هو تحديد أنواع روبيان المياه العذبة العملاق الموجود في مجرى نهر سيرايو. الطريقة المستخدمة في هذا البحث هي الطريقة الاستكشافية. تم إجراء البحث في اتجاه مجرى نهر سيرايو. مدة البحث سنة واحدة (يناير - ديسمبر 2023). وجدت نتائج البحث نوعين من الروبيان العملاق الذي يعيش في المياه العذبة، وهما *Macrobrachium rosenbergii* و *Macrobrachium spinipes*. هذا البحث هو أول سجل لـ *M. spinipes* استنادًا إلى جين السيتوكروم سي أو أكسيداز الوحدة الفرعية (CO1). كان مقطع *M. rosenbergii* mtDNA في هذه الدراسة 631 نقطة أساس (أزواج أساسية) (انضمام GenBank: LC813231.1) بينما كان الجزء الخاص بـ *M. spinipes* 616 نقطة أساس (انضمام GenBank: PP735157.1). يُظهر *M. rosenbergii* و *M. spinipes* في نهر سيرايو السفلي نفس الموقع والخصائص. أظهرت المسافة الوراثية التي أجريت على 15 نوعًا من جنس *Macrobrachium* أن روبيان المياه العذبة العملاق *Serayu* لديها قيمة مصفوفية تبلغ 0.09 - 0.18. يُظهر التحليل الوراثي الذي تم إجراؤه والمطابقات مع GenBank أن أشواك *M. rosenbergii* تختلف عن الأنواع الأخرى. يشير هذا إلى الانتشار الطبيعي ليرقات *M. spinipes* عبر التيارات عبر خط الـ *M. spinipes* هو الأقرب إلى *M. Suphanense*. ومن المهم جدًا إجراء مسح للأنهار في غرب جزيرة جاوة بإندونيسيا لدعم هذه النتائج.

الكلمات المفتاحية: الجغرافيا الحيوية، ترميز الحمض النووي، *M. rosenbergii*، *M. spinipes*، نهر سيرايو، الحياة تحت الماء

INTRODUCTION

Rivers have a very vital role in life (6). Rivers can be used for industrial, agricultural, and fisheries (55; 39). One of the rivers in Central Java Province is the Serayu River. This river has a length of 153 km, crosses 5 districts, and empties into Cilacap Regency (52). This river is commonly used to irrigate rice fields. Given the importance of this river for people's lives, it is also very important to know the biodiversity of aquatic organisms in the Serayu River. One of the aquatic organisms present in this river is the giant freshwater shrimp. Macrobrachium is a highly diverse group of freshwater shrimp found in the Indo-Pacific region (13). The genus includes approximately 240 identified species, where more than 100 are found in East and Southeast Asia (3). It is also known as one of the freshwater crustacean species with high species variation (63). The comprehensive geographical distribution of the species has shown significant morphological and life history variation, with the most differences found on both sides of Wallace Line, which divides Asian and Australasian fauna (49). Proposed that freshwater shrimps be recognized as two distinct subspecies: *Macrobrachium rosenbergii* (sensu stricto), which is distributed in eastern Indonesia, New Guinea, Australia and the Philippines, and *Macrobrachium rosenbergii dacqueti* (Sunier, 1925), which is found in Sunda Southeast Asia, Indochina and South Asia. However, Wowor and Ng (64) provided a different classification, suggesting that the two subspecies should be separately considered due to the non-overlapping geographical distributions and distinctive morphological characteristics. These include relative height of the rostral base, shape of the second to fifth pereopods in adult shrimp, the shape of the fourth thoracic sternite, and the coloration pattern in live shrimp. Wowor and Ng (65) stated the confusion in naming *Palaemon spinipes*, initially considered the same as *Macrobrachium wallacei*. To clarify the confusion, further investigation by Ng and Wowor (35) identified *P. spinipes* as species with attractive color, which is often caught and traded as an ornamental fish. This led to the division of the genus Macrobrachium into two

species based on Wallace Line, comprising west and east species, namely *M. rosenbergii* and *M. spinipes*. In Indonesia, the large number of Macrobrachium has the potential to remain molecularly unidentified, showing the need for DNA barcoding to identify cryptic and undescribed species (556). Species identification using DNA provides several benefits in biological science, where cytochrome c-oxidase subunit I (COI) is commonly used as the standard gene. COI gene has been proven to provide convenience in amplifying and providing information to identify organisms at the species level. Very little is known about freshwater shrimp in the Serayu River. According to Siregar (51), 6 species of Macrobrachium were found in the tributaries of the Serayu River (Pelus River and Banjaran River). According to Kusbiyanto (27), the last study left 4 species found in the tributary of the Serayu River (Banjaran River). Both references above identify freshwater shrimp in the upstream tributaries of the Serayu River. However, the genus Macrobrachium's existence in the river's lower reaches has yet to be widely discussed. This study aims to determine the type of freshwater prawns found in the lower reaches of the Serayu River. Information in this study is very important and will be needed in the development of science. Similar research has never been conducted at this location. However, crustacean larval DNA research has been conducted by Winarni et al. (61), whose research location is close but has a different upstream and ecology.

MATERIALS AND METHODS

Study Area: This research was conducted for one year (January to December 2023) downstream of Serayu River with coordinates -7.525722 S, 109.200998 E to -7.579252 S, 109.148062 E (Figure 1). Shrimp were captured at night (18.00-24.00) and morning (06.00-10.00), using nets, fishing, and traps. Captured shrimp were put in an aerated coolbox and taken to the laboratory for maintenance as well as morphological identification. The collection results were morphologically identified to determine giant freshwater shrimp species that were caught. Meat tissue samples (± 10 g) were preserved in separate 50 mL bottles containing 96% ethanol technical and labeled

homology matching. Based on analysis, identified sequences were arranged using CLUSTAL Omega for multiple sequence arrange. Phylogenetic analysis was performed using MEGA X software, with phylogenetic trees constructed using the maximum parsimony method. Confidence in phylogenetic tree topology was evaluated through bootstrap analysis with 1000 replicates to provide an in-depth view of the evolutionary relationships among species in the genus *Macrobrachium*.

RESULTS AND DISCUSSION

Morphological Identification

This research successfully identified two species of giant freshwater shrimp, namely *M. rosenbergii* and *M. spinipes*, as shown in [Figure 2](#). Therefore, 20 *M. rosenbergii* and 20 *M. spinipes* were successfully collected downstream of Serayu River. This is consistent with the characteristics of *M. rosenbergii* as done by Wowor and Ng (64) and *M. spinipes* Ng and Wowor (35).

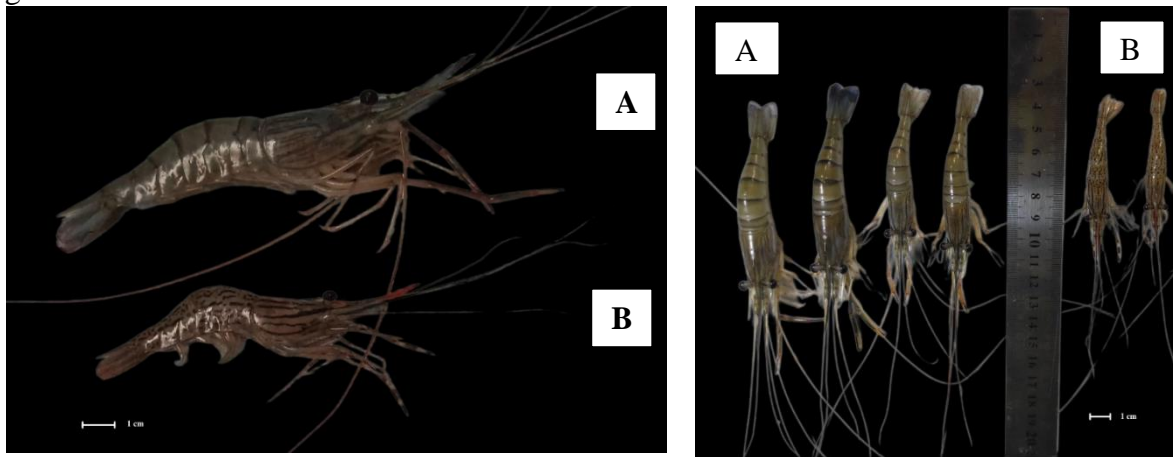


Figure 2. Giant Freshwater prawn is found downstream of Serayu River. A) *M. rosenbergii* and B) *M. spinipes*

The results of morphological identification showed significant differences in juvenile *M. rosenbergii* and *M. spinipes*, which included

rostral teeth, rostrum, cephalothorax, body, and pole claw color, as presented in [Table 1](#):

Table 1. Differences between *M. rosenbergii* and *M. spinipes* juvenile.

Characters	Juvenile <i>M. spinipes</i>	Juvenile <i>M. rosenbergii</i>
Rostral ventral teeth	6 - 16 with mode 12	6 - 15 with mode 9
Rostral dorsal teeth	8 - 15 with mode 13	8 - 14 with mode 12
Rostral	Lower rostral crest	Higher rostral peak
Color of rostrum	There is a red line from the base to the tip of the rostrum	Plain grey
Color of cephalothorax	Reddish, there is a longitudinal solid black line	Gray has faint longitudinal black lines
Body color	Reddish, there is a dotted black line longitudinal to the base of the tail	Gray with transverse lines in each segment
Claw color	There is a black and white look pattern on the tip of the claw	Grey and occasionally a slight reddish tint to the segments

Giant Freshwater prawn DNA Barcode

The results of the molecular analysis in [Table 2](#) showed that the mtDNA segment of *M. rosenbergii* in this research was 631 bp (base pairs) (GenBank accession: LC813231.1), with the percentage of nucleotides T(U): 26.49%, C: 26.49%, A: 26.67%, and G: 20.18%. Meanwhile, the mtDNA segment of *M.*

spinipes was 616 bp (GenBank accession: PP735157.1), comprising T(U): 28.60%, C: 23.68%, A: 29.12%, and G: 18.60%. The average nucleotide composition of the genus *Macrobrachium* used as a comparison was T(U): 28.05%, C: 24.97%, A: 28.04%, G: 18.95% ([Table 3](#)).

Table 2. DNA barcoding of giant freshwater prawns in the lower reaches of the Serayu River

DNA Barcoding of <i>M. spinipes</i> Serayu PP735157.1
AGTGGGCACATCTCTAAGACTACTAATCCGAGCAGAAGCTAGGCCAACCCAGGAAGACTGATTGGAAAT GACCAAATTTATAACGTTATTGTTACAGCACATGCCTTTGTAATAATTTTCTTTATAGTAATGCCTATT ATAATTGGCGGCTTTGGTAATTGACTAGTGCCCCTAATACTAGGGGCACCAGATATAGCATTTCACG AATAAACAAACATAAGATTTTGACTTCTACCACCCTCACTCACACTACTTCTATCAAGAGGAATAGTAGA GAGAGGAGTGGGTACAGGATGAACCGTATACCCCCCTCTAGCAGCAGGAAGCTGCTCACGCGGGAGCT TCAGTAGACCTAGGAATTTTCTCCCTCCACTTAGCAGGTGTTTCTTCAATTCCTAGGAGCCGTCATTTT ATTACTACTGTAATCAACATACGATCGCCAGGAATAACAATAGACCGCCTACCTTTATTTGTGTGAGC TGTATTTTAAACAGCCATTCTTCTTCTCTATCACTCCCAGTTCTAGCAGGAGCTATTACCATACTTTT AACAGACCGAAATCTAAACACATCATTCTTTGACCCGGCCGGAGGTGGTGACCCAATCCTTTACCAAC AC
DNA Barcoding of <i>M. rosenbergii</i> Serayu LC813231.1
GGTAGGTACGTCACCTAAGACTCTTAATTCGAGCAGAATTAGGGCAGCCGGGCAGACTGATCGGAAAT GACCAAATCTACAACGTAATTGTCACTGCCACGCATTTCGTAATAATTTTTCATGGTTATACCGATC ATAATTGGTGGTTTTCGGTAATTGACTAGTACCCTAATATTAGGGGCCAGACATAGCATTCCCACG CATAAACCAACATAAGATTCTGACTCCTACCCCTACTAACAACCTTCTCTCCAGAGGAATAGTAGA AAGAGGGGTTGGCAGGATGAACCTGTTTATCCACCCTAGCAGGCTGGTACCGCCACGCCGGGGCA TCGGTAGATCTAGGTATTTTTTCCCTCCACCTAGCAGGAGTTTCTTCAATCTTAGGGGCTGTCAACTTT ATTACCACAGTGATTAACATACGAGCCCCAGGAATAACTATAGATCGACTGCCCTATTCGTATGAGC CGTATTTCTAACAGCCATCTGCTTCTTCTCTCACTACCAGTTTTAGCCGGAGCCATTACCATACTCTT AACTGATCGAAACCTAAATACATCCTTTTTTCGACCCGGCGGGAGGAGGGACCTATTCTTACCAAC AC

Table 3. Nucleotide composition of the genus Macrobrachium used in this research

GenBank Code and Species	T(U)	C	A	G
PP735157.1 <i>M. spinipes</i>	28.60	23.68	29.12	18.60
LC813231.1 <i>M. rosenbergii</i>	26.67	26.49	26.67	20.18
MK782941.1 <i>M. rosenbergii</i>	26.67	26.49	26.67	20.18
MT235937.1 <i>M. sintangense</i>	27.02	24.91	28.77	19.30
MF622028.1 <i>M. suphanense</i>	30.70	22.63	28.77	17.89
MK412772.1 <i>M. nipponense</i>	29.47	22.46	29.47	18.60
MW845505.1 <i>M. lanchesteri</i>	30.88	22.28	28.77	18.07
OM273995.1 <i>M. malcolmsonii</i>	27.37	24.91	29.12	18.60
FM958058.1 <i>M. asperulum</i>	30.35	21.40	30.35	17.89
MT235944.1 <i>M. neglectum</i>	28.42	24.04	28.77	18.77
JF792432.1 <i>M. dacqueti</i>	26.67	26.49	27.37	19.47
MN526206.1 <i>M. esculentum</i>	25.96	26.67	27.72	19.65
ON753700.1 <i>M. equidens</i>	28.95	25.44	27.89	17.72
MW845507.1 <i>M. latidactylus</i>	28.60	26.49	25.61	19.30
MW845472.1 <i>M. forcipatum</i>	24.39	30.18	25.44	20.00
Average	28.05	24.97	28.04	18.95

Notes: The red color is the nucleotide composition of Macrobrachium.

Identification of Similarity

The maximum genetic divergence to the reference species in GenBank or BOLD system is less than the predetermined value of 3% (61). These results are consistent with previous decisions on the taxonomic status of the samples, which show identical characteristics to similar references in the

database. Match results with GenBank showed that *M. spinipes* in Serayu River had an identity value of 95.62%, but BOLD system showed no significant value. Meanwhile, *M. rosenbergii* with an identity value of 100% was considered true, as shown in [Table 4](#).

Table 4. Similarity of the genus Macrobrachium downstream of Serayu River

Reference and accession number	GenBank		BOLD	
	Query Coverage (%)	Identity (%)	Similarity (%)	Results Summary
<i>M. spinipes</i> PP735157.1	100	95.62	-	No match
<i>M. rosenbergii</i> LC813231.1	100	100	100	<i>M. rosenbergii</i>

Giant Freshwater Prawn Phylogenetics

Phylogenetic tree reconstruction results show that *M. spinipes* found downstream of Serayu River is most closely related to *M.*

suphanense. Similarly, *M. rosenbergii* from this research has the same bootstrap value, with *M. dacqueti* showing a close relationship with *M. malcolmsoni*, as presented in [Figure 3](#).

Genetic Distance

Genetic distance refers to the results of genetic differences between species. The analysis conducted on 15 species of the genus *Macrobrachium* shows a matrix value of 0.09 - 0.18, with the closest distance in *M.*

suphanense (0.09) and the farthest distance in *M. rosenbergii* (0.18), as shown in Table 6. Previous research showed that smaller genetic distance value correlated with more similar COI partial genes between species (8).

Table 6. Genetic distance of the genus *Macrobrachium* based on COI gene.

GenBank Code and Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PP735157.1 <i>M. spinipes</i>	*	*	*	*	*	*	*	*	*	*	*	*	*	*
LC813231.1 <i>M. rosenbergii</i>	0.18	*	*	*	*	*	*	*	*	*	*	*	*	*
MK782941.1 <i>M. rosenbergii</i>	0.18	0.00	*	*	*	*	*	*	*	*	*	*	*	*
MT235937.1 <i>M. sintangense</i>	0.09	0.18	0.18	*	*	*	*	*	*	*	*	*	*	*
MF622028.1 <i>M. suphanense</i>	0.09	0.18	0.18	0.10	*	*	*	*	*	*	*	*	*	*
MK412772.1 <i>M. nipponense</i>	0.12	0.18	0.18	0.11	0.13	*	*	*	*	*	*	*	*	*
MW845505.1 <i>M. lanchesteri</i>	0.15	0.14	0.14	0.18	0.15	0.16	*	*	*	*	*	*	*	*
OM273995.1 <i>M. malcolmsonii</i>	0.15	0.14	0.14	0.17	0.17	0.16	0.14	*	*	*	*	*	*	*
FM958058.1 <i>M. asperulum</i>	0.15	0.18	0.18	0.15	0.14	0.17	0.15	0.15	*	*	*	*	*	*
MT235944.1 <i>M. neglectum</i>	0.12	0.16	0.16	0.14	0.13	0.12	0.15	0.15	0.15	*	*	*	*	*
JF792432.1 <i>M. dacqueti</i>	0.17	0.02	0.02	0.18	0.17	0.18	0.14	0.13	0.18	0.16	*	*	*	*
MN526206.1 <i>M. esculentum</i>	0.14	0.18	0.18	0.15	0.16	0.13	0.18	0.15	0.16	0.14	0.17	*	*	*
ON753700.1 <i>M. equidens</i>	0.16	0.17	0.17	0.15	0.16	0.17	0.16	0.18	0.16	0.16	0.17	0.16	*	*
MW845507.1 <i>M. latidactylus</i>	0.15	0.18	0.18	0.17	0.18	0.17	0.19	0.17	0.19	0.16	0.17	0.17	0.18	*
MW845472.1 <i>M. forcipatum</i>	0.17	0.18	0.18	0.17	0.18	0.17	0.20	0.19	0.19	0.17	0.18	0.18	0.18	0.17

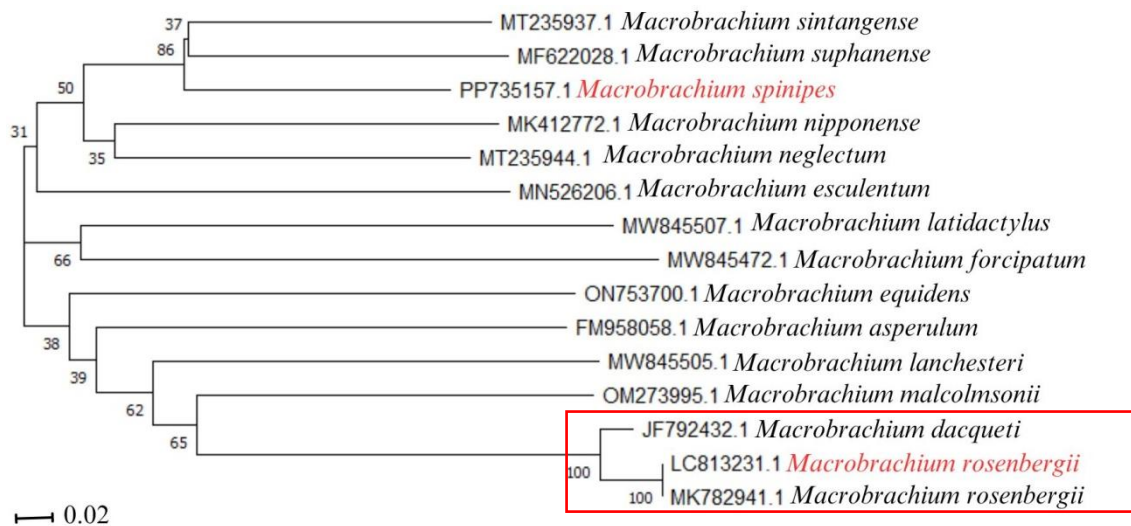


Figure 3. Phylogenetic tree of giant freshwater shrimp downstream of Serayu River, Indonesia. The red box line shows that *M. rosenbergii* is the same as *M. dacqueti*.

Habitat

M. spinipes and *M. rosenbergii* downstream of Serayu River show similar locations and characteristics. Both shrimp migrate to brackish water to spawn, and juvenile return upstream (57). Rivers with muddy and sandy

characteristics dominate habitat characteristics in freshwater downstream of Serayu River. However, the characteristics of Serayu River estuary are dominated by sandy and muddy waters, as shown in Figure 4.

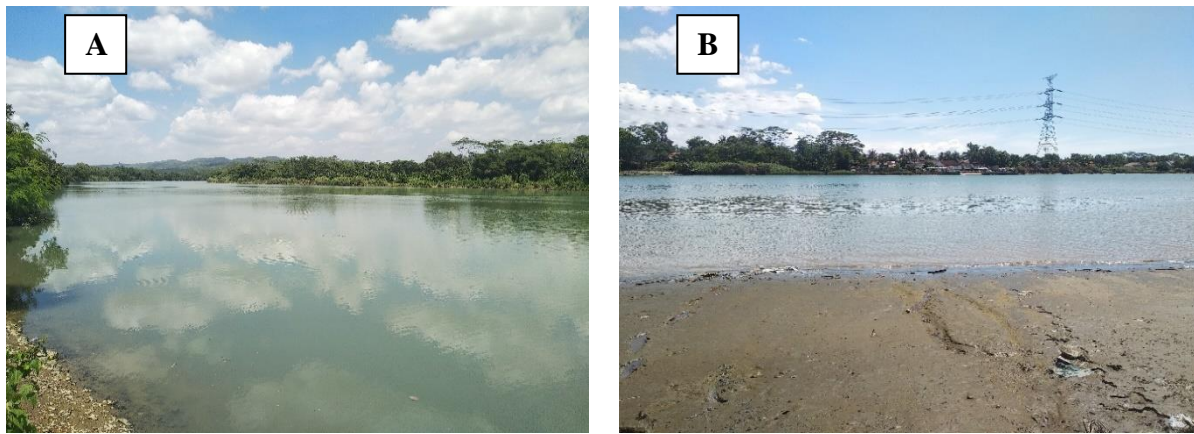


Figure 4. Habitat of *M. rosenbergii* and *M. spinipes*. A. Downstream of Serayu River with fresh water; B. Downstream of Serayu River with brackish water.

The distribution of *M. spinipes* requires detailed exploration, as previous investigations focus on the pattern by dividing *M. rosenbergii* and *M. spinipes* based on Wallace Line (50). Wallace region is an essential biogeographic zone that includes Sulawesi, Lombok, Sumbawa, and Flores. It also includes other small islands located between Sundaland such as Malay Peninsula, Sumatra, Kalimantan, Java, and Bali, as well as the Sahul region comprising Australia and Papua New Guinea (21). Wallace Line passes through the Lombok Strait, between Bali and the Lombok Islands, and through Makassar Strait, which separates Kalimantan and Sulawesi. The boundary of Wallacea is

marked by Lombok Strait, the deep, and wide Makassar Strait in the west, with Australo-Papuan Shelf in the east (26). This region comprises several islands, including Sulawesi, Lesser Sunda, Halmahera, Buru, Seram, and other small islands. *M. rosenbergii* is found west of Wallace Line and *M. spinipes* is east. The discovery of *M. spinipes* downstream of Serayu River remains complex due to the location of the river in Central Java, west of Wallace Line, compared to the expected pattern based on line separation. Based on previous research, the distribution of *M. rosenbergii* and *M. spinipes* is listed in [Table 5](#) and figure 5.

Table 5. Distribution of *M. spinipes* and *M. rosenbergii* results

<i>M. spinipes</i>		<i>M. rosenbergii</i>	
Research Location	Reference	Research Location	Reference
Australasia Eastern Wallace Line and the Philippines	Short (49); <u>De Bruyn</u> (18); Ng and Wowor (35)	Coastal freshwaters throughout India, northern Australia, and Southeast Asia	Lindenfelser (29)
Daly River in Australia's Northern Territory	Novak (38); Novak (36); Novak (37)	Western Pakistan, southern Vietnam, Southeast Asia, southern Asia, northern Australia, New Guinea, Pacific and Indian Oceans	De Bruyn (18); De Bruyn (17)
Papua New Guinea	Pickering (41)	Halmahera Island, Maluku archipelago, Indonesia	Cai (11)
Fitzroy River, Kimberley, Western Australia	Beesley (9)	East Africa	Kuguru (26)
Fitzroy River, northern Australia	Thorburn (53)	Myanmar	Cai (12)
Fitzroy River, Daly River, Mitchell River Northern Australia	Jackson (25)	South, Southeast Asia, and Indochina	Wowor (62); Wowor (64)
Serayu, Central Java	This research	Serayu, Central Java	This research

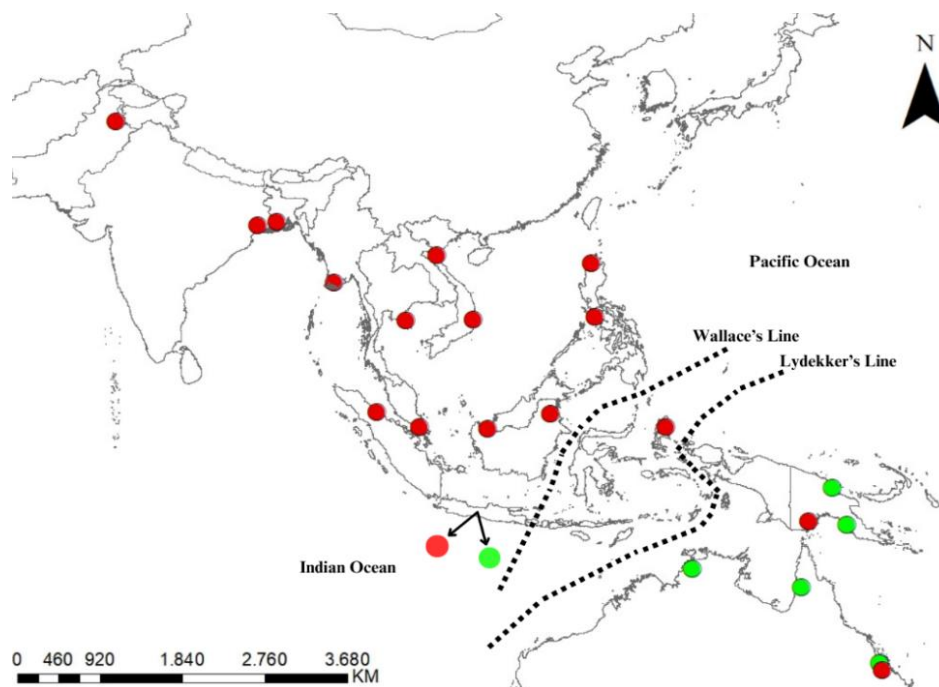


Figure 5. Distribution of Macrobrachium. Red: *M. rosenbergii*; Green: *M. spinipes*

Discussion

Giant freshwater shrimp have gained significant attention across various countries due to the great potential in aquaculture (24). Recent developments show that freshwater shrimp farming contributes to global fisheries in quality and value (34). Morphological identification showed the presence of *M. rosenbergii* and *M. spinipes* downstream of Serayu River. Specifically, *M. spinipes* juvenile have very different coloration and chromatophore patterns, namely red rostrum, transparent body with several longitudinal black lines on the carapace, and black spots on the abdomen. The difference in color patterns in juvenile in these two shrimp has also been described by Shy (50). Based on the results, *M. spinipes* juvenile from Papua Indonesia (male, 46.9 mm cl) and Sulawesi showed identical color patterns, with several longitudinal dark brown lines on the carapace, a red rostrum, and a belly decorated with dark brown spots. As described by Shy (50) in Taiwan, the body coloration of adult *M. spinipes* varied depending on individual size. The body is blue-green in more prominent individuals (carapace length ≥ 50 mm), while yellow-green is observed in smaller individuals (carapace length about ≤ 35 mm). The downstream of Serayu River at the west of Wallace Line have waters with a rocky, sandy bottom, while muddy waters are observed in the estuary. *M. rosenbergii* is distributed west

of Wallace Line, which includes Java Island, showing the natural presence in the river. Wowor and Ng (64) stated that *M. spinipes* should be found east of Wallace Line due to natural dispersal or human intervention such as trade in ornamental fish and the release of wild species (5, 33). De Bruyn (18) also stated that significant genetic differences between species in the Philippines, Indonesia, New Guinea, and Australia were influenced during the Pleistocene. The global climate change caused large sea-level fluctuations, which had a significant impact on the geography and ecology of the Indo-Australian region. At the end of the Pleistocene, approximately 20,000 years and 10,000 years after sea level rise began in the Pacific Ocean, the current level increased in 6000 - 7000 years ago. This event occurred around 7500 - 10,000 years ago for populations in Western and Eastern Indonesia. Sunda region had a shallow continental shelf, which was repeatedly to large islands due to eustatic changes in sea level (60). Therefore, the migration of marine crustaceans to and from the Indonesian Archipelago probably occurred during the last ice age (10,000 - 70,000 years ago) (21). Due to the complications of geology and climate history of transition areas such as Wallace Line regions, it is difficult to conduct an investigation (42), including several smaller biogeographic units (20). Despite the fact that this research indicated currents had an impact

on the dispersal of *M. spinipes* juvenile, it was to consider the great distance to the west Wallace Line. The results showed a strong correlation between larval dispersal as well as distance and duration of each species (23). Research has demonstrated that certain taxa can cross the Wallace Line in both directions. Notable examples include fanged frogs (19) and rodents (43) from the west, as well as avian butterflies (15), beetles (54), and more recently borer beetles (16) from the east. Pelagic larvae of marine organisms living in open water have been found to be crucial to the migration and dispersal of ocean species (45). This is due to the fact that larvae's long-distance capacity facilitates migration to new regions, which facilitates marine biodiversity. The ITF travels from the Pacific to the Indian Ocean, passing through the Makassar Strait and into Indonesia water (46). A tiny amount of the ITF also enters the South China Sea, the Karimata Strait, and the Lifamatola Strait into the Indian Ocean through Lombok, Ombai, and Timor Straits and exits through Sunda Strait in summer. Investigation has observed that the ITF reaches Sumatra (31), showing the significance of the currents on larval dispersal. Previous research established that giant freshwater shrimp larvae were capable of surviving high salinity conditions. According to Novak (38), *M. spinipes* larvae possess greater survival than other Macrobrachium except *M. amazonicum*, which can survive for approximately 11 days. Although the juvenile of *M. rosenbergii* and *M. spinipes* thrive in brackish habitats, the adults of these species are found downstream of the Serayu River in freshwater environments. Amphiboly is a stage in the life cycle of shrimp, where adults in freshwater release juvenile that float to estuarine areas. As part of a life history that is frequently observed in tropical and subtropical fish (1, 39), including crustaceans in rivers (9), these shrimp will transform into juvenile and travel upstream (48). According to Novak (37), recent research has shown that shrimp can release juvenile to approximately 400 km downstream, with the highest concentrations found downstream of Serayu River. *M. spinipes* spawn seasonally, having reproductive activity from November to April and peak in January, with gonad maturation

starting at 80 mm in length. Downstream of Serayu River, there are 435 *M. rosenbergii* and 30 *M. spinipes* recorded during the one-year research. The presence of *M. spinipes* in Segera Anakan River, Central Java, was supported by the results of Winarni et al. (61), which identified Macrobrachium at the site. This river has mangrove forests and is adjacent to Serayu River, with similar ecological characteristics. Sequences can be identified as the same species when a specimen is 98-100% similar to species found in GenBank (7). *M. rosenbergii* of Serayu River is a valid species because it has 100% similarity but not *M. spinipes*, as shown in Table 4. Since this research is the first to register *M. spinipes* in GenBank, there is difficulty in the similarity comparison with *M. spinipes* found east of Wallace Line. According to the standards the genetic distance between the shrimp of the genus Macrobrachium used for comparison with the shrimp from this research was classified as low (0.01-0.09) to medium (0.1-0.99) (Table 6). These genetic distance values showed that partial sequences of CO1 gene could provide a fast and efficient DNA barcoding method for species identification, particularly in overcoming confusion or ambiguity related to morphology (8). Genetic variation has become a source of evolutionary innovation that can facilitate reproductive isolation, thereby forming parapatric speciation (32). The existence of a significant genetic structure in giant freshwater shrimp downstream of Serayu River with other Macrobrachium genera is also influenced by the main ocean currents in each region, such as Kuroshio, Mindanao, Cross Indonesia, and East Australia Current (22). Significant differences were also observed in fiddler crabs (*Austruca perplexa*) (22) on Wallace Line explored reef fish (*Casio cuning*) (2) and found a significant genetic difference between populations in Sumatra and Java in the western part of Indonesian archipelago and populations in Maluku Islands located on the eastern side of Wallace. Previous investigations of *Polycarpa aurata* on coral reefs showed that genetic variation spread across Wallacea region. This research used microsatellite markers and suggested that genetic differences were due to the highly diverse environment

and the short duration of pelagic larvae (58). Significant population patterns have also been observed in other crustaceans in Wallacea region. For example, microsatellite analysis on ribbon crayfish (*Stenopus hispidus*) showed substantial population differences despite a relatively long pelagic larval duration of approximately 210 days (59). Several biological characteristics, such as typical reproductive mode, egg type (benthic or pelagic), variation in reproductive success, as well as the ability of adults to disperse, have been shown to influence patterns of population genetic structure in species (40). Additionally, local adaptation to specific environmental or habitat factors can lead to the evolution of genetically distinct lineages in a species without considering the potential influence of external oceanographic factors and pelagic larval duration (4). Giant freshwater shrimps often release the larvae in the water column which are frequently washed away by the current. The larvae will move and highly depend on the speed of the current, thereby causing minimal genetic differentiation in a population. The wide dispersal of larvae from different marine taxa is a significant factor that can have a major impact on genetic variation, structure, and connectivity (22). Phylogenetic relationships in the genus *Macrobrachium* remain unexplored (30). This research found that *M. rosenbergii* was similar to *M. dacqueti* and closely related to *M. malcolmsonii* (Figure 3). The result was consistent with the research conducted by Kuguru (26) in East Africa using COI and 16S markers. Wowor (63) also observed a close relationship between *M. rosenbergii* and *M. dacqueti*, *M. malcolmsonii*, and *M. lancesteri*, while *M. spinipes* formed a distinct clade closest to *M. suphanense*. The phylogenetic analysis also showed that *M. suphanense* was closely related to *M. sintangense*, as observed by Saengphan (44) in Thailand.

CONCLUSIONS

In conclusion, this research found two species of giant freshwater shrimp downstream of Serayu River were found, namely *M. rosenbergii* and *M. spinipes*. The result showed that mtDNA fragment of *M. rosenbergii* was 631 bp, while *M. spinipes* was 616 bp. The presence of *M. spinipes*

downstream of Serayu River was different from previous theories because Serayu River was located west of Wallace Line. However, there were no matching data based on the match results with GenBank and BOLD. Genetic distances performed on 15 species of the genus *Macrobrachium* showed that giant freshwater shrimp had a matrix of 0.09 - 0.18. Long genetic distances suggested natural dispersal in *M. spinipes* juvenile through currents across Wallace Line. *M. rosenbergii* was similar to *M. dacqueti* and closely related to *M. malcolmsonii* while *M. spinipes* was closest to *M. suphanense*. Additionally, it was observed that natural dispersal in *M. spinipes* juvenile was caused by larval reversal. To strengthen these results, further research was recommended on the presence of *M. spinipes* in the western part of Java Island, Indonesia.

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