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The Impact of Artificial Barriers on the *Varuna litterata* Migration Route in the Lower Serayu River, Central Java and Its Molecular Identification

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ABSTRACT

The Serayu River is one of the longest rivers in Central Java. Dams built in the lower reaches of the Serayu River are thought to block the migration paths of fish and aquatic macrocrustaceans. This study aimed to determine the effect of dam on *Varuna litterata* migration and its molecular identification. This research was conducted using the observation method. Migration observations were made on vertical paths while molecular identification of *V. litterata* in the lower reaches of the Serayu River was compared with existing data in GenBank. The study covered the freshwater area above the Serayu Dam, the open area with freshwater below the Serayu Dam, and the open area with brackish water at the estuary of the Serayu River, with a study length of 33.37 km. The results showed that the presence of dams in the lower reaches of the Serayu River inhibited the migration pattern of *V. litterata*. The vertical shape of the dam causes only a few *V. litterata* to migrate to the top of the dam. The dam in the lower reaches of the Serayu River resulted in not many *V. litterata* being able to migrate to the top of the dam, so there were very few *V. litterata* found in the population upstream of the dam (115 ind). The results of molecular identification of *V. litratata* using the cytochrome C oxidase subunit I (COI) gene show that this crab is 100% the same as the data in GenBank and is closely related to *Varuna yui* and other Varunidae crab species.

Keywords: Dams, Molecular, Migration, Serayu River, Varuna litterata***.***

ABSTRAK

Sungai Serayu merupakan salah satu sungai terpanjang di Jawa Tengah. Bendungan yang dibangun di bagian hilir Sungai Serayu diduga menghalangi jalur migrasi ikan dan makrokrustasea air. Penelitian ini bertujuan untuk mengetahui pengaruh bendungan terhadap migrasi *Varuna litterata* dan identifikasi molekulernya. Penelitian ini dilakukan dengan menggunakan metode observasi. Pengamatan migrasi dilakukan pada jalur vertikal sedangkan identifikasi molekuler *V. litterata* di bagian hilir Sungai Serayu dibandingkan dengan data yang ada di GenBank. Penelitian ini meliputi daerah air tawar di atas Bendungan Serayu (terisolasi karena adanya bendungan), daerah terbuka dengan air tawar di bawah Bendungan Serayu, dan daerah terbuka dengan air payau di muara Sungai Serayu, dengan panjang penelitian 33,37 km. Hasil penelitian menunjukkan bahwa keberadaan bendungan di bagian hilir Sungai Serayu menghambat pola migrasi *V. litterata*. Bentuk bendungan yang vertikal menyebabkan hanya sedikit *V. litterata* yang bermigrasi ke bagian atas bendungan. Bendungan yang berada di bagian hilir Sungai Serayu menyebabkan tidak banyak *V. litterata* yang dapat bermigrasi ke bagian hulu bendungan, sehingga jumlah *V. litterata* yang ditemukan pada populasi di bagian hulu bendungan sangat sedikit (115 ekor). Hasil identifikasi molekuler *V. litratata* menggunakan gen sitokrom C oksidase subunit I (COI) menunjukkan bahwa kepiting ini 100% sama dengan data yang ada di GenBank dan berkerabat dekat dengan *Varuna yui* dan spesies kepiting Varunidae lainnya. Hasil penelitian ini juga menunjukkan pentingnya pembuatan jalur perlintasan bagi organisme akuatik di Bendung Gerak Sungai Serayu.

Kata Kunci : Bendungan, Molekuler, Migrasi, Sungai Serayu, *Varuna litterata*

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1. Introduction

Rivers play an important role in ecosystems and human life (Wang & He, 2022; Biggs et al., 2017) because their formation process involves the flow of water from upstream to downstream to form a stream that eventually empties into the sea. During this process, rivers play an important role in the hydrological cycle by collecting rainwater, river water, groundwater and spring water and then transporting it to the sea. Rivers not only act as waterways, but also support the life of organisms on land and in the water (Anderson et al., 2015; Colin et al.,2016). One of the rivers that plays a very important role in Indonesia is the Serayu River. This river is 153 km long. The river crosses several districts in Central Java province, including Wonosobo, Banjarnegara, Purbalingga, Banyumas and Cilacap (Pranoto et al., 2019; Wahyudi et al., 2021; Suryaningsih et al., 2020). The inhabitants of these areas rely heavily on the Serayu River as their main source of water. Apart from its function as a water source, the river is also used for various human activities such as settlement and agriculture. Due to the importance of the Serayu River's function to the community, many development activities are being carried out on the Serayu River, one of which is the construction of the Serayu Movement Weir (Marhendi & Imron, 2022).

The Serayu Dam was built downstream of the Serayu River in Gambarsari village, Kebasen district, Banyumas regency, Central Java province (Legono et al., 2020). The presence of dams has the potential to block the migration routes of fish and aquatic macrocrustaceans (Burnett et al., 2023). The author hypothesises that Varuna litterata has difficulties in migrating above the dam. The presence of dams prevents *V. litterata* from moving from one part of the river to another. Dams disrupt the life cycle and reproduction of the crab. This may lead to a decline in the population of *V. litterata* in the downstream section of the Serayu River. This is exacerbated by the fact that *V. litterata* is a food source for the communities around the Serayu River. If crab fishing continues without sustainable management, it could threaten the sustainability of the population and even lead to extinction (Taguiam et al., 2022). Although there is no similar research in this area, previous research on the habitat of *V. litterata* in the Bengawan Solo River has been carried out by Fariedah et al. (2023), and morphometric analysis of length/width-weight relationship and condition factors has been carried out by Jumawan et al. (2022) and Mahapatra et al. (2017). By conducting this research, the information generated will be very valuable to support future development, paying more attention to the sustainability of life and migration routes of aquatic biota while considering environmental aspects.

V. litterata is a species of crustacean in the family Varunidae (Liu et al., 2021 ; Zhang et al., 2022 ; Xin et al., 2018). This crustacean is characterized by paddle-like legs that allow it to swim easily (Schmidt et al., 2020 ; Hartnoll, 1971). *V. litterata* is a species of crab that is euryhaline, meaning that it tolerates different types of salinity (Ribero et al., 2020). This crab is found throughout the Indo-Pacific region and is known for its unique life cycle (Susilo et al., 2020 ; Subang et al., 2020). This type of crab tends to inhabit vegetation-rich environments where it can find adequate shelter and food sources (Tsai et al., 2022). These crabs are also found hiding under rocks, logs and dead leaves (Fariedah et al., 2023). This crab is a fishery product that is very important to people in countries in Southeast Asia such as the Philippines, India, and China (Das et al., 2015; Susilo et al., 2020; Deyashi & Chakraborty, 2022; Taguiam et al., 2022 ; Wang et al., 2020). *V. litterata* plays a crucial role in the breakdown of organic matter in estuarine environments, serving as a natural decomposer that aids the degradation process (Devi et al., 2021; Taguiam et al., 2022).

V. litterata is a species of diadromous macroinvertebrate that relies on water or wet surfaces for navigation in aquatic environments (Burnett et al., 2023). These aquatic macroinvertebrates are known to migrate from estuaries to lowland areas. *V. litterata* is globally distributed and the megalopa (juvenile stage of the crab) moves upstream to estuaries and rivers after hatching near the coast (Ryan & Choy. 1990). The larval stage of this crab requires seawater, but develops into an adult in brackish or fresh water (Cumberlidge, 2016). *V. litterata* has a habitat that is generally distributed across different aquatic environments, ranging from mangrove forests, estuaries, fresh water to shallow tidal areas (Devi et al., 2013). The timing and duration of this migration varies across its range, and few understand the driving factors behind this migration phenomenon (Susilo et al., 2020; Mos et al., 2017).

DNA barcoding facilitates rapid and accurate species identification (Karim et al., 2015), with two main objectives: identifying known species and discovering undescribed species (Karohmatulloh et al., 2024). In this study, molecular identification was used to determine relationships between different species and gain a deeper understanding of the evolutionary relationships of *V. litterata*. This technique analyzes specific sections of mitochondrial DNA, such as the cytochrome C oxidase subunit I (COI) gene, to build phylogenetic trees and measure genetic distances between species (Suppapan et al., 2017; Zhang et al., 2022). These molecular identifications complement morphological analyses, often erroneous due to significant variability in crab shape, coloration, and size, leading to misidentification or naming (Muñoz et al., 2021; Magalh et al., 2016). Similar research on molecular identification of *V. litterata* was conducted by Winarni et al. (2023) from Segara Anakan Lagoon, and the sequences have been deposited in GenBank. Given the vulnerability of the intertidal zone to environmental changes that can indirectly affect the adaptation of organisms, including crabs, molecular identification is an essential complement to traditional morphological methods. Given the importance of more information on *V. litterata*, this study aimed to determine the effect of dam on *Varuna litterata* migration and its molecular identification.

2. Material and Methods

This study was conducted in the lower reaches of the Serayu River, which has a length of 33.37 km. The river empties into the Indian Ocean on the south coast of Java. The focus of this study was the Serayu Dam. Geographically, the dam is located about 15 km south of Purwokerto City. The construction of this dam began in 1993 and was inaugurated in 1996. To date, the dam is still operational and active. Serayu Dam plays a vital role in regulating the flow of Serayu River through 8 radial floodgates (10.70 m x 9.00 m), 273 m long floodgates (2 lanes), weir entrance (4 pieces of 2.50 m x 2.00 m each), weir exit (6 pieces of 2.50 m x 2.00 m each), operating water level ± 12.20 to 12.90 masl, and crest elevation ± 15.50 masl. The dam is 121.20 m long and 109.60 m wide. The study area includes freshwater above the isolated Serayu dam, open areas with freshwater below the dam, and open areas with brackish water (Figure 1). The geographical coordinates of the study site are between 7°18'52.5 "S -109°06'56.2 "E to - 7°41'09.2 "S 109°06'10.7 "E. The study was conducted for 4 months, using a gill net with a mesh size of 2 mm, 4 m long, and 1.5 m high for sampling. Each time we take samples, they are stored in a dry system to cool them down. This was done by placing ice cubes in a cool box. Once the samples arrived at the laboratory, the *V. litterata* obtained were separated by sex and then counted by capture station.

2.1. Migration of *V. litterata*

Observations of *V. litterata* migration were made as conducted by Burnett *et al.* (2024). Records were made by observing vertical migration paths (Zyl, 2023). Sampling was conducted at three stations. Station 01: above the dam with freshwater properties; Station 02: below the dam with freshwater properties; and Station 03: below the dam with brackish water properties. Sampling was conducted to determine the population of *V. litterata* at each station. During the study period, notes were made on how *V. litterata* migrated and the pathways used vertically. Observations of *V.litterata* migration

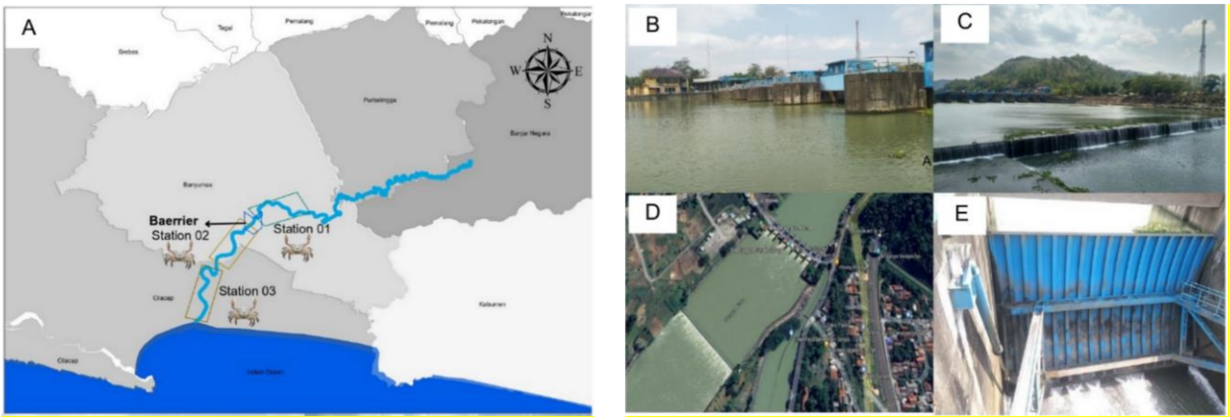

Figure 1. Research Location of the Serayu River (Banyumas and Cilacap regencies), Central Java, Indonesia Research location in the lower reaches of the Serayu River (Banyumas and Cilacap regencies), Central Java, Indonesia. A) Research Observation Station; B) Condition of the Waters Above the Dam; C) Condition of Waters Below the Dam; D) Serayu Dam Based on Google Earth; E) One of the Gates of the Serayu Dam

behavior during the study were made by evaluating the impact on *V. litterata* populations.

2.2. DNA Extraction

V. litterata samples that were obtained were stored in sample bottles containing 96% ethanol. Furthermore, tissue samples weighing ±10 grams were taken, and an extraction process was carried out to isolate DNA sample using a method that followed the Blood & Tissue Kit protocol from Qiagen.

2.3. PCR Amplification and Nucleotide Sequencing

The PCR process involves the use of primers jgLCO (5'-TIT CIA CIA AYC AYA ARG AYA TTG G -3') and jgHCO (5'-TAI ACY TCI GGR TGI CCR AAR AAY CA -3') for the amplification of the COI gene of *V. litterata* according to the method described by Geller *et al.* (2013). The PCR reaction totaled 25 µL, comprising 2 µL of the extracted DNA template, 1.25 µL of each primer at a concentration of 10 mM, 9 µL of ddH2O, and 12.5 µL of Ready mix. This mixture was amplified using an Applied Biosystems™ 2720 Thermal Cycler. The PCR protocol followed these temperature and time settings: an initial denaturation at 94°C for 3 minutes, followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 50-55°C for 30 seconds, and extension at 72°C for 60 seconds, concluding with a final extension at 72°C for 2 minutes. The PCR products were then visualized on a 1% agarose gel stained with GelRed®. Positive samples, indicated by visible DNA bands, were subsequently sequenced using the Sanger dideoxy method.

2.4. Molecular Analysis

Molecular analysis was performed using ClustalW (version 1.6) via MEGA11 (Molecular Evolutionary Genetic Analysis) software. Genetic data and information from GenBank at NCBI (National Center for Biotechnology Information) were integrated using BLAST (Basic Local Alignment Search Tool). To confirm the taxonomic status of the *V. litterata* mtDNA sequence and reconstruct a phylogenetic tree, 21 species were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/). Genetic distances (D) between species were calculated, and a phylogenetic tree of *V. litterata* was reconstructed using MEGA11, using the Neighbor-Joining (NJ)

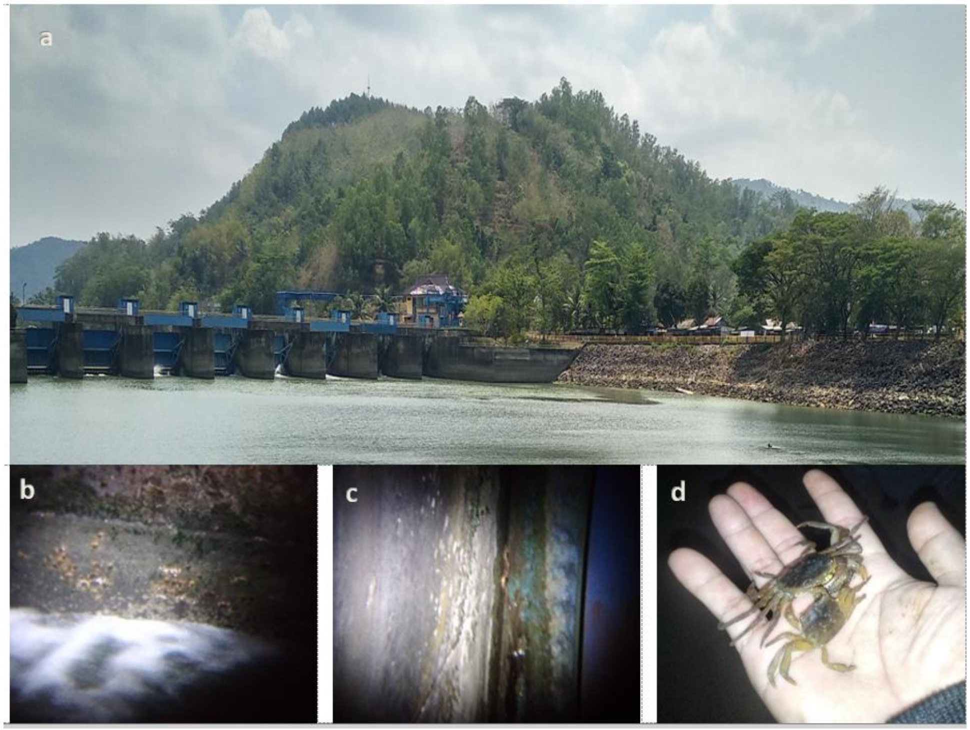

Figure 2. Migration of *V. litterata* at Serayu Dam in the lower Serayu River, Central Java. a) Serayu Dam gate. b) *V. litterata* climbs onto land to start climbing buildings. c) *V. litterata* trying to climb the dam building. d) *V. litterata* samples collected while trying to climb the dam.

method and a 2-parameter Kimura model, with a bootstrap value of 1000x.

3. Result and Discussion

3.1. Migration of *V. litterata*

Samples of *V. litterata* were found at all observation stations. The results of the field observations show that station 02 (below the dam) has the largest population of *V. litterata*. The presence of the Serayu Movement Weir is not an obstacle for *V. litterata* to migrate (Figure 2). The *V. litterata* that migrated were still juveniles and no adult *V. litterata* were found (Figure 2d). *V. litterata* climb the dam at sunset (18:00 - 05:00). This crab species has strong walking legs which give it good climbing ability. This species is also able to survive on land for some time (semi-terrestrial), making it very useful in the migration process.

Examination of the edge of the dam floor revealed that many juvenile *V. litterata* had emerged and were preparing to migrate (Figure 2b). The migration of *V. litterata* occurs at the dam during sunset (18:00 - 05:00). This event took place during the rainy season when the dam was damp and slightly wet. *V. litterata* migration was observed in vertical gaps (Figure 2d). The presence of the Serayu dam means that there are no crabs able to migrate against the current. This is because the construction of the dam blocks the entire water body and crabs wishing to migrate must climb the dam. Any *V. litterata* that falls while migrating will be carried by the current upstream to the Serayu River. The discovery of *V. litterata* above the dam shows that this crab has the ability to migrate quite far.

Research conducted by Ryan & Choy (1990) in Fiji showed that the mass migration of the megalopa *V. litterata* could cover a distance of 1.8 km. The migratory ability of *V. litterata* has also been recorded up to 31 km in Meru Betiri National Park, Indonesia (Susilo et al., 2020). Furthermore, this crab species is able to migrate to the poles up to ± 290 km. This is due to an increase in the East Australian Current, resulting in warmer waters and increased salinity in the region (Mos et al., 2017).

As a result of the Serayu dam, not many *V. litterata* were able to migrate to the top of the dam (Table 1). This is due to the vertical shape of the dam and the presence of a motorbike crossing at the top of the dam, which hinders the migration of *V. litterata*. Research by Burnett et al. (2023) also shows that vertical migration routes limit connectivity, as seen in this study. The low number of *V. litterata* caught above the dam (station 01) suggests that the presence of the dam blocks a migration route unsuitable for *V. litterata*. The same conclusion was drawn from research conducted by Mackay & Cyrus (2001), which showed that reduced river connectivity resulted in a decrease in the abundance of *V. litterata*.

The factors that influence the migration and spawning patterns of *V. litterata* towards the estuary are still unknown. According to Burnett et al. (2023), negative phototaxis and positive rheoctaxis may play a role, while according to Devi et al. (2013), moon phase and tides also affect migration patterns. Some studies mention that moon phase affects migration in *V. litterata*, but have different moon phases. The difference in this study is that the location of the Serayu River dam does not correlate with the tides. The push of the tide does not increase the importance of the water in the dam. The migration pattern of *V. litterata* found shows that this crab species is able to maintain its life cycle. The impact of the dam on the life of *V. litterata* requires further research to determine the water depths and currents that are suitable for the life of *V. litterata*. The presence of the dam changes the flow pattern in the lower reaches of the Serayu River. Changes in the habitat of *V. litterata* will alter the availability of food and the survival system of this crab species.

3.2. Characteristics of COI Sequences and Genetic Diversity of *V. litterata*

The results of the molecular analysis carried out showed that the mtDNA fragment of *V. litterata* was 688 bp. The base composition of the COI gene from 21 species in GenBank was compared with *V. litterata* in this study (Table 2). The gene base composition of *V. litterata* in this study was T(U) (33.14%), C (20.35%), A (27.62%), G (18.90%), C+G (39.24%), A+T

Table 1. Abundance of *V. litterata in the lower Serayu River, Central Java*

Information	Station 01 (On the dam of freshwater nature) (ind)	Station 02 (Under the dam of fresh-water properties) (ind)	Station 03 (Under the dam, the water is brackish) (ind)
Male	46	267	123
Female	69	375	218
Sum	**115**	**642**	**341**

Table 2. Comparison of nucleotide composition between *V. litterata* in the Lower Serayu River using the COI gene with data in GenBank.

Species	Country	T(U) %	C %	A %	G %	C+G %	A+T %
OQ852799.1 (*Varuna litterata*)	Indonesia	33.23	19.85	27.54	19.38	39.23	60.77
MW591184.1 (*Varuna litterata*)	Malaysia	34.04	20.06	28.12	17.78	37.84	62.16
MK091850.1 (*Varuna litterata*)	India	34.21	19.82	28.63	17.33	37.15	62.85
BIOSUB245.001 (*Varuna litterata*)	Serayu River	33.14	20.35	27.62	18.90	39.24	60.76
MZ832164.1 (*Varuna yui*)	Hongkong	33.77	20.82	28.03	17.38	38.20	61.80
MZ832165.1 (*Varuna yui*)	Hongkong	34.04	20.06	27.81	18.09	38.15	61.85
MW000766.1 (*Ptychognathus altimanus*)	Taiwan	35.71	17.17	29.33	17.78	34.95	65.05
MW000764.1 (*Ptychognathus altimanus*)	Taiwan	35.71	17.17	29.18	17.93	35.11	64.89
OQ852797.1 (*Ptychognathus altimanus*)	Indonesia	35.17	17.35	29.02	18.45	35.80	64.20
LC698503.1 (*Eriocheir japonica*)	Japan	36.35	18.40	27.89	17.36	35.76	64.24
LC698457.1 (*Eriocheir japonica*)	Japan	36.20	18.55	28.04	17.21	35.76	64.24
OL877140.1 (*Chiromantes haematocheir*)	South Korea	38.84	15.11	29.66	16.38	31.50	68.50
MZ832033.1 (*Chiromantes haematocheir*)	Hongkong	39.21	15.20	29.48	16.11	31.31	68.69
JX502929.1 (*Chiromantes haematocheir*)	South Korea	38.91	15.35	29.33	16.41	31.76	68.24
LC537817.1 (*Pseudosesarma edwardsi*)	Malaysia	39.06	14.89	30.09	15.96	30.85	69.15
LC537818.1 (*Pseudosesarma edwardsi*)	Singapore	38.91	15.05	30.09	15.96	31.00	69.00
MN184196.1 (*Sesarma curacaoense*)	Panama	39.51	14.44	29.79	16.26	30.70	69.30
MN183914.1 (*Sesarma curacaoense*)	Panama	39.42	14.46	29.83	16.29	30.75	69.25
KU313349.1 (*Sesarma rectum*)	Brazil	38.86	15.36	29.52	16.27	31.63	68.37
KU313344.1 (*Sesarma rectum*)	Brazil	38.67	15.41	29.63	16.30	31.70	68.30
OL877002.1 (*Orisarma dehaani*)	South Korea	37.71	15.40	29.94	16.95	32.34	67.66
OL877003.1 (*Orisarma dehaani*)	South Korea	37.71	15.40	29.94	16.95	32.34	67.66
Average		**36.91**	**16.94**	**29.09**	**17.16**	**34.23**	**65.77**

Note: The red colour is the nucleotide composition of *V. litterata* as a result of this research

(60.76%). The average gene base results of the species show T(U) (36.91%), C (16.94%), A (29.09%), G (17.16%), C+G (34.23%), A+T (65.77%).

Molecular analysis is a scientific method for understanding the evolutionary relationships between organisms by using molecular information, such as DNA or protein sequences. This approach is based on the fact that genetically similar organisms tend to have ancestors that are closer in evolutionary history. Molecular analysis is closely related to the basic principles of biological evolution, involving genetic changes over time in a population of organisms. Genetic mutation and recombination are the main mechanisms that cause genetic variation in populations. Species such as *Ptychognathus altimanus*, *Eriocheir japonica*, *Chiromantes haematocheir*, *Pseudosesarma edwardsi, Sesarma curacaoense, Sesarma rectum,* and *Orisarma dehaani* were used as comparisons to determine the evolutionary relationship with the *V. litterata* species in the lower reaches of the Serayu River.

The genetic distance of *V. litterata* (Table 3) from the lower reaches of the Serayu River, with data available in GenBank, has a genetic distance value ranging from 0.00 to 0.13. The genetic distance between *V. litterata* and *Varuna yui* ranges from 0.00 to 0.15. *V. litterata - Ptychognathus altimanus* (0.00 to 0.16), *V. litterata - Eriocheir japonica* (0.00 to 0.19), *V. litterata - Chiromantes haematocheir* (0.00 to 0.20), *V. litterata - Pseudosesarma edwardsi* (0.00 to 0.22), *V. litterata - Sesarma curacaoense* (0.00 to 0.21), *V. litterata - Sesarma rectum* (0.00 to 0.22), *V. litterata - Orisarma dehaani* (0.00 to 0.21). Species are considered identical if their genetic differences are less than 2%, while species are considered different if their genetic differences exceed 2%.

Table 3. Genetic Distance of *V. litterata* in the Lower Serayu River using the COI gene with data in GenBank

Spesies	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
OQ852799.1 (*Varuna litterata*), Indonesia	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MW591184.1 (*Varuna litterata*), Malaysia	0.14	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MK091850.1 (*Varuna litterata*), India	0.13	0.02	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
BIOSUB245.001 (*Varuna litterata*), Serayu River	0.00	0.14	0.13	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MZ832164.1 (*Varuna yui*), Hongkong	0.15	0.00	0.02	0.15	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MZ832165.1 (*Varuna yui*), Hongkong	0.14	0.01	0.02	0.14	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MW000766.1 (*Ptychognathus altimanus*), Taiwan	0.16	0.17	0.16	0.16	0.17	0.17	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
MW000764.1 (*Ptychognathus altimanus*), Taiwan	0.16	0.17	0.16	0.16	0.17	0.18	0.00	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
OQ852797.1 (*Ptychognathus altimanus*), Indonesia	0.16	0.17	0.16	0.17	0.17	0.17	0.01	0.01	*	*	*	*	*	*	*	*	*	*	*	*	*	*
LC698503.1 (*Eriocheir japonica*), Japan	0.19	0.20	0.18	0.19	0.20	0.20	0.19	0.19	0.19	*	*	*	*	*	*	*	*	*	*	*	*	*
LC698457.1 (*Eriocheir japonica*), Japan	0.19	0.20	0.18	0.19	0.20	0.20	0.19	0.19	0.19	0.00	*	*	*	*	*	*	*	*	*	*	*	*
OL877140.1 (Chiromantes haematocheir), South Korea	0.20	0.19	0.18	0.20	0.19	0.19	0.15	0.15	0.16	0.19	0.19	*	*	*	*	*	*	*	*	*	*	*
MZ832033.1 (*Chiromantes haematocheir*), Hongkong	0.20	0.19	0.19	0.20	0.19	0.19	0.15	0.15	0.16	0.20	0.20	0.00	*	*	*	*	*	*	*	*	*	*
JX502929.1 (*Chiromantes haematocheir*), South Korea	0.20	0.19	0.19	0.20	0.19	0.19	0.16	0.16	0.17	0.19	0.19	0.00	0.01	*	*	*	*	*	*	*	*	*
LC537817.1 (*Pseudosesarma edwardsi*), Malaysia	0.22	0.21	0.22	0.22	0.22	0.22	0.16	0.17	0.17	0.21	0.21	0.06	0.06	0.06	*	*	*	*	*	*	*	*
LC537818.1 (*Pseudosesarma edwardsi*), Singapore	0.22	0.21	0.21	0.22	0.22	0.21	0.17	0.17	0.17	0.21	0.21	0.06	0.06	0.06	0.00	*	*	*	*	*	*	*
MN184196.1 (*Sesarma curacaoense*), Panama	0.21	0.23	0.23	0.21	0.23	0.23	0.18	0.18	0.19	0.20	0.20	0.07	0.07	0.08	0.08	0.08	*	*	*	*	*	*
MN183914.1 (*Sesarma curacaoense*), Panama	0.21	0.23	0.23	0.21	0.23	0.23	0.18	0.18	0.19	0.20	0.20	0.07	0.07	0.08	0.08	0.08	0.00	*	*	*	*	*
KU313349.1 (*Sesarma rectum*), Brazil	0.22	0.21	0.21	0.22	0.21	0.21	0.19	0.19	0.20	0.20	0.20	0.08	0.08	0.08	0.08	0.08	0.06	0.06	*	*	*	*
KU313344.1 (*Sesarma rectum*), Brazil	0.22	0.21	0.21	0.22	0.21	0.21	0.19	0.19	0.20	0.20	0.20	0.08	0.08	0.08	0.08	0.08	0.06	0.06	0.00	*	*	*
OL877002.1 (*Orisarma dehaani*), South Korea	0.21	0.22	0.21	0.22	0.22	0.22	0.19	0.19	0.20	0.21	0.21	0.07	0.08	0.08	0.07	0.08	0.09	0.09	0.09	0.09	*	*
OL877003.1 (*Orisarma dehaani*), South Korea	0.21	0.22	0.21	0.22	0.22	0.22	0.19	0.19	0.20	0.21	0.21	0.07	0.08	0.08	0.07	0.08	0.09	0.09	0.09	0.09	0.00	*

Note: red is the sample results from this research

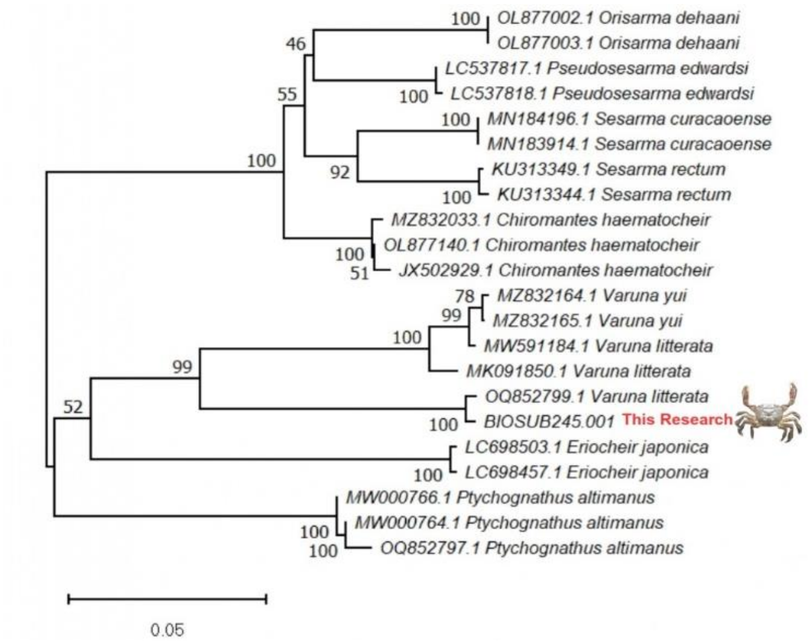

Figure 3. Reconstruction of the *V. litterata* phylogenetic tree.

The results of the phylogenetic tree of *V. litterata* found in the lower reaches of the Serayu River show that this species can be easily distinguished from other species. *V. litterata* found in the lower reaches of the Serayu River is the same as the results of research conducted by Winarni et al. (2023) in Segara Anakan Lagoon and the phylogenetic tree also shows the same as *V. litterata* in GenBank. *V. litterata* is closely related to *Varuna yui*. In general, *V. litterata* is very difficult to distinguish from *V. yui*, but Hwang & Takeda. (1986) were able to distinguish this species. Figure 3 shows that *V. litterata* found in the lower reaches of the Serayu River is closely related to other Varunidae crabs. This result is in agreement with the research conducted by Liu et al. (2021), who also showed that *V. litterata* has a sibling relationship and belongs to Grapsoidea and Sesarmidae. The phylogenetic tree (Figure 3) also shows *V. litterata*. At the family level, *V. litterata* is most closely related to the species *Eriocheir japonica* and *Ptychognathus altimanus*. This research is consistent with research conducted by Xin et al. (2018) and Liu et al. (2015). Research conducted by Schubart & Neigel (2002) also showed that *V. litterata* is closely related to other Varunidae. The phylogenetic tree generated from this study still belongs to the family Grapsoidea. Four species (*Chiromantes haematocheir, Sesarma curacaoense, Orisarma dehaani*, and *Pseudosesarma edwardsi*) show a close and clear relationship and have an independent position in the family Sesarmidae. These results are also consistent with the research of Li et al. (2019) and Schubart et al. (1998), which confirm that the Sesarmidae family is polyphyletic.

Genetic movement or gene flow between different regions can occur due to a number of factors, such as species movement and larval dispersal processes. Suppapan et al. (2017) conducted a study on genetic population structure and demonstrated the development of genetic populations of *V. litterata* along the coast of Thailand. In *V. litterata*, ocean currents act as a means of transport, facilitating the movement of larvae or genes between different water locations. This phenomenon highlights the importance of connectivity between sites in maintaining genetic diversity in *V. litterata* populations. As gene flow facilitates genetic exchange between populations, these species can maintain levels of genetic diversity that are crucial for adaptation to environmental change, while reducing the risk of extinction at the local level.

4. Conclusions

The presence of weirs in the lower reaches of the Serayu River means that not many *V. litterata* can migrate upstream. The vertical weir restricts crab migration. The dam's impact resulted in differences in *V. litterata* populations above and below the dam. The ability to migrate indicates the ability of the crab to maintain its life cycle. The mtDNA nucleotide sequence of *V. litterata* in the cytochrome oxidase subunit I gene conducted in the lower Serayu River was 688 bp. This crab is closely related to *V. litterata* and other

Varunidae crabs. The results show the importance of development that considers the sustainability of aquatic biota in the river.

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