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Research Article

Morphological and Genetic Variation of *Filopaludina javanica* (von dem Busch, 1844) (Gastropoda: Viviparidae) from Madura Island, Indonesia

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ABSTRACT

Filopaludina javanica (von dem Busch, 1844) is a freshwater gastropod species in the Viviparidae family. *Filopaludina javanica* is widely distributed in freshwater waters in Java (including Madura), Sulawesi, Sumatra, Borneo, Papua, Thailand, and Vietnam. The morphological, morphometric, and molecular characterization studies of *F. javanica* based on the COI gene originating from Madura Island are still quite limited. Therefore, this study aims to determine the morphological variations, morphometry, and molecular characters of *F. javanica* in Bangkalan, Madura. The samples used are the Taxonomy Laboratory collection from Madura Island, followed by morphological observations, morphometry, and analysis of DNA: isolation, amplification, electrophoresis, and COI gene sequencing. Morphological and morphometric variations in *F. javanica* from Madura Island showed seven types of morphological variations. Principal Component Analysis (PCA) scatterplot results showed morphometric clustering of *F. javanica* based on morphological type. Identification of *F. javanica* using BLAST and comparison with the GenBank database revealed five nucleotide base variations, with an overall genetic distance of 0.031. Therefore, the phylogenetic tree shows that *F. javanica* from Madura Island belongs to the same clade as *F. javanica* from Sarawak, West Java, and North Kalimantan.

1. Introduction

Filopaludina javanica (von dem Busch, 1844) is a freshwater gastropod species that belongs to the family Viviparidae (MolluscaBase 2024). *Filopaludina javanica* is a valid name for *Bellamya javanica*, the species name that has been used more often in the past. Besides *F. javanica*, at least three further species of the genus *Filopaludina* have been recorded from Indonesia, i.e., *F. tricostata*, *F. decipiens*, and *F. sumatrensis*. *Filopaludina tricostata* and *F. decipiens* are very uncommon, with only reports from Maluku and West Papua. Meanwhile, *F. sumatrensis* is found throughout Java and Sumatra (Benthem-jutting 1963; Taufiqurrahman and Setyaningsih 2024).

Filopaludina javanica is widely distributed in Java (including Madura), Sulawesi, Sumatra, Borneo, Papua, Thailand, and Vietnam (MolluscaBase 2024). This species lives in freshwater habitats such as

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rivers, swamps, lakes, rice fields, and ponds with slow or fast currents (Marwoto and Nurinsiyah 2009). *Filopaludina javanica* is often utilized as an alternative food source (Priawandiputra *et al*. 2020; Gofriyanti *et al*. 2024). *Filopaludina javanica* is also used as animal feed, reduces the total organic matter content in wastewater, and acts as a bioindicator (Lailiyah *et al*. 2021; Theofilius *et al*. 2021; Sriwahjuningsih and Fitri 2022; Mamonto *et al*. 2023).

Madura Island is located in the eastern part of Java Island (Agustin and Syah 2020). Madura Island consists of 4 districts: Bangkalan, Sampang, Pamekasan, and Sumenep (Ridwanah *et al*. 2022). Madura Island has many water sources, including rivers, dams, and lakes (Vidayanti *et al*. 2021). Studies on aquatic gastropods of Madura Island are generally still dominated by research in coastal areas (Islamy and Hasan 2020) and in mangrove ecosystems (Haqqi *et al*. 2024). Meanwhile, research on freshwater gastropods on Madura Island has recorded 14 species in the Gili Timur River, Bangkalan, Madura, and a limited report on *Pila virescens* in Sampang, Madura, has recently been published (Farid *et al*. 2023; Ambarwati *et al*. 2025).

Species identification based on COI genes is often done to get accurate results (Islam *et al*. 2018) and could also identify closely related species and cryptic species (Antil *et al*. 2023). Genetic diversity of the genus *Filopaludina* based on the COI gene has been carried out by Krairat *et al*. (2020) in the Mekong River basin, Thailand. Molecular characterization of *F. javanica* with the COI gene was first reported by Stelbrink *et al*. (2020) on the *F. javanica* species from the island of the Sunda region (Java, Borneo, Sumatra, and mainland of Southeast Asia). However, morphological, morphometric, and molecular characterization studies of *F. javanica* based on the COI gene originating from Madura Island are still quite limited. Therefore, this study aims to explain the morphological variation, morphometric variation, and molecular characterization of *F. javanica* from Madura Island.

2. Materials and Methods

2.1. Sample Collection

The samples of *F. javanica* were from the results of freshwater snail expeditions on Madura Island, Indonesia, in October 2023-June 2024, stored in the Taxonomy Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya. Madura Island is geographically located on coordinates between 112.6756°-114.6214° and 6.878333° (Figure 1) with a total area of 5,168 km^2 (Agustin and Syah 2020).

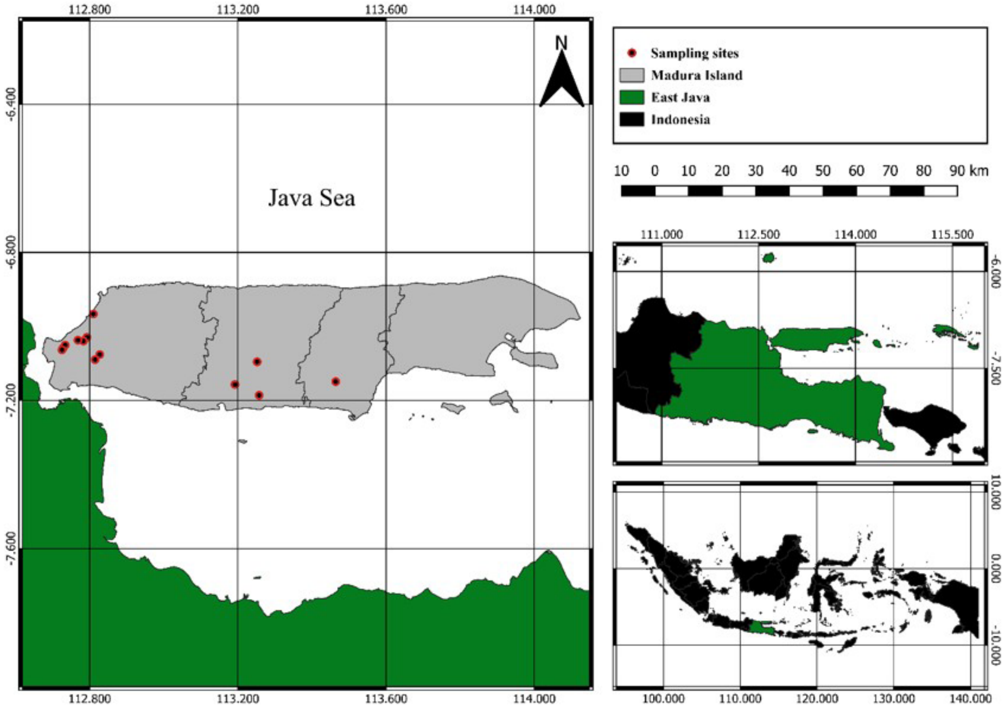

Figure 1. Map of sampling location of *Filopaludina javanica* on Madura Island, Indonesia

The large islands in the west, such as Sumatra, Borneo, Java (including Madura), and Bali, when sea levels were significantly lower than at present, merged with mainland of Southeast Asia to form a vast dry landmass called Sundaland during the Pleistocene glacial period (Tanudirjo 2014). Therefore, based on the history of changes in flow patterns and their association with ancient river systems, the characteristics of rivers in Madura that flow northward are related to the Bengawan Solo River System, which extends to South Kalimantan. Meanwhile, rivers that flow southward to the Madura Strait are part of the Brantas River System.

2.2. Identification and Morphometric Measurement

Specimens of *F. javanica* were cleaned and identified based on morphological characters according to Dharma (2005) and also based on the original description (Philippi 1845). Two hundred seventy-four individuals were observed and measured to analyze the morphological and morphometric variation of *F. javanica* on Madura Island. The measured parameters include Shell Length (SL), Shell Width (SW), Body Whorl (BW), Spire Length (SP), Aperture Length (AL), and Aperture Width (AW) (Isnaningsih and Listyawan 2010) (Figure 2). SL is defined as the perpendicular distance between the anterior and posterior of the shell, while SW is defined as the horizontal distance between the sinistral and dextral of the shell. BW includes measurements from the posterior end of the aperture perpendicular to the last whorl. SP is measured by perpendicularly measuring from the apex to the previous body whorl (BW). AL is measured by perpendicular measurement between the anterior and posterior apertures of the shell. Meanwhile, AW is derived from horizontal measurements on the aperture. All morphometric measurements of *F. javanica* were carried out using a caliper with an accuracy of 0.05 mm.

2.3. DNA Extraction

Six individuals of *F. javanica* belonging to morphological variations Type 1 (specimen code: FB1, FB2, and FB3) and Type 4 (specimen code: FS1, FS2, and FS3) were obtained from the inventory of the Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, and stored in absolute ethanol. Genomic DNA was extracted from 15-20 mg of leg muscle tissue using the NextPrep Kit according to the manufacturer's instructions with several modifications: samples were ground in liquid nitrogen before extraction and incubated at 56°C with tube inversion every 5 minutes. The extraction procedure included pre-lysis by GTI buffer and lysis by Proteinase K, ethanol precipitation, purification through a spin

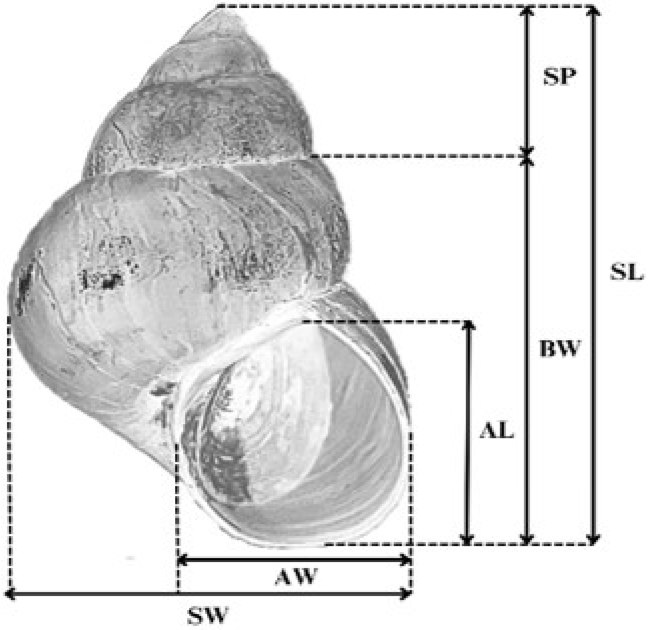

Figure 2. Measurement dimensions of *F. javanica* shells (SP-Spire Length, SL-Shell Length, SW-Shell Width, AL-Aperture Length, AW-Aperture Width, BW-Body Whorl)

column with gradual washing, and final elution in 50-100 µL Elution Buffer. The resulting DNA was stored at -20°C until further analysis.

2.4. PCR and Sequencing

Amplification of a segment of approximately 648-651 base pairs corresponding to the COI gene region of mitochondrial DNA (mtDNA) using Polymerase Chain Reaction (PCR). Amplification was conducted by using the universal primers LCO1490 (5' GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3') by Folmer *et al.* (1994). PCR was performed using a hot start technique utilizing a Kapa master mix and two Taq master mixes. Amplification consisted of 35 cycles with each cycle consisting of several stages: initial denaturation at 95°C for 3 minutes, followed by denaturation at 94°C for 45 seconds, annealing at 45°C for 45 seconds, and extension at 72°C for 2 minutes. The process was terminated with a final elongation step at 72°C for 10 minutes. The product was analyzed on a 1% agarose gel that contained 4 µL of ethidium bromide. 3 µL of PCR product and 1 µL of loading dye were mixed and subsequently loaded into the gel wells. Electrophoresis was conducted at 220 V and 400 mA for 25 minutes. After this, purification of the PCR products was performed using the Qiagen purification kit according to the manufacturer's instructions. Furthermore, the purified samples were sent to First Base in Malaysia for sequencing.

2.5. Analysis of Morphological and Morphometric Data

Morphological characters were analyzed to determine the morphological variations present in *F. javanica* on Madura Island. All values on morphological and morphometric characters were analyzed descriptively and quantitatively using Microsoft Excel software. Furthermore, the mean and standard deviation of morphometrics were calculated. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis by utilizing ntedit.exe and NTSYSpc 2.02i software was conducted to determine the distinctive morphological characters in *F. javanica* on Madura Island. Morphological and morphometric variations of the entire *F. javanica* shells were further tested with Principal Component Analysis (PCA) using Paleontological Statistics (PAST) Version 4.03.

2.6. Molecular Analysis

The DNA sequencing results of the samples were processed through the chromatogram, which was the primary analysis by using ClustalX. The similarity percentage of DNA sequencing results was validated using the Barcode of Life Data System through the BOLD System web (https://barcodinglife.org), which shows the similarity of samples with data from the BOLD System. Alignment of research sample sequences with reference sequences was carried out by retrieving sequence data from GenBank NCBI (Table 1) and performed using ClustalX and Bioedit. Phylogenetic analyses were constructed using MEGA 11 software to

Table 1. Sequences from NCBI GenBank were used as reference species

Species	Sample location	Acc number of GenBank NCBI
Filopaludina javanica	Burneh, Madura	FB1 (This study)
Filopaludina javanica	Burneh, Madura	FB2 (This study)
Filopaludina javanica	Burneh, Madura	FB3 (This study)
Filopaludina javanica	Socah, Madura	FS1 (This study)
Filopaludina javanica	Socah, Madura	FS2 (This study)
Filopaludina javanica	Socah, Madura	FS3 (This study)
Filopaludina javanica	Sarawak	MN997939.1 ZMB 112753
Filopaludina javanica	North Kalimantan	MN997940.1 ZMB 127082
Filopaludina javanica	West Java	MN997941.1 ZMB 127085
Filopaludina javanica	West Java	MN997942.1 ZMB 127086
Filopaludina javanica	North Kalimantan	MN997943.1 ZMB 127093
Filopaludina sumatrensis	Myanmar	MN997973.1 ZMB 113444
Filopaludina sumatrensis	Myanmar	MN997974.1 ZMB 113446
Filopaludina sumatrensis	Myanmar	MN997975.1 ZMB 113447b
Filopaludina sumatrensis	Thailand	MN997976.1 ZMB 114070
Filopaludina sumatrensis	Vietnam	MN997977.1 ZMB 114405
Pila ampullacea	Southeast Asia and Hawaii	EU528503.1 VN19

obtain genetic distances and phylogenetic trees. Genetic distance was analyzed using the Kimura-2 parameter model to obtain matrix calculations. Phylogenetic tree analysis used the Neighbor-Joining Tree and Maximum Likelihood methods with a bootstrap value of 1,000 replications. Similarity values were analyzed by the following: similarity percentage = (1 - Genetic distance) x 100%. The percentage of nucleotide base composition of COI gene sequences was analyzed via Jupyter Lite (https://jupyter.org) with Python (Pyodide).

3. Results

3.1. Morphological and Morphometric Characterization

Systematics
Subclass Caenogastropoda
Order Architaenioglossa
Family Viviparidae
Genus *Filopaludina*

Filopaludina javanica (von dem Busch, 1844)
(Figure 3A-G)
Diagnosis.

Conical shell with a smooth surface, featuring thin spiral and radial line patterns that are more pronounced in the last body whorl. The apex color tends to be blackish.

Description.

Conical shell, dextral shell round with radial line pattern and spiral line pattern. Shell color yellowish-brown, greenish-yellow, blackish-brown, greenish-brown, greenish-black. Umbilicus slit. The aperture shape is oval. Deep suture line. Concentric operculum. Number of whorls 5-6, with a relatively large body whorl. Shell length 20.00-51.70 mm, shell width 8.70-43.00 mm, aperture length 7.90-30.25 mm, aperture width 7.55-24.90 mm, body whorl length 10.55-43.85 mm, spire length 3.80-11.80 mm.

Observations of *F. javanica* shells from Madura Island showed seven types of morphological variation (Figure 3). The shell morphological variations are indicated by the

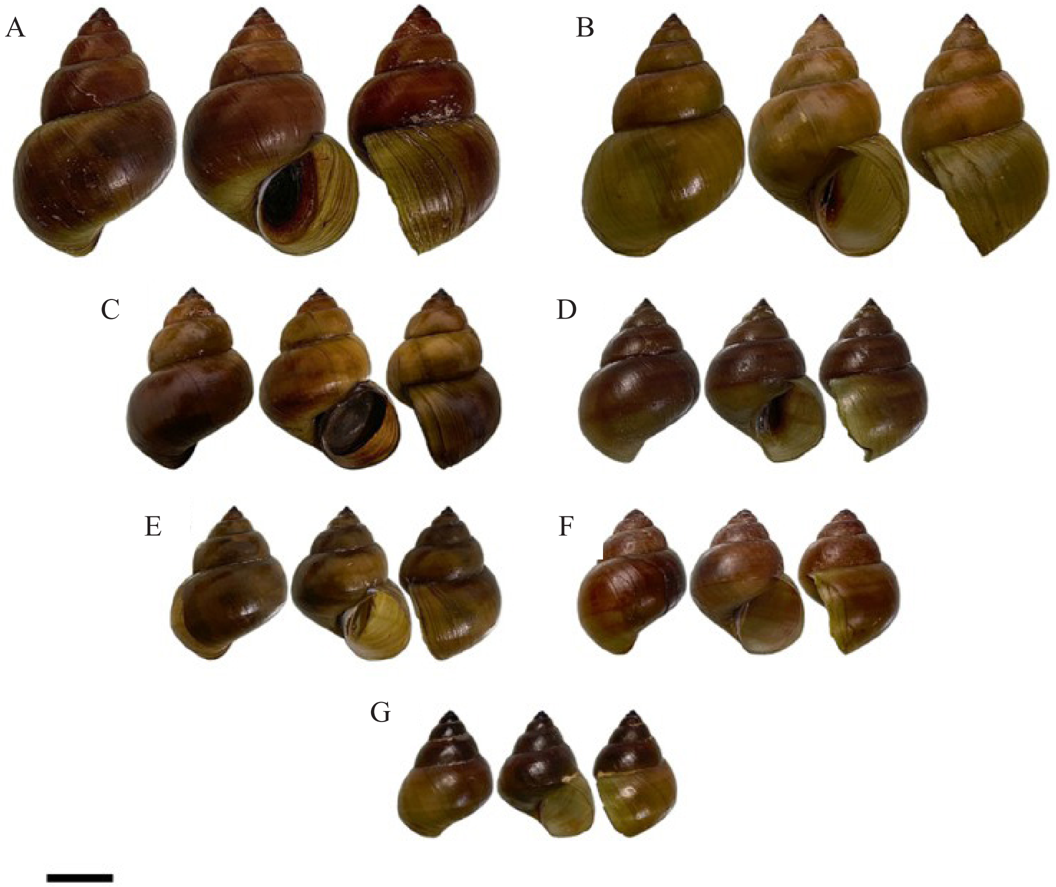

Figure 3. Morphological variation of *F. javanica* on Madura Island. (A) Type 1. (B) Type 2. (C) Type 3. (D) Type 4. (E) Type 5. (F) Type 6. (G) Type 7. Scale bar: 10 mm

presence of distinctive characters such as Spire Color (SC), Body Whorl Color (BWC), Spiral Line Pattern (SLP), Radial Line Pattern (RLP), Apex Shape (A), Apex Color (AC), Umbilicus (U), and Number of Whorls (NW) (Table 2). Besides the seven types of morphological characters of *F. javanica* on Madura Island, differences in morphological variation can also be seen in 274 individuals of shell morphometrics (Table 3).

Figure 4 shows the dendrogram of the whole morphological character relationships between the seven morphological variations of *F. javanica* shells. The results revealed that the morphological characters of *F. javanica* shells were divided into 3 clusters. Cluster 1 comprises two clades of *F. javanica*: clade 1, which includes *F. javanica* types 1 and 3, and clade 2, which is exclusively filled by *F. javanica* type 2. Cluster 2 consists of *F. javanica* types 4, 5, and 7. Meanwhile, *F. javanica* type 6 is in cluster 3.

The Principal Component Analysis (PCA) scatterplot shows variations in shell morphometry that cluster based on morphological type (Figure 5). Components 1 and 2 are a combination that separate the shell morphometry of *F. javanica* based on its morphological type. In each morphological type, *F. javanica* exhibits distinct morphometric variations and tends to cluster.

Figure 6 shows the Principal Component Analysis (PCA) scatterplot, which shows morphological variations in *F. javanica* from Madura Island. Components 1 and 2 are a combination that could group the morphology of *F. javanica* based on its distinguishing characteristics. The scatterplot shows that *F. javanica* types 4, 5, and 7 are clustered in the same quadrant characterized by NW and SC characters. *Filopaludina javanica* type 3 and type 1 are in the same quadrant. Meanwhile, type 2 and type 6 are separated in different quadrants due to differences in characters on U and RLP. PCA analysis shows that SC, SLP, A, and NW are the strongest characteristics in separating the seven morphological types. This is evidenced by the relatively large vector length and the position of the types in the same direction as the vector. Type 4 is clearly separated by vectors SC and SLP, type 6 is separated by vector A, while types 1-3 are distinguished mainly by vector NW.

3.2. Identification of Similarities using the BOLD System and BLAST NCBI

Similarity identification of *F. javanica* from Madura Island based on the COI gene was performed with the BOLD System and BLAST NCBI (Table 4 and 5). The identification results showed high similarity >98%. Similarity values obtained from this study were between 99.52% and 100% when paired with *F. javanica* from Sarawak, West Java, and North Kalimantan.

Molecular characterization was conducted based on Cytochrome C Oxidase Subunit I (COI) gene sequences analyzed from six *F. javanica* samples. Barcode sequence data among the six samples showed an average composition of G+C nucleotide bases of 35.26% and A+T nucleotide bases of 64.75%. In this study, the average percentage of A+T nucleotide base composition is higher than the composition of C+G (Table 6).

Table 7 shows the COI gene nucleotide base sequence transition and transversion mutations in the sample when compared to related species. The transition substitution of nucleotide base number 307 shows a change from base A (Adenine) to G (Guanine). A Transversion mutation is found in nucleotide base number 329, indicating a change in the nucleotide base from T (Thymine) to A (Adenine). Transition substitution of nucleotides was also observed at base number 340, indicating a change from T (Thymine) to C (Cytosine). In nucleotide base number 395, a transversion mutation from T (Thymine) to A (Adenine) is also present. Another nucleotide transition substitution was also present at nucleotide number 574, which shows a T (Thymine) to C (Cytosine) transition. This study shows automorphic nucleotide bases that are specific to *F. javanica* from Madura Island, which distinguishes this population of *F. javanica* from Sarawak, North Kalimantan, West Java, and other species.

3.3. Genetic Distance

The genetic distance value was calculated using the Kimura-2 Parameter calculation model to determine the genetic closeness between individuals. The genetic distance of *F. javanica* samples on Madura Island was compared with GenBank NCBI data to determine the matrix of gene distance values, thereby illustrating the relationship between each sample and the data from GenBank NCBI (Table 8). The results of the genetic distance comparison show that the average genetic distance of *F. javanica* on Madura Island is 0.003. Meanwhile, the average genetic distance value between *F. javanica* on Madura Island and the *F. javanica* in-group on Sarawak, West Java, and North Kalimantan is 0.031. The difference in distance value indicates the effect of geographical isolation on Madura Island.

3.4. Reconstruction of Phylogenetic Trees

The reconstruction of the phylogenetic tree using the Neighbor-Joining Tree and Maximum Likelihood

Tree methods shows 3 clusters (Figures 7 and 8). The depiction of phylogenetic relationships between species is clearly visible, as each species is associated with a distinct cluster that has a high bootstrap value. Cluster 1 consists of 2 clades, namely *F. javanica* from Madura Island in this study, with its close relative *F. javanica* from Sarawak, West Java, and North Kalimantan. Cluster 2 consists of *F. sumatrensis* from Myanmar, Thailand, and Vietnam. *Filopaludina javanica* and its close relative *F. sumatrensis* form distinct monophyletic branches on

Table 2. Type of morphological variation of *F. javanica* on Madura Island

Character	Morphology type						
	1	2	3	4	5	6	7
Spire Color	Bright	Bright	Bright	Dark	Dark	Bright	Dark
Body Whorl Color	Contrasting	Uniform	Uniform	Contrasting	Uniform	Contrasting	Contrasting
Spiral Line Pattern	Absent	Absent	Absent	Present	Present	Present	Absent
Radial Line Pattern	Present	Absent	Present	Absent	Absent	Absent	Absent
Apex Shape	Sharp	Sharp	Sharp	Sharp	Sharp	Blunt	Sharp
Apex Color	Black	Black	Black	Black	Black	Black	Black
Umbilicus	Slit	Slit	Slightly closed	Slit	Slit	Slit	Slit
Number of Whorls	6	6	6	6	6	5	6

Table 3. Morphological variation of *F. javanica* on Madura Island

Type	Morphometry (mean±SD)					
	SL	SP	BW	SW	AW	AL
Type 1 (FB)	30.12±3.97	8.15±1.55	21.97±2.53	20.9±2.27	12.11±1.38	13.26±1.45
Type 2	29.20±2.95	9.08±1.60	20.12±2.41	17.46±21.96	11.57±1.62	14.37±2.34
Type 3	25.34±3.07	7.24±1.57	18.10±2.24	17.39±2.12	9.76±1.08	11.05±1.27
Type 4 (FS)	22.77±1.52	6.21±0.58	16.56±1.31	16.38±1.11	9.63±0.73	10.75±0.73
Type 5	25.82±0.58	7.49±0.67	18.33±0.29	18.26±0.55	9.80±0.26	10.61±0.41
Type 6	41.44±7.34	9.60±11.87	35.84±6.32	34.63±6.05	20.54±3.67	25.77±3.72
Type 7	24.51±3.28	6.82±1.28	17.69±2.23	17.16±2.35	9.68±1.14	10.88±1.32
Average	25.85	7.07	18.78	18.06	10.34	11.77

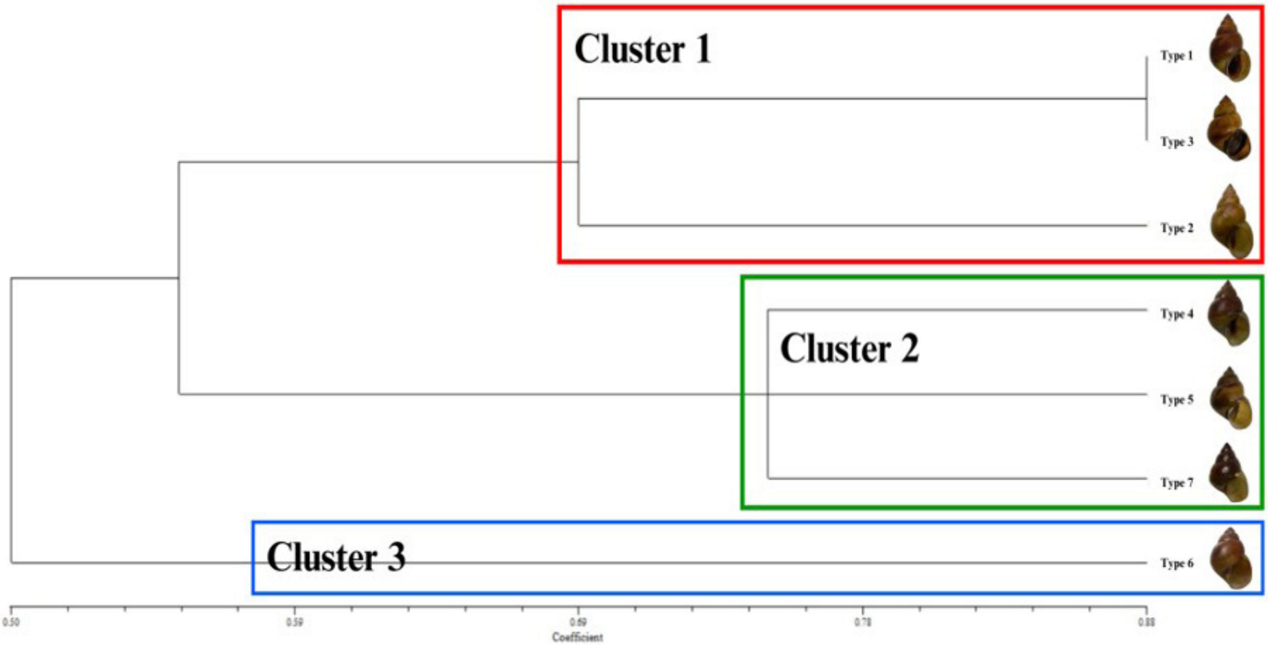

Figure 4. UPGMA cluster analysis based on morphological characteristics of *F. javanica* on Madura Island

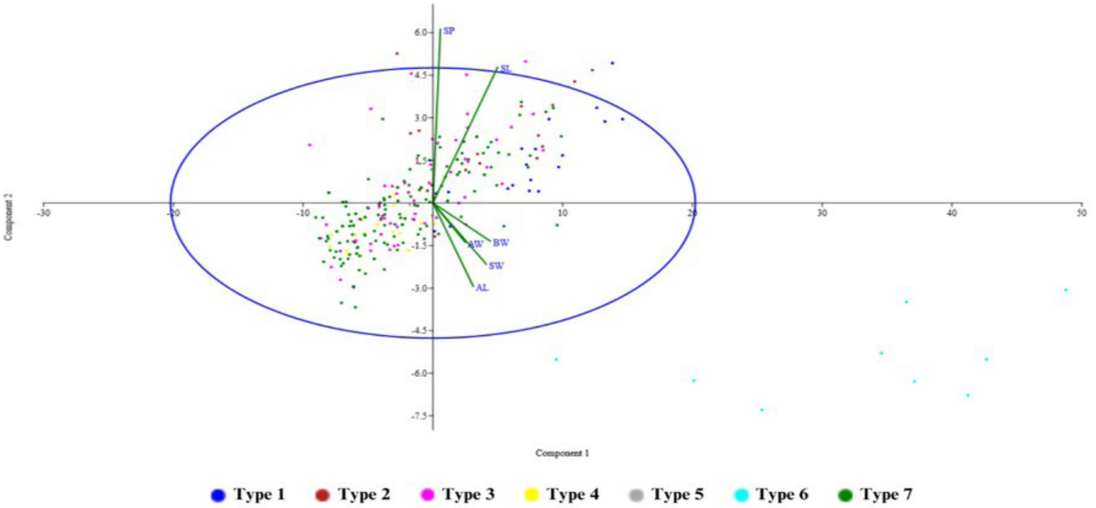

Figure 5. Principal Component Analysis (PCA) scatterplot showing morphometric variation of *F. javanica* shells on Madura Island based on morphological type. (SP-Spire Height, SL-Shell Length, SW-Shell Width, AL-Aperture Length, AW-Aperture Width, BW-Body Whorl)

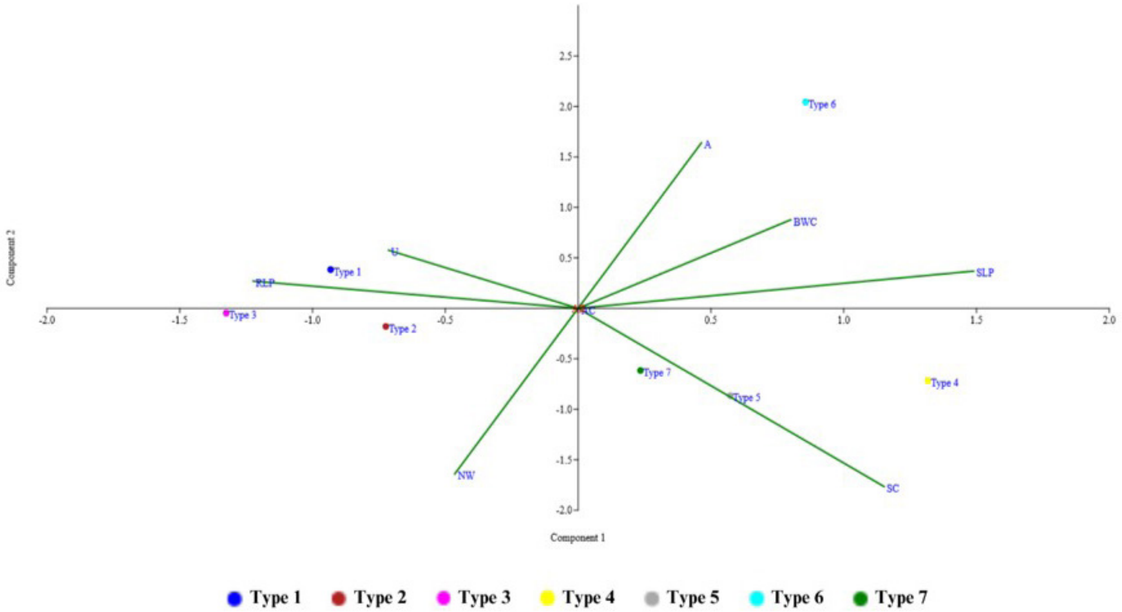

Figure 6. Principal Component Analysis (PCA) scatterplot showing morphological variation of *F. javanica* on Madura Island based on morphological type. (SC-Spire Color, BWC-Body Whorl Color, SLP-Spiral Line Pattern, RLP-Radial Line Pattern, A-Apex Shape, AC-Apex Color, U-Umbilicus, NW-Number of Whorl)

Table 4. Highest similarity pairs from identification via the BOLD System

Field code	Species name	Similarity value (%)
FB1 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	100
FB2 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	99.84
FB3 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	99.68
FS1 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	100
FS2 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	99.68
FS3 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	99.52

Table 5. Highest similarity pairs from identification via BLAST NCBI

Field code	Species name	Similarity value (%)
FB1 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	100
FB2 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	99.84
FB3 (*F. javanica* Burneh, Madura)	*Filopaludina javanica*	99.69
FS1 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	100
FS2 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	99.69
FS3 (*F. javanica* Socah, Madura)	*Filopaludina javanica*	99.53

Table 6. Nucleotide base composition of *F. javanica* on Madura Island, East Java, Indonesia

Sample	A (%)	C (%)	G (%)	T (%)	G + C (%)	A + T (%)
FB1 (*F. javanica* Burneh, Madura)	22.27	13.52	21.81	42.4	35.33	64.67
FB2 (*F. javanica* Burneh, Madura)	22.43	13.52	21.81	42.24	35.33	64.67
FB3 (*F. javanica* Burneh, Madura)	22.58	13.52	21.81	42.09	35.33	64.67
FS1 (*F. javanica* Socah, Madura)	22.38	13.58	21.6	42.44	35.18	64.82
FS2 (*F. javanica* Socah, Madura)	22.69	13.58	21.6	42.13	35.18	64.82
FS3 (*F. javanica* Socah, Madura)	22.84	13.58	21.6	41.98	35.18	64.82
Average	22.53	13.55	21.71	42.21	35.26	64.75

A: Adenine; C: Cytosine; G: Guanine; T: Thymine

Table 7. Nucleotide base variation of *F. javanica* on Madura Island

Species	Nucleotide base variation				
	307	329	340	395	574
MN997939.1 *Filopaludina javanica*	A	T	T	T	T
MN997940.1 *Filopaludina javanica*	●	●	●	●	●
MN997941.1 *Filopaludina javanica*	●	●	●	●	●
MN997942.1 *Filopaludina javanica*	●	●	●	●	●
MN997943.1 *Filopaludina javanica*	●	●	●	●	●
FB1 *Filopaludina javanica* Burneh, Madura (this study)	G	●	C	●	C
FB2 *Filopaludina javanica* Burneh, Madura (this study)	G	A	C	●	C
FB3 *Filopaludina javanica* Burneh, Madura (this study)	G	A	C	●	C
FS1 *Filopaludina javanica* Socah, Madura (this study)	G	●	C	●	C
FS2 *Filopaludina javanica* Socah, Madura (this study)	G	●	C	A	C
FS3 *Filopaludina javanica* Socah, Madura (this study)	G	●	C	A	C
MN997973.1 *Filopaludina sumatrensis*	T	●	●	●	●
MN997974.1 *Filopaludina sumatrensis*	T	●	●	●	●
MN997975.1 *Filopaludina sumatrensis*	T	●	●	●	●
MN997976.1 *Filopaludina sumatrensis*	T	●	●	●	●
MN997977.1 *Filopaludina sumatrensis*	T	●	●	●	●
EU528503.1 *Pila ampullacea*	T	●	●	●	●

● = Conserve sequence

Table 8. Genetic distance of *F. javanica* based on COI gene sequences

ACC number of GenBank NCBI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MN997939.1 *Filopaludina javanica* voucher ZMB 112753																	
MN997940.1 *Filopaludina javanica* voucher ZMB 127082	0.071																
MN997941.1 *Filopaludina javanica* voucher ZMB 127085	0.067	0.012															
MN997942.1 *Filopaludina javanica* voucher ZMB 127086	0.073	0.008	0.014														
MN997943.1 *Filopaludina javanica* voucher ZMB 127093	0.003	0.067	0.064	0.069													
FB1 *Filopaludina javanica* Burneh. Madura (this study)	0.009	0.077	0.073	0.079	0.009												
FB2 *Filopaludina javanica* Burneh. Madura (this study)	0.011	0.079	0.075	0.080	0.011	0.002											
FB3 *Filopaludina javanica* Burneh. Madura (this study)	0.013	0.080	0.077	0.082	0.013	0.003	0.002										
FS1 *Filopaludina javanica* Socah. Madura (this study)	0.010	0.077	0.074	0.079	0.010	0.000	0.002	0.003									
FS2 *Filopaludina javanica* Socah. Madura (this study)	0.013	0.077	0.074	0.079	0.013	0.003	0.005	0.006	0.003								
FS3 *Filopaludina javanica* Socah. Madura (this study)	0.014	0.079	0.075	0.081	0.014	0.005	0.006	0.008	0.005	0.002							
MN997973.1 *Filopaludina sumatrensis* voucher ZMB 113444	0.102	0.113	0.111	0.113	0.098	0.107	0.109	0.111	0.106	0.110	0.111						
MN997974.1 *Filopaludina sumatrensis* voucher ZMB 113446	0.114	0.125	0.123	0.125	0.111	0.121	0.122	0.124	0.119	0.123	0.125	0.022					
MN997975.1 *Filopaludina sumatrensis* voucher ZMB 113447b	0.109	0.124	0.122	0.123	0.105	0.115	0.117	0.118	0.114	0.117	0.119	0.020	0.008				
MN997976.1 *Filopaludina sumatrensis* voucher ZMB 114070	0.101	0.111	0.106	0.109	0.097	0.108	0.110	0.112	0.107	0.110	0.112	0.028	0.044	0.042			
MN997977.1 *Filopaludina sumatrensis* voucher ZMB 114405	0.095	0.100	0.094	0.103	0.095	0.102	0.104	0.105	0.102	0.106	0.108	0.048	0.064	0.062	0.039		
EU528503.1 *Pila ampullacea* isolate VN19	0.181	0.204	0.197	0.204	0.176	0.183	0.185	0.187	0.180	0.180	0.177	0.189	0.199	0.197	0.187	0.189	
Average overall genetic distance of *F. javanica*	0.031																

Marked sections indicate the closest genetic distance between the study sample and the in-group

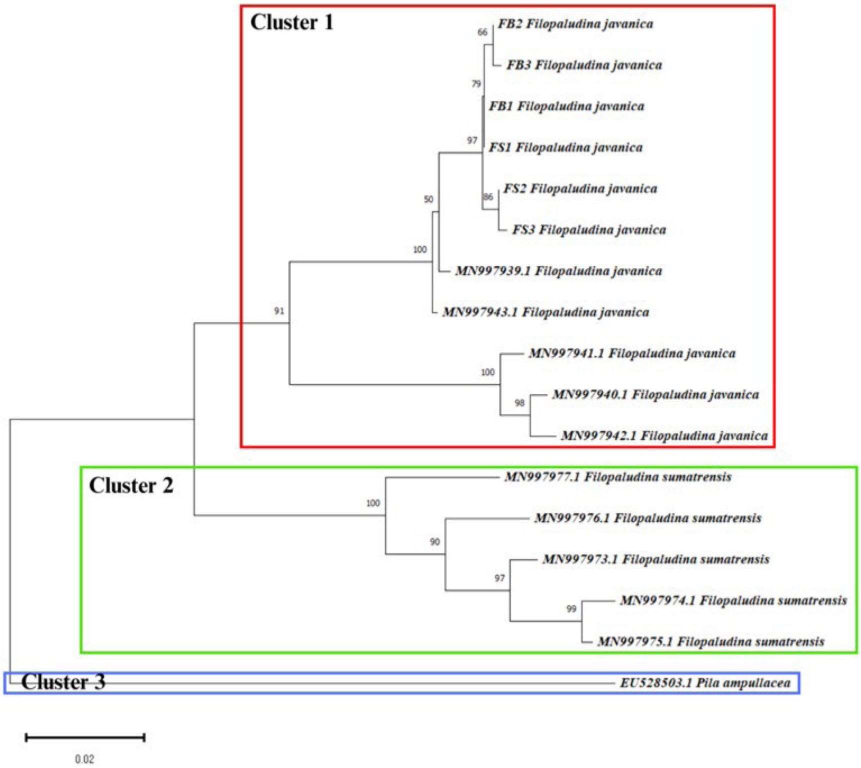

Figure 7. Phylogenetic topology of *F. javanica* on Madura Island, East Java, Indonesia, referring to the COI gene from NCBI GenBank using the Neighbor-Joining Tree method with a bootstrap of 1,000 replications

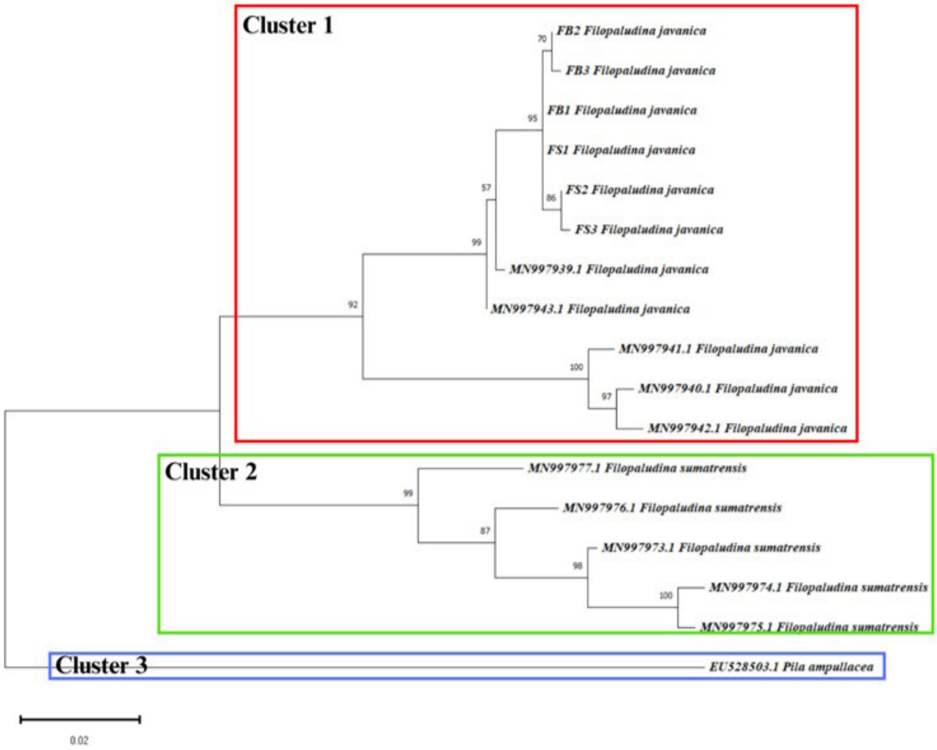

Figure 8. Phylogenetic topology of *F. javanica* on Madura Island, East Java, Indonesia, referring to the COI gene from NCBI GenBank using the Maximum Likelihood method with a bootstrap of 1,000 replicates

the phylogenetic tree. This genetic distance indicates a close evolutionary relationship between *F. javanica* from Madura Island, *F. javanica* within the in-group, and also with *F. sumatrensis*, in accordance with the genetic distance between species. *P. ampullacea* is separated in cluster 3, differentiated as an out-group.

4. Discussion

Filopaludina javanica collected on Madura Island has seven morphological variations. According to Verhaegen *et al*. (2018), variations in shape are mainly related to genetic relatives, while shell size can also be affected by environmental factors. In this study, seven morphological types are distinguished by eight-character markers on the PCA scatterplot. The morphometric scatterplot shows that shell size clusters according to morphological type variation.

Analysis of nucleotide base variation reveals transition and transversion substitutions in nucleotide bases 307, 329, 340, 395, and 574, indicating the presence of automorphic characteristics (307,340, and 574) due to the geographical isolation of *F. javanica* on Madura Island. Factors such as habitat type, climate, presence of predators, and various other environmental factors in different habitats can affect genetic structure (Dumidae *et al*. 2024). In evolutionary biology, environmental variation can create neutral and adaptive genetic variation in natural populations (Quintela *et al*. 2014). The nucleotide base composition of G+C and A+T reveals essential information about the genetic characteristics and evolutionary dynamics of *F. javanica* on Madura Island. The diversity of genetic composition of nucleotide bases in a species indicates different adaptability (Baksir *et al*. 2022). Automorphism characters are unique characters that are only owned by one species, which can be used to distinguish it from other species (Nugroho *et al*. 2017). The nucleotide base variation showed three automorphic patterns that are only owned by *F. javanica* on Madura Island.

According to Valen *et al*. (2019) on the COI gene fragment, the genetic distance matrix data obtained can be used to analyze relationships based on phylogenetic trees. A further analysis found that the average similarity value between *F. javanica* from Madura Island with *F. javanica* on Sarawak, North Kalimantan, and West Java was 97.08%. This indicates that *F. javanica* populations from Sarawak, North Kalimantan, and West Java with Madura Island are still included in one species despite variations in nucleotide bases. The average genetic distance of the study samples was 0.003 while the average genetic distance of *F. javanica* as a whole was 0.031. Environmental factors such as differences in ecological conditions may also be one of the causes of genetic and morphological variation. As Djoemharsjah *et al*. (2023) explained a certain environmental factor in a species can also change the morphology and phylogenetics of the population. The relationship between geographic differences and genetic distance indicates that basic information on genetic distance between two populations can be derived from geographic distance (Gil *et al*. 2015). Geographical location differences and potential barriers affecting gene flow between populations are correlated with variations in similarity percentages among the samples (Maulana *et al*. 2024).

Phylogenetic tree analysis showed that *F. javanica* from Madura Island is in the same cluster as *F. javanica* from Sarawak, West Java, and North Kalimantan. The presence of morphological, morphometric, and nucleotide base composition variations of *F. javanica* on Madura Island reveals a correlation between morphological and morphometric variations and nucleotide base composition. Nucleotide base composition variations create a genetic distance between *F. javanica* from Sarawak, West Java, and North Kalimantan, and *F. javanica* from Madura Island. This may be due to environmental influences and geographical isolation, which caused transition and transversion substitution of nucleotide bases in *F. javanica* from Madura Island.

A study on *F. javanica* from Madura Island revealed morphological, morphometric, and genetic diversity caused by geographic isolation by the Madura Strait. This variation is supported by morphometric diversity with seven variations in shell morphology as shown by Principal Component Analysis (PCA) scatterplot. Genetic diversity in the form of nucleotide base transition and transversion substitution is shown in the order of 307, 329, 340, 395, 574 base pairs. Phylogenetic tree analysis of *F. javanica* from Madura Island revealed that *F. javanica* from Madura Island still belongs to the *F. javanica* cluster in Sarawak, West Java, and North Kalimantan, with an average similarity value of 97.08% despite the presence of a gap in genetic distance and morphological variation. The distinctive automorphic characteristics of the *F. javanica* species on Madura Island are caused by morphological, morphometric, and genetic diversity, which may be influenced by local environmental conditions and the geographical isolation of Madura Island from the mainland of Java.

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