Utilization of COI Marker for Species Identification and Population Delineation of White Shrimp in the Demak Waters, Indonesia

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Abstract

White shrimp is one of the largest artisanal capture fisheries commodities in Demak. The utilization of these shrimps often overlooks their species category as they are mostly white in appearance. Growing trend of shrimp fishing may affect shrimp management due to population declination to the genetic level since the genetic data are insufficient. Hence, we aim to identify and study the genetic diversity of white shrimp caught in Demak Waters to provide an overview as a reference for fishery improvement project. A random purposive sampling method was used to collect a total of 90 white shrimp specimens from four fishing grounds (Babalan, Gojoyo, Menco, and Seklenting) in the Demak Waters. Specimens were extracted using Chelex 10%, amplified using PCR, and sequenced by the Sanger method based on the mtDNA COI gene. This research revealed the presence of five species of white shrimp: Penaeus merguiensis, Fenneropenaeus penicillatus, Penaeus vannamei, Metapenaeus brevicornis, and Metapenaeus ensis*. The genetic diversity studies were continued using the* Penaeus merguiensis *species only. The results showed 17 haplotypes with a genetic diversity (Hd) of 0.6936 and a nucleotide diversity of 0.00108, which express moderate genetic diversity. Population analysis using Analysis of Molecular Variance (AMOVA) indicated a non-significant difference between the four study populations (panmictic population) with an FST value of 0.00756 (P-value < 0.05). Based on these results, an adequate management of stock is important. For instance, an open-closed season is needed in order to sustain and reduce capture pressure on the white shrimp population in Demak waters.*

Keywords: *COI, Demak Waters, Haplotypes, Sustainable Fisheries, White Shrimp*

Introduction

The white shrimp (*Penaeus merguiensis*), locally known as Udang Putih, belongs to the family Penaeidae and is widely distributed across tropical waters of the Indo-West Pacific region (Vance *et al*., 2002). According to Vance and Rothlisberg (2020), this species is benthic and can adapt to various bottom types, though it predominantly inhabits silt and sandy-loam substrates. Throughout its life cycle, *P. merguiensis* utilizes mangrove estuaries as nursery grounds for larvae (Gillanders *et al*., 2003; Sheaves *et al*., 2012), later migrating offshore to mature and spawn. The newly spawned larvae subsequently return to estuarine habitats to continue the cycle (Vance *et al*., 1988; Sheaves *et al*., 2012). Similar to other penaeid shrimps, the species is a detritivore–carnivore and functions as a keystone species linking lower and higher trophic levels (Muro-Torres *et al*., 2020). In Indonesia, *P. merguiensis* is commonly found in the Java Sea, particularly in the Demak coastal waters, where nutrient-rich mangrove ecosystems provide optimal substrate conditions (Yudhistira and Arisuryanti, 2019; Umam *et al*., 2021).

The coastal fishery economy of Demak Regency is primarily supported by the capture fisheries sector, with key products including flathead grey mullet (*Mugil* sp., locally known as Belanak), mackerel, Brown shrimp, White shrimp, and crabs (Adlina *et al*., 2019). White shrimp serves as a leading species in both industrial and artisanal fisheries, with catch production reaching 50,707 kg in the first semester of 2021 and 49,844 kg in the second semester, generating a total production value of approximately 2.8 billion rupiah (Demak Regency Marine and Fisheries Service, 2021). The fishing gears used vary according to the fishing grounds: in Seklenting and Menco, where the waters are relatively calm and sheltered, fishermen commonly use dragon trap nets (bubu naga) and traditional wangkong traps. In contrast, fishermen in Gejoyo are

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more active in offshore fishing, employing trammel nets and push trawls, while in Babalan both trap nets (wangkong traps) and trammel nets are used. Artisanal fishers in Demak generally harvest shrimp without distinguishing between species, often referring to all their catch as "white shrimp," which is not always accurate. This morphological misidentification may lead to errors in catch data and consequently to mismanagement of this economically important species.

Morphological identification poses a considerable challenge for cryptic species such as shrimps. In the case of white shrimp, the presence of sibling species between *Penaeus merguiensis* and *Penaeus indicus* has been reported in previous studies, often causing misidentification among fishermen due to their similar whitish coloration (Alam *et al*., 2015). Molecular identification using the mitochondrial COI gene marker offers an effective approach to resolve this issue. Numerous studies employing COI-based analysis in penaeid shrimps have been conducted in several countries, including Malaysia (Halim *et al*., 2021), Thailand (Prasertlux *et al*., 2024), Vietnam (Huy *et al*., 2024), and China (Han *et al*., 2015). However, molecular research on shrimps in Indonesia remains limited, primarily focusing on species identification with relatively small sample sizes (Solichin *et al*., 2020; Muhammadar *et al*., 2021). Moreover, COI gene analysis can also provide valuable insights into the genetic diversity and population structure of shrimp species, particularly in the Demak waters. Therefore, this study aims to identify and assess the genetic diversity of white shrimp collected from four fishing grounds: Babalan, Gejoyo, Menco, and Seklenting based on the mitochondrial COI gene. Understanding genetic diversity and population structure will contribute to a broader comprehension of white shrimp population dynamics, enrich the regional genetic database, and serve as a scientific reference for the Fisheries Improvement Project (FIP) under the Marine Stewardship Council (MSC) Indonesia to support the sustainability of white shrimp resources in Demak waters

Materials and Methods

The research was conducted from October 2022–April 2023. The sampling locations were carried out following local artisanal fishermen in the estuary area of Demak waters, namely Babalan, Dusun Menco, Dukuh Tambak Gejoyo, and Dukuh Tambak Seklenting, since those locations are the main fishing grounds for white shrimp (Figure 1). A total of 90 specimens of white shrimp were collected from trammel nets and trap nets (wangkong) using the random purposive sampling method (Figure 2). Each white shrimp was preserved in a 50ml tube containing 96% ethanol, given an ID number, and stored in a coolbox for further laboratory analysis.

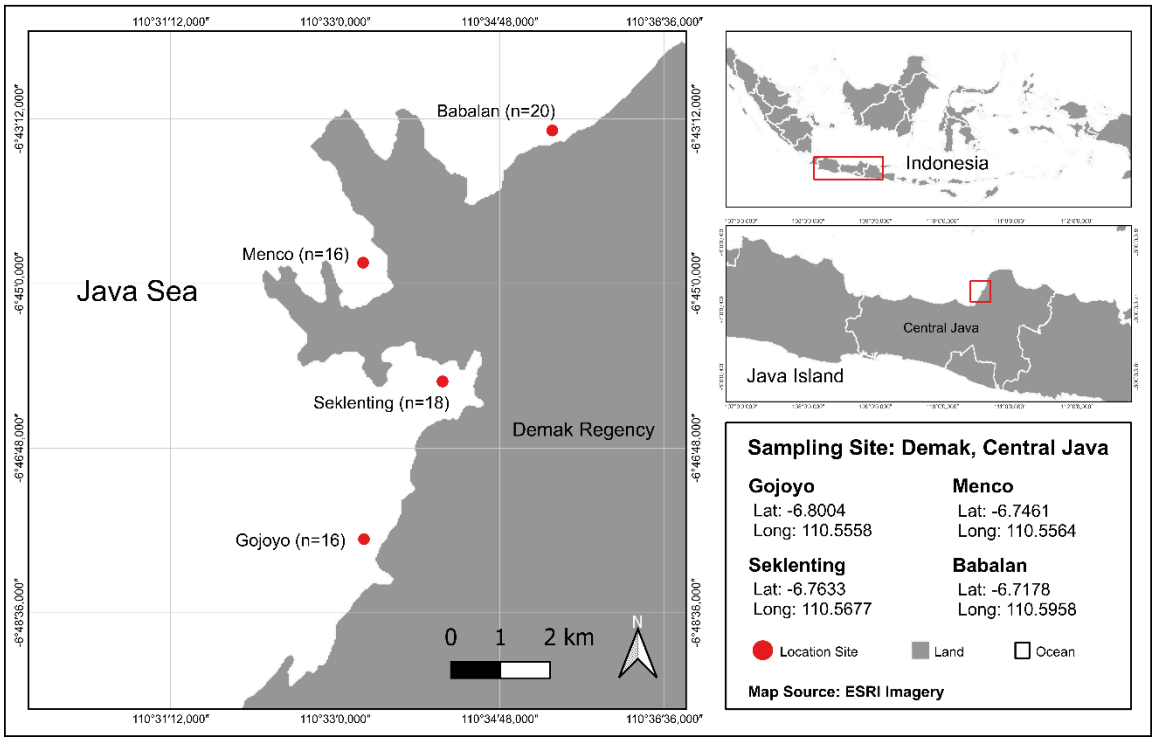

Figure 1. Sampling Location: Demak waters, Java Sea, Central Java.

Figure 2. Traditional trap net (Wangkong); a. bamboo fence nets; b. trap net (Impes/Divek) with hand nets; c. bamboo hut.

Molecular analysis

DNA extraction was carried out using a Chelex 10% solution (Walsh *et al*., 1991). White shrimp tissues were taken at approximately 1 mg and placed in a tube containing 0.5 ml of Chelex solution (Solichin *et al*., 2020). The sample was vortexed for 10–15 sec (Wijayanti *et al*., 2018), then heated using a heating block at a temperature of 95ºC for 45 min. After being heated, the sample was vortexed again for 10–15 sec, then centrifuged using a microcentrifuge at a speed of 10,000 rpm for ±3 min, which aims to precipitate the sample tissue in the Chelex solution (Wijayanti *et al*., 2018). This study targeted Cytochrome Oxidase I (CO1) locus of mitochondrial DNA (mtDNA). The PCR reaction was carried out in a 25 µL mixture containing 1.25 µL of DNA template; 12.5 µL of My TaqTM HSRed Mix PCR kit (BIOLINE: 25 µM MgCl2, 5 U.µL^{-1} Taq Polymerase, 10x Taq Buffer, and 10 µM dNTPs); 1 µL of each primer and 9.25 µL of distilled water. The primers used were according to Geller *et al*. (2013), specifically: forward primer (JgLCO1490: 5'-TITCIACIAAYCAYAARGAYATTGG-3') and reverse primer (JgHCO2198: 5'-TAIACYTCIGGRTGICCRAARAAYCA-3'). DNA amplification was carried out using a Bio-Rad™ MJ Mini 48-Well Personal Thermal cycler. The amplification process was carried out for 40 cycles, consisting of a pre-denaturation process at 80°C for 10 min, double-stranded DNA separation (denaturation) at 95°C for 30 sec, and primer attachment (annealing) at 50°C for 30 sec. Segment lengthening (extension) was carried out at 72°C for 45 sec and post-extension at 72°C for 5 min (Geba *et al*., 2021). The sequencing process aims to obtain a sequence of nucleotide bases from PCR products that have been tested by an electrophoresis process with good results. Several requirements must be fulfilled, such as the sample having been amplified and getting a single band, no smear, and sufficient base length. Samples were then sequenced using the Sanger Dideoxy Sequencing method at PT. Genetika Science Indonesia to obtain the sequence of nucleotide bases.

The sequences used in this study were trimmed, edited, and aligned using MEGA11 (Molecular Evolutionary Genetic Analysis) software (Edgar, 2004). Species identification was carried out

by comparing the sequences with those in the database using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information, National Institute for Health, USA (www.ncbi.nlm.nih.gov). The species name of each sequence was decided based on the highest percent of identity and query cover values detected from BLAST. A phylogenetic tree was constructed using 683 bp in length with the addition of *Pristipomoides multidens* (accession no. OR524596.1) as an outgroup. Reconstruction of the phylogenetic tree was carried out using the maximum likelihood, as well as a statistical approach to the bootstrap method with 1000 repetitions. In this study, genetic diversity analyses were conducted focusing on the species *P. merguiensis*. Furthermore, analysis of haplotype and nucleotide diversity, as well as genetic differentiation estimation, was carried out using DNAsp 6.0 software (Rozas, 2009). Molecular Variance Analysis (AMOVA) was performed using Arlequin 3.1 (Cao and Li, 2016). Population connectivity was visualized through haplotype network analysis using PopART v1.7 (Population Analysis with Reticulate Trees) software, with Median-joining network algorithm (Leigh and Bryant, 2015).

Results and Discussion

In total, 77 of 90 specimens were successfully amplified and sequenced, with a final edited sequence length of 683 bp (Table 1). The unsuccessful amplification is assumed to be caused by several factors, such as the degradation of the DNA sample, an insufficient amount of DNA template (a faint band), and the presence of inhibitors that contaminate the DNA samples (Nguyen *et al*., 2009). The resulting COI sequences were analyzed for homologies with sequences in the database using BLAST. The results showed that the percentage of identities are ranging from 98.56–100% and query cover values of 92–100%. The white shrimp COI sequences obtained in this study were deposited in the GenBank database with accession numbers ON259533-ON259570, ON263286-ON263303, ON332466-ON332485, and ON263409 (Figure 3).

Based on the BLAST results, the samples were detected to be close to 5 species, namely *Penaeus merguiensis, Penaeus vannamei, Fenneropenaeus penicillatus, Metapenaeus brevicornis*, and *Metapenaeus ensis* (Table 1). These species belong to the Penaeidae family and typically reside on muddy substrates and estuaries with mangrove ecosystems (Vance and Rothlisberg, 2020). The existence of species differences beyond the target species (*P. merguiensis*) indicates that morphological identification alone is not sufficient; therefore, molecular identification is used in this study to improve the validity of the data. Among all the non-target species, we identified *Penaeus vannamei*, a non-native Indonesian shrimp species. *Penaeus vannamei*, or synonym *Litopenaeus vannamei,* often called Whiteleg Shrimp and Pacific White Shrimp, is indigenous to the tropical Eastern Pacific, ranging from northern Peru to the Gulf of California and Mexico (FAO, 2014). *P. vannamei* became the most widely farmed shrimp in the world and has been cultivated in at least 27 countries, including Indonesia, since the 1990s (FAO, 2014). The occurrence of *P. vannamei* in the wild is presumed to result from escapes originating from the numerous shrimp aquaculture ponds along the northern coast of Java. Further phylogeography studies are required to confirm whether *P. vannamei* is a potentially invasive species. On the other hand, the discovery of the species M. *brevicornis, M. ensis*, and *F. penicilatus* is reasonable because they are native to Indonesian waters (Cao *et al*., 2017; Pratiwi *et al*., 2023; Wardani *et al*., 2022).

The phylogenetic tree results indicate that all samples from four populations (Babalan, Gejoyo, Menco, and Seklenting) are grouped into five clades based on species without any differences between locations (Figure 3). In the *P. merguiensis* clade, the number of sequences included in the phylogenetic tree was taken from 17 unique haplotypes from a total of 70 *P. merguiensis* samples. The grouping is based on the similarity of characters or traits that are considered to have a very close relationship and are thought to be descended from a common ancestor (Ramirez *et al*., 2021). Sequences derived from one ancestral lineage will form a monophyletic group. The result performs a monophyletic group *between P. merguiensis* and *F. penicillatus*, while performing a paraphyletic group between *P. vannamei*, *M. ensis* and *M. brevicornis*. The addition of the *Pristipomoides multidens* (accession no. OR524596.1) outgroup was done to distinguish and polarize characters or traits, which are divided into two categories, namely apomorphy and plesiomorphy. (Costa *et al*., 2007; Mondal and Mandal, 2020). In addition, Hualkasin *et al*. (2003) shows two clades that separate populations in the Gulf of Thailand from populations in the Andaman Sea. As well as Yudhistira and Arisuryanti (2019), who researched *P. monodon* in Indonesia, revealed that the genetic divergence between the two clades was not based on geographic distribution.

The average genetic distance within a clade (intraspecies) ranged from 0% to 0.15%, while the average genetic distance between clades (interspecies) ranged from 5.07% to 24.69% (Table 2). The intra- and interspecies genetic distances sampled were between the Decapoda intraspecific and interspecific thresholds. Within the Order Decapoda, the highest known intraspecies distance

Table 1. BLAST results with 683 bp sequence length

Sample ID	BLAST results	Accession number	Ident (%)	Query Cover (%)	Deposited ID
SE1	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332466
SE2	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332467
SE3	*Penaeus merguiensis*	MT178524.1	99.84	92	ON332468
SE4	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332469
SE5	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332470
SE6	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332471
SE8	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332472
SE9	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332473
SE10	*Metapenaeus brevicornis*	MT438424.1	99.39	95	ON332474
SE11	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332475
SE12	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332476
SE13	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332477
SE14	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332478
SE15	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332479
SE16	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332480
SE17	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332481
SE19	*Penaeus merguiensis*	MT876653.1	99.56	99	ON332482
SE20	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332483
SE22	*Penaeus merguiensis*	MT876653.1	99.41	99	ON332484
SE23	*Metapenaeus ensis*	MK500697	99.41	100	ON332485
M1	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263286
M2	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263287
M3	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263288
M4	*Fenneropenaeus penicillatus*	KX891351.1	98.83	100	ON263289
M5	*Penaeus merguiensis*	MT876653.1	99.41	99	ON263290
M6	*Penaeus merguiensis*	MT876653.1	99.41	99	ON263291
M7	*Penaeus merguiensis*	MT876653.1	98.56	99	ON263292
M8	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263293
M9	*Penaeus merguiensis*	MT876653.1	99.41	99	ON263294
M10	*Penaeus vannamei*	NC_009626.1	99.85	99	ON263409
M11	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263295
M12	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263296
M13	*Penaeus merguiensis*	MT876653.1	99.41	99	ON263297
M14	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263298
M15	*Penaeus merguiensis*	MT876653.1	99.41	99	ON263299
M16	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263300
M17	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263301
M19	*Penaeus merguiensis*	MT876653.1	99.56	99	ON263302
M20	*Fenneropenaeus penicillatus*	KX891351.1	98.98	100	ON263303
BA1	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259533
BA2	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259534
BA3	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259535
BA4	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259536
BA5	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259537
BA6	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259538
BA7	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259539
BA8	*Penaeus merguiensis*	MT876653.1	99.26	99	ON259540
BA9	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259541
BA10	*Penaeus merguiensis*	MT876653.1	99.26	99	ON259542
BA11	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259543
BA12	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259544
BA13	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259545
BA14	*Penaeus merguiensis*	MT876653.1	99.26	99	ON259546
BA15	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259547
BA16	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259548
BA17	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259549
BA18	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259550
BA19	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259551
BA20	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259552
G1	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259553
G2	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259554
G3	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259555

Table 1. BLAST results with 683 bp sequence length (continue)

Sample ID	*BLAST results*	Accession number	Ident (%)	Query Cover (%)	Deposited ID
G4	*Penaeus merguiensis*	MT876653.1	99.12	99	ON259556
G5	*Fenneropenaeus penicillatus*	KX891351.1	98.98	100	ON259557
G6	*Penaeus merguiensis*	MT876653.1	99.85	97	ON259558
G7	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259559
G8	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259560
G9	*Penaeus merguiensis*	MT876653.1	100	97	ON259561
G10	*Fenneropenaeus penicillatus*	KX891351.1	98.98	100	ON259562
G11	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259563
G12	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259564
G13	*Penaeus merguiensis*	MT876653.1	100	97	ON259565
G14	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259566
G15	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259567
G16	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259568
G17	*Penaeus merguiensis*	MT876653.1	99.56	99	ON259569
G18	*Penaeus merguiensis*	MT876653.1	99.41	99	ON259570

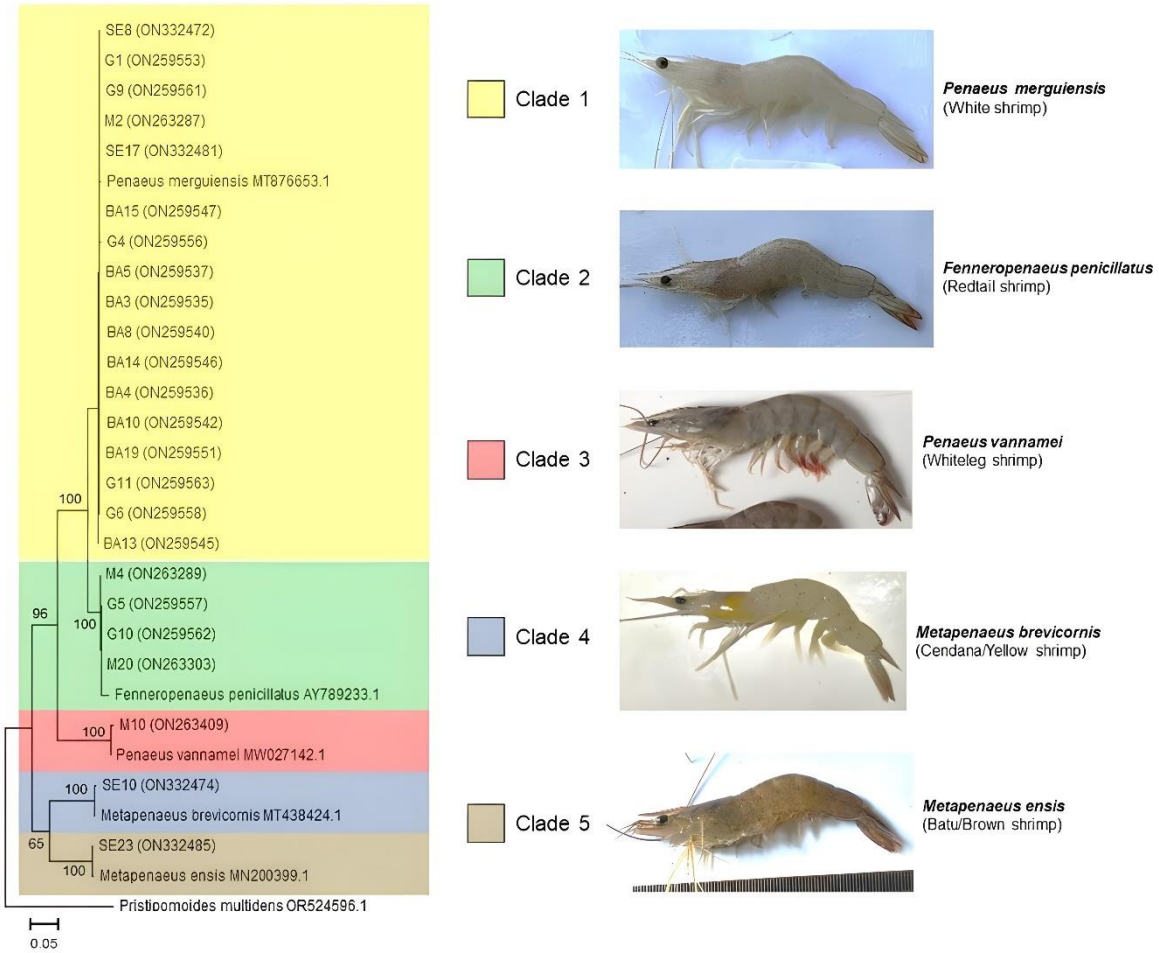

Figure 3. Phylogenetic Tree (Maximum Likelihood Tree), 883 bp, Outgroup *Pristipomoides multidens*

for the COI mtDNA gene is ~2%, while interspecies distances are usually higher than 5% (Batista *et al*., 2019) and can exceed 30% (Costa *et al*., 2007). This threshold value is also in line with the research of Penaeid shrimp Ramirez *et al*. (2021), which showed an average intraspecies distance of 1.3% and interspecies 19.7%. Nevertheless, we observed that the lowest genetic distance between *Penaeus merguiensis* and *Fenneropenaeus penicillatus* with only a 5,07% difference showed a monophyletic grouping; conversely, *Penaeus vannamei* became paraphyletic against them (Figure 3). It could be assumed that

Table 2. Pairwise genetic distance of white shrimp species

Genetic Distance	*P. merguiensis*	*M. brevicornis*	*M. ensis*	*P. penicillatus*
P. merguiensis	-	-	-	-
M. brevicornis	0.2131	-	-	-
M. ensis	0.2139	0.1815	-	-
P. penicillatus	0.0507	0.2162	0.2195	-
P. vannamei	0.1893	0.2303	0.2469	0.1915

Table 3. Genetic Differentiation Estimate of *P. merguiensis* within-location and between-location

Location	Within location mean distance	Between location mean distance		
		SE	BA	G
SE	0.00117			
BA	0.00174	0.00759		
G	0.00210	0.03536	0.01688	
M	0.00089	0.02211	0.00513	0.00513

Table 4. Haplotype and nucleotide diversity of *P. merguiensis*

Location	ns	h	Hd	π	S
BA	20	10	0.758	0.00173	8
G	16	8	0.808	0.00051	9
M	16	5	0.533	0.00089	4
SE	18	5	0.667	0.00117	3
All locations	70	17	0.6936	0.00149	16

Note: ns= Number of s equences; h= Number of haplotypes; Hd= Haplotype diversity; π= Nucleotide diversity; S= Number of segregating sites.

these two species are derived from one ancestral lineage as a sibling species. This result is in line with Lavery *et al*. (2004), revealing that *Penaeus penicillatus*, *Penaeus silasi*, and *Penaeus indicus* are sister taxons; *Penaeus merguiensis* then joined this subclade with *Penaeus chinensis* as the most outlying sister taxon. Moreover, *F. penicillatus* and *P. merguiensis* appear morphologically similar; thus, it required morphometric and meristic studies to confirm the difference between both species (Figure 3). Furthermore, there is often confusion in the use of genus names in crustacean taxonomy (for example, *Fenneropenaeus* or *Penaeus*); therefore, Flegel *et al*. (2007) proposed to include a statement in brackets after the new binomial (or sub-genus) the first time it is mentioned [*e.g., Fenneropenaeus merguiensis* (also called *Penaeus merguiensis*)].

A genetic diversity analysis was conducted using 70 sequences of *Penaeus merguiensis*. This study revealed a moderate haplotype diversity (Hd=0.6936) with low nucleotide diversity (0.00149) on average in all locations (Nei, 1987). There is a relatively low (Hd=0.533) value in the Menco fishing ground located in a closed bay, so it is assumed that there is a physical barrier that prevents gene flow. Haplotype diversity is shaped by intricate interactions among random mating, migration, mutation, substantial population size, and natural selection. Genetic diversity of a species provides the ability to adapt to environmental and climatic changes as well as disease (Liu *et al*., 2013; Wong *et al*., 2021). In contrary, low genetic diversity increases the risk of extinction as it reduces the potential of species to adapt to environmental changes (Hobbs *et al*. 2013). However, the average value of *P. merguiensis* haplotype diversity is lower than other Penaeid shrimp studies but quite high when only compared to the Gejoyo region (Vaseeharan *et al*., 2013; Alam *et al*., 2015; Cao and Li, 2016; Yudhistira and Arisuryanti, 2019). The nucleotide diversity (π) value is very low compared to other shrimp studies. Haplotype diversity and nucleotide diversity of white shrimp show low values compared to other pelagic organisms such as tuna (Menezes *et al*., 2012; Pertiwi *et al*., 2017). This study also highlighted that the overall haplotype diversity of white shrimp in Demak waters is moderate compared to other Penaeid shrimp that had been recorded within the last ten years (Vaseeharan *et al*., 2013; Alam *et al*., 2015; Cao and Li, 2016; Yudhistira and Arisuryanti, 2019; Soares *et al*., 2021).

Haplotype distribution analysis revealed 17 unique haplotypes, with 10 haplotypes found in the Babalan fishing ground (Figure 4). The haplotype network

Table 5. Analysis of Molecular Variance (AMOVA) of *P. merguiensis*

Source of Variation	d.f.	Sum of squares	Variance component	Percentage of Variation
Among population	3	1.717	0,00385 Va	0.76
Within population	66	33.340	0,50516 Vb	99.24
Total	69	35.057	0,50900	
FST	0.00756			
P-value	0.29326 ± 0.01476			

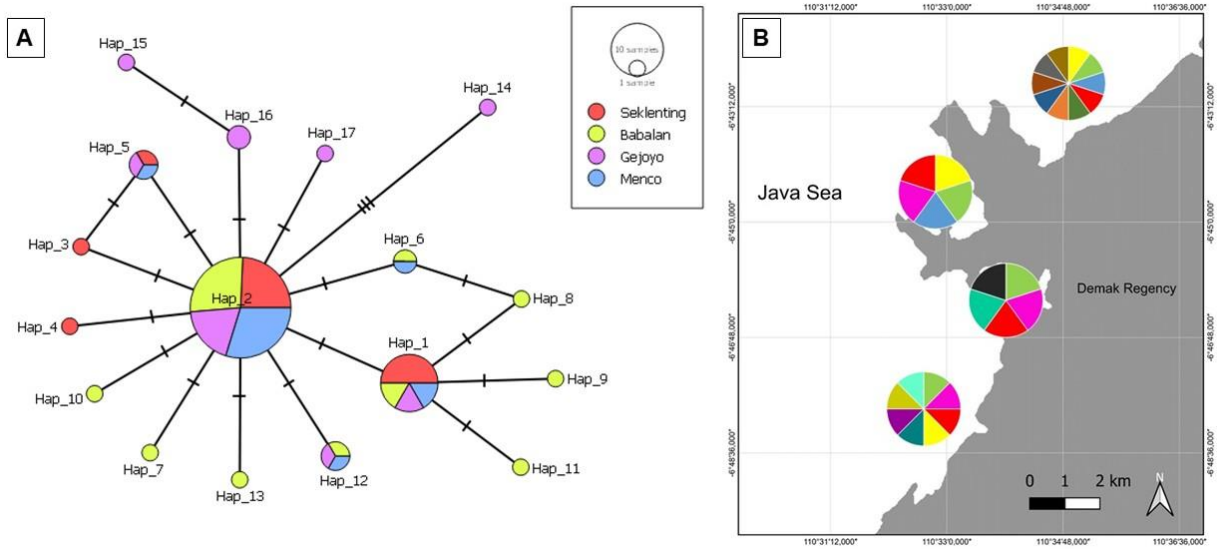

Figure 4. A. Haplotype Network in Demak, each color represents a different location; B. Overall Haplotype Distribution Map in Demak, each color represents a different haplotype.

shows one essential haplotype (Hap_2) located at the center of the network, indicating its role as a maternal ancestor, the origin of some of the recorded mutations (Bandelt *et al*. 1999). The haplotype distribution also indicated that there was haplotype sharing between populations of *P. merguiensis* on the Demak coast with 1 haplotype shared only between two populations (Babalan-Menco), 2 haplotypes shared between three populations (Babalan-Gejoyo-Menco, Gejoyo-Menco-Seklenting), and 2 haplotypes shared among all four populations (Figure 4). The overlapping haplotype from both locations may indicate that *P. merguiensis* population comes from the same gene pool. Genetic differentiation estimate (Table 3) shows all differentiation values <0.05, with all non-significant values corresponding to the Chi-square significance level (0.01<*P*<0.05), indicating almost no separation with high gene flow. The AMOVA results (Table 5) indicate the highest percentage of variation within the population, with a low FST and a *P*-value >0.05. Both of these statistical results indicate a low population structure with non-significant genetic variation based on references (Wright, 1978; Excoffier *et al*., 1992). Therefore, it can be justified that the four fishing grounds constitute a single panmictic Demak population. These results are also in line with the conditions of Demak coastal waters, which exhibit a complex geomorphological profile characterized by both erosion and accretion processes, influenced by weak tidal dynamics (Wirasatriya *et al*., 2017). These conditions may be associated with the observed moderate-to-low levels of genetic diversity in Menco and Seklenting populations, with no significant population structure detected.

P. merguiensis is a benthic species (Vance and Rothlisberg, 2020) that is generally considered to have limited mobility and migration capacity. Similar to other penaeid shrimps, *P. merguiensis* exhibits a fast reproductive rate, early sexual maturation, and a short lifespan (Dall *et al*., 1990). Nevertheless, its pelagic larval stage enables dispersal across distant populations, facilitated by surface currents (Hutabarat *et al*., 2015). To date, comprehensive shrimp catch data from the four study areas are still lacking, thus preventing further assessment of the relationship between fishing intensity and genetic diversity patterns. Although penaeid shrimps possess high fecundity that may support short-term population recovery, reduced genetic diversity can diminish their adaptive potential and long-term

sustainability, particularly under environmental fluctuations or anthropogenic stressors. The present study provides a valuable reference for white shrimp stock management, which would be further strengthened by incorporating population dynamics analyses, particularly in the Demak waters.

Conclusions

Molecular identification based on COI markers revealed five shrimp species from the collected samples: *Penaeus merguiensis*, *Fenneropenaeus penicillatus*, *Penaeus vannamei*, *Metapenaeus brevicornis*, and *Metapenaeus ensis*. The population and genetic diversity analysis of *P. merguiensis* indicated that individuals from Babalan, Menco, Gejoyo, and Seklenting form a single panmictic population with no significant genetic differentiation. Effective stock management, such as implementing open and closed fishing seasons, setting catch quotas, and regulating size limits in estuarine fishing grounds, is required to sustain and reduce fishing pressure on white shrimp populations, particularly in the Menco fishing ground. Furthermore, detailed morphological assessments are recommended to record shrimp species diversity in the Demak region.

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