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Connectivity of *Variola albimarginata* Between Marine Protected Areas and Fishing Grounds in Fisheries Management Area 714: A Molecular Approach

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ABSTRACT

This study investigated the connectivity of *Variola albimarginata* populations between marine protected areas (MPAs) and fishing grounds within fisheries management area 714 (FMA 714) using molecular approaches. DNA barcoding with the *cytochrome c oxidase subunit I (coi)* gene marker was employed to assess genetic diversity and connectivity. A total of 18 samples were analyzed, showing high genetic diversity, albeit with low genetic variation. The haplotype network analysis revealed the presence of seven distinct haplotypes, with one dominant haplotype observed in 11 samples from four different populations. The remaining six haplotypes were found in single individuals, suggesting a more limited distribution of these genetic variants. The findings indicate significant gene flow between the MPAs and fishing grounds, supporting the hypothesis of population connectivity across these areas. Despite the high haplotype diversity, the low nucleotide variation observed suggests that the populations may have undergone recent population bottlenecks followed by rapid growth. Overall, the study highlights the importance of genetic monitoring to understand the connectivity of fish populations in managing marine resources effectively, particularly in areas exposed to both conservation efforts and fishing pressures.

INTRODUCTION

Indonesia has great potential for fishery resources, with a sea area of 5.9 millionkm^2 and a coastline of 95,161km, which is the second longest in the world **(Arian, 2020)**. The potential of Indonesia's fishery resources is estimated at around 12.54 million tons/year

(Munaeni *et al.,* 2024). Meanwhile, the potential of fish resources (SDI) in Indonesia reached 12.01 million tons/year **(KKP, 2022)**. This value shows a decrease in the potential of SDI from the previous 12.54 million tons in 2017 **(KKP, 2017)**. Fish resources are seen as renewable resources so that management efforts are expected to ensure the sustainability of fish resources.

In an effort to manage fisheries resources optimally and sustainably, based on the Regulation of the Minister of Marine Affairs and Fisheries Number 18 of 2014 concerning the fisheries management areas (FMAs) of the Republic of Indonesia, Indonesian waters are divided into 11 FMAs that are based on the physical, biological, oceanographic, and ecological characteristics of the waters. One of the FMAs in Eastern Indonesia is FMA 714, which includes the waters of the Banda Sea and Tolo Bay with the potential for utilization of fish resources including squid, demersal fish, reef fish, large pelagic fish, small pelagic fish, crabs, lobsters, and penaeid shrimp. However, one of the SDI groups that has reached an over-exploited condition is the reef fish group. The potential of coral fish resources in FMA 714 in 2017 was 145,530 tons with a total allowable catch of 116,424 tons, but decreased in 2022 where the potential became 121,326 tons with a total allowable catch of 60,663. This is because the coral fish group is being exploited excessively so that the status of the condition of coral fish resources in FMA 714 which was initially fully exploited in 2017 became over-exploited in 2022.

Reef fish are a group of fish that have important economic value and are therefore vulnerable to overexploitation **(Amorim *et al.,* 2019)**. Based on the Decree of the Minister of Marine Affairs and Fisheries No. 50 of 2017, one type of fish included in this group is grouper. Grouper has a high economic value and is Indonesia's main export commodity **(Halim *et al.,* 2020; Dimarchopoulou *et al.,* 2021)**. The increasing demand for grouper exports and the high trade value encourage exploitation. This has an impact on the decline in the population of several species, including *Variola albimarginata*. Although based on the IUCN Red List Category, *V.albimarginata* is included in the Least Concern (LC) **(Damora, *et al.,* 2021)**, simultaneously this species is one of the grouper species categorized in a population decline trend **(Fadli *et al.,* 2021)**. This is because there are already signs of over-fishing in the *V.albimarginata* species **(Achmad *et al.,* 2024)**.

Understanding genetic connectivity is an important step in conservation. Connectivity is the exchange of genetics between individuals and their offspring through migration and dispersal **(Schunter *et al.,* 2011)**. Connectivity can be seen through a molecular approach with the DNA barcoding method. DNA barcoding is a method that uses short and specific DNA sequences to identify species **(Hebert *et al.,* 2003a)**. In DNA barcoding, the gene marker used is *coi* from mitochondrial DNA (mtDNA). This gene marker is used to identify species and test evolutionary relationships and relationships between and within taxa **(Madduppa *et al.,* 2021)**. In addition, mtDNA is

easy to explain and produces population connectivity relatively quickly **(Galtier *et al.*, 2009)**.

Genetic connectivity information can be used to estimate the direction/model of an organism's movement which can be used as a key to conservation, so that genetic connectivity plays an important role in maintaining populations and recovering from damage **(Almany *et al.*, 2009)**. Therefore, information related to *V.albimarginata* connectivity between marine protected areas (MPAs) and fishing grounds is important to help plan appropriate conservation strategies. This is important for the population of coral fishes, especially *V.albimarginata* to avoid experiencing further decline. The aim of this research was to analyze the genetic distance, phylogenetic relationships, as well as genetic diversity, population structure, and genetic connectivity of *V.albimarginata* between MPA and fishing ground in FMA 714.

MATERIALS AND METHODS

Sample collection

V.albimarginata (white-edged lyretai) samples were collected in the FMA 714 in April-May 2022. Samples were obtained with survey method from 4 locations, Banda and Kei, which are considered as MPAs, while Ambalau and Haja are designated as fishing ground (Fig. 1). The dorsal muscle tissue (tissue) from the samples was taken one by one as a source for genetic analysis **(Ariyanti *et al.*, 2015)**. The collected tissue samples were preserved in labeled sample tubes containing 96% alcohol **(Marwayana, 2015)**.

DNA extraction, primer and PCR

Genomic DNA was extracted using the Double Spin Column method and following the Spin-Column Protocol from the Qiagen Dneasy blood and tissue kit. Extracted DNA was quantified using the Nabi UV/Vis Nano spectrophotometer. The primers used in the PCR process are *coi* primers **(Folmer *et al.*, 1994)**, where the forward primer is LC-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and the reverse primer is HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR was set up in 50µL mixture reaction (25µL Bioline My Taq Master Mix, 1.0µL of each primer, 2µL DNA template and 21µL of nuclease free water). The PCR amplification start with initial denaturation at 95ºC for 3min, followed 35 cycles of denaturation at 95ºC for 30s; annealing at 42ºC for 30s; extension at 72ºC for 1.0min and a final extension at 72ºC for 7min.

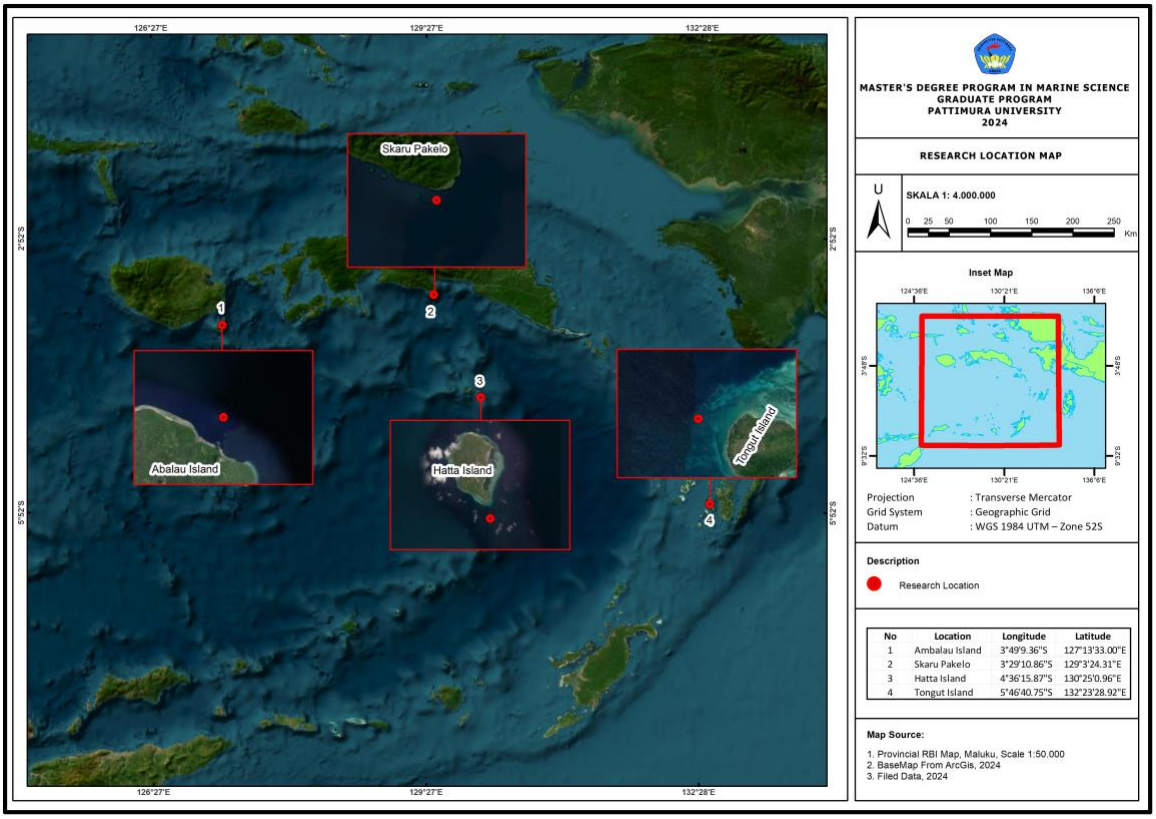

Fig. 1. Sampling site of *Variola albimarginata* in FMA 714

Gel electrophoresis and sequencing

Amplicons were separated and visualized on a 2% agarose gel stained with 5.0µL of ethidium bromide. A 5.0µL volume of PCR product mixed with 1.0µL loading dye was loaded into the agarose gel. Successful PCR products were sent to First BASE Laboratories Malaysia for the sequencing analysis, with a total of 18 samples.

Data analysis

The *coi* sequences were initially aligned using Clustal W and then edited in MEGA XI **(Tamura *et al.*, 2021)**. The corrected DNA fragment results were then saved in FASTA format and compared with the available data in GenBank on the NCBI website (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/) using the BLAST (Basic Local Alignment Search Tool) method. The aim was to assess the sequence alignment accuracy (query cover, identified, and identities) of the research DNA sequences with the reference standards available in GenBank **(Rahayu *et al.*, 2019)**. Phylogenetic analysis was then conducted on the BLAST results using the Neighbor-Joining (NJ) method, the Kimura 2-parameter (K2P) model, and 1000 bootstrap replicates **(Kimura, 1980)**. Genetic distances between species were calculated using the same software and model, and the results were described based on categories **(Nei, 1972)**.

The statistical values, including haplotype diversity (Hd) and nucleotide diversity (π) for genetic diversity analysis, as well as the fixation index (Fst) for population structure analysis, were described according to the value categories defined by **Nei (1987)** and **Excoffier *et al.* (1992)** (Table 1).

Haplotype distributions were analyzed and summarized in DnaSP 6 **(Rozas *et al.*, 2017)**. A minimum spanning network (MSN) was used to create a graphical representation of the haplotype relationships based on the median joining method, and the haplotype network plotted using the PopArt software **(Bandelt *et al.*, 1999)**.

Table 1. Criteria for genetic distance, genetic diversity and population structure

Analysis	Category			Reference
	Low	Medium	High	
Genetic Distance	0.010-0.099	0.1-0.99	1.00-2.00	**Nei (1972)**
Diversity Genetic (Hd)	0.1-0.4	0.5-0.7	0.8-1.00	**Nei (1987)**
Population Structure (Fst)	0.1-0.3	0.4-0.7	0.8-1.00	**Excoffier *et al.* (1992)**

RESULTS

1. Identification in a way molecular using BLAST (Basic local alignment and search tool)

In total, 18 *V.albimarginata* samples were successfully amplified through the PCR process then sent for analysis sequencing. The read lengths of *coi* sequences from the samples were 690- 726 bp (Table 2). The BLAST result showed that all samples were identified as *V.albimarginata* with a gibbus with a *coi* gene sequence similarity level of 99.10- 100% (query cover 91-99%).

Table 2. Results of BLAST analysis on GenBank *Variola albimarginata*

No	ID	Species	BLAST results with GenBank				
			Query Coverage (%)	Identified (%)	Identities	Fragment Length	
						Study	Genbank
1	HJA1	*V.albimarginata*	94	99.85	651/652	690	675
2	HJA2	*V.albimarginata*	99	99.54	650/653	659	707
3	HJA3	*V.albimarginata*	91	99.40	659/663	719	707

4	HJA4	*V.albimarginata*	93	99.40	659/663	705	707
5	HJA5	*V.albimarginata*	97	99.39	650/654	667	707
6	AMBL1	*V.albimarginata*	96	100	629/629	654	675
7	AMBL2	*V.albimarginata*	97	99.25	666/671	689	707
8	AMBL3	*V.albimarginata*	91	99.24	649/654	715	707
9	KEI1	*V.albimarginata*	96	100	629/629	652	675
10	KEI2	*V.albimarginata*	91	99.39	650/654	716	707
11	KEI3	*V.albimarginata*	95	99.84	628/629	657	675
12	KEI4	*V.albimarginata*	94	99.53	642/645	685	707
13	BND1	*V.albimarginata*	99	99.24	650/655	658	707
14	BND2	*V.albimarginata*	97	99.25	666/671	688	707
15	BND3	*V.albimarginata*	91	99.55	661/664	726	707
16	BND4	*V.albimarginata*	91	99.40	659/663	725	707
17	BND5	*V.albimarginata*	92	99.10	664/670	721	707
18	BND6	*V.albimarginata*	96	100	632/632	655	675

The mean base compositions were as follows: A = 23.9%, C = 26.2%, G = 17.5%, and T = 32.3%. Across all samples obtained from the *COI* gene, the proportion of AT base pairs ranged from approximately 56.0% to 56.5%, while CG base pairs ranged from 43.5% to 44.0%. Based on these results, it can be concluded that AT base pairs are more abundant than CG base pairs, though the overall composition is relatively balanced.

Nucleotide frequency refers to the relative proportion of the nucleotides A, T, G, and C. The base composition of T(U), C, A, and G across all samples is presented in Table (3).

Table 3. Composition nucleotides research samples

No	ID	Species	%T	%C	%A	%G
1	HJA1	*V.albimarginata*	32.3	26.3	23.7	17.7
2	HJA2	*V.albimarginata*	32.3	26.3	24.0	17.4
3	HJA3	*V.albimarginata*	32.4	26.2	24.0	17.4
4	HJA4	*V.albimarginata*	32.3	26.2	24.0	17.5
5	HJA5	*V.albimarginata*	32.2	26.3	24.0	17.4
6	AMBL1	*V.albimarginata*	32.4	26.2	24.0	17.4
7	AMBL2	*V.albimarginata*	32.3	26.3	23.8	17.5
8	AMBL3	*V.albimarginata*	32.4	26.2	23.9	17.6

9	KEI1	*V.albimarginata*	32.4	26.2	23.9	17.6
10	KEI2	*V.albimarginata*	32.5	26.0	24.0	17.4
11	KEI3	*V.albimarginata*	32.1	26.4	24.0	17.5
12	KEI4	*V.albimarginata*	32.3	26.2	24.0	17.5
13	BND1	*V.albimarginata*	32.4	26.2	23.9	17.6
14	BND2	*V.albimarginata*	32.3	26.3	23.8	17.5
15	BND3	*V.albimarginata*	32.4	26.2	24.0	17.4
16	BND4	*V.albimarginata*	32.5	26.0	24.0	17.4
17	BND5	*V.albimarginata*	32.5	26.0	23.9	17.6
18	BND6	*V.albimarginata*	32.3	26.3	23.8	17.5

2. Genetic distance and reconstruction tree phylogenetics

The results of the genetic distance analysis using the Kimura 2-Parameter (K2P) model, with 1,000 bootstrap replicates, are presented in Table 4. The genetic distances among the 18 sequences ranged from 0.0000 to 0.0046, which is considered low **(Nei, 1972)**.

The lowest genetic distance value (0.0000) was observed between samples from the same location, and in some cases, even between samples from different locations. This low value indicates that the individuals are closely related genetically.

Table 4. Genetic distance between the *V.albimarginata* samples studied

No		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	HJA1_V.*albimarginata*																		
2	HJA2_V.*albimarginata*	0.0031																	
3	HJA3_V.*albimarginata*	0.0031	0.0000																
4	HJA4_V.*albimarginata*	0.0015	0.0015	0.0015															
5	HJA5_V.*albimarginata*	0.0046	0.0015	0.0015	0.0031														
6	AMBL1_V.*albimarginata*	0.0015	0.0000	0.0000	0.0000	0.0015													
7	AMBL2_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000												
8	AMBL3_V.*albimarginata*	0.0031	0.0015	0.0015	0.0015	0.0031	0.0015	0.0015											
9	KEI1_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000	0.0000	0.0015										
10	KEI2_V.*albimarginata*	0.0046	0.0015	0.0015	0.0031	0.0031	0.0015	0.0031	0.0031	0.0031									
11	KEI3_V.*albimarginata*	0.0031	0.0031	0.0031	0.0015	**0.0046**	0.0015	0.0015	0.0031	0.0015	**0.0046**								
12	KEI4_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000	0.0000	0.0015	0.0000	0.0031	0.0015							
13	BND1_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000	0.0000	0.0015	0.0000	0.0031	0.0015	0.0000						
14	BND2_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000	0.0000	0.0015	0.0000	0.0031	0.0015	0.0000	0.0000					
15	BND3_V.*albimarginata*	0.0015	0.0000	0.0000	0.0000	0.0015	0.0000	0.0000	0.0015	0.0000	0.0015	0.0015	0.0000	0.0000	0.0000				
16	BND4_V.*albimarginata*	0.0046	0.0015	0.0015	0.0031	0.0031	0.0015	0.0031	0.0031	0.0031	0.0031	**0.0046**	0.0031	0.0031	0.0031	0.0015			
17	BND5_V.*albimarginata*	0.0031	0.0031	0.0031	0.0015	**0.0046**	0.0015	0.0015	0.0031	0.0015	**0.0046**	0.0031	0.0015	0.0015	0.0015	0.0015	0.0015		
18	BND6_V.*albimarginata*	0.0015	0.0015	0.0015	0.0000	0.0031	0.0000	0.0000	0.0015	0.0000	0.0031	0.0015	0.0000	0.0000	0.0000	0.0000	0.0031	0.0015	

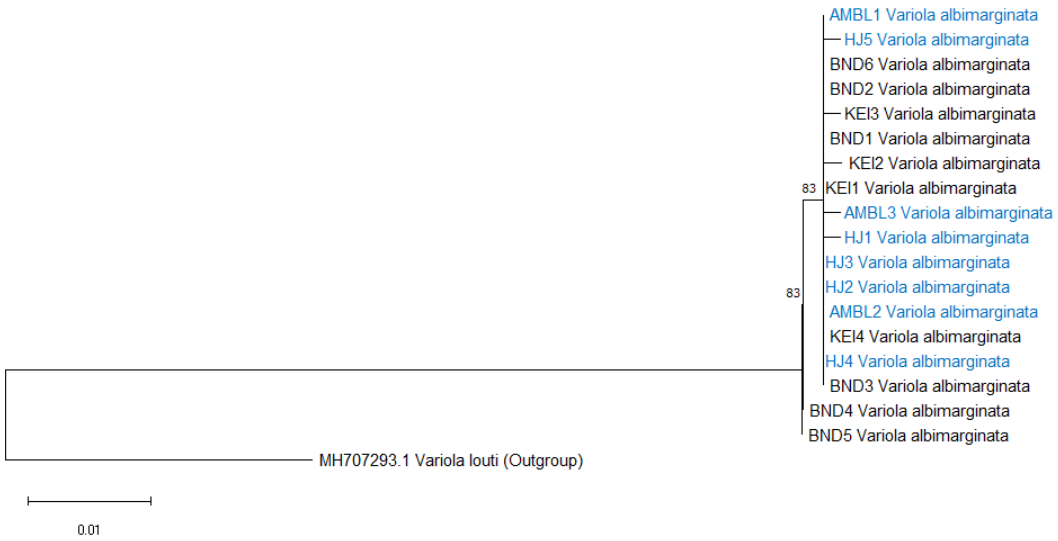

Fig 2. Tree phylogenetics of *Variola albimarginata* in WPP 714

The results of reconstruction tree phylogenetic showed that all the samples have a close relatively (Fig. 2). Based on the results of the analysis, the phylogenetic tree shows one clade consisting of 16 samples and two additional branches, each representing a single species. However, all clades originate from a common node. This suggests that all taxa included in the study share a recent common ancestor and are closely related to one another.

3. Diversity genetics, structure population, and network haplotype

Based on the analysis results, 7 haplotypes were identified from the 18 samples analyzed (Table 5). The Haja population, consisting of 5 samples, contained 3 unique haplotypes. In the Banda and Ambalau populations, 2 haplotypes were observed, with Banda comprising 6 samples and Ambalau 3 samples. In the Kei population, 3 haplotypes were found among 4 samples.

The haplotype diversity values ranged from 0.5 to 0.8. This value was classified as moderate for the Banda population and high for the other populations.

Table 5. Number of samples (n), number haplotype (Hn), diversity haplotype (Hd) and diversity nucleotide (π) *Variola albimarginata* WPP 714

Location	n	Hn	Hd	π
Hajj	5	3	0.7	0.00124
Band	6	2	0.5	0.00083
Ambalau	3	2	0.6	0.00103

Kei	4	3	0.8	0.00154
All Population	18	7	0.6	0.00118

The nucleotide diversity values obtained ranged from 0.00083 to 0.00154, indicating a low level of genetic variation. Low mark diversity nucleotide are shown in a sample of population having their own order genetics that are almost similar or vary a little. Based on results, *V.albimarginata* sample obtained a mark high haplotype diversity with diversity of low nucleotides.

Genetic connectivity between populations was assessed using Fst values. The analysis showed that Fst values were low between the Ambalau–Haja, Kei–Haja, and Kei–Ambalau populations (Table 6). These low Fst values indicate the presence of gene flow between populations of *V. albimarginata*, suggesting that genetic exchange occurs both from marine protected areas (MPAs) to fishing grounds, as well as between different fishing grounds..

Table 6. Structural analysis population (Fst) of *Variola albimarginata* at WPP 714 location

Location	Haja	Banda	Ambalau	Kei
Hajj	-	-	-	-
Band	0.09091	-	-	-
Ambalau	0.00000	0.10000	-	-
Kei	0.00000	0.08000	0.00000	-

Genetic connectivity between populations was reconstructed using a haplotype network based on the Minimum Spanning Network method (Fig. 3). Specimens with genetic similarity form the same haplotype. Based on Fig. (3), seven haplotypes were identified, among which six were represented by only a single individual. One dominant haplotype, however, consisted of 11 samples across four different populations. This pattern indicates a relatively high level of gene flow among populations.

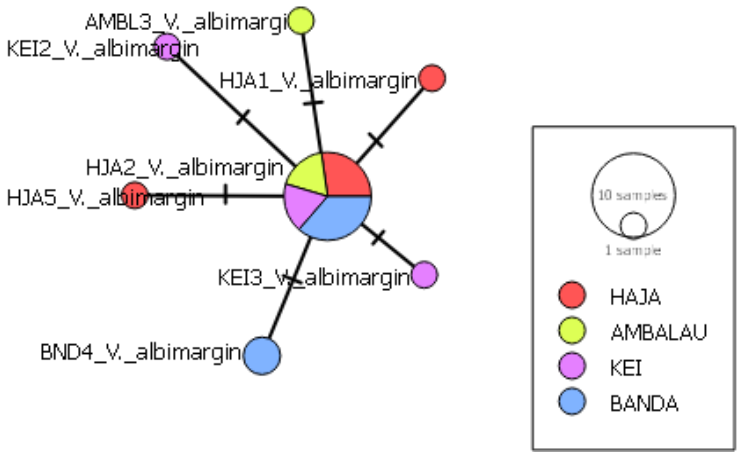

Fig. 3. Minimum spanning network (MSN) of *Variola albimarginata*

DISCUSSION

Information related to genetic connectivity is a crucial aspect of marine resource conservation. Connectivity can be assessed through molecular approaches, particularly using the DNA barcoding method. DNA barcoding is a technique that identifies species by analyzing specific gene regions **(Toha *et al.*, 2020)**. One commonly used gene marker is the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, which provides a fast and reliable way to assess connectivity. The *COI* gene is highly effective for species identification and plays a key role in reconstructing phylogenetic relationships **(Kartavtsev *et al.*, 2016)**.

Molecular approaches are considered more effective than traditional identification methods, especially when dealing with cryptic species. DNA barcoding results provide valuable genetic data that can be used to assess biodiversity, population connectivity, and phylogenetic relationships.

In this study, BLAST results confirmed that all 18 samples belong to *Variola albimarginata*, with a high similarity range (99.10–100%). Higher similarity scores indicate greater confidence in sequence identification. The length of the *COI* gene fragment from the four locations ranged from 690 to 726 base pairs, consistent with **Hebert *et al.* (2003b)**, who reported that DNA barcoding typically yields sequences of 600–700 bp in length.

The base composition analysis of the *COI* gene across all samples revealed AT base pair content of approximately 56.0–56.5% and CG content of 43.5–44.0%. Similarly, **Fadli *et al.* (2023)** reported an A+T content of 54.4% and G+C content of 45.6% for several grouper species.

Genetic distance variation, calculated using the Kimura 2-Parameter (K2P) model with 1,000 bootstrap replicates, showed a range of 0.0000 to 0.0046, indicating low

genetic divergence among the 18 sequences. According to **Slatkin (1985)**, low genetic distances are often a result of gene flow, where interbreeding between individuals from different populations causes genetic material to be shared. These results suggest that the *V. albimarginata* samples analyzed are genetically very similar, showing strong genetic connectivity.

This finding is further supported by the phylogenetic tree reconstruction. The tree revealed one major clade containing 16 samples and two separate branches, each representing a single individual. All clades shared a common node, indicating that individuals from all four locations—both marine protected areas (MPAs) and fishing grounds—originated from a common ancestor and are genetically closely related.

According to **Ketchum *et al.* (2016)**, understanding genetic diversity patterns in marine species is essential for conservation and resource management. The present study revealed a moderate to high level of haplotype diversity among *V. albimarginata* populations within Fisheries Management Area (FMA) 714. A total of 7 distinct haplotypes were identified from 18 specimens. The Banda population exhibited the lowest haplotype diversity, which, based on **Nei (1987)**, is still classified as moderate. Populations with moderate genetic diversity generally retain some adaptive capacity but may be vulnerable to environmental changes if gene flow is limited **(Palstra *et al.*, 2008)**. In contrast, the remaining three populations showed high haplotype diversity. According to **Toha *et al.* (2020)**, populations with high genetic diversity are more resilient and have better chances of long-term survival. **Pereira *et al.* (2004)** also noted that differences in sample size do not significantly affect measures of haplotype and nucleotide diversity.

mucleotide diversity in this study ranged from 0.00083 to 0.00154. According to **Grant and Bowen (1998)**, values below 0.005 are considered low for marine species. This suggests that the populations studied have very similar genetic sequences. One contributing factor to this low diversity could be overfishing. **Achmad *et al.* (2024)** reported that *V. albimarginata* is subject to overfishing. Marine species experiencing high fishing pressure tend to show reduced genetic diversity compared to stable populations **(Pinsky & Palumbi, 2014; Ketchum *et al.*, 2016)**.

Overall, *V. albimarginata* in this study exhibited high haplotype diversity alongside low nucleotide diversity. **Grant and Bowen (1998)** classified this pattern (high h, low π) as indicative of a population that has gone through a bottleneck followed by rapid expansion. This suggests that although the species may have experienced a reduction in genetic variation in the past, the current population size is relatively large. Nonetheless, intense exploitation may have contributed to the observed loss in nucleotide diversity.

Genetic connectivity between populations was also assessed using Fst values. The Fst analysis showed low genetic differentiation between the Ambalau–Haja, Kei–Haja, and Kei–Ambalau populations, indicating ongoing gene flow. This suggests that *V. albimarginata* individuals may move between MPAs and fishing grounds, facilitating

genetic exchange. The geographic proximity of these areas likely promotes larval dispersal and gene flow.

The haplotype network reconstruction for *V. albimarginata* populations in FMA 714 revealed seven distinct haplotypes. Six of these were unique to single individuals, while one dominant haplotype included 11 samples from four populations. This indicates a high degree of gene flow and shared genetic structure across populations **(Slatkin, 1985)**. Such patterns are often driven by high dispersal capability, habitat connectivity, or selection pressures **(Hedrick, 2000; Barrett & Schluter, 2008)**. As a result, identical or similar haplotypes can be found across geographically separated areas.

CONCLUSION

All samples exhibited close genetic relationships, as indicated by low genetic distance values ranging from 0.0015 to 0.0046. In the phylogenetic tree reconstruction, all samples clustered within the same clade, further supporting their genetic similarity. Haplotype diversity among the samples ranged from moderate to high (0.5–0.8), while nucleotide diversity remained low (0.00083–0.00154).

Population structure analysis using Fst values revealed low genetic differentiation among the four populations (Ambalau, Haja, Banda, and Kei).

These results indicate the presence of genetic connectivity between samples from both marine protected areas (MPAs) and fishing grounds. Gene flow appears to occur between *V. albimarginata* populations in MPAs and those in fishing grounds, as well as among different fishing ground populations.

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