Morphology and genetic diversity of the walking sharks *Hemiscyllium galei* and *Hemiscyllium henryi* in Papua Bird's Head Seascape

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Abstract. The Papua Bird's Head Seascape is a water area that has a high level of biodiversity, shallow water habitat, also endemic species of walking sharks like as *Hemiscyllium galei* and *Hemiscyllium henryi* can be found in this area. The need for data on the genetic diversity of sharks has become an urgency that can assist in considering shark protection regulations in this area. This study was conducted with the aim of knowing the morphological characters, levels of individual and populations genetic diversity, and the genetic relationship between walking sharks *Hemiscyllium galei* and *Hemiscyllium henryi* in the Papua Bird's Head Seascape. The research method used was morphometric data collection and molecular analysis. The results of this study concluded that the size of the walking sharks *H. galei* and *H. henryi* in the Papua Bird's Head Seascape area varied between 29.4 cm to 86.5 cm. Furthermore, the genetic distance between individual walking sharks has a genetic distance between 0.000 to 0.043, with the level of genetic diversity of the population belonging to the high and medium categories. In addition, the walking shark species *Hemiscyllium henryi* and *Hemiscyllium galei* in Bird's Head Seascape area of Papua are closely related to *Hemiscyllium freycinet* and *Hemyscillium halmahera*.
Key Words: epaulette sharks, Kwatisore, Lemon Island, population, Triton Bay, Tubuh Seram Island.

Introduction. The Papua Bird's Head Seascape (BHS) is a water area located in the Northwest of Papua Island which has an elevated level of biota diversity and shallow water habitat (Allen & Erdman 2009). The survey results stated that this area has more than 577 coral species, 4 or more marine turtle species, 1638 reef fish species, and 699 molluscs species (Allen & Erdman 2009, 2012; Donnelly et al 2003; Tapilatu & Tiwari 2007). The prominent level of biodiversity in this area needs to be supported by various efforts that can support the need for conservation and beauty, one of which can be done through research.

Among the thousands of species of biota that have been recorded, there are at least 41 endemic fish species that can be found in this area (Dimara et al 2010). Two of the endemic fish found in this area are the walking sharks *Hemiscyllium galei* and *Hemiscyllium henryi* (Allen & Erdmann 2008). The walking shark or commonly known in the local language as Kalabia, is a type of shark that is unique and different because it can forage for food at night (nocturnal) (Allen et al 2016; Weigmann 2016; Iriansyah et al 2021). Settling in shallow water bottom areas, lack of swimming capability, and the influence of geological history factors cause these fish species to not have a high distribution variation and make it an endemic icon in the Papua Bird's Head Seascape (Mangubhai et al 2012).

The presence of walking shark species in these water areas makes an important contribution to increasing genetic diversity and productivity of natural resources in fulfilling their ecological functions in the Papua Bird's Head Seascape ecosystem (Sihasale 2013). However, along with the development of commercial fish business enthusiasts and

the absence of regulations for shark protection, shark fishing practices continue to fulfil market needs and have the potential to threaten their sustainability in nature (Compagno 2002). On the other hand, the biological nature of walking sharks, which have limited swimming ability and oviparous reproduction, enable them to lay eggs in corals reefs habitats. But due to this instinct and behaviour, they are vulnerable to various threats such as habitat degradation, pollution, mining activities, fishing practices using cyanide, and climate change (Jutan et al 2018). If this threat continues without any conservation and protection efforts, then the walking sharks population in this area has the potential to decrease. A decrease in the population of an organism can affect the minimum number of parents and have the potential to cause inbreeding which results in low levels of genetic variation in the population (Budi & Lutfiyah 2017; Kusuma et al 2018; Sheridan et al 1997). Furthermore, the increase in inbreeding can also affect the stability of the development of locomotor organs which leads to the appearance of asymmetric and abnormal characteristics in individuals (Vøllestad et al 1999), so this results in increased individual vulnerability in adapting to the surrounding environment. This statement is supported by the opinion of Clarke (1992), which states that the growth rate and stability of the pair of locomotor organs in an individual has a close relationship with the level of genetic diversity, where the higher the biodiversity, the healthier the population will be and vice versa. In this regard, the need for detailed and accurate data on the genetic diversity of walking sharks in clarifying the genetic diversity of a population can help in considering the formulation of regulations for the protection of walking sharks in this area (Fahmi et al 2018; Turan et al 2004; Zamroni et al 2016).

Research on the genetic diversity of walking sharks of the genus *Hemiscyllium* has been conducted by Dugeon et al (2020) and Maduppa et al (2020). However, research on the genetic diversity of walking sharks *Hemiscyllium galei* and *Hemiscyllium henryi* in the BHS area has never been carried out. Based on this, it is important to conduct this research to support the need for data on the genetic diversity of the two species in the Bird's Head Seascape area of Papua.

Material and Method

Description of the study sites. The Bird's Head Seascape (BHS), Indonesia is known as an area with a high diversity of marine biota species. The Bird's Head Seascape (BHS) Papua has an area of 183,000 km^2, which spans several areas covering the waters of the West Papua Province and the Cendrawasih Bay National Park. This area is a marine conservation priority area at the regional, national and world levels, because it is rich in marine biodiversity and has the highest diversity of coral reefs and marine life in the world. In this area there are several types of marine biota that have high economic value, and some are endemic, like the *Hemiscyllium* genus. Regarding this, study samples were taken at various locations representative of the Papua Bird's Head Seascape, such as: Manokwari (Lemon Island), Fakfak (Tubu Seram), Kaimana (Triton Bay), and Nabire (Lemon River). In this case, the determination of the research location was taken based on data on the distribution of the genus *Hemiscyllium* in the Indopacific region by Allen et al (2016).

Sample collection. A total of 11 samples of walking sharks *Hemiscyllium galei* (6 individuals) and *Hemiscyllium henryi* (5 individuals) were collected during the period of January 2021 until August 2021 from four different locations in Papuan Bird's Head Seascape: Manokwari (Lemon Island), Fakfak (Tubuh Seram Island), Kaimana (Triton Bay), and Nabire (Kali Lemon) (Figure 1).

All tissue samples were collected from right pectoral fin by modified hog ear notch pliers during the night time and were preserved using 96% ethanol. After the samples were secured, they were then placed in a refrigerator at 3°C until the time of molecular analysis. Based on the 11 samples of walking sharks analysed, we also strengthened the results of our study by combining other 17 nucleotide from walking sharks of genus *Hemiscyllium* from Gen Bank (Corrigan et al 2017). The 17 sequences that we used represent 9 species of walking sharks in the Indo Pacific region.

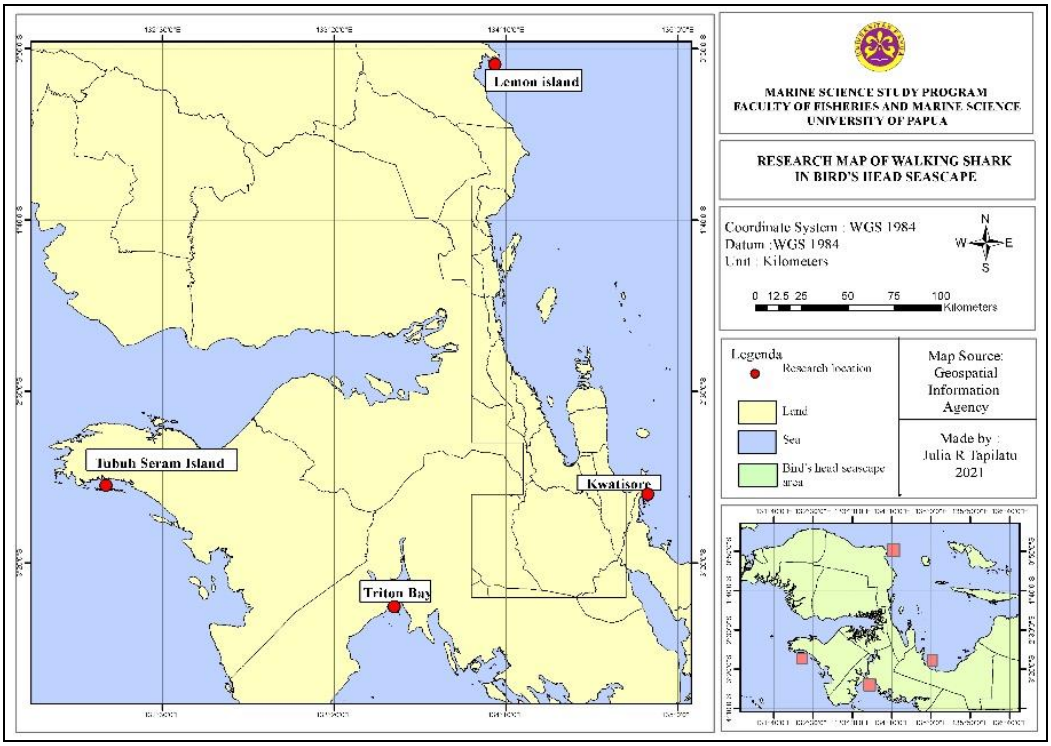

Figure 1. The location of *Hemiscyllium henryi* and *Hemiscyllium galei*, Bird's Head Seascape, Indonesia (map generated using ArcGIS 10.4).

Morphological assessment. Both of this species can be can distinguished by morphological identification and molecular identification. The morphological identification can be done by observing the different patterns for the head of the walking shark as an identification key (Figure 2), which is then combined with the data of length and sex of each sample. Differences in pattern shape in *Hemiscyllium henryi* are characterized by the presence of a double ocellus on the back of the head, while the unique pattern of *Hemiscyllium galei* is characterized by a brown oval shape on the back of the head, dorsal fin, and tail (Allen & Erdmann 2008).

Figure 2. Comparison of unique patterns of *H. henryi* (left) and *H. galei* (right).

Molecular analysis. In this case, the process of extracting shark fin samples was conducted at the Genetics Laboratory of Bengkulu University, using the "GsyncTM DNA Extraction Kit" animal tissue extraction kit. At the end of the extraction stage, the samples were stored in a freezer at a temperature of -18°C.

The primer types used in this study is a specific primer for epaulette sharks with the combination of ND4-F: 5' – CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC - 3' (Arevalo et al 1994) and H12293-Leu-R: 5' – TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC - 3' (Inoue et al 2001). The PCR conditions were set as follows: pre-denaturation at 95°C for 5 minutes, followed by denaturation at 95°C for 15 seconds, annealing at 56°C

for 30 seconds, extension at 72°C for 1 minute and the final extension temperature was 72°C for 7 minutes with 30 cycles (Allen et al 2013).

The electrophoresis will begin by transferring the amplified PCR sample results as much as 5 L into 1% agarose media, then proceed with the preparation of an electrophoresis machine with a setting of 100, 400 mA, for 30 minutes. Next, all DNA molecules separated in ethidium bromide solution can be visualized with the help of UV light. The final result of electrophoresis is a positive molecule shaped like DNA bands (Pratiwi et al 2001).

The sequencing stage was started by extending the primer using DNA polymerase along with the four types of Deoxyribonucleoside Triphosphate (dNTP) and low concentration breaking nucleotides (ddNTP). Furthermore, ddNTP will be attached to the dNTP mold, then added reagents that can produce fluorescence labelled fragment lengths of different sizes (Hidayah et al 2021). At the end of sequencing, the results will be separated based on size criteria and edited and aligned to obtain species identification and kinship relationships (França et al 2002; Maulid 2015).

Data analysis. Morphological data was analysed descriptively and presented in the form of tabulations and images. The molecular data was analysed by various software in several stages. Sequencing data were edited and aligned using MEGA (Molecular Evolutionary Genetics Analysis) version 10 application. The data was then analysed online through BLASTN (Zhang et al 2000; Morgulis et al 2008) on the NCBI website to look for similarities in the nucleotide arrangement of the walking shark species *H. galei* and *H. henryi.* Furthermore, analysis of genetic diversity such as the number of haplotypes, nucleotide diversity, and haplotype diversity were then analysed by the application of DnaSP and will be continued using the Network 10.2 application to determine the relationship of genetic diversity in the form of haplotypes.

Results

Morphological diversity. This study has identified 11 walking shark individuals, 6 of which are *Hemiscyllium galei* species and 5 of them have been categorized as *Hemiscyllium henryi* species. From the sample we also identified the gender and total length of each individual that are presented in Table 1.

Table 1

Morphometric data of walking shark

No.	*Sample code*	*Species*	*Location*	*Total length (TL)(cm)*	*Gender*
1	4323250_1_Hh	*H. henryi*	Triton Bay	50.5	Male
2	4323252_2_Hh	*H. henryi*	Triton Bay	72.5	Male
3	4323254_3_Hh	*H. henryi*	Triton Bay	86.5	Female
4	4323266_9_Hh	*H. henryi*	Triton Bay	83,5	Female
5	4323268_10_Hh	*H. henryi*	Tubuh Seram Island	81	Female
6	4323256_4_Hg	*H. galei*	Lemon Island	65	Male
7	4350941_X1C_11_Hg	*H. galei*	Lemon Island	73.5	Female
8	4323258_5_Hg	*H. galei*	Kwatiore	43.8	Male
9	4323260_6_Hg	*H. galei*	Kwatiore	35.6	Male
10	4323262_7_Hg	*H. galei*	Kwatiore	37.5	Female
11	4323264_8_Hg	*H. galei*	Kwatiore	29.4	Male

Genetic diversity. Sequencing analysis showed that the length of DNA fragments from each sample was 678 bp for 11 individuals, with an average nucleotide sequence of T (U) = 31.6; C = 10.9; A = 31.4; G = 26.1 and T(U) = 32.4; C = 10.3; A = 31.6; G = 25.7 for *H. henryi* and *H. galei*, respectively. We identified 22 numbers of variable sites. The variable sites of the walking sharks *H. galei* and *H. henryi* are listed in Table 2.

Table 2

The variable sites of the walking sharks *H. galei* and *H. henryi* in the BHS

Sample code, species	*Variable sites of the walking sharks H. galei and H. henryi*																					*Haplotype*
1. *H. henryi*	C	G	A	A	C	G	A	C	A	A	G	G	C	A	A	G	G	T	G	A	G	A	1
2. *H. henryi*	C	G	A	A	C	G	A	C	A	A	G	G	C	A	A	G	G	T	T	A	A	T	2
3. *H. henryi*	C	G	A	A	C	G	A	C	A	A	G	G	C	A	A	G	G	T	T	A	A	T	2
9. *H. henryi*	C	G	A	A	C	G	A	C	A	A	G	G	C	A	A	G	G	G	T	A	A	T	3
10. H. henryi	C	G	A	G	C	G	A	C	G	A	A	G	C	A	A	G	G	T	T	A	A	T	4
4. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	A	G	A	A	T	T	G	A	T	5
5. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	G	A	A	A	T	T	A	A	T	6
6. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	A	A	A	A	T	T	A	A	T	7
7. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	A	A	A	A	T	T	A	A	T	7
8. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	A	A	A	A	T	T	A	A	T	7
9. *H. galei*	T	A	G	A	T	A	T	T	G	G	A	A	T	G	A	A	A	T	T	A	A	T	8

In total, there are 8 haplotypes of the walking sharks *H. galei* (4 haplotypes) and *H. henryi* (4 haplotypes). The number of haplotypes per species are listed in Table 3.

Table 3

The number of haplotypes per species

Species	*Number of samples*	*Number of haplotypes*
H. henryi	5: 4 (Triton Bay), 1 (Tubuh Seram Island)	4: 3 (Triton Bay), 1 (Tubuh Seram Island)
H. galei	6: 2 (Lemon Island), 4 (Kwatiore)	4: 2 (Lemon Island), 2 (Kwatiore)

In addition to primary sample data, this study also utilized 17 individuals from 9 *Hemiscyllium* species, secondary data from NCBI as a reference in determining the relationship between species in the Hemiscyllium genus. The list of NCBI sequences can be seen in the Table 4.

Table 4

The list of NCBI sequences

No.	*Species*	*Location*	*Accession number*	*Source*
1	*H. halmahera*	Indonesia: Halmahera waters	MF740834	Corrigan et al 2017
2	*H. halmahera*	Indonesia: Halmahera waters	MF740833	Corrigan et al 2017
3	*H. halmahera*	Indonesia: Halmahera waters	MF740832	Corrigan et al 2017
4	*H. ocellatum*	Australia: Great Barier Reef	MF740846	Corrigan et al 2017
5	*H. ocellatum*	Australia: Great Barier Reef	MF740845	Corrigan et al 2017
6	*H. ocellatum*	Australia: Great Barier Reef	MF740844	Corrigan et al 2017
7	*H. freycinet*	Indonesia: Raja Ampat	MF740831	Corrigan et al 2017
8	*H. freycinet*	Indonesia: Raja Ampat	MF740830	Corrigan et al 2017
9	*H. freycinet*	Indonesia: Raja Ampat	MF740829	Corrigan et al 2017

10	*H. hallstromi*	Papua New Guinea: Papua Bay	MF740841	Corrigan et al 2017
11	*H. michaeli*	Papua New Guinea: Milne Bay	MF740842	Corrigan et al 2017
12	*H. strahani*	Indonesia: Depapre Bay	MF740836	Corrigan et al 2017
13	*H. trispeculare*	Indonesia: Arafura Sea	MF740840	Corrigan et al 2017
14	*H. trispeculare*	Australia: Darwin	MF740839	Corrigan et al 2017
15	*H. trispeculare*	Australia: Kimberley	MF740838	Corrigan et al 2017
16	*H. henryi*	Indonesia: Triton Bay	MF740835	Corrigan et al 2017
17	*H. galei*	Indonesia: Kwatisore	MF740843	Corrigan et al 2017

Based on the genetic analyses, the results shows that genetic diversity is closely related to the number of haplotypes, haplotype diversity, nucleotide diversity and polymorphic sites. The results of the analysis show that from the total 11 samples found, there are 8 haplotypes from 4 locations. In this case, several individuals who have the same haplotype consist of 3 individuals of the *H. galei* species at the Kwatisore location (Sample code 6-8) and 2 individuals of the *H. henryi* species at the Triton Bay location (Sample code 2-3).

In addition to individual diversity, genetic diversity can also be reviewed based on population. Data related to the genetic diversity of the walking sharks population in this study are arranged in a table that includes several values to indicate the number of samples per population (n), the number of haplotypes per population (Hn), haplotype diversity (Hd), and nucleotide diversity per population and between species in Table 5 and Table 6.

Table 5

Genetic diversity of each population

Location	*Species*	*n*	*Genetic diversity of each population*		
			Hn	*Hd*	*∏*
Triton Bay	*H. henryi*	4	3	0.8	0.0029
Lemon Island	*H. galei*	2	2	1	1
Kwatisore	*H. galei*	4	2	0.5	0.5
Tubuh Seram Island	*H. henryi*	1	1	0	0

Table 6

DNA divergence between species

Species	*Number of sequences*	*Number of polymorphic sites*	*Total number of mutations*	*Average number of nucleotide differences, k*	*Nucleotide diversity, ∏*
H. henryi	5	7	7	2.80	0.004
H. galei	6	3	3	1.20	0.002
Total	11	22	22	9.16	0.013

The haplotype relationships of each location can be seen in Figure 3.

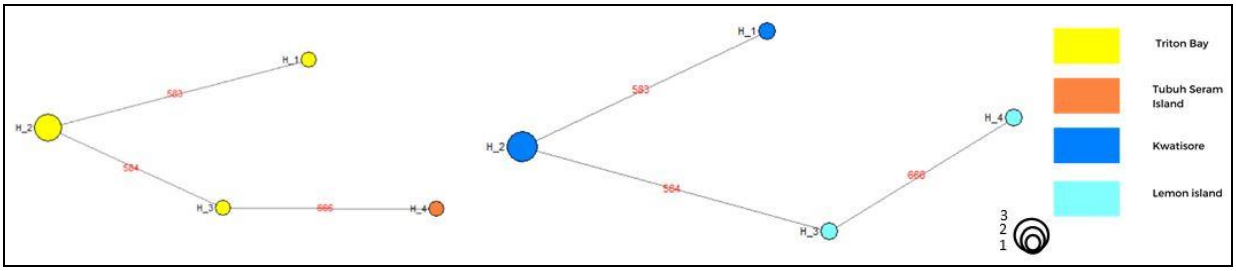

Figure 3. Networks of *H. henryi* (right) and *H. galei* (left) haplotypes in the BHS.

The phylogenetic tree analysis was formed from 11 individual sequences obtained from several waters in the BHS, as well as 17 individuals in the *Hemiscyllium* genus (9 species) from NCBI sequences found in various locations (Figure 4).

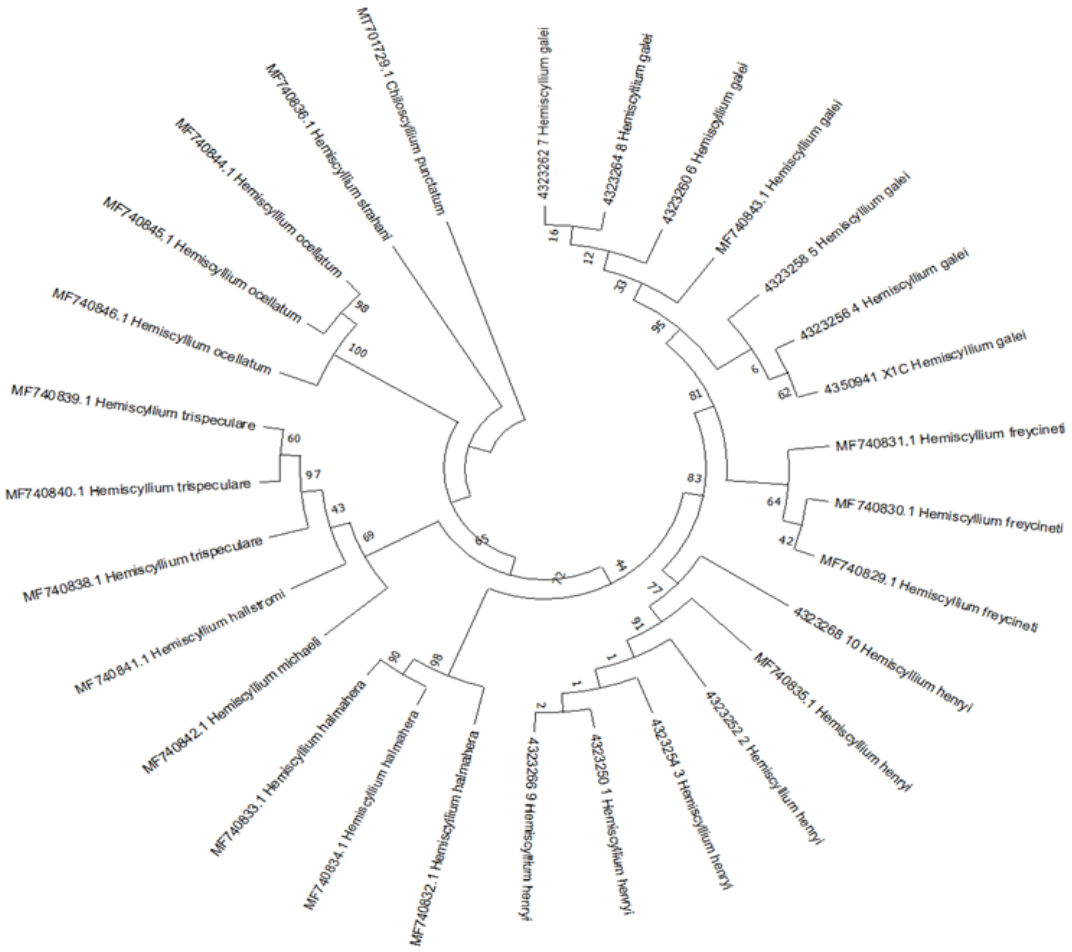

Figure 4. Phylogenetic tree.

Discussion. Comparisons of morphological characteristics between species in this study was conducted by observing differences patterns between the two species. Differences in pattern shape in *H. henryi* are characterized by the presence of a double ocellus on the back of the head, while the unique pattern of *H. galei* is characterized by a brown oval shape on the back of the head, dorsal fin, and tail (Allen & Erdmann 2008). Further morphological characterization is focusing on the size and gender of walking sharks. The result shows that the size range of sample collected were in between of 29.4 cm to 86.5 cm. Furthermore, the results of the measurement of the total length of the individual indicate that the male sex has a larger size than the male. Based on the result, the main factor that causes females to tend to be larger in size than males, is mostly because female sharks require more energy to reproduce and maintain individual shark health after hatching (Laili & Sudibyo 2017).

Molecular analysis of this study was conducted using CO1 markers to obtain a greater chance of interspecific results in differentiating species or population levels within a genus (Allen & Erdmann 2008; Allen et al 2013). Further molecular characteristic was conducted using the BLAST feature on NCBI. The use of this feature produces some related data, including species name, query cover (%), similarity, and accession number (Madduppa et al 2020).

Based on the analysis that has been conducted, it shows that query cover (%) of primary data ranges from 93% - 96%, where the higher the query cover value, this indicates that the order being compared is more effective (Berchtold et al 1996). In addition to the cover query, the BLAST results for individual samples of two species, *H. galei* and *H. henryi*, have similarities between 99.38% - 100%. The high percentage level of similarity that ranged from 97% -100% was included in the significant category (Bhattacharjee et al 2012). This can be concluded that samples of walking shark species

H. galei and *H. henryi* obtained from four locations have effective and significant results with the data in the GenBank database. In this case, the approximate location of each individual is recorded on the NCBI and distribution map of the walking shark genus *Hemiscyllium* by Dudgeon et al (2020).

One of the factors that can affect the genetic diversity of each population of *H. galei* and *H. henryi* in the BHS can be influenced by the diversity of the number of haplotypes that can be found in this area. According to Nei (1972), the value of genetic diversity ranging from 0.8-1 is included in the high category, 0.5-0.7 is in the medium category, while values 0.1 - 0.4 are included in the low category. In relation to the calculation results of the genetic diversity of walking sharks in the waters of the BHS is divided into two groups of diversity, namely: high and medium. In this case, the population of Lemon Island is the area that has the highest genetic diversity with a value of 1, the population of Teluk Triton (0.8333) is in the high category, Kwatisore (0.5) is in the medium category, while Tubuh Seram Island has no diversity in the population with a value of 0. However, if all the samples of this study were combined and compared between species, the nucleotide diversity populations of *H. henryi* (0.004) and *H. galei* (0.002) species in the Papuan Bird's Head Seascape were in the low category.

Although different locations and types of haplotypes, each individual in one genus still has haplotype relationships between each other. This relationship can be visualized in the form of a network, which can connect haplotypes within and between populations through a branching system. Reconstruction of the haplotype network between populations showed that each population had a different haplotype, however, several individuals at the same location had the same haplotype. Large circle of haplotype in Figure 3 indicates an increasing number of individuals and a small circle indicates a smaller number of individuals. In this regard, 2 individuals of *H. henryi* species coded 2-3 at the Triton Bay location had the same haplotype, while 3 individuals of *H. galei* species coded 6-8 at the Kwatisore location had the same haplotype.

Furthermore, based on the analysis of the genetic diversity of each population (Table 4) and the haplotype network (Figure 3), it shows that the highest genetic diversity was found in the population of *H. galei* on Lemon Island. This is evidenced by the number of haplotypes of this population equal to the number of individual samples collected. This means that each individual in the population is different from one another. Comparison results of the Kwatisore and Lemon islands are quite significant because in the value of population diversity in Kwatisore area only has a diversity value of 0.5. In addition to haplotype diversity, environmental conditions can also be a factor that can affect differences in the level of diversity between populations. In this regard, variations in topographical differences and habitat conditions in each population can affect the number of population dispersal diversity (Madduppa et al 2020).

The habitat conditions of the waters of Lemon Island have good average parameter values and sloping bottom topography with coral reefs and seagrass as basic substrates that can support the life of various types of biotas in this area (Leatemia et al 2017; Iriansyah et al 2021). In contrast to Lemon Island, the population of *H. galei* in Kwatisore lives in areas with limited shallow coral reefs where giant clam can be found in this shallow area (Tapilatu et al 2021) and has a steep barrier reef section (Suruan et al 2018). Significant differences between populations can also be seen in the study of Akbar et al (2019), where the Loleo location which has sloping beach conditions can help the process of forming good individuals compared to other populations that are less sloping.

Furthermore the low value of population diversity of 1 individual of *H. henryi* in the population of Tubuh Seram Island which is worth 0, indicated by the number of haplotypes in this population is only one and there is no individual distinguishing data that can be found in the population of Tubuh Seram Island. The low genetic diversity of walking sharks in a population is not only influenced by the conditions of the aquatic environment, but also because of the limited number of individuals analysed and the unavailability of variation in diversity data. This is also in accordance with a previous study by Madduppa et al (2020) which stated that the small number of individuals found could affect the level of diversity of walking sharks in the Tidore and Ternate areas with a value of 0.

The results of the analysis of the phylogenetic tree of walking sharks of the genus *Hemiscyllium* (Figure 4), formed class groups based on the type of similarity between individuals. The genetic distance between individuals in the *H. galei* and *H. henryi* species is close to each other and is divided into several small sub-classes based on the sampling location. Two sequences of *H. galei* and *H. henryi* from NCBI also formed a group of subclasses that were close to each other with the resulting sequences from the Kwatisore and Triton Bay locations, so they may have come from the same or adjacent locations. Furthermore, the results of the phylogenetic tree analysis showed that there was a close relationship between *H. galei* and *H. freycineti* (Raja Ampat), as well as a close relationship between *H. henryi* and *H. Halmahera* (Halmahera waters). The genetic relationship is one of the supporting genetic data that can be achieved by using a description of the evolutionary process of the relationship of DNA composition between individuals that resembles a tree (Baldauf 2003).

Research related to the estimation of close kinship between these four species has been investigated by Dudgeon et al (2020). In this case, there is a close kinship, presumably due to the unification of the mainland from the western part which approached the Bird's Head peninsula in the period between 2 and 10 million years ago (Pandolfi 1993). When this event occurs, shallow water is created in the Bird's Head area which allows migration of *H. halmahera* to the BHS (Hall 2009). Furthermore, variations in species and populations of walking sharks are formed due to differences in habitat and growing places, which can then affect variations in genetic material for each individual (Freeland 2005).

Conclusions. This study found 11 individual walking sharks of two species, *Hemiscyllium galei* and *Hemiscyllium henryi* in the Bird's Head Seascape (BHS). The morphological characteristics of walking sharks in this area are characterized by a unique pattern that is unique and has a size range that varies between 29.4 cm to 86.5 cm. In this regard, the genetic distance between individual walking sharks has a genetic distance between 0.000 to 0.043, where the genetic diversity of the population in this area is in the high and medium categories. The population that has the highest level of genetic diversity can be found in the population of *H. galei* species on Lemon Island, with a population diversity value of 1. The value of genetic diversity is indicated by the number and diversity of haplotypes, which then categorized *H. galei* and *H. henryi* in BHS are both in the low levels of nucleotide diversity. This is presumably due to the low swimming capability of walking sharks, the limited distribution area, and the small population size in this area. Furthermore, the walking sharks *H. galei* and *H. henryi* in the BHS are closely related to other species, namely *H. freycinet* and *H. halmahera*. In this case, the close kinship of *H. galei* and *H. henryi* with other species in the *Hemiscyllium* genus is due to the migration process of biota in the past.

However, the genetic diversity between individuals and populations of *H. galei* and *H. henryi* walking sharks is still relatively low, even being included in the vulnerable category at the IUCN (2020) (Vander et al 2022). In response to this, conservation efforts for the protection of *H. galei and H. henryi* species in the BHS need to be conducted to support survival and reduce the risk of threats to these two species.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Akbar N., Tahir I., Baksir A., Paembonan R. E., Ismail F., 2019 [Morphological description of the endemic species of Walking Shark (*Hemiscyllium halmahera*, Allen & Erdmann, 2013) in the waters of the North Maluku Sea]. Indonesian Journal of Ichthyology. Indonesia, 19(2):297-314 [in Indonesian].

Allen G. R., Erdmann M. V., 2008 Two new species of bamboo sharks (Orectolobiformes: Hemiscylliidae) from Western New Guinea. Aqua (Miradolo Terme). 13(3–4):93–108.

Allen G. R., Erdmann M. V., 2009 Reef fishes of The Bird's Head Peninsula, West Papua, Indonesia. Check List, 5(3):587-628.

Allen G. R., Erdmann M. V., 2012 Reef fishes of The East Indies. Volumes I-Iii. Tropical Reef Research, Perth Australia, 1292 Pp.

Allen G. R., Erdmann M. V., Dudgeon C. L., 2013 *Hemiscyllium halmahera*, a new species of bamboo shark (Hemiscylliidae) from Indonesia. Aqua, International Journal Of Ichthyology, 19(3):123-136.

Allen G. R., Erdmann M. V., White W. T., Dudgeon C. L., 2016 Review of the bamboo shark genus Hemiscyllium (Orectolobiformes: Hemiscyllidae). Journal Of The Ocean Science Foundation, 23:51-97.

Arevalo E., Davis S. K., Sites J. W., 1994 Mitochondrial-DNA sequence divergence and phylogenetic relationships among eight chromosome races of the Sceloporus-Grammicus Complex (Phrynosomatide) in Central Mexico. Systematic Biology 43:387-418.

Baldauf S. L., 2003 Phylogeny for the faint of heart: a tutorial. genetics 6(19):345-351.

Bhattacharjee S., Rajaraman P., Jacobs K. B., Wheeler W. A., Melin B. S., Hartge P., Gliomascan Consortium, Yeager M., Chung C. C., Chanock S. J., Chatterjee N., 2012 A subset-based approach improves power and interpretation for the combined analysis of genetic association studies of heterogeneous traits. The American Journal of Human Genetics, 90(5):821-835.

Berchtold S., Keim D. A., Kriegel H. P., 1996 The X-tree: An index structure for high-dimensional data. In very large data-bases. pp. 28-39.

Budi D. S., Lutfiyah L., 2017 [Asymmetric fluctuations of tilapia (*Oreochromis niloticus*) at the Marine and Fisheries Independent Training Center (P2MKP) Water World, Banyuwangi]. Journal of Aquaculture Science, 2(1):72-80 [in Indonesian].

Clarke G. M., 1992 Fluctuating Asymmetry: A technique for measuring developmental stress off genetic and environmental origin. Acta Zoology Fennica 191:31-35.

Compagno L., 2002 Shark of the world an annotated and illustrated catalogue of shark species known to date Volume 2. Fao, Rome. 278 pp.

Corrigan S., Yang L., Allen G. R., Erdmann M. V., Fahmi F., Sugeha H. Y., White W. T., Naylor G., 2017 *Hemiscyllium* phylogenetics. Unpublished. https://www.ncbi.nlm.nih.gov/nucleotide/MF740835.1?report=genbank&log$=nucltop&blast_rank=1&RID=7FDXTNMP016.

Dimara R., Fauzan A., Lazuardi M., Pada D., Allen G. R., Erdmann M. V., Huffard C. L., Katz L. S., Winterbottom R., 2010 Pisces, Teleostidae, Gobiidae, Illustrated list of additions to the fauna of The Raja Ampat Islands, Indonesia. Check List, 6:619–625.

Donnelly R., Neville D., Mous P. J., 2003 Report on a rapid ecological assessment of the Raja Ampat Islands, Papua, Eastern Indonesia Held October 30 – November22, 2002. The Nature Conservancy, Bali.

Dudgeon C. L., Corrigan S., Yang L., Allen G. R., Erdmann M. V., Sugeha H. Y., Naylor G. J., 2020 Walking, swimming or hitching a ride? Phylogenetics and biogeography of the walking shark genus Hemiscyllium. Marine And Freshwater Research, 71(9):1107-1117.

Fahmi M. R., Hayuningtyas E. P., Zamroni M., Nur B., Sinansari S., 2018 [Genetic diversity of tiger fish (*Datnioides* Sp.) from Kalimantan and Sumatra]. Journal of Aquaculture Research, 13(3):191-199 [in Indonesian].

França L. T., Carrilho E., Kist T. B., 2002 A review of DNA sequencing techniques. Quarterly Reviews of Biophysics, 35(2):169-200.
Freeland R., 2005 Molecular ecology. John Wiley & Sons. Ltd., England, Uk. 402 pp.
Hall R., 2009 Southeast Asia's changing palaeogeography. Blumea 54:148–161.
Hidayah A. S. A., Utomo B., Mustofa I., 2021 [Follicle stimulating hormone (rfsh) receptor gene profile in madrasin-crossed cattle]. Veterinary Medical Journal, 4(1):91-97 [in Indonesian].
Inoue J., Miya M., Tsukamoto K., Nishida M., 2001 A mitogenomic perspective on the basal teleostean phylogeny: resolving higher-level relationships with longer DNA sequences. Mol Phylogenet Evol Biol 9:235.
Iriansyah I., Tapilatu R. F., Hendri H., 2021 Abundance, distribution patterns and habitat conditions of giant clam (family: Tridacnidae). Musamus Fisheries and Marine Journal. 3(2)95-106.
Jutan Y., Retraubun A. S. W., Khouw A. S., Nikijuluw V. P. H., Pattikawa J. A., 2018 Study on the population of Halmahera walking shark (*Hemiscyllium halmahera*) in Kao Bay, North Maluku, Indonesia. International Journal of Fisheries and Aquatic Studies, 6(4):36-41.
Kusuma A. B., Dietrich G. B., Hawis M., Beginer S., Dondy A., 2016 [Genetic diversity of soft coral *Sarcophyton trocheliophorum* in Java Sea, Nusa Tenggara and Sulawesi Sea Populations]. Enggano Journal. 1(1):89-96 [in Indonesian].
Laili N., Sudibyo M., 2017 [*Chiloscyllium hasselti* based on morphological and morphometric characters]. Journal of Biosciences, 3(2):103-111 [in Indonesian].
Leatemia S. P., Kopalit H., Pakilaran E. L., 2017 [The abundance of macrozoobenthos in vegetated (seagrass) and non-vegetated areas in Doreri Bay, Manokwari]. Indopacific Journal of Aquatic Resources, 1(1):15-26 [in Indonesian].
Madduppa H., Putri A. S., Wicaksono R. Z., Subhan B., Akbar N., Ismail F., Arafat D., Prabuning D., Muksin L. M. I., Srimariana E. S., Baksir A., Bengen D. G., 2020 Morphometric and DNA barcoding of endemic Halmaheran walking shark (*Hemiscyllium halmahera*, Allen, 2013) in North Maluku, Indonesia: Morphogenetic of endemic Halmaheran walking shark. Biodiversitas Journal of Biological Diversity, 21(7).
Mangubhai S., Erdmann M. V., Wilson J. R., Huffard C. L., Ballamu F., Hidayat N. I., Hitipeuw C., Lazuardi M. E., Muhajir, Pada D. N., Purba G., Rotinsulu C., Rumetna L., Sumolang K., Wen W., 2012 Papuan Bird's Head Seascape: emerging threats and challenges in the global center of marine biodiversity. Marine Pollution Bulletin, 64(11):2279-2295.
Maulid D. Y., 2015 [DNA barcoding to authenticate mackerel (*Scomberomorus* sp.) products]. Aquatic Journal, 6(2) [in Indonesian].
Morgulis A., Coulouris G., Raytselis Y., Madden T. L., Agarwala R., Schäffer A. A., 2008 Database indexing for production MegaBLAST searches. Bioinformatics 24:1757-1764.
Nei M., 1972 Genetic distance between population. American Nature. 106:283- 292.
Pandolfi J. M., 1993 A review of the tectonic history of New Guinea and its significance for marine biogeography. In '7th International Coral Reef Symposium', 22–27. p. 718-728.
Pratiwi R., 2001 [Get to know the electrophoresis method]. Oseana, 26(1):25-31 [in Indonesian].
Sheridan L., Pomiankowski A., 1997 Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. heredity, 79(5):515-523.
Sihasale D. A., 2013 [Biodiversity in the coastal area of Ambon City and the consequences for the development of coastal tourism]. Journal Of Indonesian Tourism and Development Studies, 1(1):20-27 [in Indonesian].
Suruan S. S., Kamal M. M., Bawole R., Tania C., Mulyadi M., 2019 [Population distribution of whale sharks (*Rhincodon typus*, Smith 1828) in Kwatisore waters, Nabire Regency, Papua Province]. Proceedings of the Fisheries Research Center, 1(1):23-32 [in Indonesian].

Tapilatu J. R., Siburian R. H., Tapilatu M. E., 2021 Species identification, density, and type of substrate of clam (Tridacnaidae) in Kali Lemon coastal water-Kwatisore, Cenderawasih Bay, Papua, Indonesia. Aquaculture, Aquarium, Conservation & Legislation, 14(5):2662-2671.
Tapilatu R. F., Tiwari M., 2007 Leatherback turtle, *Dermochelys coriacea*, hatching success at Jamursba-Medi and Wermon Beaches in Papua, Indonesia. Chelonian Conservation And Biology, 6:154–158.
Turan C. D., Ergüden F., Turan, Gurlek M., 2004 Genetic and morphologic structure of *Liza abu* (Heckel, 1843) populations from the rivers Orontes, Euphrates and Tigris. Turkish Journal of Veterinary And Animal Sciences, 28(4):729-734.
VanderWright W. J., Dudgeon C. L., Erdmann M. V., Sianipar A., Dulvy N. K., 2021 Extinction risk and the small population paradigm in the micro-endemic radiation of epaulette sharks. Imperiled: The Encyclopedia of Conservation, 1-11. https://doi.org/10.1016/B978-0-12-821139-7.00130-6.
Vøllestad L. A., Hindar K., Møller A. P., 1999 A meta-analysis of fluctuating asymmetry in relation to heterozygosity. Heredity 83(2):206-218.
Weigmann S., 2016 Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. Journal of Fish Biology, 88(3):837-1037.
Zamroni A., Suwarso S., Nugroho E., 2016 [The genetic structure of the population of Blue Malalugis fish (*Decapterus macarellus* Cuvier, 1833) around Sulawesi based on the Mt-DNA Marker]. Indonesian Journal of Fisheries Research, 20(1):31-41 [in Indonesian].
Zhang Z., Schwartz S., Wagner L., Miller W., 2000 A greedy algorithm for aligning DNA sequences. J Comput Biol 7(1-2):203-14.

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