Population genetic structure and genetic diversity of a gastropod (*Telescopium telescopium*) from the geothermal waters of coastal Jailolo, West Halmahera, North Maluku, Indonesia

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Abstract. *Telescopium telescopium* gastropod of the Potamididae family is found in abundance in the geothermal waters of Jailolo, Indonesia. Genetic information was determined for conservation purposes. Environmental parameters were determined *in situ*. The samples collection was carried out along the geothermal water flow (number of sumples in locations: Payo=10 and Bobo=10) and a normal location (Tuada=5). The meat was extracted from the shells and placed in a container with 95% ethanol. Extraction was performed using Neasy Blood & Tissue Kit. Two primers were used: forward LCO1490, GGTCAACAAATCATAAAGATATTGG and reverse HC02198, TAAACTTCAGGGTGACCAAAAAATCA. PCR (polymerase chain reaction) was performed. The extraction results were amplified at the COI locus using the Gold method (KAPA). Sequencing analysis was conducted using the Sanger method. The value of soil temperature in the geothermal water environment reached 45-54°C. The soil temperature in the normal environment in Tuada village was 28°C. The water temperature was 50.5-50.7°C in locations that were affected by geothermal water flow. Water and soil pH were neutral. Salinity was 0-0.5‰ in geothermal water locations and 34‰ in the normal environment. The molecular characteristics results (n=25) provided 599 bp. Phylogeny trees were not grouped according to the location, but mixed with individuals from other locations. *Telescopium telescopium* inter-population and intra-population genetic distances based on the mtDNA locus control region show low genetic disparities between populations. Amova pairwise (Fst) found high genetic flow between populations. The genetic diversity was moderate (0.533-0.667) and high (0.800). The distribution of the haplotype network found 5 specific haplotypes, 1 mixed haplotype, and 2 similar haplotypes. Minimal spawning networks (MSN) and genetic diversity (Bobo, Payo and Tuada) were in stable condition.

Key Words: differentiation, fixation index (Fst), *Gastropoda*, genetic variation, heat energy.

Introduction. One of the geothermal potentials of North Maluku is the geothermal field in Jailolo, West Halmahera Regency (Baksir et al 2018). Baksir et al (2020) reported that the geothermal source in the area has a fluid enthalpy of 1100 kJ kg^{-1} with a minimum fluid temperature of 179°C. The Jailolo coastal area also presents geothermal potential. The geothermal potential found in represented by hot springs. Geothermal water naturally flows out of the underground in the coastal areas of Payo and Bobo villages. Apart from having geothermal water sources, this area is also overgrown with mangrove ecosystems. The geothermal water flows through the widespread mangrove ecosystem. The area of geothermal water sources in this coastal area is also a habitat for aquatic organisms.

Gastropods have an important role in ecological functions and as aquatic bioindicators (Romdhani et al 2016; Abubakar et al 2018). Some gastropod species also live in the geothermal water sources that flow through the mangrove area. The most common gastropods are *Telescopium telescopium* of the Potamididae family. This species has a wider distribution in large numbers and is associated with mangrove ecosystems affected by geothermal water flows. Kurniawati et al (2014) said that some of the allegations related to this interaction were due to the good utilization of mangroves by *T. telescopium*. This species was found in the substrate and roots of mangroves and is distributed from the middle to the back of the mangrove ecosystem (Isnaningsih & Patria 2018).

Research on geothermal utilization in the coastal area of Jailolo has been carried out by Baksir et al (2018) regarding fish processing using geothermal sources in Idamdehe Village, Baksir et al (2019) regarding the use of geothermal energy sources for drying fish in Idamdehe Village. Research on the aspects of biota in the coastal area of Jailolo has been reported by Arahala et al (2015) on density, distribution patterns and morphometry of *T. telescopium*. Research on the structure of gastropod communities in Indonesia has been conducted by Ernanto et al (2010) at the mouth of the Batang Ogan Komering River, Ilir South Sumatra, Central Java. Romdhani et al (2016) conducted research on the diversity of gastropods in Baban Village, Gapura District. Isnaningsih & Patria (2018) conducted studies on the role of the mollusk community in supporting the function of the mangrove area in Tanjung Lesung, Pandegelang, Banten. The research activities that have been carried out showed that gastropods are an attractive biota to investigate.

Research on *T. telescopium* was conducted by Kurniawati et al (2014) regarding its characteristics in the mangrove ecosystems of Segara Anakan, Cilacap, and by Husein et al (2017) on the density and distribution in Kalidupa District, Wakatobi Regency. Research on the genetics of the gastropods was carried out by Lavie & Nevo (1986) on *Cerithium rupestre* and *C. scabridum* in the Mediterranean Sea, Guzman et al (2011) on *Crepipatella dilatata* and *C. fecunda* at two locations in South America, Gu et al (2015a) on *Bellamya purificata*, Attwood et al (2019) on *Neotricula aperta* and others. However, research on the genetic population structure of *T. telescopium* in Indonesia is scarce. Genetic information can infer conditions, chances of survival, adaptation to environmental quality disturbances and describe the migration rates among sub-populations (Akbar et al 2018a; Attwood et al 2019). Genetic diversity is used for the sustainability of fish and other resources and to assess the conservation status of local populations and species as a whole (Akbar et al 2014; Gu et al 2015b).

The existence of T. telescopium gastropods in the mangrove affected by the flow of hot springs should be studied to determine information about the characteristics and structure of the genetic population. The genetic characteristics obtained can be used as a consideration for DNA conservation, because it is possible to obtain unique genetic characteristics and qualities. Unique gene characteristics can be developed in gene engineering activities for the purposes of conserving resources. The availability of genetic data can be used for the effectiveness of conservation measures, improving genetic quality (Arifin et al 2009; Timm et al 2017; Madduppa et al 2018).

Material and Method

Location. Research was carried out in geothermal water areas (Payo and Bobo) and normal areas (Tuada), on the coast of Jailolo, West Halmahera Regency, North Maluku Province, Indonesia, in June 2020 (Figure 1).

Samples collection. *T. telescopium* hand-collection sampling based on morphology was conducted in geothermal water areas (number of sumples in locations: Payo=10 and Bobo=10) and normal locations (Tuada=5). The meat was extracted and placed in a tube containing 95% ethanol (Guzman et al 2011; Gu et al 2015a; Nehemia & Kochzius 2017; Akbar et al 2018a).

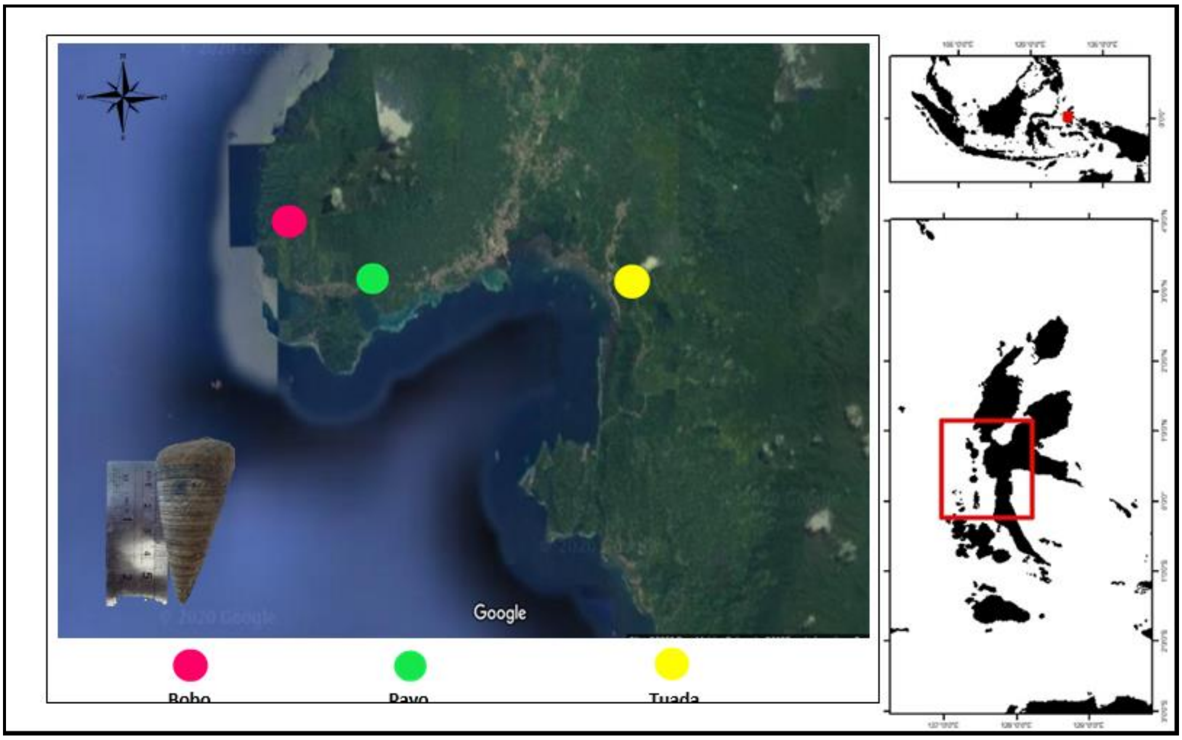


Figure 1. Research site in Coastal Jailolo (pink - Bobo; green - Payo; yellow - Tuada).

Extraction, amplification, electrophoresis. Sample extraction was performed using the Neasy Blood & Tissue Kit. 25 mg of tissue sample was collected using tweezers and placed into a 1.5 mL tube. Before and after the tissue was collected, the tweezers were dipped in 95% ethanol and burned with Bunsen fire. 180 uL ATL Buffer and 20 uL proteinase K was added to the sample tissue in a 1.5 mL tube. Then it was vortexed and centrifuged for 20 sec, and heated in a heating block with a temperature of 56°C overnight. 200 uL Buffer AL were added, the mix was vortex and incubated at 56°C for 10 min. 200 uL 96% ethanol were added and it was vortexed again. The sample and reagent mixture was then transferred to a DNeasy Mini spin column, which was placed in a 2 mL collection tube. Samples were centrifuged at 8000 rpm for 1 min. The disposal of the liquid in the collection tube was carried out. The spin column was placed on a new 2 mL collection tube, and 500 uL Buffer AW1 was added. It was centrifuged at 8000 rpm for 1 min. The liquid was discarded. The spin column was placed on a new 2 mL collection tube and 500 uL Buffer AW2 was added. It was centrifuged at 14000 rpm for 3 min. The liquid and collection tube were discarded. The spin column was transferred to a new 1.5 mL tube. DNA was eluted by adding 100 uL ddH2O to the center of the membrane spin column. It was incubated at room temperature, then centrifuged at 8000 rpm for 1 min. The final step was to add 100 uL ddH2O, so that the final volume was 200 uL. The extraction solution was ready to use for amplification.

DNA amplification was carried out by the PCR method. The extracted sample was amplified at the COI locus (cytochrome oxidase I) using the Gold (Bioline) method. The parameters used in this method were as follows: pre-denaturation at 94°C for 3 min, denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 1 min, and final extension at 72°C for 2 min. The PCR process (denaturation, annealing and extension) was repeated for 38 cycles (Barber et al 2006). In this method, two primers were used, namely the front primer (forward) jgLCO1490 with the nucleotide sequence TITCIACIAAYCAYAARGAYATTGG and the back primer (reverse) jgHCO2198, with the nucleotide sequence TAIACYTCIGGRTGICCRAARAAYCA (Geller et al 2013).

Sequencing. Sequencing was conducted by the method of Sanger et al (1977), using a wet approach.

Data analysis. Sequenced DNA was verified with the Blast application (Basic Local Alignment Tools). The data similarity between the species was determined using the DNA weight Matrix clustalw (1.6) and Translation Weight (0.5) on the software MEGA5. The mtDNA sequence of control regions was analyzed using the application MEGA5 (Molecular Evolutionary Genetic Analysis) (Tamura et al 2011). DnaSP 4.0 was used to determine the diversity of haplotype (Hd) (Nei 1987; Rozas et al 2003) and nucleotide (п) diversity (Lynch & Crease 1990). Network 4.6 was used for the reconstruction of haplotype spreads found.

Results and Discussion

Environmental parameters. There were differences between environmental variables from the geothermal water location and the normal environment (Table 1). The value of soil temperature in the geothermal water environment reached 45-54^{o}C, indicating that the soil in the area is geothermal. The soil temperature in the normal environment in Tuada village was 28^{o}C. The water temperature was 50.5-50.7^{o}C in locations affected by geothermal water, while in the normal water environment was 30^{o}C. Geothermal water that flows to the surface affects the surrounding soil temperature. Nainggolan et al (2018) noted that this type of fluid is formed due to heating of groundwater by geothermal steam. Budhyastoro et al (1979) stated that soil parameters that affect temperature include specific heat capacity, heat conductor, heat diffusivity, source and internal heat output at a certain time. Factors that affect soil temperature are divided into two, namely internal and external factors. External factors are the influence of solar radiation, natural conditions such as cloudiness, which results in increased water humidity, rain and wind. External factors affect soil, soil structure, soil water content, organic matter content, and geothermal potential. Research on earth's soil temperature was also carried out by Baksir et al (2018) in Idamdehe village, West Halmahera Regency, where the results of the study found that the geothermal soil temperature in the area reached 100^{o}C.

Table 1

Environmental parameters

Location	*Environmental parameters*					*Note*
	water temperature (^{o}C)	land temperature (^{o}C)	water pH	soil pH	Salinity ‰	
Payo	50.7	54	6.4	8	0	Geothermal water
Bobo	50.5	45	6.3	7	0.5	
Tuada	30.9	28	7	7.5	34	Normal environment

The pH environmental parameter values obtained fall into the normal category, in accordance with the requirements of the Ministry of Health (1990) regarding water quality. Additionally, it was in accordance with Government Regulation Number 82 of 2001 concerning water quality management and water pollution control. Payo geothermal water is fresh water that flows from the ground. Geothermal water appears to the surface, then flows towards the sea. This area has not yet had the influence of heavy pollutant input from the ground. Environmental conditions are supporting the quality of the water. Tatangindatu et al (2013) also revealed that temperature, brightness, pH, dissolved oxygen and nitrate were in good condition in Lake Tondano, Paleloan Village, Minahasa Regency. Mulyaningsih et al (2014) found pH ranging between 6-7 in the geothermal fluid in the Beta field, Ambon.

The soil pH value showed that the soil conditions in this area fell into the normal/neutral category. This was because there was no effect of geothermal water sources on soil pH conditions. In addition, various kinds of chemical liquids have minimal influence on soil pH conditions. The salinity was between 0-0.5‰ in locations affected by geothermal water flow, indicating that geothermal water was fresh water with the influence of sulfur (Table 1). The normal environment had a salinity level of 34‰, because it was not influenced by fresh water. The salinity levels found were in the normal or neutral category for fresh and marine water. The results are in accordance with the Government Regulation Number 82 of 2001 concerning water quality management and water pollution control, ranging from 6-9 for fresh water and 30-35‰ for seawater.

Molecular characteristics. Molecular characteristics obtained in 25 (primary data) and 1 (sample out group) *T. telescopium* samples presented 599 fragment lengths (bp) in the locus control region (Table 2). The fragment length found was the normal size in *T. telescopium*. Palanisamy et al (2020) found *T. telescopium* base lengths of 637-652 (bp). Similar lengths of DNA bases were also found in other gastropods, as reported by Ramirez & Ramirez (2013), Borges et al (2016), Saleky et al (2016), Leatemia et al (2018) and Attwood et al (2019) (Table 2). The differences in base pairs (bp) can be due to the use of primers, PCR processes, contamination of organic matter, number of samples, DNA quality, primary base composition, environment, food and heredity (Williams et al 1990; Shizuka & Lyon 2008; Nuryanto & Kochzius 2009; Akbar et al 2014; Jefri et al 2015; Saleky et al 2016; Akbar et al 2018b).

Table 2

Base pairs length of some molluscs

Names	*Total Specimens*	*Base pairs (bp)*	*Location*	*Literature*
Telescopium	25	599	Coastal Jailolo	Current study
Telescopium	10	637-652	Parangipettai coast, Bay of Bengal and India	Palanisamy et al 2020
Bostryx aguilari	11	826	Amancaes, Atocongo and Iguanil	Ramirez & Ramirez 2013
Bellamya aeruginosa	182	709	Lakes and one river in China	Gu et al 2015a
Patella ulyssiponensis, Patella Aspera, Gibbula pennanti, Tectura virginea, Calliostoma virescens, Jujubinus pseudogravinae, Berthella plumula, Ocenebra erinaceus, Siphonaria pectinata, Littorina littorea, Hexaplex Trunculus, Nassarius reticulatus	109	600	Coastal Portugal	Borges et al 2016
Turbo sparverius and Turbo bruneus	179	656	Bird Seascape Papua, Indonesia	Saleky et al 2016
Turbo sp.	12	650	Manokwari Waters West Papua	Leatemia et al 2018
Neotricula aperta	38	505	Cambodia, Laos and Thailand	Attwood et al 2019

Phylogenetic relationship. *T. telescopium* phylogenetic analyses derived from Tuada, Bobo and Payo found two main clades with bootstrap support (77-97) (Figure 2). High bootstrap value affects the consistency and stability of phylogeny tree formation (Wirdateti et al 2015). Clade 1 is mixed with 3 groups of individuals from Tuada, Payo and Bobo and clade 2 showed the separation of 2 individuals from Bobo. The phylogeny tree showed that *T. telescopium* individuals were not grouped by location, but mixed with individuals from other locations. The obtained phylogenetic typology indicated that the *T. telescopium* population on the coast of Jailolo is not genetically different. Genetic mixing resulted in no genetic boundaries among *T. telescopium* individuals. The mixing of individual groups, even though they were far apart, shows a high genetic flow. Saleky et al (2016) stated that genetic closeness between individuals occurs due to high sequence similarity.

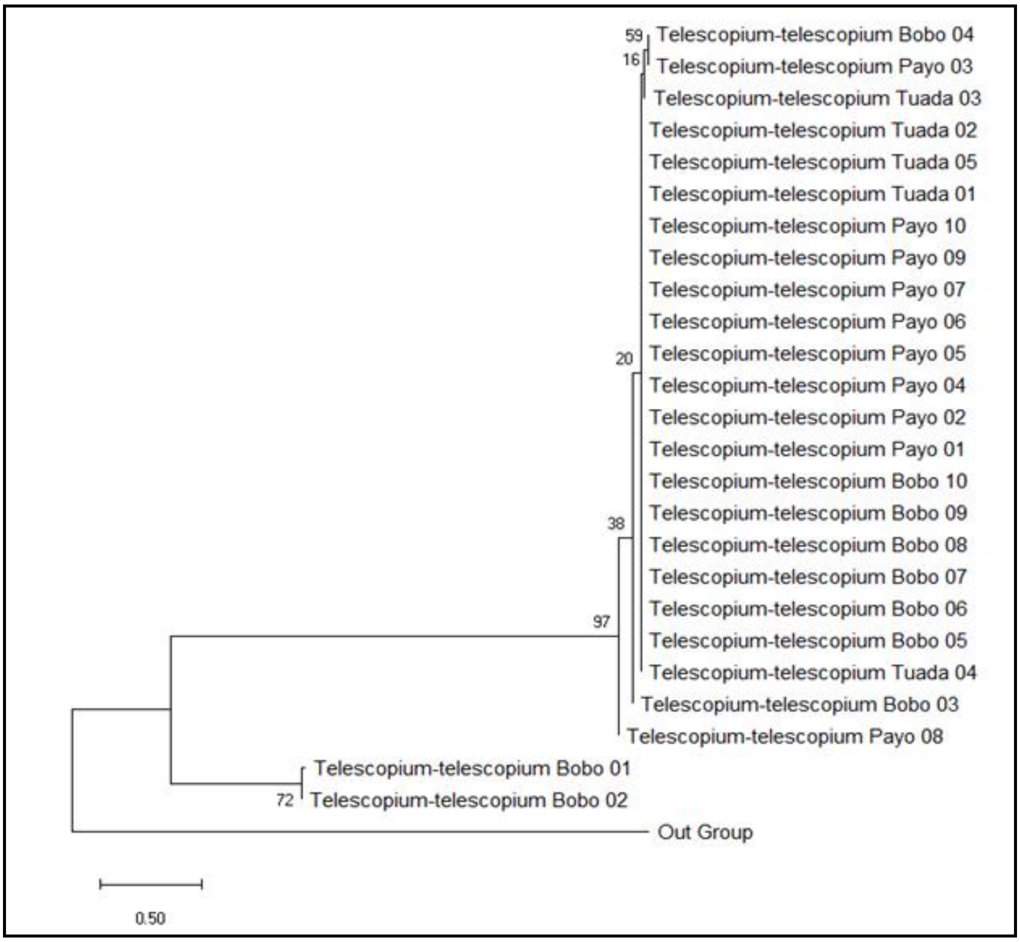


Figure 2. Phylogenetic tree of *Telescopium telescopium* in Coastal Jailolo.

The history of evolution was deduced using the Neighbor-Joining method (Figure 2). Phylogenetic tree reconstruction was carried out with the number of branches length equal to 6.38886767. The phylogenetic tree was contructed with the bootstrap test (500 replicates) (Figure 2). Trees were constructed to a scale, with the length of the branches in units equal to the evolutionary distance used to deduce the phylogenetic tree. The evolutionary distance was calculated using the 2-parameter Kimura method and was in units of the number of base substitutions per site. The rate variation between sites was modeled by the gamma distribution (shape parameter = 1). This analysis involved 26 nucleotide sequences. The codon position entered is 1 + 2 + 3 + Noncoding. All positions containing gaps and missing data were eliminated (complete delete option). Evolution analysis obtained a total of 560 base pairs (bp) performed on MEGA X.

The separation of individuals to form clade 2 was likely the result of a process of genetically significant mutations (Figure 2). Phylogenetic trees were supported by genetic distance analysis between populations (Table 3).

Table 3

Genetic distance inter and intra population in *Telescopium telescopium*

Name	*Location*	*Bobo*	*Payo*	*Tuada*
Inter population	Bobo	-	-	-
	Payo	0.607		-
	Tuada	0.602	0.011	-
	Location	Bobo	Payo	Tuada
Intra population	Bobo	1.063	-	-
	Payo	-	0.014	-
	Tuada	-	-	0.0080

Low genetic flow may result in differences between individuals genetically, without affecting morphology. Geographical distance between sampling locations and differences in habitat can result in morphological and genetic differences (Leatemia et al 2018). Morphological studies involving shell structure can be carried out to support the genetic research results found. The long evolutionary process may result in two individuals being isolated and forming different genetic characters. Genetic differences in individuals result in limited breeding crosses, which result in two groups (De Queiroz 2007). Natural conditions, such as circulation and current patterns, historical geographic aquatic environments, mating cycles, recruitment, population dispersal abilities result in genetic differences intra-population and between populations (Waycott et al 2006; Pertiwi et al 2019). Phylogenetic reconstruction of this gastropod is supported by Palanisamy et al (2020), who noted that there was mixing of individuals from different locations and the separation of individuals from the same group was found. Research on gastropod phylogenetic reconstruction reported by Guzman et al (2011) found differences in clades between *Crepipatella dilatata* and *C. fecunda* species, but sub-clades occurred among individuals in South America. Borges et al (2016) found clade formation between species, however, with genetic mixing and separation between individuals in a gastropod population on the Portuguese coast. Leatemia et al (2018) show close relationships between individuals in the same species, and form a clade of each species. Attwood et al (2019) indicated based on phylogenetic topology that there was separation of the genetic distance between north and south Asia (Cambodia, Laos and Thailand) and Ran et al (2020) found species-based clade formation on the island of Hainan, China.

Genetic distance and AMOVA population pairwise (Fst). Genetic distance provides information on intrapopulation and interpopulation genetic proximity. The value of genetic distance obtained from the distance method in pairwise differences found that all samples had a genetic distance value lower than 1 (Table 4).

Table 4

Genetic diversity, population structure and genetic distance

	Category			
Analysis	*Low*	*Middle*	*High*	*Literature*
Genetic diversity (*H_d*)	0.1-0.4	0.5-0.7	0.8-1.00	Nei (1987)
AMOVA population pairwise (*Fst*)	0.1-0.3	0.4-0.7	0.8-1.00	Excoffier et al (1992)
Genetic distance (*D*)	0.010-0.099	0.1-0.99	1.00-2.00	Nei (1972)

The inter and intrapopulation genetic distances of *T. telescopium* based on the mtDNA locus control region showed a low genetic disparity between populations (Table 4). The genetic distance between individuals from Bobo and Payo was 0.605, Tuada and Bobo was 0.602 and Payo and Tuada was 0.011. The analysis results showed that the closest genetic distance was found between the populations from Tuada and Bobo. The analysis showed that the farthest genetic distance was found between Bobo and Payo, and Tuada and Payo locations. The difference in genetic distance between populations was in the medium category, which allowed for the mixing of the population. Intrapopulation genetic distance showed genetic differences between individuals in the *T. telescopium* population from Bobo. Genetic distance was found to be within the criteria for height among individuals in the Bobo population. The genetic distance between individuals in the Tuada and Bobo populations was considered low. The phylogenetic tree reconstruction supports the analysis of the genetic distances found (Figure 2). The low value of genetic distance indicated low genetic separation and the possibility of dispersal gene flow, allowing genetic connectivity between populations. Species that have close genetic distance could share their haplotypes through the hybridization process (Hidayani et al 2020). High genetic similarity can occur due to the conditions and direction of the flow of gene transport mediation among populations, and the similarity of habitat in each population (Saleky et al 2016).

Palanisamy et al (2020) found large differences between populations of *T. telescopium* along the southwest coast of India and Australia showing limited gene flow between the two continents. Research in the gastropod class shows genetic proximity despite geographic distances (Svin'ina & Kartavtsev 2009; Gu et al 2015b; Leatemia et al 2018; Ran et al 2020). However, different results were found by Attwood et al (2019) in *Neotricula aperta* (Gastropoda: Pomatiopsidae), where well-differentiated populations were geographically far apart. Low levels of genetic differentiation between populations suggested little restriction of gene flow.

Genetic differences between individuals in the population indicated a genetic mutation. The shifting of nucleotide bases can result in genetic differences in low, medium and high scales. The process of genetic mutation causes individual disparities in the population. The mutation site can be a nucleotide base that undergoes genetic changes in the form of insertion, deletion and transversion (Tindi et al 2017). Kombong & Arisuryanti (2018) said that it appears that differences in the composition of nucleotides in gene sequences lead to evolution and result in genetic variations. Extreme ecological health causes species morphological and genetic changes due to natural selection (Bickford et al 2007). Species that have undergone a strong selection of behavioral and physiological characters for adaptation to certain conditions usually do not show changes in phenotypic characters (Hidayani et al 2020). Species can adapt to extreme environmental situations and pass strong selections combining physical characteristics and speciation through habitat fragmentation.

AMOVA population pairwise (Fst) found high genetic flow between populations (Table 5).

Table 5

AMOVA population pairwise (*Fst*) in *Telescopium telescopium*

Location	*Bobo*	*Payo*	*Tuada*
Bobo	-	-	-
Payo	0.093	-	-
Tuada	0.105	0.019	-

The value of Fst=0 indicates low genetic divergence between populations and the value of Fst=1 shows differences in genetic characters (Excoffier & Lischer 2010). The Fst values ranged from 0.019 to 0.105, distributed across the three locations (Table 5). The Fst analysis was supported by the results of genetic distance analysis and phylogenetic tree reconstruction, showing that the *T. telescopium* population (Payo, Tuada and Bobo) was very closely related (Table 3 and Figure 2). Geographical location has no influence on genetic flow between populations. The research locations (Payo, Tuada and Bobo) were on the coast of Jailolo, West Halmahera Regency, North Maluku, Indonesia. The distances between locations were 93.4 km between Bobo and Tuada, 1.5 km between Bobo and Payo, and 88 km between Payo and Tuada (88 km). A low Fst value indicated that *T. telescopium* come from a single stock population. The planktonic phase in marine organisms affects the distribution patterns globally, because they do not have the morphological ability to move. Local ocean current transport helps the dispersal of marine organisms and forms a small sub-population in each region. This spread allows genetic connectivity between populations. Haws & Elis (2000) explained that the distribution of marine organisms occurs in the planktonic phase, so they have high genetic similarity.

High genetic flow indicated that closely related populations may occur due to species dispersal patterns in the aquatic environment. The research area is affected by tides and by the movement of local currents and waves. The ebb and flow of seawater can be suspected as a factor causing dispersal of *T. telescopium* larvae. Genetic similarities indicated that the three populations are separate groups due to the influence of oceanography. The haplotype distribution indicated that the Payo population was the parent population (Table 6 and Figure 3).

Table 6

Haplotype distribution between species into population *Telescopium telescopium*

No	*Haplotype (H_d)*
1	Hap_1: 2 [Telescopium-telescopium_Bobo_01 Telescopium-telescopium_Bobo_02]
2	Hap_2: 1 [Telescopium-telescopium_Bobo_03]
3	Hap_3: 1 [Telescopium-telescopium_Bobo_04]
4	Hap_4: 16 [Telescopium-telescopium_Bobo_05 Telescopium-telescopium_Bobo_06 Telescopium-telescopium_Bobo_07 Telescopium-telescopium_Bobo_08 Telescopium-telescopium_Bobo_09 Telescopium-telescopium_Bobo_10 Telescopium-telescopium_Payo_01 Telescopium-telescopium_Payo_02 Telescopium-telescopium_Payo_04 Telescopium-telescopium_Payo_05 Telescopium-telescopium_Payo_06 Telescopium-telescopium_Payo_07 Telescopium-telescopium_Payo_09 Telescopium-telescopium_Payo_10 Telescopium-telescopium_Tuada_01 Telescopium-telescopium_Tuada_04]
5	Hap_5: 1 [Telescopium-telescopium_Payo_03]
6	Hap_6: 1 [Telescopium-telescopium_Payo_08]
7	Hap_7: 2 [Telescopium-telescopium_Tuada_02 Telescopium-telescopium_Tuada_05]
8	Hap_8: 1 [Telescopium-telescopium_Tuada_03]

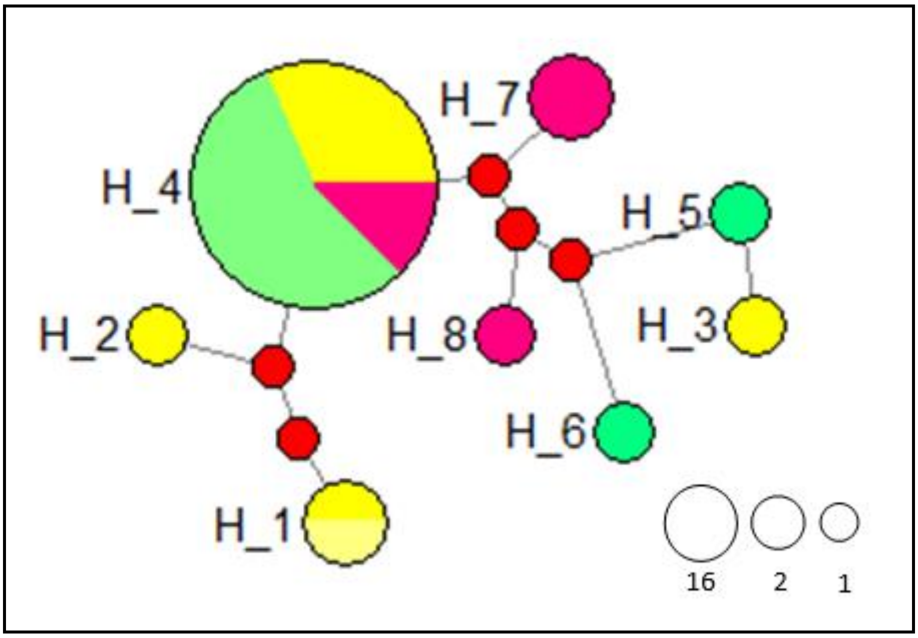


Figure 3. Haplotype distributions *Telescopium telescopium* using Network 5.0.1.1. Note; green - Payo; pink - Tuada; yellow - Bobo.

The Payo population was indicated as the source of the spread of *T. telescopium* larvae in the Bobo and Tuada populations. The Bobo population was geographically close to Payo, where it was possible to obtain a supply of *T. telescopium* larvae. Habitat conditions in the Payo area such as mud substrate, mangrove composition and tidal influence support the existence of *T. telescopium*. Although the Payo and Bobo areas were affected by geothermal water flow, it was not a barrier to the life of *T. telescopium*. The location description showed that the Payo and Bobo populations live in more indented areas (such as bays). The Tuada population was found on open beaches without the influence of geothermal springs. The level of genetic separation was low, indicating that the population was the same descendant. Geographically, Tuda is far from Payo and Bobo.

The results of the analysis showed that there was no genetic boundary based on geographic location. The distribution of animals may be due to geographic expansion events with the effect of genetic distribution (Ramirez & Ramirez 2013).

Genetic diversity. The genetic diversity of *T. telescopium* was moderate (0.533-0.667) and high (0.800) (Table 7). Genetic variation indicated differences between populations. The mean genetic diversity values obtained were included in the moderate criteria. The Bobo and Payo populations have moderate genetic diversity, indicating that inbreeding has occurred in the population. The inbreeding process occurs due to the limited movement of the population. The decrease in genetic diversity from generation to generation is due to inbreeding (Arifin et al 2017). Toha et al (2016) explained that low genetic diversity is likely due to regional populations. Low genetic diversity indicated that the population has a low migration ability resulting in the population closing the intersection of populations. Gastropods have sessile properties that result in limited movement. A decrease in the level of genetic diversity was closely related to a decrease in gene heterozygosity (Arifin et al 2017).

Table 7

Genetic diversity in *Telescopium telescopium*

Location	*N*	*H_n*	*H_d*	*Π*	*Base pairs*
Bobo	10	5	0.667	0.211	599
Payo	10	4	0.533	0.014	
Tuada	5	3	0.800	0.008	
All locations	25	8	0.593	0.097	

Note: n - number; Hn - haplotype number; Hd - haplotype diversity; Π - nucleotide diversity.

Moderate genetic diversity was probably influenced by environmental conditions. The environmental character of Payo and Bobo's locations has the influence of geothermal water flow. Past geological processes and environmental adjustments have resulted in *T. telescopium* being able to survive extreme conditions. Baksir et al (2020) found a geothermal water temperature of 50°C in the mangrove ecosystem area in Payo village. High hydrothermal temperatures indicated that the area (Payo-Bobo) was a geothermal spot. Genetic diversity can be compromised by extreme environmental conditions. Extreme temperatures can result in physiological disturbances and passive movement of the biota. Biota at extreme temperatures adapts genetically through genetic mutations. The shifting of nitrogenous bases (A, G, C and T) has an impact on the process of adjusting the body's morphology to extreme environments. Extreme temperatures can cause stress and limit the movement of organisms. Hydrothermal temperatures of 25°C for a long time lead to growth deficits, decreased genetic diversity and species loss (Tardieu 2013; Maher et al 2013). Combined research shows that genetic diversity differs between species (Table 8).

Table 8

Gastropod class genetic diversity

Species	*Genetic diversity*	*Literature*
Telescopium telescopium	0.533-0.800	The result study
T. sparverius	0.657-0.705	Saleky et al 2016
T. bruneus	0.739-0.816	
Bellamya purificata	0.889-1.000	Gu et al 2015a
Bellamya aeruginosa	0.867-0.978	Gu et al 2015b

The genetic diversity of *T. telescopium* is low when compared to the results of previous studies in the gastropod class, namely *Bellamya purificata*, *Bellamya aeruginosa* and *T. bruneus*, but higher than that of *T. sparverius* (Table 8). The genetic variability found between species ranged from 0.533 to 1000, indicating that each species has a different genetic composition and adaptability. The similarities and differences in the value of genetic diversity are caused by different numbers of samples used at the time of the study (Akbar et al 2014a). Genetic diversity can occur due to changes in the nucleotides that form DNA, which can affect the phenotype of organisms or affect the phenotype of an individual in a certain environment. In general, the genetic diversity of a population can occur due to mutations, recombinations, or gene migration (Arifin et al 2017).

The minimum spawning networks (MSN) found 8 haplotype networks from a total of 25 samples. The distribution of the haplotype network found 5 specific haplotypes, 1 mixed haplotype among locations (mixing population) and 2 haplotypes that were the same (Figure 3). Haplotype 1 consists of 2 individuals of the Bobo population; haplotype 2 contains 1 individual of the Bobo population; haplotype 3 had 1 individual in the Bobo population; haplotype 4 consisted of 16 individuals, 6 from the Payo population, 8 from the Bobo population and 2 from the Tuada population; haplotype 5 had 1 Payo individual; haplotype 6 had 1 Payo individual; haplotype 7 consisted of 2 Tuada individuals and Haplotype 8 had 1 Tuada individual. The haplotype networks found were supported by the results of genetic diversity analysis and AMOVA population pairwise (Fst), which showed a strong genetic flow. High polymorphism increases population ability in abnormal environment. Diverse types of haplotype composites increase genetic diversity and adaptability to environmental change, climate and disease (Smith & Chesser 1981; Akbar et al 2014; Kusuma et al 2016).

Haplotype linkages indicate genetic relationships between individuals and ancestral population. The genetic diversity and distribution of the haplotypes explain the genetic structure of the *T. telescopium* population. Minimal spawning networks (MSN) and genetic diversity of *T. telescopium* (Bobo, Payo and Tuada) were in stable condition. Moderate genetic diversity in Bobo and Payo requires a special approach. A genetic maintenance program (conservation) needs to be carried out to maintain the genetic stability of the population. An approach to increase genetic diversity through crossbreeding can also be carried out. The life cycle in extreme areas and low displacement results in population confinement limits. *T. telescopium* populations were globally distributed so that genetic flow was significant between populations. Geographical boundaries are a natural barrier to gastropod distribution, even though the planktonic phase is the beginning of gastropod life. The planktonic phase makes genetic distribution wide and can cause divergences between regions.

The genetic connectivity of the three populations suggested that the *T. telescopium* population from Payo was the original population. The Payo population was the center of genetic distribution for Bobo and Tuada (Figures 3 and 4). Genetic distribution occurred because it could be influenced by aquatic environments such as currents, waves and historical geology. Oceanographic conditions that dominate the Payo location were tides. The mangrove ecosystem, which was the *T. telescopium* habitat, gets the maximum tidal effect. The location of the mangroves is more indented inland, resulting in the dominant distribution of gastropods in the mangrove area. The ebb and flow of seawater that affects the mangrove ecosystem was thought to be a medium for spreading *T. telescopium* larvae. The larvae that were carried by the tides were distributed through the current that moves into the bay. Local currents dominate and move into the bay at high tide, then out of the bay when the water recedes.

Daruwedho et al (2016) explained that Indonesia has a monsoon, which blows in a certain direction in some periods and blows opposite in other periods. In the northern hemisphere, the wet season is in December-February, while the southern part enters the summer period. This difference causes the wind from the center of the mainland (Asia) with high pressure to blow to low pressure areas (Australia). This event is commonly called the West Season (West Monsoon). Another monsoon period is in July-August, when winds blow from high pressure areas (Australia) to low pressure areas (Asia). This condition is commonly called the East Season (East Monsoon). West Halmahera Regency globally experiences the pattern of the west wind monsoon, transition I, east wind and transition II. The current pattern based on the seasons shows that the current movement in the west, east, transition I and transition II monsoons has the same pattern, namely the inflow into the Jailolo bay area (Figure 5).

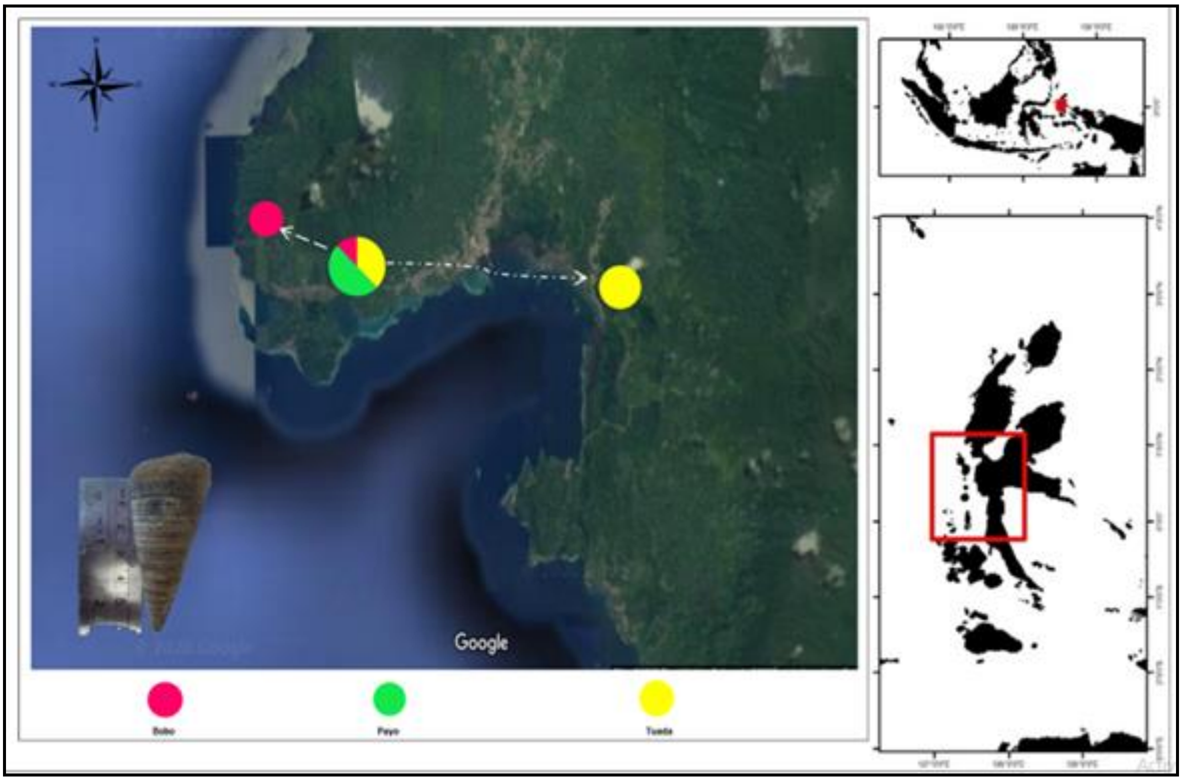
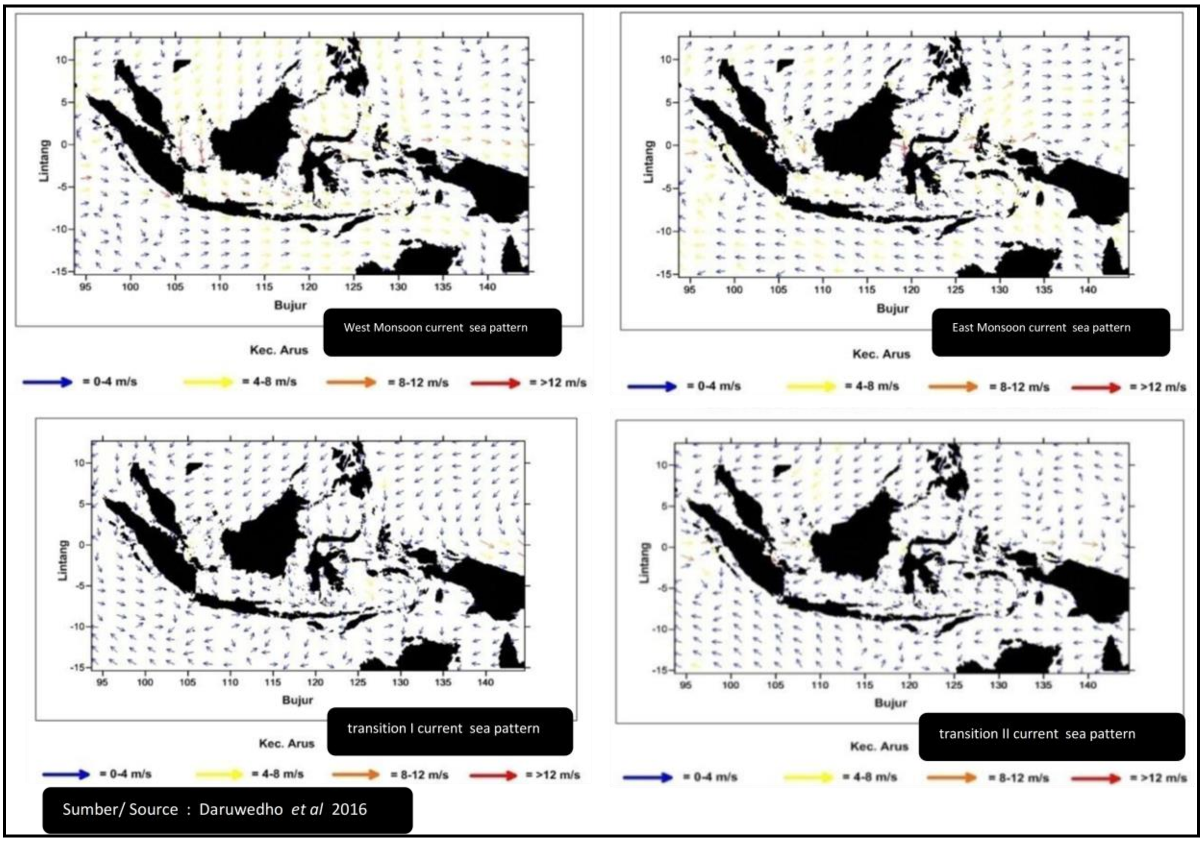


Figure 4. Genetic connectivity *Telescopium telescopium* from Bobo (pink), Payo (green) and Tuada (yellow) on control region locus.

Oceanographic patterns have an impact on the genetic distribution at the three locations. Current circulation patterns will provide information about the distribution of larvae, and their connectivity patterns can be determined (Botsford et al 2009). The flows facilitate larval dispersal, gene flow and geographic isolation of each region (Saleky et al 2016). Marine environment and genetic data can make important contributions to understanding population connectivity, as well as conservation and management strategies, including marine reserve design. Previous studies have reported genetic connectivity in the gastropod class at several locations in the world (Guzman et al 2011; Ramirez & Ramirez 2013; Crocetta et al 2020), where divergences and genetic similarities were found.

The gastropod life cycle originates from the planktonic, larval, juvenile and adult phases. Gastropods are a group of zooplankton. The zooplankton life cycle is divided in meroplankton and holoplankton. Gastropods are included in the meroplankton group, namely aquatic animals that spend part of their life as plankton, especially at the larval stage; therefore, they are also referred to as temporary plankton (Mahipe et al 2017). Previous research on the planktonic phase of gastropods was reported by Dody (2012), where the ability of gastropods to live in the planktonic phase for 4 days. This study supports the results of genetic analysis, which showed the connectivity between populations. The long-lasting ability helps in the spread of gastropod larvae over a wide area.



West Monsoon current sea pattern

East Monsoon current sea pattern

transition I current sea pattern

transition II current sea pattern

Sumber/ Source : Daruwedho *et al* 2016

Figure 5. Ocean current circulation patterns based on seasons.

Conclusions. There were several differences among environmental variables at the location of geothermal water and the normal environment. Molecular characteristics of *T. telescopium* showed a 599 fragment length (bp) in the locus control region. Phylogenetic reconstruction obtained two clades, where the separation of individuals to form clade 2 was likely due to a process of significant genetic mutations. The inter-population and intra-population genetic distance showed that there was a low genetic disparity between populations. AMOVA population pairwise (Fst) found high genetic flow between populations. The genetic diversity of *T. telescopium* was found to be moderate (0.533-0.667) and high (0.800). The distribution of the haplotype network found 5 specific haplotypes, 1 mixed haplotype between locations (mixing population) and 2 same haplotypes. Genetic information is required for management purposes and conservation strategies.

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

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