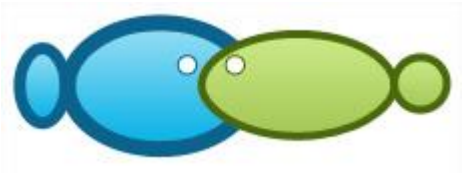

A pilot study on the genetic diversity of tropical eel (*Anguilla* spp.) in the Pacific region of North Maluku Sea, Indonesia

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Abstract. Eel (*Anguilla* spp.) is a fish with economic importance in the fisheries sector. High-quality eels (*Anguilla japonica*, *A. marmorata*, *A. interioris*) are fished in Asia. Increased demand of eel can lead to threatened populations. The research aimed to determine the genetic fragility of eel in the North Maluku Sea. The eel samples were collected from 3 locations (Bacan, Tidore and Gane Barat) and DNA was analyzed in the laboratory. The molecular phases include extraction, amplification, electrophoresis and DNA sequencing. Amplification in the locus DNA Control Region with the Gold (Bioline) method uses the L15923 (forward) and H16498 (reverse) primers. The results of DNA amplification in the mtDNA Locus control region found 603 base pairs, from a total of 14 samples (Bacan=4, western Gane Barat=5, and Tidore=5). Based on the results of the analysis, the genetic diversity of eel is high, with various nucleotides and variability. Haplotype distributions show that there is a high diversity of haplotypes overall. The fixation index (Fst) fshowed that the eel populations of Bacan, Tidore and Gane Barat were not distinct.
Key Words: adaptation, behavior, ecology, enviromental, marine biota.

Introduction. The eels (*Anguilla* spp.) are catadromous species with unique characteristics, including a migration to deep sea for reproduction (Triyanto et al 2008). The eel starts its life in the sea, migrates to freshwater where it grows into adult, and returns to the sea for spawning (Fahmi 2015). Eels have a high market demand, and the fishing efforts increased. The eel is very popular in Asia and Europe. The crude protein content and protein quality makes the eel a fish with high economic value. Thus, the production technologies are advanced, with cultivation activities with seed supply from the wild (Fahmi 2015). Eels are sold in international markets (Japan, Hongkong, Germany and Italy), being an important export commodity (Affandi 2005). The market demand for eel is very high, especially in Japan and South Korea (50000 tons per year) (Sembiring et al 2015). Demand for eels for the global market is 268342 tons per year, with a price range between 13-16 USD per kg (Montcini 2014).

The over utilization of eel resources can lead to a decrease in population. Data shows that from 2009 to 2018, the export value of eels fluctuated (Noor & Abidin 2019). Loss of population can lower the survival ability of an organism in nature, resulting in low genetic diversity. High exploration of eels should be followed by a conservative approach. This requires a strategy in preventing the loss of fish resources (Akbar & Labenua 2018). The eel is an important and strategic resource today, both for market and research purposes (Fahmi 2015). Genetic conservation is a strategy of management and preservation of resources. Genetic research on eels has been previously conducted (Triyanto et al 2008; Fahmi 2015; Arai et al 2015; Arai et al 2016; Arai & Abdul Kadir 2017; Arai & Chino 2018; Zan et al 2020). Genetic information is important in describing the state of the eel populations. Knowledge of genetic aspects of a species is important in

its conservation and monitoring (Schwartz et al 2006; Muchlisin et al 2012; Kusuma et al 2016). The morphology of *Anguilla* spp. is similar, making the morphological identification at species level difficult. Thus, DNA barcoding is required for an accurate identification (Madduppa et al 2020). Previous research reports are scarce when the genetic diversity of eel is concerned in the Pacific region of North Maluku. This study aims to be a pilot study of the genetic diversity of eels in Maluku Sea, Indonesia.

Material and Method

Sample collection. Eel tissue samples were collected from 3 locations (Bacan, Tidore and Gane Barat, Indonesia) (Figure 1), from the estuary of rivers (brackish water), from June 2018 to December 2019. The number of eels captured were 4 in Bacan, 5 in Gane Barat, and 5 in Tidore. Eels were captured using fishing nets in estuaries at incoming tide. 3 cm of pectoral fins were sampled from each fish for the purpose of DNA analysis. The samples were placed in individual containers with ethanol 90% and labeled. The DNA samples were sent to the BIONESIA Laboratory (Indonesia biodiversity) in Bali, for the extraction, amplification, electrophoresis and DNA sequencing.

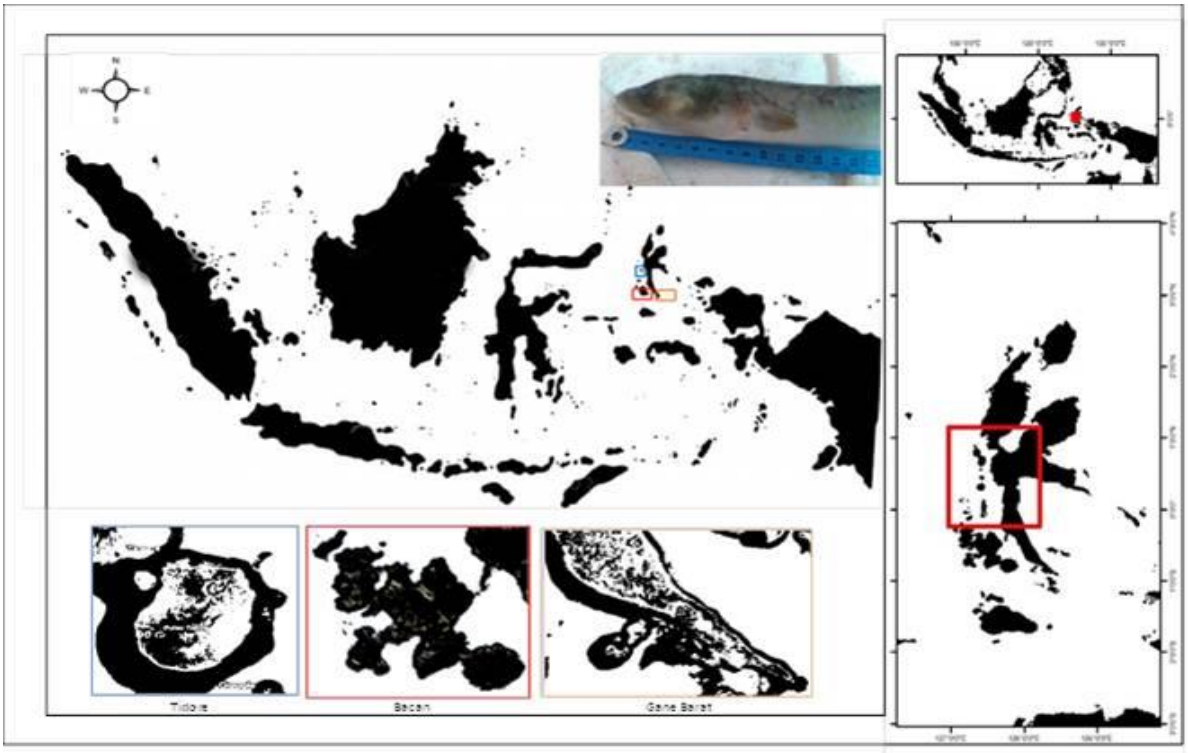

Figure 1. Research site in North Maluku Sea; Tidore - blue; Bacan - red; Gane Barat - orange).

Extraction, amplification, electrophoresis. Sample extraction was conducted using Chelex. 25 mg of sample tissue was placed with tweezers into a 1.5 mL tube. Before and after the tissue was touched, tweezers were dipped in ethanol 95% and burned with bunsen fire. 180 uL of ATL buffer and 20 uL of proteinase K were added. The sample tube was vortexed and placed in a centrifuge for 20 seconds at 14000 rpm. It was then heated in a heating block with a temperature of 56^{o}C for 45 minutes. 200 uL of AL buffer were added, vortexed for 1 minute and incubated at a temperature of 56^{o}C for 10 minutes. 200 uL of 96% ethanol and vortex were added. 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 14000 rpm for 1 minute. The liquid was removed and the spin column extract was placed in a new 2 mL collection tube. 500 uL of AW1 buffer was added. The mix was centrifuged at 8000 rpm for 1 minute. The liquid is removed and

the spin column extract was placed in a new 2 mL collection tube. 500 uL of AW2 buffer was added. The new tube was centrifuged at 14000 rpm for 3 minutes. The liquid was again removed, and the spin column extract was placed in a new 1.5 mL tube. 100 uL of ddH2O was added to the center of the spin column. Incubation was done at room temperature. The extract was centrifuged at 8000 rpm for 1 minute. The step was repeated by adding 100 uL of ddH2O, so that the final volume was 200 uL. Thus, the extraction solutions were prepared for amplification. Amplification was conducted by PCR (polymerase chain reaction). DNA samples were amplified on the control region by the Gold (Bioline) method. The steps used in this method are: pre-denaturation at 94^{o}C for 3 minutes, denaturation at a temperature of 94^{o}C for 30 seconds, annealing at a temperature of 50^{o}C for 30 seconds, and the extension at a temperature of 72^{o}C for 1 minute. The final extension takes place at a temperature of 72^{o}C for 2 minutes. The PCR process (denaturation, annealing and extension) was repeated by 38 cycles (Barber et al 2006). This method used two primers, namely the forward L15923 and the reverse H16498 (Geller et al 2013).

Sequencing. The DNA samples amplified using the PCR method were then sequenced to obtain their nucleotide sequence using the Sanger sequencing method (Sanger et al 1977). Eel DNA sequences were identified from the GenBank database with the BLAST feature.

Data analysis. The DNA sequences are then verified with the Blast application (Basic Local Alignment Tools), with the intention of obtaining the data similarity using the DNA weight Matrix clustalW (1.6) and Translation Weight (0.5) in the MEGA5 software. The mtDNA sequence of control regions was analysed using the application MEGA5 (Molecular Evolutionary Genetic Analysis) (Tamura et al 2011) for DNA sequences, DnaSP 4.0 (Rozas et al 2003) was used to determine the diversity of the haplotype (Nei 1987) and nucleotide (π) diversity (Lynch & Crease 1990) and the Network 4.6 software was used for the reconstruction of haplotype spreads found. The fixation index (Fst) was determined using the Arlequin 3.5 software with the description of the gene flow and population structure (Excoffier & Lischer 2009). Fakhri et al (2015) state that if the value of Fst≥0<0.5, then the population is not distinct; and if Fst>0.5≤1, the population is distinct.

Results and Discussion

Molecular characteristics. The results of identification based on the similarities of the eel samples from Maluku waters showed the compatibility with the species *A. marmorata*, with a similarity level of 97-100% and *A. interioris*, with a similarity level of 100%. The eel species identified in Bacan was *A. marmorata*, in Tidore it was *A. marmorata*, and in Gane Barat it was *A. marmorata* and *A. interioris*. The results of the identification showed that the spread of eel has been dominated by *A. marmorata*, found in each sampling location, whereas *A. interioris* was found only in one location: West Gane.

The results of DNA amplification in the mtDNA locus control region found 603 base pairs (bp) in 14 samples (Bacan=4, western Gane Barat=5, Tidore=5) (Table 1). The (bp) of the eels are similar to those found by Triyanto et al (2008), 500-600 bp in the waters of Poso, and to those found by Fahmi (2015) in Indonesian waters, with 620-795 BP. Edwin et al (2018) also found similar results, 620 bp in the Cimandiri River estuary, Port Ratu, Indonesia. The length difference can be caused by different sample quantities and use, the quality of DNA extracted, the specific primers, the size of the primer length, the composition of primer bases, the environment, food and heredity (Williams et al 1990; Shizuka & Lyon 2008; Akbar et al 2014; Jefri et al 2015; Akbar et al 2018). The bp difference could also result from gene mutations (Akbar & Aris 2018).

Table 1

Molecular characteristic of eel (*Anguilla* spp.) in the Pacific region, North Maluku Sea

Location	*Samples*	*Base pairs*
Bacan	4	603
Gane Barat	5	
Tidore	5	
Total	14	

Genetic diversity. Based on the results of the analysis, the eel genetic diversity is high, with various nucleotides and variability (Table 2). The value of the nucleotide diversity at the Bacan site was 0.289, with 4 haplotypes, at Gane Barat it was 0.305, with 5 haplotypes and at Tidore it was 0.026, with 5 haplotypes. The genetic diversity in Bacan, Gane Barat and Tidore has a total of 1. The value shows high genetic diversity, likely to be caused by large eel populations in every water and high migration capability. Large migrations and population patterns lead to interpopulation intersections. This process resulted in a mix between populations and different molecular characteristics, therefore increasing the population's genetic variation. Akbar & Lacontinents (2018) say that the similarity of the value of genetic diversity may be caused by the nature of the migration of marine fish, thus giving the opportunity to meet other groups in various waters. Diverse haplotypes demonstrate a high level of genetic diversity in a population (Fakhri et al 2015).

Table 2

Genetic diversity of eel (*Anguilla* spp.) in the Pacific region, North Maluku Sea

Location	*n*	*Hn*	*Hd*	*nd*	*Base Pairs*
Bacan	4	4	1.00	0.289	
Gane Barat	5	5	1.00	0.305	603
Tidore	5	5	1.00	0.026	
All Populations	14	13	0.98	0.197	603

Note: n - total number of samples; Hn - haplotype number; Hd - haplotype diversity; nd **-** nucleotide diversity.

A population with a high degree of genetic diversity can persist in extreme environmental conditions. Low genetic diversity will result in the emergence of negative traits, such as decreased growth, diversity of size, instability of organ development, low survival rate, as well as lower adaptation to the changing environment (Arifin & Kurniasih 2007). According to Akbar et al (2014), the high value of haplotype diversity is alleged to be caused by two factors. First is the periodic pressure of arrest against a large size of the population. The second is the migration capability, which causes crossbreeding and mixing of genes between populations. Akbar et al (2014) and Sakai et al (2001) said that populations with high genetic diversity have a better chance of living because each gene has a different response to environmental conditions.

High genetic diversity in eels is reported Triyanto et al (2008) in the waters of Poso, Fahmi (2015) in some waters in Indonesia, and Edwin et al (2018) in the Cimandiri Ono, Pelabuhan Ratu, Sukabumi. Hakim (2015) stated that species sustainability can be maintained by preserving the nucleotide diversity of the species and, subsequently, the high genetic diversity. The same author also said that a decline in genetic diversity could occur naturally through the avoidance of random genetic drift.

Haplotype distributions show that there is a high diversity of haplotypes in the overall sample (Figure 2). The high degree of haplotypes affects the genetic value of the eel, because genetic polymorphisms will increase. The results of the analysis found 13 different haplotypes and 1 mixed haplotype. Mixing is found in Haplotype 3, from samples from the waters of Bacan and Tidore. Regarding haplotype similarities, Arifin & Kurniasih (2007) indicate that a population is derived from genetic sources with the proximity of relatives. Nugroho et al (2007) said that every combination of genes has a

different response to environmental changes, the variety of genes providing a better opportunity to respond to environmental changes.

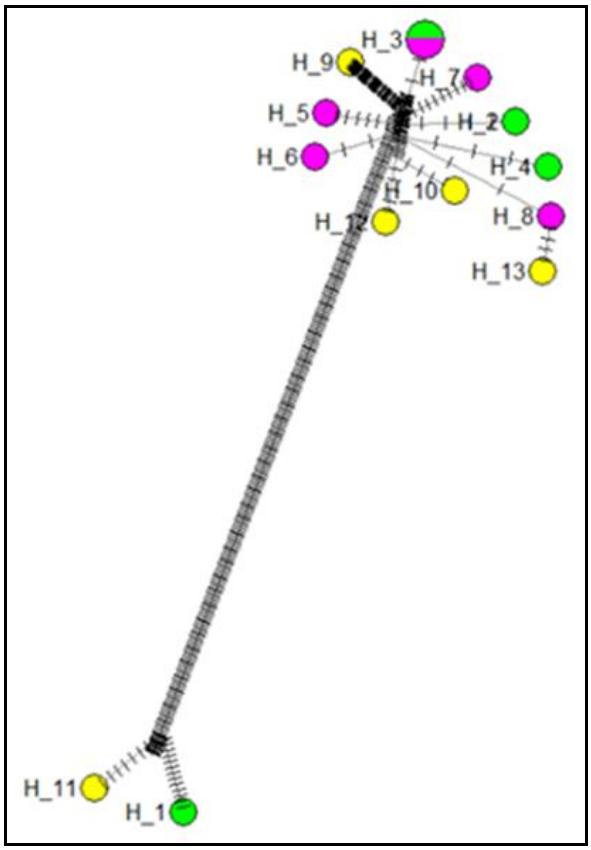

Figure 2. Haplotype distribution of eels (*Anguilla* spp.) in the Pacific region, North Maluku Sea; green - Bacan; pink - Tidore; yellow - Gane Barat.

Fst analysis. The Fst analysis found that the eel populations of Bacan, Tidore and Gane Barat were not distinct (Table 3). The values of the Fst indicate that the haplotype spread in the 3 locations does not reveal structural differences between populations (Fakhri et al 2015). The value of Fst showed that there was a strong genetic flow among the populations, despite the geographic location. The genetic flow associated with geographical isolation is influenced by the geographical distance and biodiversity of the complexes (Wu et al 2010). A strong genetic flow among the populations suggests that there is no inbreeding process. The spread of larvae also affects genetic differences between populations. The parent population may be dispersed due to the oceanographic factors that result in the formation of sub-populations. Genetic composition changes in the population result in variations that create small differences. Widespread larvae lead to the formation of sub-populations resulting in genetic mutations. However, the genetic washout of the parent continues to occur and is retained in the population. Thus, genetic kinship persists. Ramadhaniaty et al (2018) state that more differences produce more mutations that cause genetic distances.

Table 3

Fst (fixation index) analysis of eels (*Anguilla* spp.) in the Pacific region, North Maluku Sea

Location	*Bacan*	*Tidore*	*Gane_Barat*
Bacan	-	-	-
Tidore	0.033	-	-
Gane_Barat	-0,161	0.092	-

The eel populations did not form clades, but were mixed. Polarization was found, explaining that kinship occurs between populations due to genetic proximity. A distant genetic differentiation illustrates that all 3 populations present kinship. The geographic distance between Bacan and Gane Barat is 72.6 km, between Bacan and Tidore is 136 km, and between Tidore and Gane Barat is 170 km. The 3 locations are geographically dispersed, but the genetic flow between populations is strong. The genetic proximity also explains that the three populations are derived from the same one. The low-ethical convergence also explains the mixing of populations with other populations affected by the migration factor. Fakhri et al (2015) said that the interpopulation mixing at the time of migration in a wide range of seas is called panmictic. The panmictic population has a high genetic variation, enhancing the body's immune system. Nugroho et al (2007) said that every combination of genes has a different response to environmental changes.

The results of the research are different from those of Triyanto et al (2008), where there are differences between populations of *A. marmorata* from Solokaya, and Pandolo and Tentena ($p<0.05$), suspected to be caused by haplotype. The high genetic diversity need to be supported by proper management and strategies for the protection of eel populations. This is because the eel is a commercial fish target for trading. According to Fahmi (2015), genetic conservation is a strategy to maintain the genetic diversity of a population and genetic diversity of a species. IUCN recommends the preservation of genetic diversity as one of the three priorities of global conservation, alongside species diversity and extinction rate (Fahmi 2015). Akbar & Labenua (2018) say that the management and conservation actions must be conducted to minimize the occurrence of the genetic quality decrease of fish.

Conclusions. The high genetic diversity of eels at all three locations (0.98-1.00) and the Fst values show (0.092) that the three populations close relatives because of their strong genetic flow.

Acknowledgements. The authors would like to thank Khairun University, Ternate, North Maluku, Indonesia.

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Received: 02 July 2020. Accepted: 14 September 2020. Published online: 21 February 2021.
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How to cite this article:
Akbar N., Achmad M. J., Supyan, Subur R., Ismail F., Wahab I., Arai T., 2021 A pilot study on the genetic diversity of tropical eel (*Anguilla* spp.) in the Pacific region of North Maluku Sea, Indonesia. AACL Bioflux 14(1):309-316.