Species composition of eels larvae (Anguillidae) in Mentawai Island waters, Indonesia based genetic data

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Abstract. The genetic variation of eels larvae Anguilla spp. which had migrated to the estuaries of North Pagai Island at Mentawai Islands was studied from September 2016 to January 2017. The objective of the present study was to validate the species composition of eels larvae that migrated to estuary of North Pagai Mentawai Islands, West Sumatra Province, Indonesia. The eels larvae were collected from Mabola, Simpungan, Saumanganya, and Taikako estuaries. A total of 10 samples from every location were used to analyze the genetic variation of eels. The result showed that the heterozygosities of Anguilla marmorata and A. bicolor pacifica are highest in Saumanganya estuary, 0.1863 and 0.1840, respectively, while A. bicolor bicolor is the highest in Taikako estuary (0.1518). Gene flow (Nm) values of eels A. marmorata, A. bicolor bicolor, and A. bicolor pacifica were 0.3656, 0.5309, and 0.4679, respectively. The genetic differentiation value (Gst) of A. marmorata, A. bicolor bicolor, and A. bicolor pacifica were 0.8677, 0.4418, and 0.5686, respectively. It is concluded that the genetic variation of inter-populations and intra-populations of A. marmorata was higher than A. bicolor bicolor and A. bicolor pacifica.

1. Introduction
Tropical eels (Anguilla spp.) are one of the commercial fish and its have been intensively exploited worldwide. Sugha and Suharti [1] stated that there are 19 species and three of subspecies of eels have been recorded in the world, where nine species and subspecies were reported in Indonesia waters, namely, Anguilla bicolor bicolor, A. bicolor pacifica, A. nebulosa nebulosa, A. interioris, A. borneensis, A. celebesensis, A. marmorata, A. megastoma, and A. obsecura. Meanwhile Muchlisin et al. [2] have reported three species of tropical eels in northern part of Sumatra Island in Aceh waters, Indonesia: A. bicolor bicolor, A. marmorata, and A. bengalensis bengalesis where A. bengalensis bengalesis is a new record for Aceh waters. Hence there were totally 10 species of eels in Indonesian waters. In addition, Fahmi [3] reported that A. marmorata, A. bicolor bicolor, and A. bicolor pacifica had high genetic diversity and wide distribution in Indonesia waters.
Presently, eels culture has been initiated in Indonesia, but the eels larvae are mostly from the wild population making the quantity and quality of larvae seasonally dependent [4,5]. The main problem in the eels culture is high mortality at larvae stage; at this stage they have morphologically resembled mature eels but not yet have a body pigment and translucent [6]. In Sumatra, the eels larvae are distributed widely from Aceh waters to Mentawai Islands [7,8]. However, no comprehensive studies were conducted to evaluate the species composition in the Mentawai Island waters.

The Mentawai Islands are situated in the Indian Ocean at the west coast of Sumatra Island (Figure 1). Based on the initial field survey, we recorded three species of eels, namely, *A. marmorata*, *A. bicolor bicolor*, and *A. bicolor pacifica*. However, this preliminary identification requires further validation by genetic data. The precise identification of taxonomic status of the fish is crucial to plan a better management and conservation strategies [9-11]. Hence, the objective of the present study was the species composition of eels in Mentawai Island waters using the Restriction Fragment Length Polymorphism (RFLP) method.

2. Materials and Methods

2.1 Location and time

The sampling was conducted during September and October 2016 at four estuaries representing the condition of Mentawai Island, namely, North Pagai Island, namely, Mabola (-02.675°S; 100.186°E), Simpungan (-02.643°S; 100.165°E), Saumanganya (-02.573°S; 100.066°E), and Taikako (-02.795°S; 100.165°E) (Figure 1).

![Figure 1. Map of sampling sites eels larvae Anguilla spp. in North Pagai Island](image)

2.2 Sampling Procedures and DNA Extraction

A total of 1118 eel larvae samples were collected using fish trap and the samples were preserved in 95% alcohol and transported to Laboratory of Genetics and Biology Cell, Department of Biology, University of Andalas, Padang, for further analysis.

A total of 40 eels larvae samples were taken randomly and processed for DNA extraction. The DNA was extracted using Invitrogen kit, DNA amplification used BION-02, BION-40, and BION-35 primers [12]. DNA isolation was amplified with PCR after mixing GoTaq Green 12.5 µl + 6.5 µl nuclease-free water + 4 µl Isolate DNA + 2 µl primer to determine the success of primers amplification with electrophoresis on agarose gel 2% (w/v).
2.3 Data analysis
Genetic variations were analyzed according to the percentage of polymorphic loci (P), genetic diversity (H), the diversity index of phenotypic Shannon (I), heterozygosity in a subpopulation (Hs) and heterozygosity of total population (Ht), coefficient of genetic differentiation (Gst), and gene flow (Nm). The analysis was performed using POPGENE 3.20 software program [13].

3. Results and Discussion
The differences of genetic variation are present within population in each *Anguilla* species at different estuaries (Saumanganya, Mabola, Simpungan, and Taikako) (Table 1). There are two valid species of eels detected, namely *Anguilla marmorata* and *A.bicolor*, where the last species is composing of two sub-species, namely; *A.b. bicolor* and *A. b. pasifica*. The larvae of *A. marmorata* and *A. bicolor pacifica* have a higher genetic variation at the estuary of Saumanganya compared to other estuaries. Genetic variation of eels larvae of *A. marmorata* and *A. bicolor pacifica* is lower than *A. b. bicolor*, but both of *Anguilla* have a high gene flow (Table 2).

Table 1. Genetic variation analysis of within population of eels larvae of *A. marmorata*, *A.bicolor bicolor* and *A. bicolor pasifica* in the several estuaries in North Pagai Island

| Species               | Location | Samples | H     | I     | N  | PP % |
|-----------------------|----------|---------|-------|-------|----|------|
| *A. marmorata*        | MB       | 2       | 0.1827| 0.2668| 15 | 44.12 |
|                       | SP       | 2       | 0.1096| 0.1601| 9  | 26.47 |
|                       | SY       | 3       | 0.1863| 0.2824|18  | 52.94 |
|                       | TK       | 4       | 0.1528| 0.2312|15  | 44.12 |
| *A.bicolor bicolor*   | MB       | 6       | 0.0893| 0.1374|10  | 29.41 |
|                       | SP       | 5       | 0.1385| 0.2128|15  | 44.12 |
|                       | SY       | 1       | 0      | 0     | 0  | 0    |
|                       | TK       | 4       | 0.1518| 0.2340|16  | 47.05 |
| *A.bicolor pasifica*  | MB       | 2       | 0.0731| 0.1067| 6  | 17.65 |
|                       | SP       | 3       | 0.1048| 0.1582|10  | 29.41 |
|                       | SY       | 6       | 0.1840| 0.2837|20  | 58.82 |
|                       | TK       | 2       | 0.0365| 0.0534| 3  | 8.82 |

Remarks: MB: Mabola estuary, SP: Simpungan estuary, SY: Saumanganya estuary, TK: Taikako estuary, H: genetic diversity, I: the diversity index of phenotypic Shannon, N: polymorphic loci, PP: percentage of polymorphic loci

Table 2. Genetic variation analysis of among populations of eels larvae of *Anguilla* spp. in the several estuaries in North Pagai Island

| Species      | Sample | Ht     | Gst   | Nm   |
|--------------|--------|--------|-------|------|
| *A.marmorata*| 11     | 0.2489 | 0.3656| 0.8677|
| *A.b.bicolor*| 16     | 0.2023 | 0.5309| 0.4418|
| *A.b.pasifica*| 13    | 0.1872 | 0.4679| 0.5686|
Remarks: Ht: Heterozigosity of total population, Hs: Heterozigosity in a subpopulation, Gst: Genetic differentiation, Nm: Gene flow

The genetic distance of the eels larvae of *A. marmorata* and *A. bicolor pacifica* from all estuaries is low, while the genetic distance of *A. marmorata* located in Mabola and Simpungan was 0.1687 (Figure 4). On the other side, the genetic distances of *A. bicolor pacifica*, from Simpungan and Taikako, were lower than from Mabola 0.1393 (Figure 6). This is supported by high value of gene flow (Nm) and low value of genetic differentiation (Gst) while the nearest genetic distance was recorded between *A. bicolor bicolor* is from Simpungan and Taikako at 0.3121 (Figure 5). This is supported by low value of gene flow (Nm) and high value of genetic differentiation (Gst).

Based on direct observation in the field it is showed that the estuary of Saumanganya is the largest and fast-flowing river estuary compared to other estuaries. We suspected that eels larvae of *A. marmorata* and *A. bicolor pacifica* which entered the estuaries came from the different spawning ground and they have a good adaptability to this estuary condition. The genetic variation of eels larvae of *A. bicolor bicolor* at Taikako is higher than any other estuaries. Probably, the differences in the genetic variation in *A. bicolor bicolor* from different estuary are influenced by the strong currents of ecological factors such as waves, tides, salinity, and river water penetration into the sea which affect the migration of eels larvae of each species of eels from the sea to the estuary.

![Figure 2](image2.jpg)

**Figure 2.** There are 40 samples were analysed by RAPD technique using BION 35 as a primer

![Figure 3](image3.jpg)

**Figure 3.** There are 40 samples were analysed by RAPD technique using BION 40 as a primer
The study of genetic variation using PCR-RAPD technique on other species such as fish *Osteochilus kelabau* showed low genetic variation in the value of heterozygosity is from 0.0100 to 0.1051 [14]. The other study on *Ancistroides hypostomus* fish (Teleosti: Loricariidae) showed low genetic variation in the value of heterozygosity is 0.1230 to 0.1526 [15]. The similar report was also recorded on fish *Epinephelus awoara* where the high genetic variation is 0.2189 to 0.4616 [16]. Furthermore, the eels larvae of *A. bicolor bicolor* have high Gst value, and gene flow value is low; this indicates that the genetic variation among populations of *A. bicolor bicolor* is higher than within the population.

**Figure 4.** Dendogram of the eels larvae of *A. marmorata*

**Figure 5.** Dendogram of the eels larvae of *A. bicolor bicolor*

**Figure 6.** Dendogram of the eels larvae of *A. bicolor pasifica*

Chandra *et al.* [17] state that if the Gst value is lower, indicating the gene flow (Nm) value will be higher. According to Halliburton [18], the gene low value is higher than ≥ 0.5. In this situation *A. b. bicolor* has a something condition to investigate, because the Gst value is higher than Nm value. Based on Carvalho and Hauser [19], the lower Gst value is influenced by several factors such as the small population size, geographic isolation, and limited distribution. In addition, Maes and Volckaert [20] reported that in *A. anguilla* Gst value of 0.014 indicates that the overall genetic differentiation in the population is low.

Lehmann *et al.* [21] reported the genetic distance between populations *A. anguilla, A. rostrata, A. japonica,* and *A. reinhardtii* showed the average genetic distances were high ranging from 0.384 to 0559. The close genetic distance is between *A. anguilla* and *A. rostrata* at 0.384 and the higher genetic distance is between *A. reinhardtii* and *A. anguilla* at 0.559. According to Yoon [12] the lowest genetic distance value of *A. japonica* population was at 0.430. Jia-Jing and Ping-Li [22] reported the genetic distances between populations of *A. japonica, A. anguilla,* and *M. cinereus* are low, it was ranged from 0.25 to 0.32.
4. Conclusions
It is concluded that three valid species and sub-species of eels are detected in Mentawar waters, namely:
*Anguilla marmorata*, *A. bicolor bicolor* and *A. bicolor pacifica*. The Genetic variation within and among populations of *A. marmorata* is higher than *A. bicolor bicolor* and *A. bicolor pacifica*. The highest genetic variation was found in *A. marmorata* and *A. bicolor pacifica* sample from Saumanganya and *A. bicolor bicolor* from Taikako.

Acknowledgements
We would like to thank the Faculty of Mathematics and Natural Sciences of Andalas University for Research Award 2016. We are indebted to the staff of Fisheries Mentawai (Hardimansyah) and staff of Genetics and Biomolecular Laboratory of Biology Department, Andalas University, for helping in the field and laboratory during this project.

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