

# Haplotype network of three species of *Anguilla* (freshwater eels) in West Sumatra, Indonesia, based on *Cytochrome b* gene

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**Abstract.** A study on haplotype network of three species of *Anguilla* from West Sumatra has been conducted from April to August 2021. *Anguilla* samples were collected from four locations including Padang, Lubuk Basung, Pesisir Selatan and Pasaman Barat, and DNA of glass eels was isolated from South Pagai Island in Mentawai. The PCR technique based on *Cytochrome b* gene and the Network app version 5.0.0.3 were applied to haplotype network analysis. The 12 haplotype of 22 *Anguilla* species showed a value of haplotype diversity of 0.294 and 1.0 for *Anguilla marmorata* and *Anguilla bicolor bicolor*, respectively.

**Key Words:** *Anguilla*, *cytochrome b* gene, haplotype diversity, PCR.

**Introduction.** Freshwater eel (*Anguilla* spp.) of the Anguillidae family is a catadromous fish that generally found in temperate, tropical and sub-tropical areas (Arai 2016). About 19 species of *Anguilla* have been identified in the world (Sugeha et al 2008; Sugeha & Suharti 2008), while in Indonesian waters, approximately 5 to 7 species of *Anguilla* were found (Wouthuyzen et al 2009). However, several studies stated that there were 9 species/sub-species of *Anguilla* found in Indonesian waters including *Anguilla celebensis*, *Anguilla marmorata*, *Anguilla borneensis*, *Anguilla interioris*, *Anguilla obscura*, *Anguilla bicolor bicolor*, *Anguilla bicolor pacifica*, *Anguilla nebulosa* and *Anguilla megastoma* (Sugeha & Arai 2010). There were 2 species of *Anguilla* found in West Sumatera, specifically in Mentawai waters including *A. marmorata*, *A. bicolor*, both *A. bicolor bicolor* and *A. bicolor pasifica* (Syaifullah et al 2019).

*A. bicolor* and *A. marmorata* are of interest for the research and development because they have the largest distribution and highest abundance (Fahmi 2013). However, freshwater eels have shown drastic decreases worldwide for the last three decades. This happened because of the aquaculture of freshwater eels is still dependent on the availability of freshwater eels in nature (Widyasari 2013). Nonetheless, future strategies for the production of eel require data on the haplotype diversity for successful broodstock and genetic resources management. One of future strategies for sustainability of freshwater eels is to provide the genetic data, particularly on the haplotype of freshwater eels. There were 129 different haplotypes of *Anguilla* species that were identified from Indonesian waters, most of them for the first time, showing haplotype and nucleotide diversities of 0.98 and 4.57%, respectively. *A. bicolor* was clearly found to be composed of two subspecies, *A. b. bicolor* and *A. b. pacifica*. The first subspecies was split into two major clades supported by a high bootstrap value, with each clade characterized by two diagnostic nucleotides (Fahmi et al 2015). But the haplotype diversity of freshwater eels especially from West Sumatra is still limited.

Haplotype networks are used in the analysis of population genetic data to visualize genealogical relationships at the intra-specific level, as well as to make inference about

bio-geography and history of population (Leigh & Bryant 2015). Mitochondrial DNA is widely used as molecular markers because it's inherited maternally and can produce data quickly and consistently (Arab et al 2017). One of the mitochondria genes that encoded protein is the *cytochrome b*. *Cytochrome b* gene has also a high variation among the 13 genes coding for the mitochondrial gene (Satoh et al 2016). Analysis of mitochondrial *cytochrome b* sequences successfully confirm the differentiation between *Anguilla* species (Fahmi et al 2015). Previous research about *Anguilla* spp. includes the species composition of eels larvae (Anguillidae) in Mentawai Islands by Syaifullah et al (2019), but it does not cover freshwater eels at adult stages in West Sumatra. This research was conducted in Sungai Bangek (Padang), Sungai Batang Antokan (Lubuk Basung), Sungai Batang Maligi (Pasaman Barat) and Sungai Batang Tarusan (Pesisir Selatan), because these four DAS are a potential habitat for the *Anguilla* spp. This research aimed to obtain information about the haplotype diversity of *Anguilla* spp. in West Sumatra. The information can be used as a basis for the study of *Anguilla* spp. conservation and aquaculture.

## Material and Method

**Description of the study sites.** *Anguilla* spp. samples were collected from April to August 2021 at four locations representing every region of the western part of West Sumatra, Sungai Bangek (Padang), Sungai Batang Antokan (Lubuk Basung), Sungai Batang Tarusan (Pesisir Selatan) and Sungai Batang Maligi (Pasaman Barat) in West Sumatera (Figure 1). DNA isolated from Pagai Utara, Mentawai Islands, recorded by Syaifullah et al (2019) from Mentawai, was also used in this study.

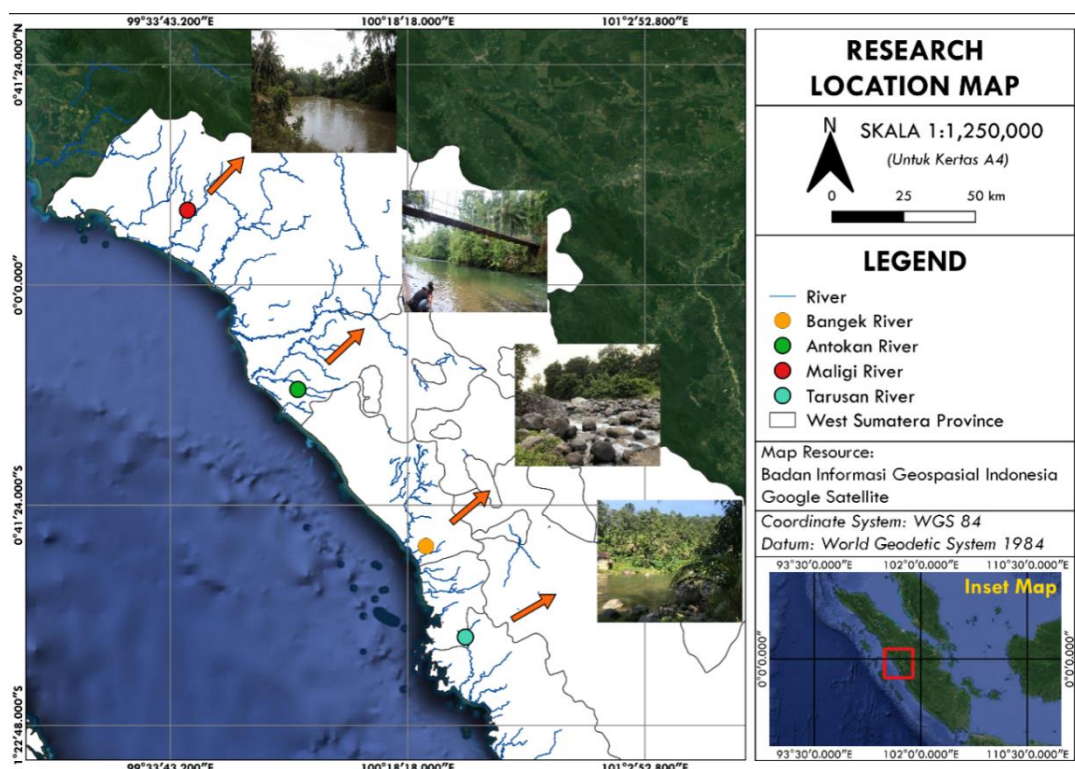


Figure 1. Location sampling site of *Anguilla* at West Sumatra.

**Sampling procedures.** The fish samples were collected using fishing nets, then labeled and photographed prior to preserving them into 10% formalin solution, and they were transported to the Laboratory of Genetics and Cell Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Padang. Freshwater eels were identified using morphological characters referring to the freshwater eels identification book (Anguillidae) (Silfvergrip 2009; Tesch 2003). Morphometric measurements were carried out according to Hakim et al (2015).

Table 1

## List of freshwater eel samples from West Sumatra

Locality	Sample type	Sample total	Sample codes
Sungai Bangek (Padang)	Liver	4	SB01-SB04
Sungai Batang Antokan (Lubuk Basung)	Liver	3	ANT01-ANT03
Sungai Batang Tarusan (Pesisir Selatan)	Liver	3	TRS01-TRS03
Sungai Batang Maligi (Pasaman Barat)	Liver	5	PSM01-PSM05
South Pagai Island (Mentawai)	Isolated DNA (Syaifullah et al 2019)	7	MB02, MB07, SY06, SY07, SP06, TK09, TK10

**DNA isolation and amplification.** Genomic DNA samples were isolated from liver tissue samples of freshwater eels, in each population, using the Invitrogen Kit. Amplification of *cytochrome b* gene used universal primers. *Cytochrome b* (Cyt b-1) forward: 5' TGCTAACGATGCCCTAGTGG-3' and Cyt (b-2) reverse: 5'-CTAGTCAACCTACT-AATGGG-3' (Han et al 2002). Amplification was carried out using the Polymerase Chain Reaction (PCR) method with 35 cycles of pre-denaturation at 94°C for 2 minutes, denaturation at 94°C for 1 minute, annealing at 55°C for 1 minutes, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. The PCR results were analyzed by electrophoresis. Then, the gel was visualized using a UV trans-illuminator (BIO-RAD) and photographed with BIOSTEP DNA. The amplified PCR product was then used as a template for DNA sequencing.

**Data analysis.** The results of the forward and reverse DNA sequences are combined using the DNA STAR program (Burland 2000). DNA sequences of *cytochrome b* genes of freshwater eels are comparing with other species in the other location using the BLAST (Basic Local Alignment Search Tool) program on the NCBI website. All DNA sequences of *cytochrome b* genes of freshwater eels and compared species are aligned using the Clustal X version 1.8 programs. The alignment results are checked using the Bioedit program (Hall 1999). Amino acids in the DNA sequence of *cytochrome b* gene of freshwater eels are adjusted using a DNA to Protein Translation program, accessed on the website <http://insilico.ehu.es/translate/>. Haplotype network tree analysis based on *cytochrome b* gene is carried out using the Median Joining Networks application version 5.0.0.3 (Bandelt et al 1999).

**Results and Discussion.** Based on the study obtained, a total of 22 freshwater eels were used, belonging to three species of *Anguilla* found in West Sumatra, namely: *A. marmorata*, *A. bicolor bicolor*, *A. interioris*. *A. marmorata* was recorded from four locations: Padang, Pesisir Selatan, Lubuk Basung and Mentawai. *A. bicolor bicolor* was recorded from Padang, Pasaman Barat and Mentawai, while *A. interioris* was only recorded from Mentawai (Table 2).

Table 2

## Freshwater eel samples obtained from West Sumatra

Species	Location					Total
	SB	TRS	ANT	PSM	MNT*	
<i>A. marmorata</i>	3	3	3	-	4	13
<i>A. bicolor bicolor</i>	1	-	-	5	2	8
<i>A. interioris</i>	-	-	-	-	1	1
Total	4	3	3	5	7	22

SB-Sungai Bangek (Padang); TRS-Sungai Batang Tarusan (Pesisir Selatan); ANT-Sungai Batang Antokan (Lubuk Basung); PSM-Sungai Batang Maligi (Pasaman Barat); MNT-Mentawai; \*Syaifullah et al 2019.

The electrophoresis results of DNA amplification of freshwater eels, based on *cytochrome b* gene visualized on 2% agarose gel, were successfully obtained, with clear DNA bands. The length of DNA sequences that have been amplified started from 1,000 bp. The DNA fragments that have been amplified were compared with the the DNA ladder of 100 bp. All of the sequencing results were used in haplotype analysis, where the length of DNA sequence fragments of 22 individuals includes comparison species of 1,029-1,129 bp, according to Syaifullah et al (2019). According to Minegishi et al (2008), the length of the complete *cytochrome b* gene in *Anguilla* species was of 1,140 bp. Satoh et al (2016) stated that the complete length of the *cytochrome b* gene in fish ranged from 1,110-1,190 bp. Based on the polymorphic sequences analysis using the DNA Sequence Polymorphism 5.10 (Rozas et al 2003), it can be concluded that there were 90 haplotypes of 124 eels samples (Table 3).

Table 3

Haplotypes of freshwater eel samples in West Sumatra

Haplotype	Species	Location
Haplotype 1	<i>A. marmorata</i>	Padang 1*, Padang 3*, Tarusan 2*, Tarusan 3*, Antokan 1*, Antokan 2*, Antokan 3*, Mentawai MB02*, Mentawai TK09*, Mentawai SY06*, Mentawai SP06*, AB279505.1 Papua New Guinea, AB279504.1 New Caledonia, AB279516.1 Sumatra, HG965562.1 Mentawai, HG965560.1 Mentawai, HG965557.1 Bengkulu_Mentawai_Pelabuhan Ratu_Aceh
Haplotype 2	<i>A. bicolor bicolor</i>	Padang 2*
Haplotype 3	<i>A. marmorata</i>	Padang 4*, AB279488.1 Tahiti, HG965556.1 Ambon
Haplotype 4	<i>A. marmorata</i>	Tarusan 1*, AB279512.1 Sumatra Pasaman 1*, HG965513.1 Pelabuhan Ratu, HG965512.1 Pangandaran, HG965510.1_Cilacap_Padang_Pangandaran_Bali_Pelabuhan Ratu
Haplotype 5	<i>A. bicolor bicolor</i>	Pasaman 2*, HG965478.1 Pelabuhan Ratu, HG965475.1 Aceh_Pangandaran
Haplotype 6	<i>A. bicolor bicolor</i>	Pasaman 3*
Haplotype 7	<i>A. bicolor bicolor</i>	Pasaman 4*, HG965482.1 Padang_Pelabuhan Ratu
Haplotype 8	<i>A. bicolor bicolor</i>	Pasaman 5*, HG965516.1 Pelabuhan Ratu
Haplotype 9	<i>A. bicolor bicolor</i>	Mentawai MB07*
Haplotype 10	<i>A. bicolor bicolor</i>	Mentawai SY07*
Haplotype 11	<i>A. interioris</i>	Mentawai TK10*
Haplotype 12	<i>A. marmorata</i>	HG965564.1 Poso
Haplotype 13	<i>A. marmorata</i>	HG965561.1 Papua
Haplotype 14	<i>A. marmorata</i>	HG965559.1 Aceh
Haplotype 15	<i>A. marmorata</i>	HG965555.1_Bengkulu
Haplotype 16	<i>A. marmorata</i>	HG965553.1_Bengkulu_Pelabuhan Ratu
Haplotype 17	<i>A. marmorata</i>	HG965550.1_Papua
Haplotype 18	<i>A. marmorata</i>	HG965549.1_Mentawai
Haplotype 19	<i>A. marmorata</i>	HG965548.1_Poso
Haplotype 20	<i>A. marmorata</i>	HG965537.1 Lombok_Bali_Mentawai_Poso_Obi, HG965534.1 Palu, HG965533.1 Bali_Lasusua_Lombok_Papua_Sangata_Pelabuhan Ratu_Poso
Haplotype 21	<i>A. marmorata</i>	

<i>Haplotype</i>	<i>Species</i>	<i>Location</i>
Haplotype 22	<i>A. marmorata</i>	HG965532.1_Lasusua
Haplotype 23	<i>A. marmorata</i>	HG965543.1 Amurang, HG965542.1 Papua
Haplotype 24	<i>A. marmorata</i>	HG965535.1_Bali
Haplotype 25	<i>A. marmorata</i>	HG965531.1_Bali_Amurang, HG965526.1_Poso_Bali_Lombok_Lasusua
Haplotype 26	<i>A. marmorata</i>	HG965539.1_Sangata
Haplotype 27	<i>A. marmorata</i>	HG965546.1_Pelabuhan Ratu
Haplotype 28	<i>A. marmorata</i>	HG965544.1_Lasusua
Haplotype 29	<i>A. marmorata</i>	HG965541.1_Lasusua_Poso
Haplotype 30	<i>A. marmorata</i>	HG965540.1_Palu
Haplotype 31	<i>A. marmorata</i>	HG965536.1_Poso
Haplotype 32	<i>A. marmorata</i>	HG965529.1_Palu
Haplotype 33	<i>A. marmorata</i>	HG965527.1_Ambon_Lasusua_Palu_Papua_Bali_Poigar _Inobont
Haplotype 34	<i>A. marmorata</i>	HG965523.1_Papua_Sangata
Haplotype 35	<i>A. marmorata</i>	HG965545.1_Poigar
Haplotype 36	<i>A. marmorata</i>	HG965530.1_Obi
Haplotype 37	<i>A. marmorata</i>	HG965525.1_Papua
Haplotype 38	<i>A. marmorata</i>	HG965522.1_Lombok
Haplotype 39	<i>A. marmorata</i>	HG965528.1_Bali
Haplotype 40	<i>A. marmorata</i>	AB279523.1_Reunion_(Western Indian)
Haplotype 41	<i>A. marmorata</i>	AB279521.1_Reunion (Western Indian), AB279520.1_Reunion (Western Indian)
Haplotype 42	<i>A. bicolor</i> <i>bicolor</i>	HG965507.1_Pelabuhan Ratu
Haplotype 43	<i>A. bicolor</i> <i>bicolor</i>	HG965507.1_Pelabuhan Ratu
Haplotype 44	<i>A. bicolor</i> <i>bicolor</i>	HG965492.1_Aceh
Haplotype 45	<i>A. bicolor</i> <i>bicolor</i>	HG965491.1_Padang
Haplotype 46	<i>A. bicolor</i> <i>bicolor</i>	HG965515.1_Padang
Haplotype 47	<i>A. bicolor</i> <i>bicolor</i>	HG965514.1_Bali
Haplotype 48	<i>A. bicolor</i> <i>bicolor</i>	HG965511.1_Pangandaran
Haplotype 49	<i>A. bicolor</i> <i>bicolor</i>	HG965509.1_Bengkulu
Haplotype 50	<i>A. bicolor</i> <i>bicolor</i>	HG965508.1_Padang
Haplotype 51	<i>A. bicolor</i> <i>bicolor</i>	HG965506.1_Cilacap
Haplotype 52	<i>A. bicolor</i> <i>bicolor</i>	HG965502.1_Pelabuhan Ratu_Padang
Haplotype 53	<i>A. bicolor</i> <i>bicolor</i>	HG965500.1_Pelabuhan Ratu, HG965495.1 Mentawai
Haplotype 54	<i>A. bicolor</i> <i>bicolor</i>	HG965494.1_Pelabuhan Ratu
Haplotype 55	<i>A. bicolor</i> <i>bicolor</i>	G965493.1_Cilacap
Haplotype 56	<i>A. bicolor</i> <i>bicolor</i>	HG965503.1_Pelabuhan Ratu
Haplotype 57	<i>A. bicolor</i> <i>bicolor</i>	HG965499.1_Padang
Haplotype 58	<i>A. bicolor</i>	HG965498.1_Padang

<i>Haplotype</i>	<i>Species</i>	<i>Location</i>
	<i>bicolor</i>	
Haplotype 59	<i>A. bicolor</i>	HG965496.1_Padang
	<i>bicolor</i>	
Haplotype 60	<i>A. bicolor</i>	HG965504.1_Aceh
	<i>bicolor</i>	
Haplotype 61	<i>A. bicolor</i>	HG965501.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 62	<i>A. bicolor</i>	HG965490.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 63	<i>A. bicolor</i>	HG965488.1_Bali, HG965486.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 64	<i>A. bicolor</i>	HG965483.1_Poso
	<i>bicolor</i>	
Haplotype 65	<i>A. bicolor</i>	HG965481.1_Pangandaran
	<i>bicolor</i>	
Haplotype 66	<i>A. bicolor</i>	HG965480.1_Cilacap
	<i>bicolor</i>	
Haplotype 67	<i>A. bicolor</i>	HG965476.1_Bengkulu
	<i>bicolor</i>	
Haplotype 68	<i>A. bicolor</i>	HG965474.1_Padang_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 69	<i>A. bicolor</i>	HG965472.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 70	<i>A. bicolor</i>	HG965489.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 71	<i>A. bicolor</i>	HG965485.1_Aceh
	<i>bicolor</i>	
Haplotype 72	<i>A. bicolor</i>	HG965479.1_Padang
	<i>bicolor</i>	
Haplotype 73	<i>A. bicolor</i>	HG965473.1_Bali
	<i>bicolor</i>	
Haplotype 74	<i>A. bicolor</i>	HG965484.1_Pelabuhan Ratu
	<i>bicolor</i>	
Haplotype 75	<i>A. interioris</i>	HG965570.1_Mentawai
Haplotype 76	<i>A. interioris</i>	HG965569.1_Mentawai
Haplotype 77	<i>A. interioris</i>	HG965566.1_Papua
Haplotype 78	<i>A. interioris</i>	HG965565.1_Mentawai
Haplotype 79	<i>A. interioris</i>	HG965565.1_Mentawai
Haplotype 80	<i>A. interioris</i>	HG965574.1_Papua
Haplotype 81	<i>A. interioris</i>	HG965573.1_Mentawai
Haplotype 82	<i>A. interioris</i>	HG965575.1_Mentawai
Haplotype 83	<i>A. interioris</i>	HG965572.1_Mentawai_Poso
Haplotype 84	<i>A. interioris</i>	HG965571.1_Papua
Haplotype 85	<i>A. celebesensis</i>	HG965457.1_Poso
Haplotype 86	<i>A. obscura</i>	AB021781.1_Pacific
Haplotype 87	<i>A. borneensis</i>	HG965452.1_Mahakam
	<i>A. bicolor</i>	
Haplotype 88	<i>pacifica</i>	HG965467.1_Mahakam
	<i>A. nebulosa</i>	
Haplotype 89	<i>nebulosa</i>	HG965518.1_Pelabuhan Ratu
	<i>A. subrostratus</i>	
Haplotype 90		KF862974.1 India

\* = samples obtained from West Sumatra.

Based on this study, there were 12 haplotypes recorded for *Anguilla* species from West Sumatra (Table 3). A total of 3 haplotypes were from Padang, two haplotypes were from Pesisir Selatan, one haplotype from Lubuk Basung, 5 haplotypes from Pasaman Barat and

four haplotypes were from Mentawai. Haplotypes of *A. marmorata* consisted of 3 haplotypes namely haplotype 1, haplotype 3, and haplotype 4. Haplotypes of *A. bicolor bicolor* consisted of eight haplotypes namely haplotype 2 and haplotype 5-11. Haplotypes of *A. interioris* consisted of one haplotype, namely haplotype 12, where this species was only recorded in Mentawai. Based on the haplotype network that was analyzed, the freshwater eels consisted of three haplogroups, according to their species, as in Figure 2. In the mentioned figure, the haplogroup I was *A. marmorata*, the haplogroup II was *A. bicolor bicolor* and the haplogroup III was *A. interioris*. The haplotype network consisted of round shapes, branches and mutation numbers. The round shape indicates the number of individuals in the haplotype. The branches on the haplotype network represent the mutations.

There were three haplotypes of *A. marmorata* identified in West Sumatra. Haplotype 1, *A. marmorata*, was found in four of five populations in West Sumatra, namely Padang, Lubuk Basung, Pesisir Selatan and Mentawai. Those four populations had the same haplotype, in absence of variation of their sequences. The haplotype 3, *A. marmorata*, which was only found in Padang, has mutations occurring in 679 bp, changing Guanine to Adenine; haplotype 4 *A. marmorata*, only found in Mentawai, with mutations of nucleotide occurring in 106 bp, hanging Adenine to Guanine.

Haplotype 1 *A. marmorata* was found in four populations, which means that their sequences were not varied. It can be assumed that they might come from the same ancestor. Haplotype 1 *A. marmorata* is also recorded from South Pacific geographical areas (Papua New Guinea and New Caledonia), Mentawai and Sumatra. This might be assumed that they have the same spawning ground around the Pacific Ocean. This is also supported by their varied color of haplotype network, where each color represents the location of comparison species' haplotype. Xu & Guan (2014) stated that sharing haplotype occurred in two diploid individuals coming from the same ancestor. Arai & Taha (2021) also found some *A. marmorata* haplotypes which were found in several localities. For example, there were haplotypes occurring in Indonesia (Aceh and Bengkulu on Sumatra Island and Java Island), French Polynesia and Vietnam, in areas located at a considerable distance.

Haplotype 3, *A. marmorata* found in Padang 4 has the same haplotype as *A. marmorata* AB279488.1 Tahiti (South Pacific) and HG965556.1 Ambon, while haplotype 4 *A. marmorata* found in Pesisir Selatan 1 has the same haplotype with *A. marmorata* AB279512.1 Sumatra. It can be assumed that *A. marmorata* from Padang 4 might come from the Pacific geographical area, while *A. marmorata* from Pesisir Selatan 1 might come from the Indian Ocean due to the comparison. So, it can be concluded that *A. marmorata* from West Sumatra has come predicted from the spawning area at the Pacific Ocean. According to Minegishi et al (2008), *A. marmorata* has a population distribution, as follows: (i) in the North Pacific (from Japan to Sulawesi), (ii) in the South Pacific (from Papua New Guinea to Tahiti), (iii) in the Indian Ocean (from Sumatra to Madagascar) and (iv) in the Guam (including Micronesia). *A. marmorata* is the most widespread species and is distributed from the east coast of Africa to the Indo-Pacific Ocean (Tesch 2003; Watanabe et al 2008). According to Arai & Taha (2021), the oceanic currents in the Indo-Pacific region, especially those flowing to Sumatra, might be from the Indian Ocean (Equatorial Counter Current), southwest monsoon current and Pacific Ocean (South Equatorial Counter current). It might explain the fact that the haplotype of *A. marmorata* was found in several locations, because they were coming from the same ancestor that flow through the currents. Based on this study, *A. marmorata* from West Sumatra might come from one spawning site, particularly from the Pacific Ocean: all haplotypes of *A. marmorata* are grouped into one sub-haplogroup. This result is in accordance with Arai & Taha (2021), who stated that *A. marmorata* has a panmictic population, while it is in disagreement with previous studies, whereby *A. marmorata* was found to have a multiple structure population structure (Gagnaire et al 2009; Ishikawa et al 2004; Minegishi et al 2008). Arai & Taha (2021), stated that *A. marmorata* on the Sumatra and Java islands might be transported from spawning site(s) in the Pacific Ocean, because the dominant species found in Sumatra islands is *A. bicolor bicolor*, instead of *A. marmorata*. These findings suggest that the transportation of the leptocephali of *A. marmorata* to the

Sumatra and Java islands was likely not from the Indian Ocean but from the Pacific Ocean instead.

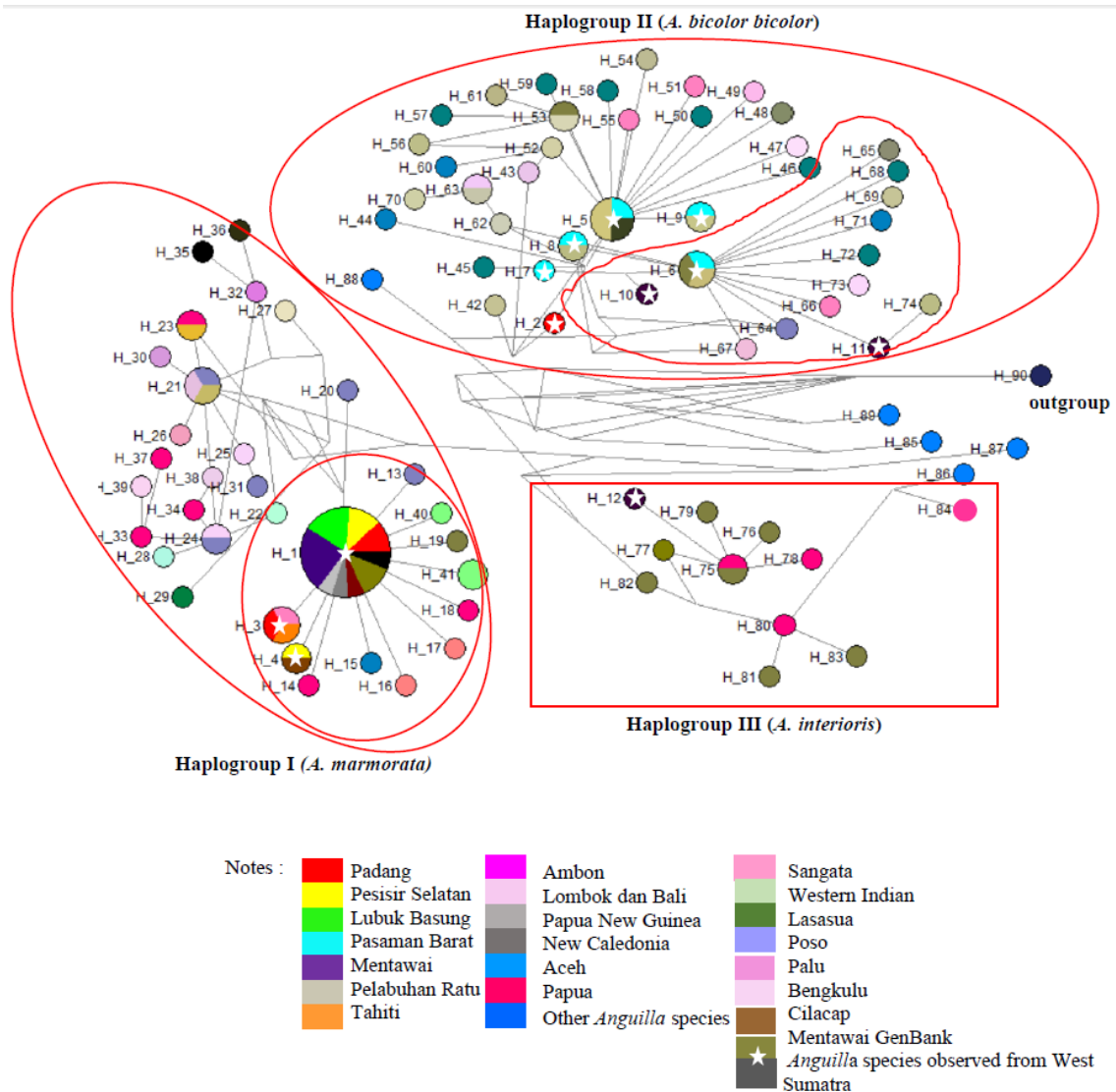


Figure 2. Haplotype network of *Anguilla* spp. based on *Cytochrome b* gene using *Network* 10.0 software (Haplogroup I: *Anguilla marmorata*, Haplogroup II: *Anguilla bicolor bicolor*, Haplogroup III: *Anguilla interioris*).

The haplogroup II consists of 8 different haplotypes of *A. bicolor bicolor*, in West Sumatra. This study also found that the haplotype of *A. bicolor bicolor* varies more than *A. marmorata*. All of the species of *A. bicolor bicolor* shown different haplotypes, for example, haplotype 2 was only found in Padang, meanwhile, haplotypes 5-9 were found in Pasaman Barat. All species in Pasaman Barat had a mutation in a fragment of 253 bp where adenine changed into guanine. *A. bicolor bicolor* from Pasaman Barat 1 has the same haplotype as *A. bicolor bicolor* HG965513.1 from Pelabuhan\_Ratu, HG965512.1 from Pangandaran and HG965510.1 from Cilacap, Padang, Pangandaran, Bali and Pelabuhan Ratu.

*A. bicolor bicolor* Pasaman Barat 2 had the same haplotype as *A. bicolor bicolor* HG965475.1 from Aceh and Pangandaran and HG965478.1 from Pelabuhan Ratu. *A. bicolor bicolor* from Pasaman Barat 4 had the same haplotype as *A. bicolor bicolor* HG965482.1 from Padang and Pelabuhan Ratu. *A. bicolor bicolor* from Pasaman Barat 5 had the same haplotype as *A. bicolor bicolor* HG965516.1 from Pelabuhan Ratu. Mentawai had 2 haplotypes of *A. bicolor bicolor*, namely Haplotype 10 and Haplotype 11, where they do not have the same shared haplotype, compared to the haplotype from the



study of Fahmi et al (2015). It might be assumed that *A. bicolor bicolor* from West Sumatra comes from a different ancestor and has multiple spawning sites, due to their several haplotypes, found in West Sumatra. Based on this study, the spawning sites of *A. bicolor bicolor* from West Sumatra might be located in the Java Indian Ocean, due to the haplotype mostly shared with *A. bicolor bicolor* from Pelabuhan Ratu. According to the haplotype network, there were two sub-haplogroups supposed to come from different spawning sites, one coming from Pangandaran and the other from Bali. So, it might be assumed that the spawning sites of *A. bicolor bicolor* are located around the Java Indian Ocean, but the exact locations remains still unclear. Arai & Taha (2021b) stated that there is still no strong evidence supporting the identification of the spawning sites of *A. bicolor bicolor*, unlike *A. marmorata*, whose spawning sites are found in the North Pacific Ocean, where have been recorded its eggs, smaller leptocephali and fully matured adults. The haplotype of *A. bicolor bicolor* from West Sumatra are different from the others and from those observed also in West Sumatra, in previous research, conducted by Fahmi et al (2015). This results also supported by haplotypes of *A. bicolor bicolor* recorded by Fahmi et al (2015) presenting a high variability with the geographical distance. It can be assumed that *A. bicolor bicolor* has several spawning sites. The spawning sites of *A. bicolor bicolor* are thought to be located in the area of West Sumatra Island, in the Eastern Indian Ocean, and around the Madagascar Island, in the Western Indian Ocean (Jespersen 1942; Robinet et al 2003). According to this study, the spawning sites of *A. bicolor bicolor* might be assumed to be the Indian Ocean, instead of the Pacific Ocean, due to their haplotype shared with *A. bicolor bicolor* from Pelabuhan Ratu, Pangandaran and Bali. These results were in disagreement with Sugeha et al (2010), who stated that the spawning sites of *A. bicolor bicolor* come from the Pacific Ocean, showing a significant genetic variation in this area. According to Minegoshi et al (2011), a genetic divergence occurred between *A. bicolor* from the Indian versus the Pacific Oceans. However, Arai & Taha (2021a) stated that two distinct haplogroups can also be found in *A. bicolor bicolor* originated from the same spawning sites, so further studies are needed to determine the *A. bicolor bicolor* population's genetic structure. Sugeha & Arai (2010) also stated the possibility of a panmictic population (single spawning population) for *A. bicolor bicolor*, based on the mtDNA D-loop.

The haplotype of *A. bicolor bicolor* from West Sumatra systematically varied with the location. The haplotype differences of *A. bicolor bicolor* from West Sumatra might occur due to their life-history strategies. It might happen because of the migration of eels larvae from the sea to the estuary, mixing larvae from several spawning sites. According to Arai et al (1999), *A. bicolor bicolor* in the eastern Indian Ocean has a long duration of the leptocephalus stage (larval age), 119-171 days, while the spawning season is year-round. It might enable *A. bicolor bicolor* to disperse widely across the Indian Ocean. Pous et al (2010) stated that, due to the seasonal changes in the Indian Ocean, the long migration of larval might be the cause of the mixing of *A. bicolor bicolor* larvae from different spawning sites. It can be speculated that the *A. bicolor bicolor* found in West Sumatera originated from different spawning sites. There were no species of *A. bicolor pacifica* found in five location includes Padang, Lubuk Basung, Pasaman Barat, Pesisir Selatan and Mentawai. This might happen because of the distribution of *A. bicolor pacifica* that tend to dominate in the center and eastern Indonesian waters, while *A. bicolor bicolor* dominated in the western Indonesian waters (Sugeha et al 2008). There is also a possibility for *A. bicolor pacifica* larvae from eastern migrate to the western of Indonesian waters, which is worth considering since *A. interioris* that usually occur in eastern Papua New Guinea have been found in waters around North Sulawesi Island and west Sumatra Island (Sugeha et al 2008; Aoyama et al 2007).

The haplotype 12 (Haplogroup III, *A. interioris*) was identified only from Mentawai in the glass eels stage. This haplotype differs of the *A. interioris* HG965570.1 from Mentawai and HG965567.1 from Papua. It can be assumed that *A. interioris* from Mentawai had a new haplotype, never observed before. According to Fahmi (2015), *A. interioris*, *A. celebesensis* and *A. borneensis* were distributed narrowly and limited to certain areas in Indonesia. Sugeha et al (2008) also stated that *A. interioris*, which are usually found in eastern Papua New Guinea, have been found in waters around North

Sulawesi Island and Sumatra Island. Following Ege (1939), those three species were endemic to Indonesian waters, while *A. marmorata* and *A. bicolor* were widely distributed. Despite being narrowly distributed, these three species had a limited abundance (small population). Frankham et al (2002) also stated that animals that live in small populations tend to have a single panmictic population spawning pattern. Based on this study, the localization of spawning sites of *A. interioris* remains unclear, due to the small size of the sample obtained, but it can be assumed that they might come from Mentawai and Papua. Their haplotype network is grouped into one haplogroup. *A. interioris* had a trend in which most yellow/silver eels have been observed from New Guinea, while glass eels have been observed in several western islands of Indonesia (Aoyama et al 2007; Fahmi et al 2013). The amount of this species found is limited. The presence of glass eels of *A. interioris* at a low frequency in western Indonesia suggests random dispersal of glass eels for *A. interioris* (Wibowo et al 2021). Due to this knowledge gap on distribution and biology, *A. interioris* is currently listed as data deficient in the IUCN Red List (Jacoby et al 2015). *A. interioris* tended to be found in large islands of Indonesia. This is supported by a research conducted by Fahmi et al (2015), where *A. interioris* was recorded from Papua Barat, Lombok, Poso and Mentawai. The highest number of *A. interioris* individuals was recorded in Papua Barat then Mentawai, Lombok and Poso respectively. This might also explain why there were no individuals of *A. interioris* found in Java. Wibowo et al (2021) also stated that *A. interioris* was mainly observed in the northern rivers of New Guinea, but the low presence of this species in western Indonesia showed the very low recruitment of *A. interioris* outside New Guinea.

Haplotype diversity values of 22 individuals of eel samples in West Sumatera ranged from 0-1 (Table 4). The highest levels of haplotype diversity were of 0.667 for *A. marmorata* from Padang and Pesisir Selatan, of 1 for *A. bicolor bicolor* from Pasaman Barat and of 1 for the *A. interioris* from Mentawai. The lowest levels, 0, of the haplotype diversity of *A. marmorata*, was found in Lubuk Basung and Mentawai *A. bicolor bicolor* in Padang. The total haplotype diversity (Hd) value of *A. marmorata* was 0.294, while for *A. bicolor bicolor* it was 1. The haplotype diversity (Hd) value showed that the genetic variation of *A. marmorata* in West Sumatera was included in the low category, while for *A. bicolor bicolor* it was included in the high category.

Table 4

Haplotype diversity analysis of *Anguilla* species in West Sumatera

<i>Species</i>	<i>Locations</i>	<i>n</i>	<i>Hn</i>	<i>Hd</i>	$\Pi$
<i>A. marmorata</i>	Padang	3	2	0.667	0.00094
	Pesisir Selatan	3	2	0.667	0.00094
	Lubuk Basung	3	1	0	0
	Mentawai	4	1	0	0
	$\Sigma$	13	3	0.294	0.00043
<i>A. bicolor bicolor</i>	Padang	1	1	0	0
	Pasaman	5	5	1.000	0.0087
	Mentawai	2	2	1.000	0.00423
	$\Sigma$	7	7	1.000	0.00423
<i>A. interioris</i>	Mentawai	1	1	0	0

n-number of samples obtained; Hn-number of haplotypes; Hd-haplotype diversity;  $\Pi$ -nucleotide diversity.

Nei & Kumar (2000) stated that a haplotype diversity value (Hd) ranging between 0 and 0.5 is considered in the low category, while a Hd value in ranging from 0.5 to 1 is considered in the high category. A high haplotype diversity will be followed by a high genetic diversity and vice versa. Haplotype diversity and nucleotide diversity of mtDNA are two important indicators for assessing the population polymorphism and genetic differentiation. According to Fahmi et al (2015), in Indonesia, there were 44 haplotypes of *A. marmorata*, with  $Hd=0.937\pm 0.013$  and  $\Pi=0.861\pm 0.002(\%)$ , based on *cytochrome b* gene.

The highest value of nucleotide diversity ( $\pi$ ) in the eel samples from West Sumatera was found for *A. marmorata* from Padang and Pesisir Selatan, of 0.00094 (0.09%), and for *A. bicolor bicolor* from Pasaman Barat, of 0.0087 (0.8%), while the least value was found for *A. marmorata* from Lubuk Basung and Mentawai, of 0 (0%), and for *A. bicolor bicolor* from Padang, of 0 (0%). The total nucleotide diversity of *A. marmorata* was 0.00043 (0.04%), while for *A. bicolor bicolor* it was 0.00423 (0.4%). Thus, it can be concluded that the genetic variation of *A. marmorata* in West Sumatera was included in the low genetic variation category while for *A. bicolor bicolor* it was included in the high genetic variation category. A relatively high level of genetic diversity was determined in the mtDNA D-loop region of the Japanese eel *A. japonica*, the tropical giant mottled eel *A. marmorata* and the European eel *A. anguilla* (Ishikawa et al 2001; Ragauskas et al 2014; Tseng et al 2012). Differences might occur because of the small size of sample.

The nucleotide diversity of the population of *A. marmorata* in West Sumatera ranged from 0 to 0.0004 (0-0.04%) (Table 5), while the nucleotide diversity of the population of *A. bicolor bicolor* was ranged from 0.0070 to 0.0121 (0.7-1.2%) (Table 6). The lowest nucleotide diversity value of *A. marmorata*, of 0, was found between Lubuk Basung and Mentawai, while the lowest nucleotide diversity value, of 0.0009 (0.09%), was found between Padang and Pesisir Selatan. The highest nucleotide diversity value of *A. bicolor bicolor*, of 0.0012 (1.2%), was found between Padang and Mentawai, while the lowest nucleotide diversity value, of 0.00727 (0.7%), was found between Pasaman Barat and Mentawai.

Table 5

Nucleotide diversity ( $\pi$ ) value between the population of *Anguilla marmorata* in West Sumatra

Locations	Padang	Lubuk Basung	Pesisir Selatan	Mentawai
Padang	-	-	-	-
Lubuk Basung	0.00047	-	-	-
Pesisir Selatan	0.00094	0.00047	-	-
Mentawai	0.00047	0	0.00047	-

Table 6

Nucleotide diversity ( $\pi$ ) value between the population of *Anguilla bicolor bicolor* in West Sumatra

Locations	Padang	Pasaman Barat	Mentawai
Padang	-	-	-
Pasaman Barat	0.00855	-	-
Mentawai	0.01214	0.00727	-

The genetic variation of *A. marmorata* was included in the low category due to a value of the haplotype diversity of 0.294. This result was also in accordance with a haplotype network analysis where *A. marmorata* was grouped into a haplotype that was found at several locations. It might be assumed that they come from the same ancestor, due to the same haplotype that is shared among several locations. The genetic variation of *A. bicolor bicolor* was included in the high category due to a value of haplotype diversity of 1. These results are also supported by the varied haplotype found in West Sumatra. This might also support the assumption that *A. bicolor bicolor* has a multiple population structure.

Sequence divergence values of three species of *Anguilla* in West Sumatra ranged from 0 to 0.218. The sequence divergence value between *A. marmorata* and *A. bicolor bicolor* was 0.218, between *A. marmorata* and *A. interioris* of 0.125 and between *A. interioris* and *A. bicolor bicolor* of 0.196.

The sequence divergence value can indicate the relationship among the species. The lowest sequence divergence value indicated two closely related species. Sequence

divergence value increased in accordance with the taxonomic level. The genetic divergence values of populations (based on the *cytochrome b* gene) were grouped into five groups: (1) intraspecies (1.38±0.30), (2) taxa of different ranks, subspecies or/and sibling species (5.10±0.91), (3) species within a genus (10.31±0.93), (4) species from different genera within a family (17.86±1.36) and (5) species from separate families within an order (26.36±3.88) (Kartavtsev 2011).

Fahmi et al (2015) stated that there were seven species inhabiting the Indonesian waters, including *A. bicolor bicolor*, *A. bicolor pacifica*, *A. marmorata*, *A. celebesensis*, *A. interioris*, *A. borneensis* and *A. bengalensis bengalensis*, while in West Sumatra there were recorded only three species, including *A. bicolor bicolor*, *A. marmorata* and *A. interioris*. Aoyama et al (2001) stated that *A. bicolor bicolor*, *A. interioris* and *A. marmorata* were in a group of Indo-Pacific lineage, where *A. interioris* was related closer with *A. marmorata* than with *A. bicolor bicolor*.

**Conclusions.** 12 haplotypes of 22 *Anguilla* species were observed in West Sumatra, based on the *cytochrome b* gene. The haplotype diversity of *A. marmorata* from West Sumatra, based on the *cytochrome b*, was of 0.294, showing that the genetic variation of *A. marmorata* in West Sumatra was included in the low category, while the genetic variation of *A. bicolor bicolor* was included in the high category, due to a value of haplotype diversity of 1.

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