



## Comparison in the phylogenetic pattern of Java eel (*Anguilla bicolor bicolor*) from Java to Western Indian Ocean: Monophyletic fact and migration loop possibility

Nur Taufiq-Spj<sup>1,4,\*</sup>, Gunawan Widi Santosa<sup>1</sup>, Agus Trianto<sup>1</sup>, Denny N. Sugianto<sup>2,4</sup>, Anindya Wirasatriya<sup>2,4</sup>, B. Budimawan<sup>3</sup>, Muh Yusuf<sup>2</sup>, Agus Indarjo<sup>1</sup>, Muhammad Helmi<sup>2,4</sup>, Andrianus Sembiring<sup>5</sup>, Ni Putu D. Pertiwi<sup>5</sup>, Mostafa I. Ighwerb<sup>6</sup>

<sup>1</sup>Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Indonesia

<sup>2</sup>Department of Oceanography, Faculty of Fisheries and Marine Science, Diponegoro University, Indonesia

<sup>3</sup>Department of Marine Science, Faculty of Marine and Fisheries Science, Hasanuddin University, Indonesia;

<sup>4</sup>Center for Coastal Disaster Mitigation and Rehabilitation Studies, Center of an Excellence Research, Diponegoro University, Indonesia

<sup>5</sup>Yayasan Biodiversitas Indonesia (BIONESIA), Bali Indonesia

<sup>6</sup>College of Marine Resources, Dept. of Marine Science and Aquaculture, Asmarya Islamic University, Libya

\*Corresponding author: [taufiqspj\\_1999@yahoo.com](mailto:taufiqspj_1999@yahoo.com)

### ARTICLE INFO

#### Article History:

Received: Nov. 16, 2021

Accepted: Dec. 11, 2021

Online: July 2, 2022

#### Keywords:

Phylogenetic;

Java eel;

*Anguilla bicolor bicolor*;

Monophyletic;

Indian Ocean

### ABSTRACT

The phylogenetic data of the Java eel population is crucial for future catadromous aquaculture in Indonesia. The study aims to know how eel species from Java and West India differ in their evolutionary patterns. Four river estuaries in Central Java and one estuary in West Java, Indonesia, were chosen as samples of Javanese eel origins. Phylogenetic comparison data from five regions of the Western Indian Ocean and one region of the Philippines (Pacific Ocean) were examined, taken from GenBank® data. The results showed that BLAST from 25 eels from Central and West Java was *Anguilla bicolor bicolor* (homology value 96 – 100%). The phylogenetic tree revealed that samples from Java nested into the West Indian Ocean with two Clades but were isolated from the Pacific Ocean eels. There are 25 pairs of monophyletic samples estimated to be the most recent common ancestor among the Indian Ocean eel samples from west to west, east to east, and west to east.

### INTRODUCTION

The phylogeny study of the Java eel population is crucial to the future of Indonesian catadromous aquaculture. The problem is that seeds are still primarily taken from estuaries where they are recruited. Phylogenetic analysis for diadromous species cannot be isolated from the long process of biological evolution, in which species evolve into complex ones throughout several generations. Because inheritance is an evolutionary process, its features will differ from those of its ancestor (Rozenfeld *et al.*, 2019, Walsh, 2019). Information is required to understand the evolutionary pattern of diadromous species, such as life cycle, species origin and identification, and locus comparison.

*Anguilla bicolor bicolor*, classified as a mid-scale (1,000-3,000 km) migratory species based on its life cycle, which is associated with migration (either leptocephalus or silver eel) (Arai & Abdul Kadir, 2017). However, *A. b. bicolor* occurrences, discovered in the Western

Indian Ocean along the East African coast (Minegishi *et al.*, 2012 and Miller *et al.*, 2019a) and along the western coast of Malaysia (Arai & Abdul Kadir, 2017). Sumatera, Java, and Bali were also homes to this species (Fahmi *et al.*, 2015). The species' breeding region was off West Sumatera (Aoyama, 2009 and Miller *et al.*, 2019b) and along the Mascarene Plateau of the West Indian Ocean, as evidenced by the emergence of freshly hatched leptocephalus (Pous *et al.*, 2010 and Miller *et al.*, 2019b). These distribution figures will help guide some migration options, given the distance from the eastern coast of Africa to Bali is almost 7,000 km. These distances may be related to the spawning area above, where they mated as a panmictic species.

The phylogenetic pattern discovered, particularly in monophyletic couples, will indicate if they have a most recent common ancestor. The main feature of diadromous species is that they return to their origin during the spawning and recruitment periods. Catadromous species have a migratory loop unique to their life history, including migration pathways linked to spawning regions and growth niches (Aarestrup *et al.*, 2009; Aoyama, 2009 and Miller *et al.*, 2019a).

Despite a lack of understanding of how eels' life histories interact with ocean current patterns and intricate seasonal changes in the circulation in some areas (Miller *et al.*, 2019a). *Anguillid leptocephali* were discovered most common in the South Java Current (SJC) along west Sumatera in a distinctive distribution study of *Elopomorpha leptocephali*, meanwhile they were not detected along the Southern Java nor Northern Java Island (Miller *et al.*, 2019b). Fahmi & Hirnawati (2010) highlighted the distribution of *A. nebulosa nebulosa* in both locations of Sri Lanka and the southern waters of Java Island in their earlier study. It is most likely due to the eastern Indian Ocean's backward current or Jet Wyrky or Jet equator, which transports eel larvae (leptocephalus) to the southern Java Sea. As a result, when leptocephalus (the leaf form of this larvae stage) drifts to the coast, marine gyres and other currents may play a role (Naisbeth-Jones *et al.*, 2017 and Miller *et al.*, 2019b). Eastward Equatorial Current (EEC) or Equatorial Counter Current (ECC), Indian Monsoon Current (IMC) may impact the Java eel leptocephali, but the silver eel may be affected by South Java Current (SJC), Westward Java Current (WJC), and South Equatorial Current (SEC) (Pous *et al.*, 2010 and Fahmi *et al.*, 2015). Another possibility is that the central Indian Ocean subtropical gyre will play a significant role in leptocephali drift (Miller *et al.*, 2015 and Miller *et al.*, 2019b). However, the Pacific Indian conveyor, known as Indonesian Through-Flow (ITF) plays a limited impact on the migration of *A.b.pacifica* near the Pacific Ocean and *A. marmorata* in Northern Celebes Island. The phylogenetic pattern may explain whether they have a prospective correlation among Clades or any current correlation dependencies based on this phenomenon.

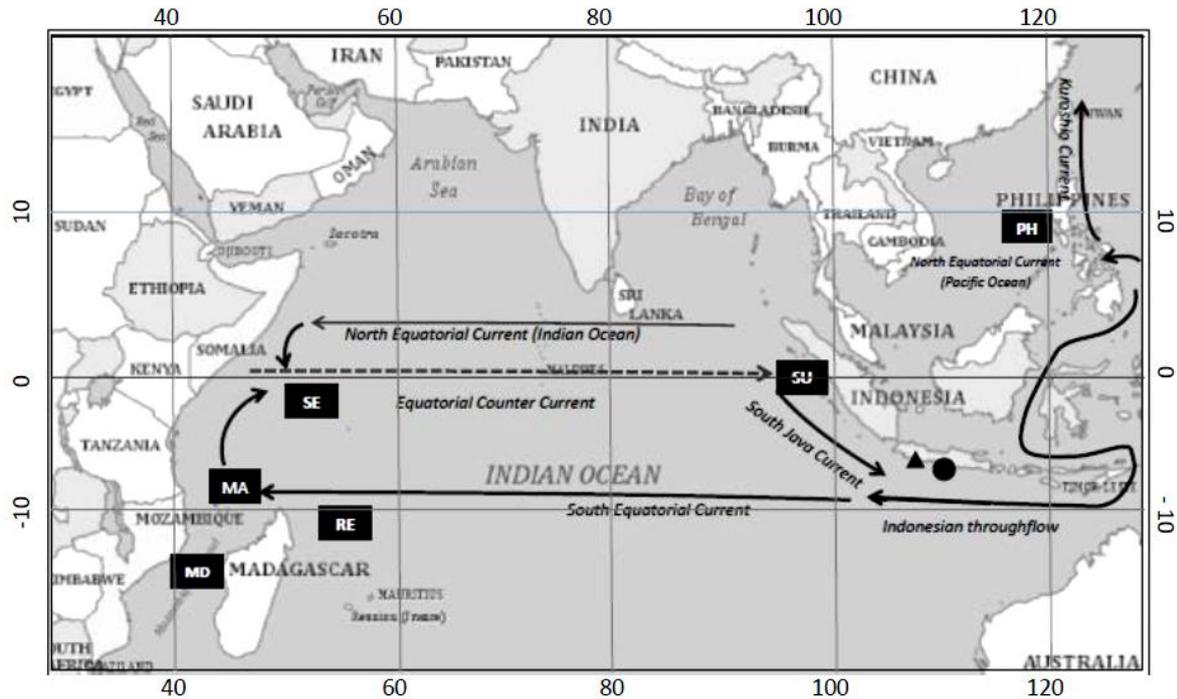
Based on the above mentioned, the purpose of this study is to determine the clade and its monophyletic variations of phylogenetic pattern of *A. bicolor bicolor* originating from Java and Western Indian Ocean, as well as the likelihood of their migration loop.

## MATERIALS AND METHODS

### The study area

The study areas (Fig. 1) consist of five locations, including one riverine estuary located at Ciwulan (GE) of Tasikmalaya-West Java, four riverines of Cilacap viz. Citandui of Segoro Anakan (SA) at Patimuan, Ciberem of Kaliwungu (KW) at Sidareja, Gatel of Kroya (KR) at Nusawungu, and Butuh-Dlanggu at Purwareja (PR) of Central Java – Indonesia. The

other four areas of the sample taken from Reunion, Madagascar, Mayotte, Seychelles, and Sumatera (Indian Ocean), and one area is in the Philippines (of the Pacific Ocean for another comparison) based on the data conducted by **Minegishi *et al.* (2012)**.



**Fig. (1).** General description of the study areas with the schematic major current systems (SY: Seycelles; MY: Moyotte; RE: Reunion; MD: Madagascar; SU: Sumatera; PH : Phillipine (**Minegishi *et al.*, 2012**); ▲ : West Java (GE); ● : Central Java (SA, KW, KR, and PR)

Java eel samples collected from the local fisherman (5 specimens at each location) were cut off into pieces of tissue at the caudal fin and stored in 96 % ethanol for further analysis. Samples from GE were in the size of glass eel with weight ranging between 0.5 – 1.0 g. SA samples were in early yellow eel (20 – 40 g), whilst samples taken from KW, KR, and PR were still in the elver stages (5 – 15 g).

## Procedures

Mitochondrial DNA extraction has proceeded with a 10% Chelex solution (**Walsh *et al.*, 1991**). A portion of the D-loop control region was amplified via Polymerase Chain Reaction (PCR) using the primers L15923 5'-TTA AAG CAT CGG TCT TGT AA-3' and H16498 5'-CCT GAA GTA GGA ACC AGA TG-3' (**Iguchi *et al.*, 1997**; **Ishikawa *et al.*, 2004**). The PCR was carried out in 25  $\mu$ L volumes, using 1.0  $\mu$ L of a template. Each reaction included 4.0  $\mu$ L 10 x PCR buffer (Applied Biosystems), 2.5  $\mu$ L 10.0 mM dNTPs, 1.25  $\mu$ L of each primer at 10.0 mM, 2.0  $\mu$ L of 25 mM  $MgCl_2$  solution (0.125  $\mu$ L AmplyTaq Gold™ (Applied Biosystems) and 14.5  $\mu$ L ddH<sub>2</sub>O. The thermo-cycling profile for D-loop included an initial denaturation of 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension of 72 °C for 2 min. The PCR product was examined on 1% agarose gels and then stained with nucleic acid gel stain (GelRed®). The PCR products then send to the Sequencing facility using Sanger Sequencing standardized analysis.

Sequenced data were aligned using MEGA5 Program (**Tamura *et al.*, 2011**), then compared to the sequence data from GenBank® to identified species of the sample. Method of identification used The Basic Local Alignment Search Tools (BLAST). How evolutionary history affects genetic diversity in *Anguilla bicolor*, a phylogenetic tree was made and

calculated using Neighbor-Joining (NJ) with a p-distance model with a 500 bootstrap replication in MEGA. Clade, which pronounces as similar species, will have a BLAST value of more than 96%.

Determination of phylogenetic tree was taken by comparing 86 sequence data from GenBank®, samples data of *Anguilla bicolor* taken from the Indian Ocean viz. Reunion (RE), Madagascar (MD), Mayotte (MA), Seychelles (SE), and Sumatera (SU) (**Minegishi et al., 2012**). Additional data of Pacific eel (*Anguilla bicolor*) from the Philippines (PH) (**Minegishi et al., 2012**) were also used to convince whether or not the Java eel has any correlation to the Pacific Clade as mentioned by **Sugeha & Suharti (2009)**. The data of GenBank® were then analyzed and compared to 25 sequence data eel from Southern Java Estuaries, i.e., West Java at Ciwulan (GE), Central Java at Citandui (SA), Ciberem (KW), Gatel (KR), and Butuh-Dlanggu (PR) riverine. Determination of species decree was by clustering (grouping) from a phylogenetic tree. Four sampling locations, i.e., Reunion (RE), Madagascar (MD), Mayotte (MA), and Seychelles (SE), were categorized as Western Indian Ocean, while Sumatera (SM), West Java (GE), and Central Java (SA, KW, KR, and PR) as Eastern Indian Ocean.

### Data analysis

Monophyletic pairs were identified manually from the formation of the phylogenetic tree then classified as the closest sample of the most recent common ancestor (MRCA) (Fig. 2). These obtained bounds were as a general base figure of the most recent migration loop (MRML). Hypothetical spawning location of **Kuroki et al. (2007)**, **Aoyama (2009)** and **Arai & Abdul-Kadir (2017)** was assumed as spawning areas both in the eastern Indian Ocean (off West Sumatera) and the western Indian Ocean or along the Mascarene basin (**Pous et al., 2010; Miller et al., 2015** and **Miller et al., 2019b**).

To draw the map of eels spawning areas were estimated by the depth and ocean basin topography found at 120 m (**Miller et al., 2019b**), at 150 – 180 m for *A. japonica* (**Aoyama et al., 2014**), or at 150 – 200 m (**Tsukamoto et al., 2011**), or 200 – 250 m (**Higuchi et al., 2020**). Hereafter to compose the map of the spawning area, ocean data sources were used global land map (Esri), ocean basin topography (GEBCO), NOAA, National Geographic, GPS maps (Garmin), and NOAA NGDC.

Prediction of eel origin was based on drift time compared to the age of the recruited glass eel or the age of leptocephalus at all ages and locations. Drift time and spawning area distance were calculated using the age of leptocephalus or early eels based on previous studies (**Arai et al., 1999; Setiawan et al., 2001; Aoyama et al., 2007; Budimawan & Lecomte-Finiger, 2007; Kuroki et al., 2007; Robinet et al., 2008; and Budiharjo et al., 2009**). Other studies on spawning grounds and recruitment models were also considered (**Pous et al., 2010; Minegishi et al., 2012; Miller et al., 2015; Drouineau et al., 2016** and **Miller et al., 2019a**). Current pattern data obtained from monthly surface currents from “Global–Reanalysis–Phy–001–025”. The Mercator reanalysis data set with lattice intervals of 0.25° (**Garric & Parent, 2017 & 2018**). One-year data is used mainly in the east (June, July, and August) and the west (December, January, and February) seasons, namely in 2015.

Distance mapping was digitized using a GIS approach. All data were taken and calculated during the 2019/2020 period. To draw a possible line for the downward migration

direction of eels (silver eels) from the sampling area (Java) to their spawning grounds (off West Sumatera or the Mascarene Basin) following possible current patterns both to the west and east. All hypothetical data on spawning areas, distance to location, current model, estimated drift time of leptocephalus, calculation of time, and eel migration distance supported the Most Recent Migration Loop (MRML) prediction graph.

## RESULTS

### Species identification

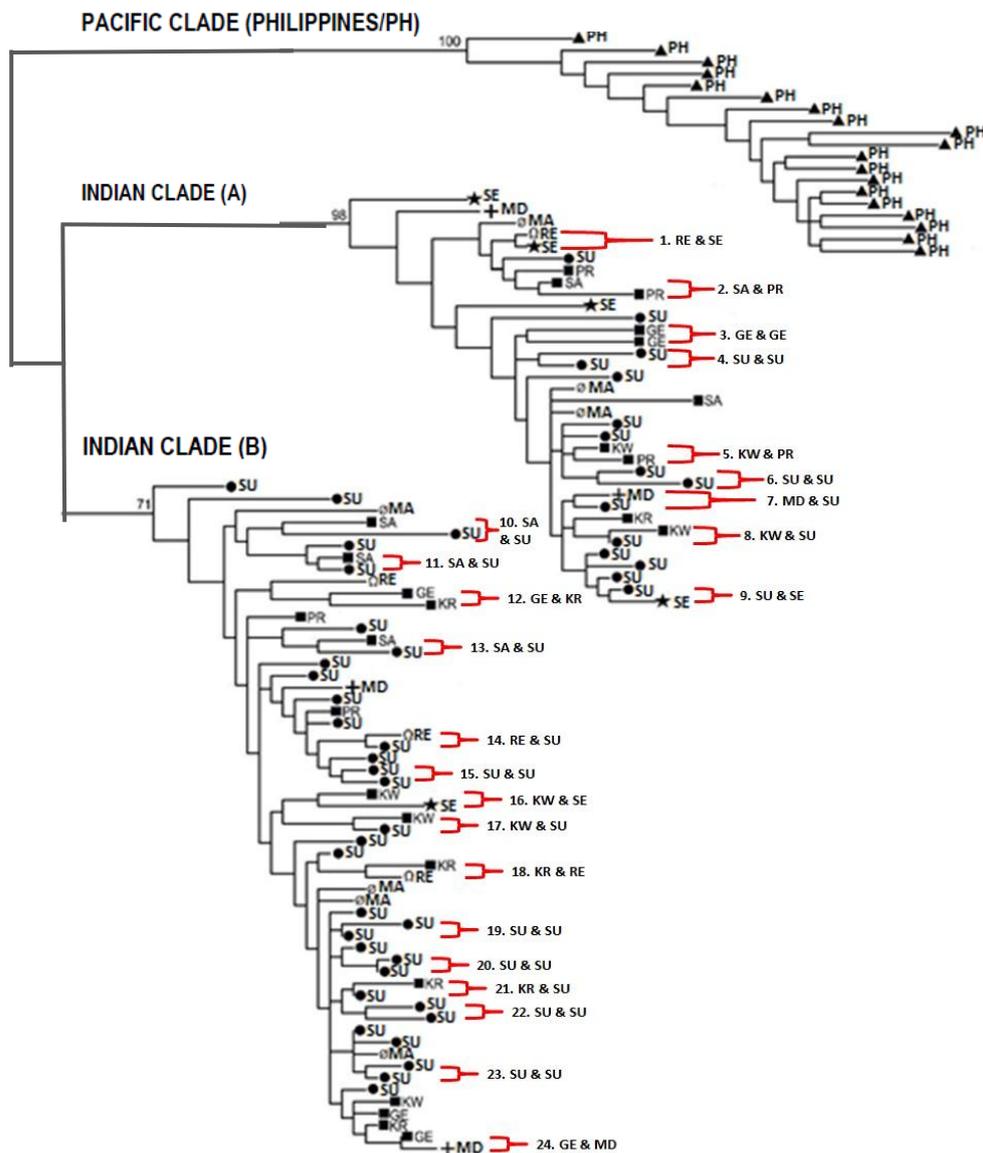
The BLAST identification shows that all the samples belong to the species of *Anguilla bicolor bicolor* with a similarity value of more than 96% (96 – 100%). The deposited haplotype sequences were in the NCBI GenBank database with accession numbers: MN630495 to MN630519 (**Table 1**).

**Table (1).** BLAST Homology of the Eel sample taken from different Riverine/estuaries of West and Central Java, Indonesia.

No	Sample/ Loc. ID	Riverine/ Estuaries	Province/ Country	Species name after BLAST	Homology (%)	Accession Number
1	GE 1.1	Ciwulan	West Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630495
2	GE 2.1	Ciwulan	West Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630496
3	GE 3.1	Ciwulan	West Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630497
4	GE 4.1	Ciwulan	West Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630498
5	GE 5.1	Ciwulan	West Java Indonesia	<i>Anguilla bicolor bicolor</i>	96	MN630499
6	SA 1.1	Citandui	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630500
7	SA 2.1	Citandui	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630501
8	SA 3.1	Citandui	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	97	MN630502
9	SA 4.1	Citandui	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630503
10	SA 5.1	Citandui	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630504
11	KW 1.1	Ciberem	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630505
12	KW 2.1	Ciberem	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630506
13	KW 3.1	Ciberem	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630507
14	KW 4.1	Ciberem	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	97	MN630508
15	KW 5.1	Ciberem	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630509
16	KR 1.1	Gatel	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630510
17	KR 2.1	Gatel	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	97	MN630511
18	KR 3.1	Gatel	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630512
19	KR 4.1	Gatel	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630513
20	KR 5.1	Gatel	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630514
21	PR 1.1	Dlanggu	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630515
22	PR 2.1	Dlanggu	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630516
23	PR 3.1	Dlanggu	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630517
24	PR 4.1	Dlanggu	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	100	MN630518
25	PR 5.1	Dlanggu	Cent Java Indonesia	<i>Anguilla bicolor bicolor</i>	99	MN630519

### Phylogenetic and monophyletic fact

The phylogenetic result shows that data schooling in different clades of Pacific and Indian Ocean. The eel *A. bicolor bicolor* from Southern Java estuaries attaches in two clades (A and B) of the Indian Ocean. Clade B has more diverse in monophyletic bound than in clade A (Fig. 2).



**Fig. (2).** The closest sample of monophyletic bonds (red lines) was found in the phylogenetic tree of *A. bicolor bicolor* from five locations in Estuary of Southern Java where samples were taken from West Java, namely the Ciwulan (GE) estuary; Estuary of Central Java, namely Citandui (SA), Ciberem (KW), Gatel (KR), and Butuh-Dlanggu (PR) compared to eel *A. bicolor bicolor* from five locations in the Indian Ocean, namely Reunion (RE), Madagascar (MD), Mayotte (MA), Seychelles (SE), and from Sumatera (SU), and *A. bicolor pacifica* from the Philippines (PH).

The phylogenetic tree suggested some closest samples at monophyletic bounds (c-mp) between Western to Western, Eastern to Eastern, and Western to Eastern Indian Ocean. The c-mp includes western to western Indian eel, i.e. Reunion and Seychelles (1 c-mp) found in clade-A but no western to western closest sample at clade-B. There are six couples of c-mp in eastern to eastern at clade-A and 11 c-mp at clade-B. Meanwhile, the two couples of closest samples between western and eastern Indian eels are in clade-A and four c-mps in clade-B. However, the closest sample found in the monophyletic bound of *A. bicolor bicolor* within-population of the estuaries has also occurred in two locations of the Eastern Indian Ocean, either schools in A and B of Indian clades (**Fig. 2** and **Table 2** signed with ▲).

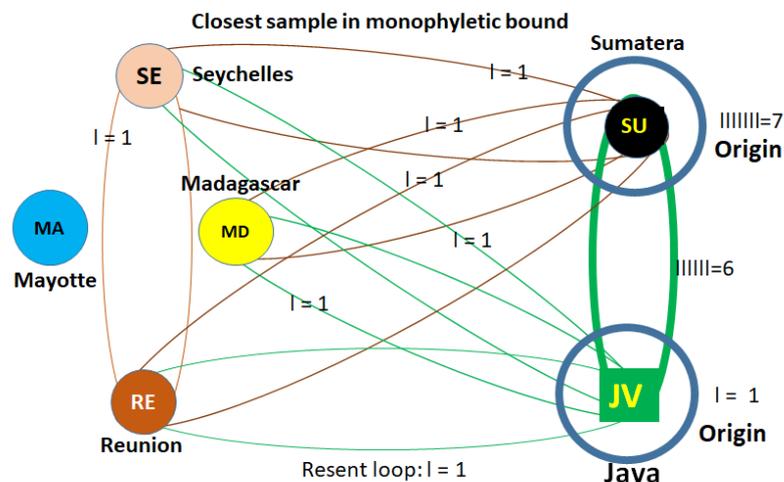
**Table (2).** Number of Eel samples distributed in Clade A and B ( $Ax+Bx$ ) and number of the pairs  $A^{or B}(x)$  among locations.

Locations	PR (A3+B2)	KR (A1+B4)	KW (A2+B3)	SA (A2+B3)	GE (A2+B3)	SU (A15+B32)	RE (A1+B3)	MD (A2+B2)	MA (A3+B4)	SE (A4+B1)
PR			* <sup>A</sup> (1)	* <sup>A</sup> (1)			* <sup>A</sup> (1)			
KR					* <sup>B</sup> (1)					
KW										
SA										
GE					<sup>A</sup> (1)▲					
SU		* <sup>B</sup> (1)	* <sup>A</sup> * <sup>B</sup> (2)	* <sup>B</sup> * <sup>B</sup> * <sup>B</sup> (3)		AABBBBB(7)▲	* <sup>B</sup> (1)	* <sup>A</sup> (1)		
RE		* <sup>B</sup> (1)								
MD					* <sup>B</sup> (1)					
MA										
SE			* <sup>B</sup> (1)			* <sup>A</sup> (1)	* <sup>A</sup> (1)			

Notes: PR (Butuh-Dlanggu); KR (Gatel); KW (Ciberem); SA (Citandui); GE (Ciwulan); SM (Sumatera); RE (Reunion); MD (Madagascar); MA (Mayotte); SE (Seychelles)

The couple bounds distribution pattern of the closest sample (share ancestor) in each clade is shown in **Fig. (2)** and summarized in **Table (2)**. The schematic migration loop of those fact couples of closest sample in monophyletic bound (c-mp) of eels where they have shared ancestor is presented in **Fig. (3)**.

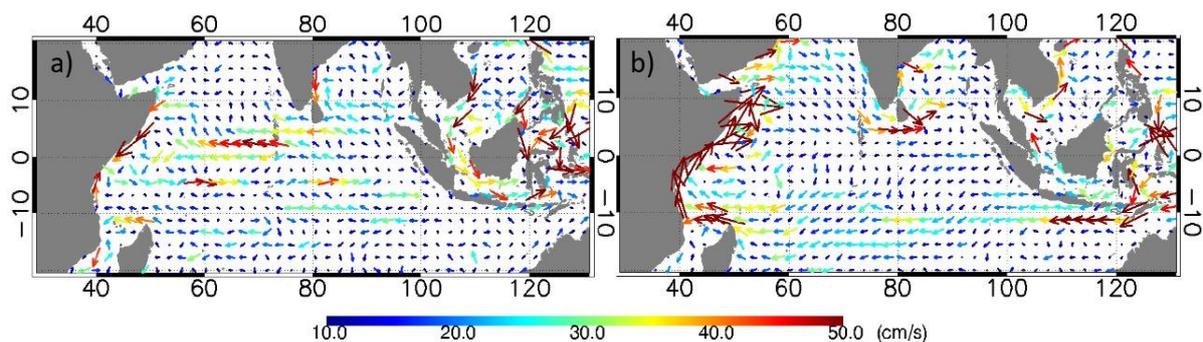
Results shows that the eel from Java and Sumatera have a very strong share in MRCA (with 6 c-mp). They have a similar pattern to the western Indian eels (SE, MD, and RE). Unlike Seychelles and Reunion samples, the Western Indian group seems not to have a specific closest sample to each other (**Fig. 3**).



**Fig. (3).** Schematic of Migration Loop Development and panmictic occurrence (2012– 2019) among Western and Eastern Indian Eel populations based on the closest sample in the monophyletic pairs of *Anguilla bicolor bicolor* found in the phylogenetic tree (share ancestor).

### Current pattern, spawning region, and distance among areas

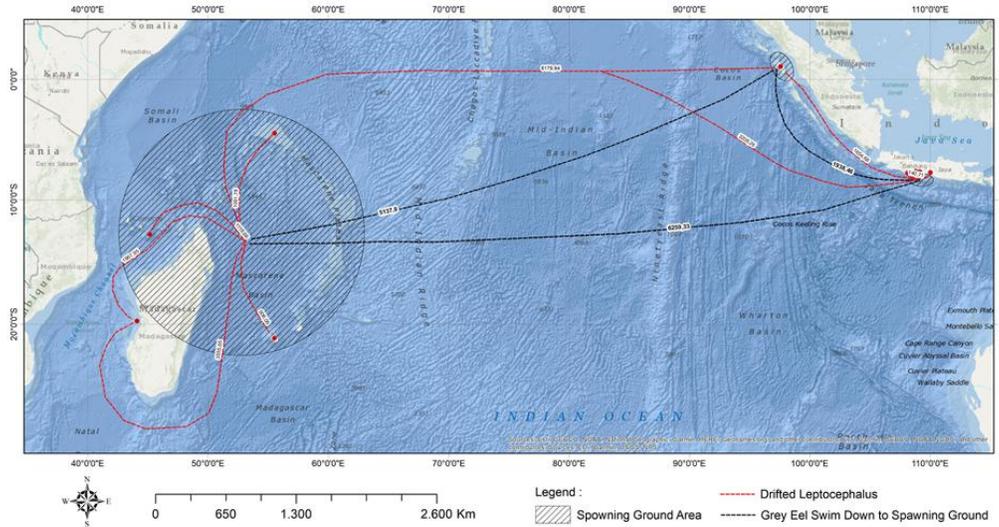
Based on Global-Reanalysis-Phy-001-025 Western Pacific to the Indian Ocean during 2015 simulation, the equatorial currents system in the Indian Ocean consists of 3 main patterns, namely the South Equatorial Current (SEC) to the West, the Equatorial Counter Current to the east (ECC), and the North Equatorial Current to the West (NEC). These currents flow along years with a current velocity between 12.51–53.94  $\text{cm}\cdot\text{s}^{-1}$  (**Fig. 3**). In certain places, the peak of the NEC occurs in the rainy season (February and March) can reach more than 80  $\text{cm}\cdot\text{s}^{-1}$ , especially along 0 – 5 °N and 50 - 80 °E. The peak of the SEC occurs in the east monsoon (June, July, and August) can reach more than 80  $\text{cm}\cdot\text{s}^{-1}$ , especially along 10 °S, 100 – 125° E (South Sea of Java Island). Based on the current simulation, the average ECC value is about 52  $\text{cm}\cdot\text{s}^{-1}$  throughout the year. However, the SEC is weaker than the NEC and ECC throughout the year, but most current speeds can reach 50  $\text{cm}\cdot\text{s}^{-1}$  (**Fig. 4**). The western Pacific Current (NEC of Pacific) mostly moves from the east then diverges north and south before approaching the Philippines. The southward current passes through the Celebes Strait to become the Indonesian Through-flow (ITF) then turns northeast of Banda and turns downward before and after East Timor to the eastern Indian Ocean (**Figs. 1 & 4**).



**Fig. (4).** Current pattern during <sup>a)</sup> East seasons (June, July, and August) and <sup>b)</sup> West seasons (December, January, and February). Data simulated from Marine Copernicus year 2015.

Results showed that *A. bicolor bicolor* from the eastern Indian Ocean (Java and Sumatera) attached to two Clades (A and B), but they are not related to *A. bicolor* from the Pacific Ocean (Philippines). Based on these results, the Javanese eel has similar descent to eel from the western Indian Ocean indicates that the eel has certain places for spawning “migration loop” (**Fig. 2**).

It may be related to the distance and age of drifting leptocephalus. Estimated spawning locations based on several studies, the distance and spawning areas are presented in **Fig. (5)**. There are three spawning areas: Mascarene Basin, West Sumatera, and possibly the South Java Ocean. However, this last spawning area (Southern Java Ocean) depends on the age of the glass eels found in the recruitment area. The digitized possible distance base on the current pattern in either direction and its current speed; there are eight possible distances for *A. bicolor bicolor* leptocephalus drifted from their were spawned attaining (drifted) into the coastal area where they will be metamorphoses into glass eel either the silver eels swim down to spawning area is presented in **Fig. (5)** and **Table (3)**.



**Fig. (5).** Cartography of distance of migration by silver eel, leptocephalus drifts of *Anguilla bicolor bicolor*, monophyletic bounds, and predicted spawning time data. Red lines are leptocephalus drifted routes, and black lines are silver eels migration routes.

**Table (3).** Distance approximation of eel's leptocephalus drifted, and the silver eels swim down to their spawning area (migration loop)

Spawning Grounds	Distance to the coastal area where metamorphosis occurs (km)						
	Central Java	West Java	West Sumatera	Reunion	Madagascar	Mayotte	Seychelles
Mascarene basin	7,834.62	7,693.91	6,179.94	906.06	3,555.65	1,118.06	1,091.78
West Sumatera	1,654.68	1,513.97	100 - 800	7,086.00	8,829.53	6,244.53	5,152.75
Southern Java Ocean	86.38	140.71	1,654.68	-	-	-	-
Closest distance the grey eel swim down to spawning area (km)							
Mascarene basin	6,259.33	6,150.33	5,137.80	906.06	1,967.95	1,118.06	1,091.78
West Sumatera	1,654.68	1,476.58	100 - 800	7,086.00	8,829.53	6,244.53	5,152.75

### Estimated drifting time, migration time, and previous data on eel recruitment

Estimated leptocephalus drifting time (days) based on the yearly mean current speed (50 cm. s<sup>-1</sup>) and possible direction of the current pattern, especially the eastward ECC found varies in every location. At the same distance, the grey eel that has 80 cm of body length with a swim speed of 0.4 body length per second (BL.s<sup>-1</sup>) reached spawning areas slower than the leptocephalus reached recruitment area (**Table 4**).

Though the study of recruitments was not easy to get, metamorphose or recruitment age of the eels varied among locations. While, the hatching date based on otolith identification showed that the eel spawned and hatched all year round (**Table 5**).

**Tabel (4).** Estimated leptocephalus drifting time based on current pattern along with the distance and time period of the silver eel's swim down to their migration loops.

Spawning Grounds	Leptocephalus drifted time (days) ( $v = 50 \text{ cm.s}^{-1}$ )						
	Central Java	West Java	West Sumatera	Reunion	Madagascar	Mayotte	Seychelles
Mascarene basin	181.36	78.10	143.05	20.97	82.31	25.88	25.27
West Sumatera	38.30	35.05	2.31 - 18.52	164.03	204.39	144.55	119.28
Indian Ocean	2.00	3.26	38.30	-	-	-	-
Silver eel migration time (days) by using 80 cm of BL and swim speed $0.4 \text{ BL s}^{-1}$							
Mascarene basin	226.39	222.45	185.83	32.77	71.18	40.44	39.49
West Sumatera	59.85	53.41	3.62 - 28.94	256.29	319.36	225.86	186.37

Notes:  $v$  = mean of current speed, BL: eel's body length

**Table (5).** A previous study on *A. bicolor bicolor* larval periods and age at recruitment by using the otolith determination, and approximations of hatching date

Sampling Locations	TL of Lc & Ge (mm)	Age to metamorph/ Larval periods (d)	Age at recruitment (d)	Hatching Date	References
West Java (Cimandiri)	45.5 - 52.3 (Ge)	119 - 171	148 - 202	Nov 10 <sup>th</sup> - Jan 3 <sup>rd</sup>	Arai <i>et al.</i> (1999)
West Java (Cimandiri)	$52.7 \pm 2.1$	$148.4 \pm 26.6$	$182.8 \pm 28.0$		Setiawan <i>et al.</i> (2001)
West Java (Cimandiri)	50 - 58 (Ge)	$85.2 \pm 12.8$	$106.4 \pm 11.1$	Jan - March	Budimawan & Lecomte-Finiger (2007)
West off Sumatera	44.1 - 55.5 (Lc)	114 - 158 (Lc)	after 158	Jan - Feb & May - June	Kuroki <i>et al.</i> (2007)
West off Sumatera (Mentawai)	44.1 - 55.5 (Lc)	-	-	-	Aoyama <i>et al.</i> (2007)
Mauritius	50 - 56	87 - 139	108 - 179	Oct 7th - Dec 12nd	Robinet <i>et al.</i> (2008)
Mayotte (1)	51-62	87-117	120-157	Oct 8-Dec 12	Robinet <i>et al.</i> (2008)
Mayotte (2)	163 - 205	92-100	127-134	March 1-August 14	Robinet <i>et al.</i> (2008)
Réunion	53	151	183	Sept 10th	Robinet <i>et al.</i> (2008)
Seychelles	364 - 644	87-128	117-158	Nc**	Robinet <i>et al.</i> (2008)
Central Java (Progo)	Ge	-	58 - 157	Jul, Aug to Dec, Jan	Budiharjo <i>et al.</i> (2009)

Notes: (TL: total length of Lc: leptocephalus or Ge: glass eel, Nc\*\* not conducted).

## DISCUSSION

The homology values (**Table (1)**) indicated that the population has a tight shared ancestry between each sample by the locus of the mtDNA control region. For these results, even though **Minegishi et al. (2012)** used the species name with *Anguilla bicolor*, the homology value of the eel from Western Indian Ocean and Sumatera directed to the species name as *Anguilla bicolor bicolor*.

In the phylogenetic tree (**Fig. 2**), Java eels attached in two Indian Ocean Clades A and B, and found some monophyletic pairs/bound (c-mp) either of within, between, and among population groups. Hence, those pairs (c-mp) of *A. bicolor bicolor* inferred the most recent common ancestor (MRCA). According to the phylogenetic concept of **Aldhebiani (2018)**, Indian Clades have branches of monophyletic and polyphyletic. These indicated that all of the eel samples of *A. bicolor bicolor* taken from the Indian Ocean have been considered as a descent of similar ancestors. Due to randomly being in pairs of monophyletic, they have been assumed as a “panmictic” species. However, this pattern of phylogenetic tree might be able to be used as an archive that during 2012 data taken by **Minegishi et al. (2012)** and the present study (2019) still have correlations (Clades) among Western Indian and Java eels niches.

Shared ancestor observed in *Anguilla bicolor bicolor* from Java also Western Indian Ocean has some monophyletic pairs but no correlation with the Pacific Clade. As they have a unique migration loop, their life history includes migration routes connecting to spawning areas and growth habitats (**Tsukamoto et al., 2002**). The European eel, *Anguilla anguilla*, was considered to have a spawning migration from Europe to the Sargasso sea undertaken at 5,000 km distances or more (**Righton et al., 2016; Cresci et al., 2017** and **Sandlund et al., 2017**). Using a similar monitoring method (PSATs) with long-life archival tags (Cefas Technology Ltd G5), **Righton et al. (2016)** suggested that observed migration ability of the eel in between 3–47 km. d<sup>-1</sup>. However, most figured out between 10 to 30 km.d<sup>-1</sup> or 0.25 – 0.5 body lengths per second (BL.s<sup>-1</sup>).

According to the phylogenetic concept of **Aldhebiani (2018)**, there was a similar ancestor of Central and West Java eel with Western Indian Ocean eel, where KR and Reunion, KW and Seychelles, and GE and Madagascar considered as a recent descent. The approximation distances, the routes follow the direction of South Equatorial Current (SEC). Calculated time of the adultery silver eel migration from Central and West Java to Mascarene basin found a bit long time: 226.39 and 222.45 days, respectively. The closest area around the basin e.g: Reunion took 32.77 days (**Table 4**). This pathway of peculiar routes for Java silver eel may be too far if using the swim speed of 0.4 BL. s<sup>-1</sup>. Pop-up satellite for identified *A. anguilla* migration speed can attain 16.5 km.d<sup>-1</sup> in the Mediterranean Sea and 9.7 km. d<sup>-1</sup> in the Atlantic Ocean (**Amilhat et al., 2016**). The monophyletic bounds between Java and the Western Indian Ocean's eel may have bred at closer areas such as the off West Sumatera basin or eastern part of Mascarene ridge. It was the peculiar migration loop of the eel, where they can measure the distance and time for their breeding.

According to **Righton et al. (2016)**, silver eels migrate along the shortest road to spawning areas without any adverse or beneficial currents and adjust their speed to swim up to their spawning ground. However, for this distance where the Java eel has to migrate to the Mascarene basin, the westward SEC may take a role in accelerating the eel speed become twice or even more i.e., the swim speed to be 1 time or > 1 x BL. s<sup>-1</sup>. Hence the current speed will push up the

eel to swim twice faster or only need 115 and 111 days to spawn area of the Mascarene basin from Central and West Java. Besides that, the eel has a magnetic compass to its spawning destination (Cresci *et al.*, 2017). In addition, the eel has an adaptive magnetic map that will tune to large-scale features of ocean circulation and facilitate the vast oceanic eel's migrations (Naisbett-Jones *et al.*, 2017).

Due to the eel's ability to detect a magnetic map; hence, the silver eels from Java or Sumatera will use the south equatorial current (SEC) to fasten their migration to the Mascarene basin. Hereafter, the duration time of the eels for their migration becomes much shorter and faster. In terms of the stage of maturation of the silver eel caught from "Segoro Anakan" (Central Java), Arai *et al.* (2016) found that eel spawns all year-round. The months of April to September as a peak season of *Anguilla bicolor bicolor* attaining stage V of gonadal maturation, though, it still found some stage V on the rest of months from October to March. Rachmawati *et al.* (2017) use the silver eel of *Anguilla bicolor bicolor* with total length (TL) approximately 67 cm found in Serayu estuary (Central Java) have attaining mature stage. In addition, Arai & Abdul-Kadir (2017) categorized that *A. bicolor bicolor* as mid-scale migratory species together with *A. marmorata* and *A. bengalensis bengalensis* with a distance of 1,000 to 3,000 km. However, the fact that appearance of MRCA between Java, Sumatera, and Western Indian eel and calculated distance from Java and Sumatera to the Mascarene basin are more than 7,000 km and 6,000 km, respectively (Table 3). These facts should be taken into account that species of *Anguilla bicolor bicolor* may belong to either the long- or mid-scales migratory type species.

Leptocephalus drifts passively across the ocean until reaching coastal water then metamorphose to glass eel (Cresci *et al.*, 2017). Growth of leptocephalus starts from hatch to metamorphosis. It is the time of the eel larvae to drift to the coastal areas where larvae will swim up upstream to a new environment with lower salinity. According to Aoyama *et al.* (2007) the spawning area of *Anguilla bicolor bicolor* occurs off West Sumatera to Southern Central Java estuaries is 1,654.68 km away, and Central Java to Mascarene basin is 7,834.62 km. Hence the drifted time of leptocephalus from Sumatera to Central Java took 38.30 days, while from the Mascarene basin to Central Java took 181.36 days. Budiharjo *et al.* (2009) found a wider age variety at recruitment glass eel between 58 – 188 d in Progo River Jogjakarta (Table 5). These ages' variations may correspond with distance (after calculating age and drifting time) before hatching. The first distance of Central Java to eel's spawning ground off West Sumatera (59.85 vs. 58 d) seems to be related to monophyletic pairs (c-mp). Hence, spawning areas at the Southern Ocean of Java might be ignored due to a lack of data. The distance to the hypothetical spawning area at Mascarene basin, where in monophyletic tree have any correlations i.e., KR and Reunion/RE (1 c-mp), KW and Seychelles (1 c-mp) as well as GE and Madagascar (1 c-mp) in Clade B seems to correlate according to the drifted time (by using mean water current of 50 cm s<sup>-1</sup>) and the age of eel at recruitment.

The metamorphosis stage differs from site to site or either species-dependent (Arai *et al.* (1999); Setiawan *et al.* (2001); Robinet *et al.* (2003); Kuroki *et al.* (2007); Robinet *et al.* (2008) see Table 5). These indicated that the duration of the eel's larvae growth and the monthly mean speed of ocean current, the spawning area of *A. bicolor bicolor* is presumed to occur along the surrounding waters of Madagascar and the Mascarene basin. Arai (2014) reported that spawning areas of *A. celebesensis* and *A. borneensis* would have a very short distance from their growth freshwater habitat (less than 100 km).

Even though ocean current patterns and circulations are mostly similar all-around years, the Indian Ocean circulation pattern varies by inter-annual variability (**Schott *et al.*, 2009** and **Phillips *et al.*, 2021**). The simulation year of 2015 noticed that the peak current can reach more than  $80 \text{ cm.s}^{-1}$  in some months. Hence, the Indian Ocean will have a mean current speed at a certain time that can reach  $50 \text{ cm.s}^{-1}$  or more. This average current found has a similar value pattern to the average currents measured by ocean drifters from 1985 to 2013 conducted by **Miller *et al.* (2019b)**. This current drifts the leptocephalus from the Mascarene basin to West Java estuaries for 178.10 days. This is very close to the time of recruitment ( $182.8 \pm 28.0$  days) of the eel found at the estuary of West Java (**Setiawan *et al.*, 2001** see **Table 5**).

**Minegishi *et al.* (2012)** stated allopatric isolation of *A. bicolor* of Indian and the Pacific Ocean based on Bayesian demographic history- where the population developed in each ocean after the Indo-Pacific divergence. To compare with the current study, based on the phylogenetic tree (differ Clades populations between Indian and the Pacific Ocean) and the current pattern of Pacific and Indian Ocean, the westward Pacific current diverges to the south and north before southern part of Visayas Island. Hence, even though the eel can swim counter-current, *A. bicolor* of Pacific Clade seems not to go down far away to the Indian Ocean. This is following **Serdiati *et al.* (2013)** stated none of *A. bicolor bicolor* was found in the Palu river- where the ITF current has passed through the Celebes strait. Another phenomenon, found by **Taufiq-Spj *et al.* (2021)** that the eel from Central Java has a very short dorsal fin with an ano-dorsal (AD) negative value of -6.56 % and is predicted belonging to *A. bicolor pacifica*.

## CONCLUSION

There are three patterns of the Clades among eastern, western Indian Ocean's eel and the western Pacific Ocean's eel of *Anguilla bicolor bicolor*, where the Java eel have attached in two Clades of Indian Ocean, but no correlation with Pacific (Philippines) Clade. It is most likely that the population between Pacific and Indian is currently not breeding with one another that proved by the westward Pacific current pattern, which diverged geographically to the south (ITF) and north before the Philippines eel of *A. bicolor* locations. The phylogenetic tree of this study found some monophyletic bound either: within, between, and among population groups. It indicates that *A. b. bicolor* from Java inferred has a most recent common ancestor (MRCA) with the same species from the Western Indian Ocean and is concerned to be a panmictic species. The current pattern of south equatorial current SEC and northern equatorial counter-current ECC play important roles for silver eels migration loop and the leptocephalus drifts. For this fact, *A. b. bicolor* either from western Indian (Reunion, Madagascar, Mayotte, and Seychelles) or the eastern Indian Ocean (Sumatera and Java) categorized as mid and long scale migration types of species.

## ACKNOWLEDGEMENTS

This work was financially supported by the DRPM, Indonesian Minister of Research, Technology, and High Education on Applied Research scheme year of 2019/2021. Thanks to every single person who involved during field and laboratory works.

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