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Morphometric and Meristic Diversity in Eel (*Anguilla bicolor*) in South Coast of Java

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Abstract

Indonesia is the largest exporter of eel to consumer countries such as European, America, Taiwan, Japan, South Korea, and the Middle East. Eels are catadromous fish that are in the growth phase in fresh waters and the development phase is in marine waters. Meristic measurement is done by counting the spine (vertebrates). Eel morphometric measurements include TL (Total Length), HL (Head Length), PDL (Pre-Dorsal Length), PAL (Pre-Anal Length) and ADL (Ano-Dorsal Length). The purpose of this study was to analyze the morphological, morphometric, and meristic diversity of intraspecies between populations. Based on the research, the samples identified were morphologically almost the same between the populations, this was seen from the color patterns in the form of plain black or brown and short fins. The morphometric and meristic measurements in the 3D distribution plot still appear to be clustered in one population, while in the dendrogram clade, one species is still congregated in each. The results of the interpretation of the matrix plot of the lowest diversity are the length and number of anodorsal vertebrate segments. This observation can be concluded that the TL and TV of the eel varied, while the PDL, PAL, PDV, PAV, and ADV showed the intraspecies character of *A. bicolor*. This is because the distribution of A. bicolor eels that inhabit the waters of Java Island is widely distributed and random (panmitic).

INTRODUCTION

Indonesia is considered an ancestral area and a center for eel biodiversity, it has eel resources spread along the southern coast of Java Island (Aoyama, 2009). Market demand for *A. bicolor* eel is very wide open for Indonesia to become an eel exporting country (Widiantoro, 2020), so Indonesia has become the largest eel exporter to consumer countries such as Japan, Europe, America, Taiwan, South Korea, and the Middle East (Achmad *et al*., 2022). National production for eel exports in the first semester of 2019 reached 5,186 tons and increased compared to the same period last year at 4,142 tons (KKP, 2020).

The *A. bicolor* eel is the preferred second choice if *A. japonica* is not available in Japan. The *A. bicolor* eel texture and taste are similar, so it is economically important in terms of market demand. Eel is popular as a luxury food because it contains 65% protein and 28% fat which is good for stamina in the body (Shiraishi and Crook, 2015).

The use of eel resources by cultivators still comes from natural fishing efforts in public waters (Tanaka *et al*., 2014). Eels are catadromous fish that migrate between laughing waters and ocean waters (Hakim *et al*., 2015). Growth is carried out in freshwater while breeding is carried out in the sea by producing leptocephalus larvae. The larvae are carried by turbulent currents to the coast and develop into glass eels. Glass eel begins to turn pigment into elver. The fairy then begins to enter the river or estuary area until it turns into a yellow eel. During maturation, the eels develop into silver eels and return to the sea to lay eggs and die (Tesch, 2003).

The morphometric character uses the measurement method on certain parts of the fish body structure or measurement method while the meristic character is related to the calculation of the number of fish body parts or counting method (Elawa, 2004). Meristic measurement is done by counting the spine (vertebrates). The morphometric measurements of the eel included total length (TL), head length (HL), pre-dorsal length (PDL), pre-anal length (PAL), and ano-dorsal length (ADL) (Wahju *et al*., 2020). Samples of *A. bicolor* eels were identified by a morphological method based on body shape and fish composition known as morphometric techniques (Putri and Madduppa, 2020). The morphological characters can separate based on the morphology of the eel species. Separation is based on special characteristics, namely skin motif, pre-anal length, predorsal and number of segments. Morphometric measurements and meristic counting are considered the easiest and most authentic methods for specimen identification which are called morphological systematics (Langer *et al*., 2013).

Differences in specific characters, namely the comparison of preanal and predorsal lengths. Then eels are grouped into two major groups, namely short fin, a class of fish with short dorsal fins consisting of 14 species and long fin, a class of fish with long dorsal fin consisting of five species. For the character of the number of segments commonly used in larval-size fish, the number of segments increases with the increase in the latitude of the live eels. Generally, the number of eel spines is 100-119, eels that live in the tropics have fewer spines than eels that live in subtropical areas (Fahmi and Hirnawati, 2010). The purpose of this study was to analyze the morphological, morphometric, and meristic diversity of a species between populations. This research needs to be done to determine the characteristics of eels in the adult phase. Each eel phase has a variety of shapes and sizes, therefore analysis is needed for each phase. The waters of the island of Java are one of the waters where many species of eel *A. bicolor* are found. In addition, it becomes a place for eel migration for the growth phase.

METHODOLOGY

Place and Time

Sampling was carried out from April to May 2022. Observations and measurements were carried out at the Anatomy and Aquaculture Laboratory, Faculty of Fisheries and Marine Affairs, Universitas Airlangga. Sampling locations are presented in Table 1 Locations for sampling eel *A. bicolor* from river waters and Figure 1 Locations known to spread *A. bicolor* eels.

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Figure 1. Locations known to spread *A. bicolor* eels from Pandeglang Regency, Banten; Cilacap Regency, Central Java; Tulungagung Regency, East Java; Banyuwangi Regency, East Java.

Table 1. Locations for sampling *A. bicolor* eels from river water.

Location Coordinates	Location of River Water	Number of Samples	Sample code
-6.4822, 105.8256	Sukaresmi, Pandeglang, Banten	10	SBT
-7.6396, 109.3712	Nusawungu, Cilacap, Center Java	10	SCP
-8.0406, 111.9236	Kedungwaru, Tulungagung, East Java	10	STG
-8.1143, 114.3491	Kalipuro, Banyuwangi, East Java	8	SBW

Research Materials

Eel samples taken were samples from natural catches in the South of Java. The sampling locations were in four locations originating from the river flow. The number of each sample used in morphological observations of all eels was obtained with an average fish weight size of 200-300 grams with a length range of 20-50 cm. Consumption of eels is obtained after 5-7 months of age with a length of 40-50 cm and a weight of 200-300 grams (Baskoro *et al*., 2016). The eel samples taken were samples from natural catches from rivers. Catching eels are taken using environmentally friendly tools namely "Bubu". This tool is made of rattan or polyethylene so it doesn't damage the ecosystem. Bubu is paired with baits such as shrimp, small fish and chicken intestines. The tool is placed on the bank of the river and the most effective time is at night. Eels are carnivorous fish and are active at night looking for food (Muryanto and Sumarno, 2015).

Research Design

The research was conducted using a laboratory exploration method with direct sampling, followed by morphological and molecular approaches and analysis related to the diversity of eels *A. bicolor* at several sampling locations. The research design used was a cross-sectional study, namely the measurement of sample variables at the same time within a certain period of time.

Work Procedure

The eel obtained was put into a coolbox which was given crushed ice cubes to lower the temperature in the coolbox. Setting the temperature when traveling long distances so that the eel is not damaged (Maulana *et al*., 2020). Early morphological observations identified fish in general. The general morphology of eels includes body shape, mouth shape, mouth location, body color, and fin location. The morphometric and meristic measurements were carried out in a preserved state with the addition of 70% alcohol. Morphometric measurements using a meter while meristic by dissecting the body and manually counting the number of vertebrae (Apriani *et al*., 2021).

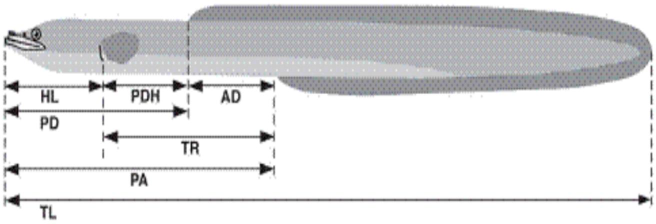

Figure 2. Morphometric measurements of *A. bicolor* specimens. Description: HL (Head Length): head length; PDL (Predorsal Length): predorsal length; PAL (PreAnal Length): preanal length; TL (Total Length): total length; SL (Standard Length): Standard Length (Fahmi and Hirnawati, 2010).

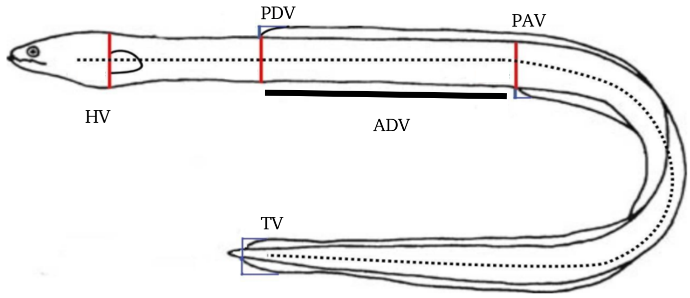

Figure 3. Meristic measurements of *A. bicolor* specimens. Description: HV (Head Vertebrae): the number of vertebrae in the head, PDV (Pre-Dorsal Vertebrae): the number of predorsal vertebrae, PAV (Pre-Anal Vertebrae): the number of vertebrae in the preanal, TV (Total Vertebrae): the number of vertebrae in total length (Amrullah *et al.*, 2019).

Data Analysis

Morphological data in the form of morphometric and meristic were then processed using SPSS 25 software using a one-way ANOVA test and PAST 4.70b software using PCA (Principal Component Analysis) (Ayyub *et al.*, 2019). PCA analysis includes 3D Plot Analysis to determine the distribution of the diversity of the plots that have been marked. Matrix plot analysis to determine the level of diversity is used as a particular characteristic. Cluster analysis to determine the level of morphological relationship of species using a Hierarchical Cluster method in the form of a dendrogram (Jolliffe and Cadima, 2016).

RESULTS AND DISCUSSION

Morphology of *Anguilla bicolor*

The morphological characteristics of eels in the form of morphometric and meristic can be influenced by their habitat environment. Measurement of morphometric and meristic parameters is usually carried out as a database to support molecular analysis. Morphometric and meristic measurements were carried out on the sample by taking into account the dominant characteristics of the eel that distinguish it from the eel, namely the presence of fins located at the back of the head such as the auricle and the specific characteristics of the eel species. Morphometric is the ratio of the relative size of fish body parts, while meristic is a countable fish part which is the number of fish body parts.

Morphological observations in the form of morphometric and meristic were carried out visually by observing the special characteristics of *A. bicolor* eels. Eel samples were taken randomly from the catch in the consumption phase. Characteristics refer to several morphological characteristics observed by eels, including the shape that appears on the eel's body. Figure 4 shows the morphology of the eel of the *A. bicolor* species.

Figure 4. Morphology of eel *Anguilla bicolor* (a) Pandeglang Regency, Banten (b) Cilacap Regency, Central Java (c) Tulungagung Regency, East Java (d) Banyuwangi Regency, East Java.

Determination steps based on McClelland (1844) description of external morphology include scales not visible on the skin surface, free tongue, nostrils above the muzzle, pectoral fin; dorsal fin; anal fin; well-developed caudal fin; dorsal base far behind gill openings. The gill openings are wide slits. The base of the dorsal fin is above the anus, the oral cleft reaches the orbit and the head length is 4 times the eye diameter. Sarwono (2007) added that eels called leaf-eared eels have pectoral fins on the right and left sides of the body. The color of the dorsal part of the body is plain dark while the belly is white with white pigmentation. The head of the eel is triangular. The eyes of eels cannot be in direct contact with the sun, therefore eels are nocturnal. The eyes are on the side with black pupils and golden irises with gray around them.

Based on its morphological characteristics, it was confirmed that samples from Pandeglang Regency, Banten, Cilacap Regency, Central Java, Tulungagung Regency, East Java, Banyuwangi Regency, East Java were species of *A. bicolor*. Visible characteristics include an elongated body shape, terminal mouth shape, lower jaw 1/3 of the length of the head, and round eyes on the sides of the head close to the back of the mouth. The dorsal and anal fins extend backward and merge with the caudal fin. The pectoral, dorsal, anal and caudal fins are well-developed. The left and right pair of pectoral fins are located behind the head or gill covers. The gill openings are wide slits. Plain black body skin color with silvery gray chest or belly (ventral). Scales are not visible on the surface of the body and there are no lateral lines on the surface. This is in accordance with Teng *et al.* (2009) explained that the morphology of A. bicolor eels has an elongated body like a snake with a pair of pectoral fins.

The head of the eel is triangular, and has round eyes, a tongue, and nostrils in front of the eyes, the mouth is slightly tilted, and the gill cover is on the underside of the head or in front of the pectoral fins. The three dorsal fins, anal fins, and caudal fins are fused. Linea lateralis well formed. *A. bicolor* eels have an elongated and smooth body with plain black color on the dorsal. The gills can be wide open with a triangular head shape. Abdomen away from head, mouth terminal, jaw not elongated in particular (Arai *et al.*, 2012).

The specialty of *A. bicolor* eels has short fins, namely the short distance ratio between the length of the dorsal and anal fins, known as ano-dorsal. The dorsal fin is located from the middle of the body to the back. The base of the dorsal fin is far behind the gill openings just above the anus adjacent to the base of the anal fin.

Sugeha and Genisha (2015) described the proportion of ADL of *A. bicolor* in the form of the PDL compared to the PAL. Between species of eel can also be determined by several characteristics including head shape, length of pre-anal and per-dorsal, and the number of vertebrae. This difference in character can be used to distinguish the adult eel. Based on specific characteristics, namely pre-dossal and pre-anal comparisons, eels can be grouped into two types. Shortfin group of eels with short dorsal fins and long fin groups of long dorsal fin eels. Furthermore, the spine character generally amounts to 100-119 vertebrae. Tropical eels have fewer spines than subtropical eels (Fahmi and Hirnawati, 2010).

Morphological characteristics of eels such as skin patterns and types of fin length can be used as the basis for the early identification of eel species (Jamandre *et al*., 2007). Morphological diversity can be influenced by several factors such as environmental conditions, topography, and different habitats (Solomon *et al*., 2015).

Morphometric Analysis

Eel morphometric measurements using SPPS 25 software were analyzed using one-way ANOVA and PCA analysis using PAST 4.07 software. The number of calculated morphometric parameters is 6 parameters. The results of the morphometric analysis in Figure 5a show 3D distribution of intraspecies diversity. Based on the plot of the four samples, shows that the points spread and converge between populations. It is suspected that the *A. bicolor* sample still has a close relationship between the four populations. The morphometric diversity can also be seen from the matrix in Figure 5b. Based on the color pattern, the highest morphometric diversity was indicated by the TL (Total Length) morphometric parameter 57,1 cm is the highest length and the lowest diversity was indicated by the ADL parameter.

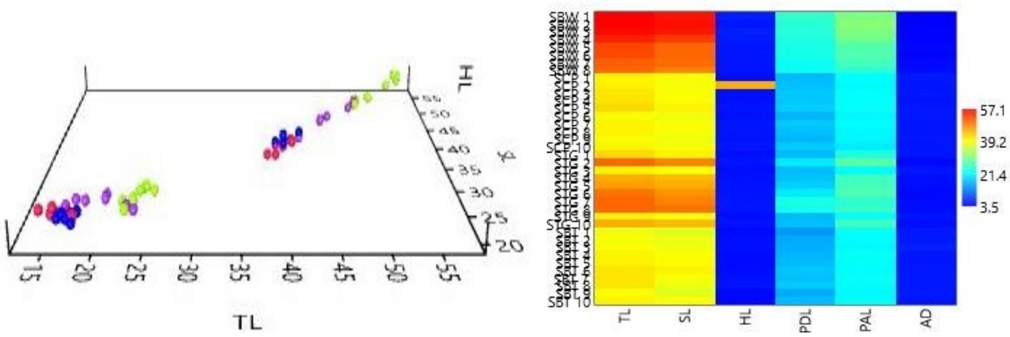

Figure 5. 3D plots (a), and matrix plots of intraspecies morphometric diversity of *Anguilla bicolor* eel (b). Descriptions: Pandeglang Regency ●, Cilacap Regency ●, Tulungagung Regency ●, Banyuwangi Regency, TL (total length), SL (standard length), HL (head length), PDL (pre-dorsal length), PAL (pre-anal length), AD (ano-dorsal).

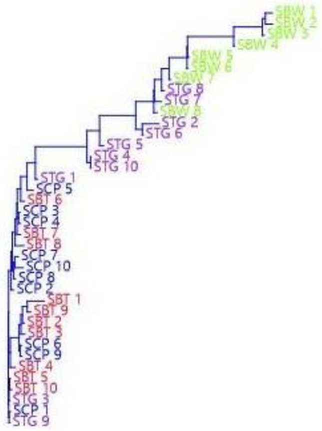

Figure 6. Diversity of morphometric using Neighbor Joining intraspecies *Anguilla bicolor* eel. Descriptions: SBT (Banten sample), SCP (Cilacap sample), STG (Tulungagung sample), SBW (Banyuwangi sample).

Table 2. Morphometric measurements of *Anguilla bicolor* eel.

Characteristics	Banten (N=8)	Tulungagung (N=10)	Cilacap (N=10)	Banyuwangi (N=10)	F Value
Total Length	38-41	40-50	38-41	50-57,1	55,648
	53,76±2,97	40,20±0,91	45,70±4,03	39,80±1,03	
Standard Length	37-40	39-49	38-40	48-56	56,614
	38,40±0,96	44,30±3,83	38,85±0,58	52,25±3,25	
Head Length	4-4,8	4,4-5	4,5-5,1	4,9-5,7	16,801
	4,31±0,28	4,77±0,24	4,75±0,25	5,2±0,29	
Pre-dorsal	14-17,7	16,5-23,4	16-18	22-25	36,419
Length	16,21±1,10	18,76±2,57	17,080,68	23,37±0,97	
Pre-anal Length	21-22	21,3-28	20,2-21,8	24,5-31	38,427
	21,46±0,40	24,9±2,24	21,16 ± 0,45	28,21±2,30	
Ano-dorsal Length	2-2,1	2-2,1	2-2,1	2-2,1	0,704
	2,06±0,09	2,03±0,04	2,04±0,06	2,06±0,05	

Several characteristics that can distinguish *A. bicolor* species significantly are based on the calculated F value which is greater than the F table ($p < 0.05$). In the one-way ANOVA test results, the significance value is also presented in the table. The following is a table of data for the results of measuring morphometric parameters. Based on differences in specific characters, namely the ratio of pre-anal and pre-dorsal lengths, *A. bicolor* eels are grouped into short fin types. Shortfin with dorsal fin shorter than anal fin, Banten with PDL 14-17.7 cm shorter than PAL 21-22 cm ; Cilacap PDL 16.5-23.4 cm < PAL 20.2-21.8 cm; Tulungagung PDL 16.5-23.4 cm < PAL 21.3-28 cm; Banyuwangi PDL 22-25 cm < PAL 24.5-31 cm. This is in accordance with the statement of Sugianti *et al*. (2020) on *A. bicolor* eel seeds found in the Cikaso River had a morphometric equation of PD value 1.4-1.9 cm < PA value 1.7-2.1 cm with AD value 0.2-0.4 cm. The results of the PCA matrix show that AD produces low diversity values so that it can be used as a reference for the character of A. bicolor eels. The AD value obtained from the results of the study was 3.5% in accordance with Watanabe *et al.* (2004) research, which was 0% - 3.7%.

The morphometric proximity is also shown through the Neighbor-Joining Cluster in Figure 6. for the identified eel species. Species of *A. bicolor* seen from four districts namely Pandeglang, Cilacap District, Tulungagung District, and Banyuwangi District are still closely related between populations. The Neighbor-Joining sample from Banyuwangi Regency appears in several clades in the clade sample from

Tulungagung Regency. Several sample clades from Tulungagung Regency also appeared in clades from Cilacap Regency and Pandeglang Regency. Samples from Pandeglang Regency also appear in the Cilacap Regency clade. This was probably because the *A. bicolor* eel population was randomly distributed in the four locations. As is known, eels have a wide distribution in Indonesian waters. In addition, eels have high mobility for spawning randomly, so that between eel populations are still closely related (Wilujeng *et al*., 2018).

Meristic Analysis

Meristic measurement is to count the number of eel vertebrae. Meristic analysis was carried out using the one way ANOVA test by calculating the F value for each meristic parameter tested referring to Fahmi (2015). The following is Table 3 of the meristic measurement results of *A. bicolor* eels.

Table 3. Meristic measurements of *Anguilla bicolor* eel.

Characteristics	Banten (N=10)	Tulungagung (N=10)	Cilacap (N=10)	Banyuwangi (N=8)	F Value
Total Vertebrae	105-110 2,78±0,10	106-110 2,44±0,22	105-110 2,75±0,07	107-111 2,08±0,15	41,671
Pre-dorsal Vertebrae	32-36 0,71±0,02	32-36 0,77±0,09	32-36 0,87±0,05	33-36 0,66±0,06	20,650
Pre-anal Vertebrae	32-39 0,80±0,25	32-29 0,82±0,07	32-36 0,87±0,03	32-38 0,66±0,58	3,46
Ano-dorsal Vertebrae	2-3 0,06±0,12	2-3 0,55±0,007	2-3 0,05±0,12	2-3 0,04±0,008	3,061

Meristic eel measurements using SPPS 25 software were analyzed using one way ANOVA and PCA analysis using PAST 4.07 software. The number of meristic parameters calculated is 4 parameters. The results of PCA analysis, among others, show the distribution pattern of intraspecies meristic diversity in *A. bicolor* eels which is presented in Figure 7a. The samples measured were 38 samples taken from the smallest number of samples to determine the pattern of species morphometric diversity. Another result of using this software is to find out the most different meristic parameters through the matrix shown in Figure 3b. Based on the meristic data, it can be seen the intraspecies kinship pattern from the sampling locations in several sub-districts using Neighbor Joining Clustering on PAST 4.07b software as shown in Figure 8.

The results of the meristic analysis in Figure 7a show a 3D distribution of intraspecies diversity. Based on the plot of the four samples, shows that the points spread and converge between populations. It is suspected that the *A. bicolor* sample still has a close relationship between the four populations. This is because the colored dots or plots between populations are still gathered together. If the points or plots between populations are spread out there is variation in eel species. Meristic diversity is also seen in the matrix in Figure 7b. Based on the color pattern, it can be seen that the highest meristic diversity is shown in the TV (Total Vertebrae), Banten 105-110, Tulungagung 106-110, Cilacap 105-110, Banyuwangi 107-111. The high diversity of eels in the total vertebrae is due to the varying length of the body. The fertility productivity of the waters where eels migrate affects the growth of eels in the form of length and weight (Wilujeng *et al*., 2018).

This is supported by research by Muthmainnah *et al*. (2015) *A. bicolor* eels between Cilacap and Bengkulu have variations in length and weight which are influenced by water conditions. The length-weight relationship between species can be influenced by individual fitness conditions. Feed availability and growth are dynamic and varied. Eels within one species can have different conditions between individuals and populations that provide variations in length relationships. The lowest meristic diversity shown in ADV (Anodorsal Vertebrae) from four locations is 2-3. This can be used as a special reference that the characteristics of *A. bicolor* have short fins and ensure that the eel seeds survive when migrating.

Observations of the variability of the distribution of ADV numbers strengthen the possibility of more than one species (Kusuma *et al*., 2016). The larvae will metamorphose into eel fry and migrate to the coast. This is adjusted to the eel seeds that enter the estuary, some of which continue their path to the upper reaches of the river. Eels change pigment shortly after entering estuaries and rivers. Eels that enter fresh water will spend most of their lives before turning into young eels and adult eels (Cresci *et al*., 2019).

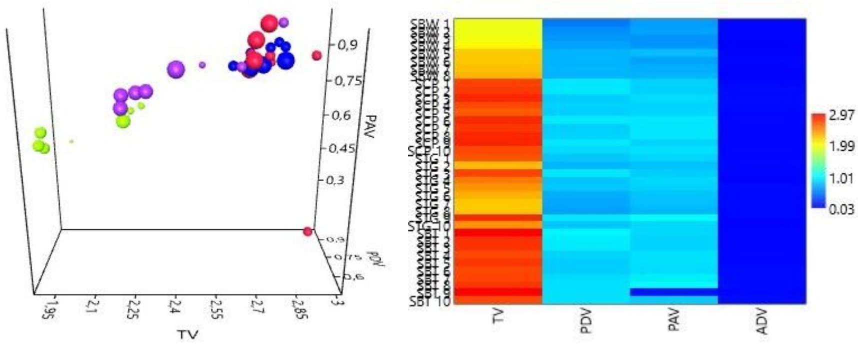

Figure 7. Pattern of intraspecies meristic diversity of *Anguilla bicolor* eel. Descriptions: Pandeglang Regency ●, Cilacap Regency ●, Tulungagung Regency ●, Banyuwangi Regency , TV (total spine), PDV (total pre-dorsal spine), PAV (total pre-anal spine), ADV (total anodorsal spine).

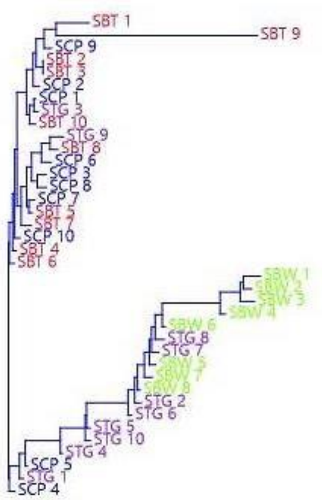

Figure 8. Meristic Diversity using Neighbor Joining intraspecies eel *Anguilla bicolor*. Description: SBT (Banten sample), SCP (Cilacap sample), STG (Tulungagung sample), SBW (Banyuwangi sample).

The results of the cluster analysis are presented in the form of a dendrogram with Euclidean distance that describes the distance of kinship. Meristically close kinship relationships are also shown through the Neighbor Joining Cluster in Figure 8 for the identified eel species. Species of *A. bicolor* can be seen from four locations, namely Pandeglang Regency, Cilacap Regency, Tulungagung Regency, and Banyuwangi Regency which are still closely related between populations. The

Neighbor Joining sample from Banyuwangi Regency appears in several clades in the clade sample from Tulungagung Regency. Several sample clades from Tulungagung Regency also appeared in clades from Cilacap Regency and Pandeglang Regency. Samples from Pandeglang Regency also appear in the Cilacap Regency clade. This may also be due to the random distribution of the *A. bicolor* eel population at the four sites.

The number of segments that are commonly used in larval-size fish, the number of segments increases with the increasing latitude of live eels. Generally, the number of spines eels is 100–119 (Fahmi and Hirnawati, 2010). Based on the research that has been done, the total spine average of eels from Pandeglang Regency (106.9±1.96), Cilacap Regency (107±1.94), Tulungagung Regency (107.6±1.57), Banyuwangi Regency (108.7±1.48). The data presented show the relationship between the number of total vertebrae. Watanabe *et al.* (2005) in the phylogenetic tree diagram between populations has similarities by describing the number of *A. bicolor* vertebrae as much as 105–111 pieces.

CONCLUSION

Based on morphology, morphometric, and meristic eel between populations have similarities. This inter-population eel is an intraspecies *A. bicolor*. TL morphometrics have different length variations between populations. However, it does not affect PDL, PAL and ADL. Meristic TV has a different number of variations between populations and has no effect on PDV, PAV, ADV. The influence of water fertility that causes length and weight to produce varied morphometric and meristic. Pre-Dorsal, Pre-Anal, Anodorsal have intraspecies similarities which are the key features of short-finned *A. bicolor* eels. This is supported by eels, which are fish that have high mobility and are widely distributed so that the populations are still closely related. and p38 MAPK indicate stressful fish.

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