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Morphological characteristics and population structure of Marbled Eel (*Anguilla marmorata*) in Thua Thien Hue, Vietnam

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ABSTRACT

The Marbled Eel (*Anguilla marmorata*) has a widespread distribution with a complex migration life cycle. There were 350 individuals of sizes from 120 to 1137 mm were analysed on characteristic morphology at 4 developing stages: juvenile, fingerling, pre-adulthood and adulthood. Morphological indicators: total length (TL), head length (HL), dorsal fin origin (PD), anal fin origin (PA), distance from dorsal to anal fin (AD), distance from pectoral fins to dorsal fin (PDH), body length (TR), eye diameter (E), tail length (T) and eye distance (IO), colour characteristics and 10 characters were standardized against the LT or LH ratios determine 95.664% of morphological differences between individuals in the study population based on principal component analysis and hierarchical cluster analysis tools. Phylogenetic tree with five morphological clusters with distance > 2.0 built a high genetic diversity. The difference of clusters is related to the morphology at different stages. Cluster 1 corresponds to the morphological characteristics at the fingerling stage (TL = 260–552.0 mm); cluster 3 is the pre-adult stage (TL = 324.6–533.9); cluster 4 is the juvenile stage (TL = 120.0–255.0); and clusters 2 and 5 for pre-adult and adult at reproductive migration (TL > 400).

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1. Introduction

Marbled Eel (*Anguilla marmorata*) of the genus *Anguilla* Schrank (1798) includes 16 species and 3 subspecies which have been identified (Ege 1939; Watanabe et al. 2004; Watanabe et al. 2005; Watanabe et al. 2006), *A. marmorata* is a large species (Jacoby and Gollock 2014), the body can reach 2 m in length (Pike et al. 2019). They have a complex migration life cycle between oceans and inland water bodies (Arai 2016) with distances from a few hundred to thousands of kilometres (Arai (2014)). They are found in freshwater, brackish and saltwater environments (Lin et al. 2012). *A. marmorata* breeds in the sea and grows to adulthood in rivers, estuaries or the sea (Tsukamoto et al. 2011). *Anguilla* eels spawn at a depth of about 200 m during the new moon. The fertilized eggs slowly float to the surface (Tsukamoto 2009). About 24 h later, they hatch into tiny pre-larvae with 5 mm of total length, then transform into leptocephalus larva (Arai et al. 2001). Leptocephalus grows during migration along ocean currents to become glass eels when entering freshwater, estuarine or coastal growth habitats where they grow as yellow eels (Hagihara et al. 2012). The growth period (yellow eel) of Marbled Eel can be as short as 2–3 years in warm breeding environments, while as long as 6–20 years or more in northern latitudes (Pike et al. 2019). And then, they transform into silver eels that start migration to the ocean for spawning (Tesch and Thorpe 2003; Tsukamoto 2009; Pike et al. 2019). The metamorphosis of *Anguilla* eels during complex migrations (Arai and

Chino 2018) and adaptation to habitats (Arai and Abdul Kadir 2017) has prompted studies concerning the morphological characteristics of the eels through different growth stages.

In Vietnam, five species of the *Anguilla* genus were identified, namely *A. nebulosa* (McClelland, 1844), *A. japonica* (Temminck & Schlegel, 1984), *A. marmorata* (Quoy & Gaimard, 1824), *A. celebensis* (Kaup, 1856) and *A. bicolor pacifica* (Schmidt, 1928)(Nguyen 2001). In the Central region, there are three species: *A. marmorata*, *A. bicolor pacifica* and *A. japonica* (Nguyen et al. 2018). Thua Thien Hue located to the south of the North Central region, Vietnam, has been identified with the presence of two species of the *Anguilla*: Marbled Eel (*A. marmorata*) and Ebony Eel (*A. bicolor*) (Kieu et al. 2020) of which the Marbled Eel is more popular related to time of occurrence and productive (Nguyen 2015). In fact, Marbled Eel are being exploited to serve both commercial needs as well as fingerling needs of farms (Kieu and Vo 2015). The pressures of environmental changes during migration and factors affecting their natural habitats, such as environmental pollution, construction of lakes, dams and hydroelectricity have led to an increase in the risk of resource decline. A morphological classification system based on the morphology of the Eels was conducted by Ege (1939), Watanabe (2003), Watanabe et al. (2004, 2005). Some information on the developmental stages of Marbled Eel was provided (Leander et al. 2012; Hagihara et al. 2012), morphological indices were also used to analyse the population structure and phylogenetics of the species (Watanabe et al. 2009). However, in Vietnam,

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information about Marbled Eel is still very limited, these studies only determine the species composition, there are no in-depth studies on the change in morphological characteristics of Eel related to their stages of growth. Recently, the analysis of the genetic structure of the Marbled Eel population in Thua Thien Hue by molecular markers has shown its close relationship with the Eel populations in the Indo-Pacific region (Kieu and Nguyen 2020; Arai et al. 2020), genetic variants were also found here (Arai and Hussein 2021). Therefore, Thua Thien Hue, Vietnam should be considered as an important conservation unit for the species in the region. The study was conducted with the aim of analysing the change and diversity of morphological characteristics of *A. marmorata* in Thua Thien Hue related to the growth stages of their life cycle, therefore, providing information on the adaptive characteristics of the species in Thua Thien Hue, Vietnam and as a basis for growth strategies for species development and conservation.

2. Material and methods

All morphological indicators were observed and measured on all 350 individual eels (*A. marmorata* Quoy & Gaimard, 1824) with sizes from 3.0 to 4500 g (120.0–1136.9 mm) collected from water bodies in Thua Thien Hue province (Figure 1 and Table 1) during the period from September 2017 to August 2018. Based on the examination of the colour of the pectoral fins and the body colouration according to the metal index stages described by Okamura et al. (2007), and Hagihara et al.

(2012) and their migration, the growth stages of eels were determined to four stages: juvenile, fingerling, pre-adulthood and adulthood. The number of specimens of each period was collected based on the actual number in the study areas to show their distribution in the wild (Table 3).

The colour and appearance of the *A. marmorata* were observed and classified in the field. Eleven out of 16 indicators were measured using the left side of the body or the dorsal side of the head as described by Watanabe et al. (2006), total length (TL), head length (LH), dorsal fin origin (PD), anal fin origin (PA), eye diameter (E) and eye distance (IO) were measured with a ruler with an accuracy of 1.0 mm and a calliper with an accuracy of 0.01 mm. Dorsal and anal (AD) distance, pectoral to dorsal fin distance (PDH), caudal fin length (T), body length (TR) are calculated from PA, PD, LH and LT. A total of 10 characters were standardized against the LT or LH ratios for comparison between the *A. marmorata* sample locations (Ege 1939; Watanabe et al. 2009). The collected data were processed according to the biological statistics method on Excell 2016.

SPSS Ver.20.0 software was used to analyse morphological data and morphological differences between groups with principal component analysis (PCA) and hierarchical cluster analysis (HCA) tools. PCA was used to describe and evaluate the variability of morphological features. A correlation matrix was used in the analysis of morphological features. Graphical depictions of specimen locations along the vector were surveyed for separate groups. The PCA technique derives the eigenvalues and variances from the correlation matrix of the original

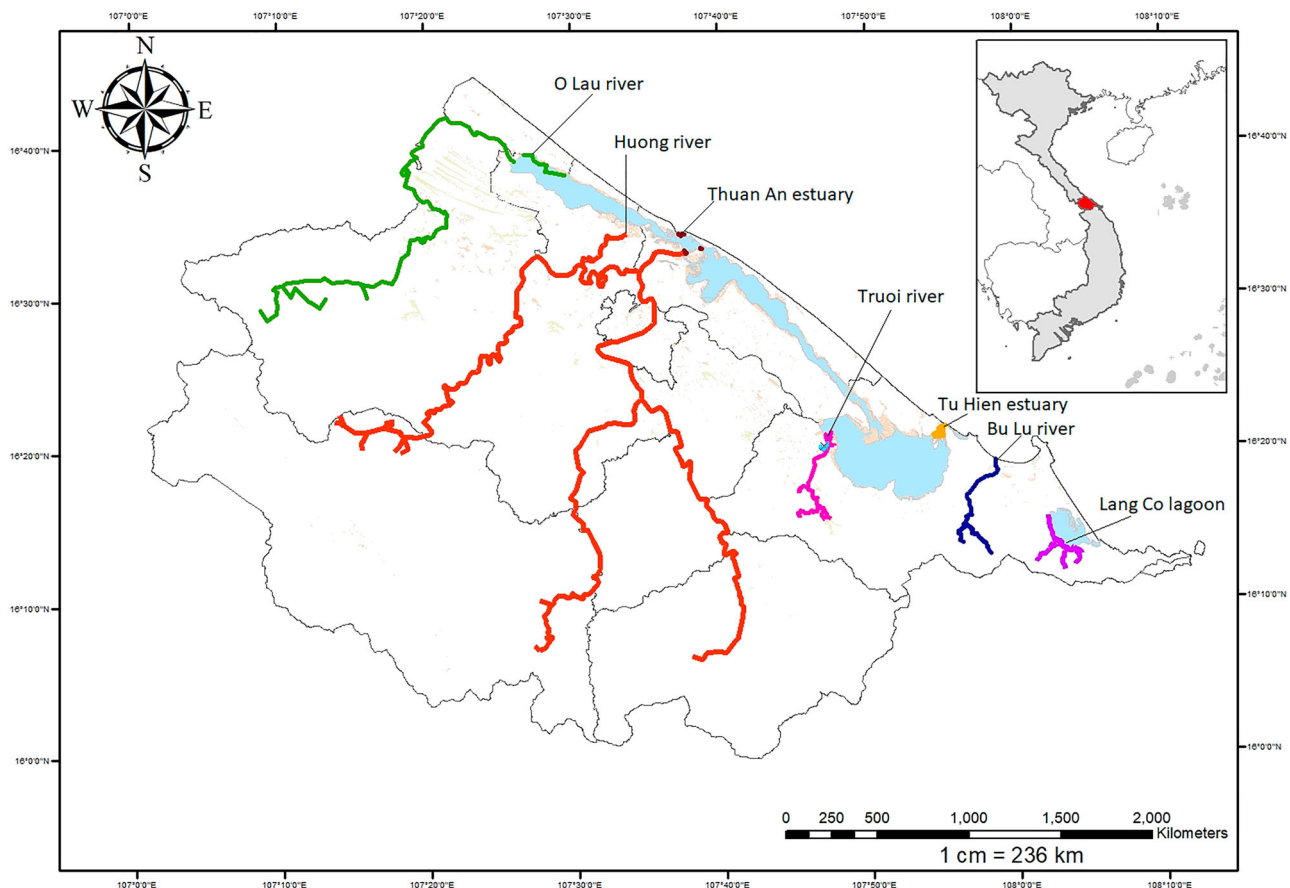


Figure 1. The geographic coordinates of sample location.

Table 1. Number of Marbled Eel samples collected from study sites.

No	Sample location	Amount	Ratio %
1	O Lau River (OL)	34	9.6
2	Huong river (SH)	105	30.0
3	Truoi river (STR)	49	14.0
4	Bu Lu river (SBL)	57	16.3
5	Lang Co (LC)	45	12.9
6	Thuan An (TA)	30	8.6
7	Tu Hien (TH)	30	8.6
Total		350	100

variables. The factors of the main components are performed by the factor extraction method. The main factor rotation is performed according to the original rotation method of factors to minimize the number of variables with large coefficients at the same factor (Vanimax with normal Kaiser) (Nguyen et al. 2017). In this study, all the relationships among parameters with correlation coefficients greater than 30% contribute to determining the characteristic morphological parameters of the study population. This method was chosen because it does not require any prior assumptions about the taxonomic identity of the specimen (Reyment and Joreskog 1993). HCA by Ward's method using the criterion of minimum variance was used to evaluate the relationship between study individuals. The nearest sequence algorithm is used to determine groups, which are temporally proportional to the size of the input distance matrix and spatially linear to the number of points analysed. The initial cluster distance in Ward's method of least variance is defined as the squared Euclidean distance between the points: $d_{ij} = d(\{X_i\}, \{X_j\}) = ||X_i - X_j||^2$, in which, X_i and X_j are the research points; d_{ij} is the square of the Eculid distance between the points X_i and X_j (Ward 1963).

3. Results

3.1. Description of morphological characteristics of the Marbled Eel *A. marmorata*

By observing 350 Marbled Eels with size 120–1136.9 mm ($W = 3.0$ – 4500.0 g) distributed in water bodies in Thua Thien Hue, we found that fish have the morphological characteristics as follows: long cylindrical body, tapering off afterward, smooth and scaleless. Dorsal and anal fins are soft, elongated and banded connected to caudal fin, without spines. Pectoral fins are small, almost circular, the number of rays is 18, the colour of the pectoral fins changes from almost transparent pale yellow to black, black to red according to development stages and habitat conditions. Body colour of the adult Marbled Eels is yellow with various spots from greenish-brown to black on the back, the belly is milky white close to

the natural colour, the back of baby eels is slightly grey to yellow. The spots on the Marbled Eel's back appear more and more clearly along with the development of the fish. The results in Table 2 show the values of the observed morphological indicators. Accordingly, the ratio of the indicators with the body length and head length is highly stable, including HL/TL: 11.2–16.6%, PD/TL: 21.2–29.3%, PA / TL: 37.5–48.1%, TR/TL: 22.7–34.3%, AD/TL: 11.9–21.3%, PDH/TL: 7.3–15.5%, T/TL: 51.9–62.5%, E/HL: 5, 9–11.9 and IO/HL: 16.0–25.0.

The change in morphology of the Marbled Eel occurs strongly when there is habitat change during the migration. In the glass eel stage when the Eels migrate from the sea to inland freshwater, their colour and morphology change rapidly. They are light yellow, brown banding on their backs, small spots, unspecified spots, almost transparent pale yellow fins, size from 120.0 to 228.5 mm (weight: 3.0–20.7 g) at this juvenile eel period accounting for 17.4% of the number of collected populations. After that, body colour of the Marbled Eel changes to yellow or grey with dark grey spots on back, yellowish-grey or black yellow on fins, and white belly. This stage is called the yellow eel. In which, fish with sizes between 187.0 and 410.0 mm with the yellow body colour, clear grey spots, yellow fins are often used as seed in commercial farms (29.7%) and called Fingerling eels; fish sized TL > 387 mm ($W > 143.5$ g) with brown back, clear spots with grey colour, grey-white belly, yellow-brown fins, can be used for food, called pre-adults (38.6%). After that, body colour of the Marbled Eel changes to yellow or grey with dark grey spots on back, yellowish-grey or black yellow on fins, and white belly. At this stage, they are called adult Marbled Eel (silver eel) accounting for 14.3% of the observed population (Table 3).

3.2. The Eel population structure in Thua Thien Hue based on morphological indicators

Key component analysis was used to evaluate the variability and contribution of traits to the degree of variation in studied individuals. The first five principal components (PC) have an eigenvalue greater than 1.0 and related parameters are shown in Table 4. The PC 1 account for 56.38% of the difference, including the morphological indicators: total length (TL), head length (HL), dorsal fin origin (PD), anal fin origin (PA), distance from dorsal to anal fin (AD), distance from pectoral fins to dorsal fins (PDH), body length (TR), eye diameter (E), T (tail length) and eye distance (IO) and colour characteristics, appearance. The PC 2 accounts for 18.72% of the difference, including the indicators PD/TL, PA/TL, TR/TL, PDH/TL and T/TL. The PC 3

Table 2. The body colour of the Marbled Eel in the population in Thua Thien Hue.

Characters	Mean ± SD	Min–Max	Characters	Mean ± SD	Min–Max
W (g)	470.5 ± 788.19	3.0–4500.0	TL (mm)	452.9 ± 231.85	120.0–1136.9
HL (mm)	62.3 ± 27.25	16.0–160.0	HL/TL (%)	13.9 ± 0.64	11.2–16.6
PD (mm)	117.8 ± 51.71	29.1–315.0	PD/TL (%)	26.2 ± 1.35	21.2–29.2
PA (mm)	190.6 ± 83.14	51.7–500.0	PA/TL (%)	42.4 ± 1.97	37.5–48.1
TR (mm)	128.3 ± 56.42	32.7–340.0	TR/TL (%)	28.5 ± 2.04	22.7–34.3
AD (mm)	72.8 ± 32.21	18.5–190.0	AD/TL (%)	16.2 ± 1.57	11.9–21.3
PDH (mm)	55.5 ± 25.21	11.7–160.0	PDH/TL (%)	12.3 ± 1.43	7.3–15.5
T (mm)	257.7 ± 110.85	66.3–614.6	T/TL (%)	57.6 ± 1.97	51.9–62.5
E (mm)	6.0 ± 2.90	1.4–18.0	E/HL (%)	9.4 ± 1.27	5.9–11.9
IO (mm)	13.5 ± 6.13	3.2–35.0	IO/HL (%)	21.0 ± 1.86	16.0–25.0

Table 3. The values of morphological indicators of the Marbled Eel distributed in Thua Thien Hue.

Groups	Characters	W (g)		TL (mm)		Number (n)	Ration (%)
		Mean ± SD	Min–Max	Mean ± SD	Min–Max		
Juvenile	The body is yellow, the back is brown, the spots are not clear; The fins are pale yellow almost transparent.	10.9 ± 4.91	13.0–20.7	180.1 ± 30.58	120.0–228.5	61	17.4
Fingerlings	The body is yellow, with clear grey spots; The fins are yellow.	71.5 ± 39.47	13.4–148.8	313.9 ± 60.81	187.0–410.0	104	29.7
Pre-adulthood	The back is brown, the spots are clear, the colour is grey, the belly is grey -white; the fins are yellow-brown.	657.4 ± 805.41	143.5–4,500.0	574.7 ± 170.61	387.0–1136.9	135	38.6
Adulthood	The back is golden brown, with black spots, yellow belly and tail; The fins are black or red	1,310.0 ± 1,101.29	165.4–4,500.0	726.4 ± 196.19	410.0–1136.9	50	14.3
Total					350	100	

Table 4. The PCA of the morphological characteristics of *A. marmorata*.

Characters	PC1	PC2	PC3	PC4	PC5
TL (mm)	0.954				
HL (mm)	0.947				
PD (mm)	0.973				
PA (mm)	0.982				
TR (mm)	0.986				
AD (mm)	0.965				
PDH (mm)	0.963				
T (mm)	0.920				
HL/TL					0.676
PD/TL		0.382			
PA/TL		0.767			
TR/TL		0.839			
AD/TL				0.526	
PDH/TL		0.474			
T/TL		0.767			
IO (mm)	0.949				
E (mm)	0.926				
E/HL			0.610		
IO/HL			0.555		
Morphological characteristics	0.772				
Eigen value	11.839	3.930	1.718	1.597	1.005
Variability (%)	56.378	18.716	8.181	7.603	4.786
Cumulative (%)	56.378	75.094	83.275	90.878	95.664

accounts for 8.18% including two indicators: the ratios of eye diameter to head length (E/HL) and eye distance to head length (IO/HL). The PC 4 and PC 5 account for 7.60% and 4.78%, respectively, with the remaining two indicators including AD/TL and HL/TL.

Hierarchical cluster analysis was used to determine the differences and genetic relationships among 350 individuals in the Marbled Eel population collected in Thua Thien Hue. The results show that the genetic similarity coefficients based on the Euclidean distance range from 0.1 to 25.0, indicating that the Marbled Eel’s samples have a high genetic diversity (Figure 2). Accordingly, at the position where the genetic distance is 25.0, the phylogenetic tree is divided into two main branches: branch I and branch II. In which, where the genetic distance is 3.0, branch I is divided into two cluster (cluster 4 and cluster 1); Branch II consists of 2 sub-branch (IIa and IIb) that are isolated at the genetic distance of 9.0. In which, sub-branch IIb, is divided into two subclusters (cluster 2 and cluster 5) with a genetic distance of 2.0. Cluster 3 is located on sub-branch IIa of branch II on phylogenetic tree. Morphological characteristics of the 5 clusters are shown in Table 5.

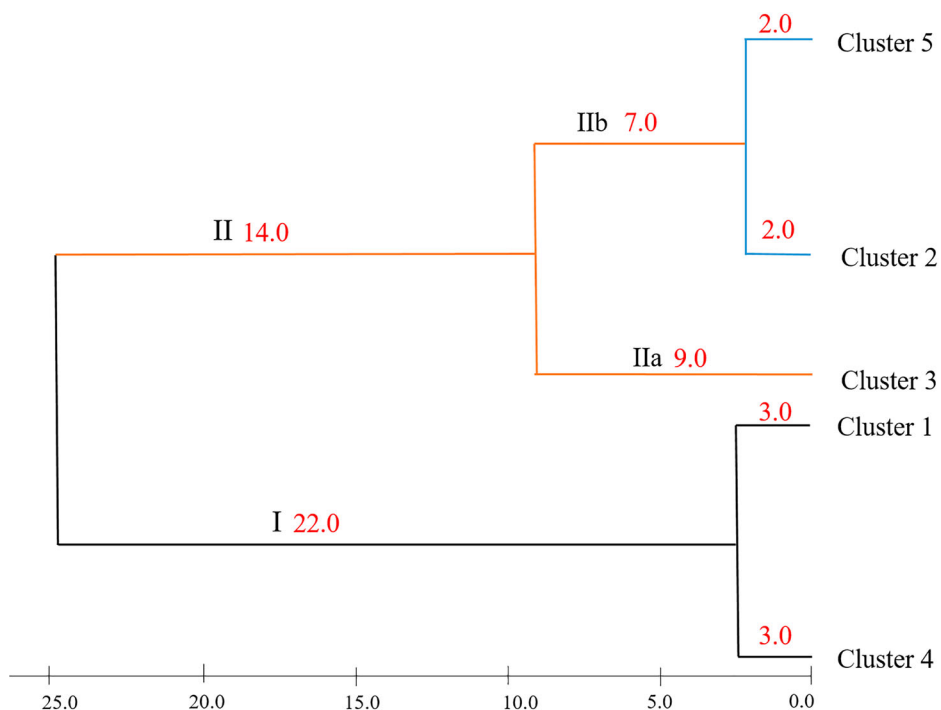


Figure 2. Phylogenetic tree of Marbled Eel population in Thua Thien Hue based on Euclidean distance using Ward method.

Table 5. Morphological characteristics of *A. marmorata* in five clusters using Ward method based on Euclidean distance.

Characters Mean ± SD (Min–Max)	Cluster				
	1 (n = 76)	2 (n = 70)	3 (n = 83)	4 (n = 85)	5 (n = 36)
W (g)	160.8 ± 93.30 (29.0–469.5)	731.6 ± 297.01 (374.9–1540.3)	195.4 ± 81.84 (52.3–369.0)	14.8 ± 8.05 (3.0–34.0)	2,406.1 ± 1025.45 (143.5–4500.0)
TL (mm)	401.3 ± 74.21 (260.0–552.0)	647.3 ± 74.80 (540.0–814.0)	435.5 ± 56.73 (324.6–532.9)	193.4 ± 35.04 (120.0–255.0)	915.5 ± 160.81 (407.0–1136.9)
HL (mm)	65.0 ± 16.31 (35.0–91.2)	82.7 ± 10.83 (58.0–122.4)	78.9 ± 4.18 (64.0–91.2)	26.7 ± 5.36 (16.0–39.0)	94.4 ± 29.99 (45.0–160.0)
PD (mm)	124.1 ± 30.89 (63.4–175.0)	154.7 ± 19.70 (112.0–210.6)	150.6 ± 7.37 (135.7–175.0)	49.7 ± 9.46 (29.1–68.0)	179.1 ± 57.83 (90.0–315.0)
PA (mm)	199.5 ± 48.74 (112.0–265.6)	254.3 ± 30.50 (188.0–341.3)	241.0 ± 10.99 (221.5–265.6)	80.1 ± 15.05 (51.7–106.0)	288.3 ± 88.91 (150.0–500.0)
TR (mm)	134.5 ± 33.28 (71.9–182.6)	171.6 ± 22.46 (130.0–232.4)	162.1 ± 10.92 (140.1–182.6)	53.5 ± 10.05 (32.7–72.0)	193.8 ± 59.62 (105.0–340.0)
AD (mm)	75.4 ± 18.95 (38.9–112.4)	99.6 ± 14.30 (70.5–140.0)	90.4 ± 8.05 (70.1–112.4)	30.4 ± 6.24 (18.5–43.1)	109.1 ± 32.37 (60.0–190.0)
PDH (mm)	59.1 ± 15.88 (22.0–90.0)	72.0 ± 11.47 (45.4–103.2)	71.7 ± 8.13 (58.9–90.0)	23.0 ± 4.66 (11.7–33.0)	84.7 ± 29.08 (45.0–160.0)
T (mm)	268.2 ± 67.44 (147.0–391.4)	338.4 ± 40.37 (240.0–488.3)	327.0 ± 16.61 (275.0–391.4)	113.3 ± 20.48 (66.3–158.0)	395.6 ± 118.56 (192.0–614.6)
HL/TL (%)	13.9 ± 0.68 (11.2–16.6)	13.9 ± 0.65 (13.0–15.9)	13.9 ± 0.69 (11.2–15.1)	13.8 ± 0.61 (11.2–15.9)	13.8 ± 0.43 (13.0–14.8)
PD/TL (%)	26.5 ± 1.26 (23.1–29.2)	26.1 ± 1.41 (21.2–28.7)	26.5 ± 1.24 (23.3–29.2)	25.7 ± 1.26 (21.2–28.6)	26.1 ± 1.50 (21.2–29.2)
PA/TL (%)	42.7 ± 1.85 (37.5–47.6)	42.9 ± 2.37 (37.8–48.1)	42.4 ± 1.84 (38.9–45.8)	41.4 ± 1.49 (38.0–44.7)	42.2 ± 1.87 (38.0–46.3)
TR/TL (%)	28.8 ± 1.92 (22.7–33.9)	29.0 ± 2.50 (23.1–34.3)	28.5 ± 1.89 (24.6–32.0)	27.7 ± 1.59 (23.7–31.1)	28.4 ± 1.93 (23.7–31.5)
AD/TL (%)	16.2 ± 1.47 (11.9–20.4)	16.8 ± 1.87 (12.4–21.3)	15.9 ± 1.40 (12.3–19.3)	15.7 ± 1.47 (12.3–20.4)	16.0 ± 1.26 (13.7–18.4)
PDH/TL (%)	12.6 ± 1.42 (7.3–15.5)	12.2 ± 1.42 (8.0–14.5)	12.6 ± 1.42 (9.4–15.5)	11.9 ± 1.32 (8.0–15.5)	12.4 ± 1.47 (8.0–14.8)
T/TL (%)	57.3 ± 1.85 (52.4–62.5)	57.1 ± 2.37 (51.9–62.2)	57.6 ± 1.84 (54.2–61.1)	58.6 ± 1.49 (55.3–62.0)	57.8 ± 1.87 (53.7–62.0)
E (mm)	14.1 ± 4.15 (7.0–20.2)	17.9 ± 2.46 (13.0–27.6)	17.6 ± 1.52 (13.8–20.2)	5.7 ± 1.36 (3.2–8.8)	20.7–6.26 (9.0–35.0)
IO (mm)	6.2 ± 2.00 (2.6–9.9)	8.2 ± 1.55 (5.0–13.1)	7.9 ± 1.06 (4.9–9.9)	2.5 ± 0.60 (1.4–4.0)	9.3 ± 3.16 (4.0–18.0)
E/HL (%)	9.2 ± 1.34 (5.9–11.9)	9.5 ± 1.27 (6.1–11.7)	9.5 ± 1.23 (5.9–11.9)	9.4 ± 1.17 (7.3–11.5)	9.5 ± 1.16 (7.7–11.6)
IO/HL (%)	20.9 ± 1.67 (16.7–24.2)	20.9 ± 1.67 (17.6–24.1)	21.2 ± 1.61 (16.7–24.2)	21.2 ± 2.20 (16.0–25.0)	21.2 ± 2.10 (17.6–24.2)
Outward appearance	Yellow, clear mottled colour, yellow pectoral fins (69.7%); brown back, clear mottled greyish-white abdomen, brownish-yellow fins (23.7%) and yellow-brown back, with black spots, yellow abdomen, black fins or red-black (6.6%)	Brown back, clear mottled colour, grey and white abdomen: 65.7%; brown yellow back, black spots, yellow abdomen and tail, black or red fins: 34.3%	Yellow, clear mottled colour, yellow pectoral fins: 32.5%; brown back, clear mottled, grey and white abdomen: 62.7%; brown yellow back, black spots, yellow abdomen and tail, black or red fins: 4.8%	Yellow, not clear mottled colour, pectoral fins are pale yellow, almost transparent (71.8%) and Eel is yellow, clear mottled colour, yellow pectoral fins (28.2%)	Brown back, clear mottled colour, grey and white abdomen: (52.8%), brownish-yellow back, black spots, yellow abdomen and tail, black or red fins: (47.2%)

4. Discussions

The first comprehensive morphological descriptions of *Anguilla* eels were made by Kaup (1856), Günther (1870) and Ege (1939). According to the description of Ege (1939), the *Anguilla* genus is divided into 16 species and 3 subspecies. According to Watanabe (2003) and Watanabe et al. (2004), Marbled Eel belongs to group 2 the classification systems with variegated body marking, narrow maxillary bands of teeth. At the dermal pigmented vitreous stage on the caudal fin of *A. marmorata* of group 3 together with *A. luzonensis* or *A. celebesensis*, there is a large patch of diffuse melanophores on the caudal bud, $ADL/\% TL > 13$ (13.27–20.35) (Leander et al. 2012). Twenty-one morphological characteristics of 686 specimens (LT = 115–1,069 mm) were collected from 13 representatives within the geographic distribution areas of *A. marmorata* in the Pacific and Indian Oceans analysed by Watanabe et al. (2009). The morphological values in Table 2 of Marbled Eel collected in Thua Thien Hue are similar to these studies.

Based on characterization by the colourations of pectoral fins and ventral skin, Okamura et al. (2007) and Hagihara et al. (2012), described four stages for *A. japonica* and *A. marmorata* as follows: Y1 – yellow eel without a metallic at the base of pectoral fins, Y2 – late yellow eel with a metallic hue at the base of the pectoral fins but without melanization at the tip of pectoral fins, S1 – silver eel with complete melanization at the tip of pectoral fins but without full pigmented belly in black or dark brown, and S2 – late silver eel with black or dark brown belly. In this study, 4 stages have been proposed for *A. marmorata* population in Thua Thien Hue, Vietnam, namely: juvenile eel, fingerling eel, yellow eel (pre-adulthood) and silver eel (adulthood) (Table 3). In which, fingerling eel, pre-adulthood and adulthood stages of Marbled Eel population in Thua Thien Hue, Vietnam (Table 3) are similar to Y1, Y2 and S1 described by Hagihara et al. (2012).

During the migration, living and growth of Marble Eels in Thua Thien Hue water bodies, individual adaptations through changes in body colour and morphological indicators, but characters were standardized as a proportion of TL or HL did not change significantly when fish developed through different stages. It is shown in the analysis results in Tables 2 and 3 with the value of indicators, including HL/TL, PD/TL, PA/TL, TR/TL, AD/TL, PDH/TL, T/TL, E/HL and IO/HL, show their stability with the growth of eel and do not depend on the growth stages (Tables 2 and 3). The contribution of the morphological characteristics to the differences of the PCs (Table 4) and the distribution of individuals in the clusters (Figure 2 and Table 5) indicated that there was homogeneity on these morphological characteristics related to growth stages of the Marbled Eel living in Thua Thien Hue, Vietnam. This result is consistent with PCA analysis for morphological indices to determine the degree of difference of morphological features between *A. marmorata* populations by Watanabe et al. (2009) shown that there were no clear differences in 15 proportional and 6 vertebral characters using PC analysis.

The clustering on the phylogenetic tree based on HCA was characterized by the occurrence of a majority of the Marbled Eel samples in each growth stages. Specifically, cluster 4 was characterized of juvenile (71.8%); cluster 1 characterizes

of fingerlings (67.9%); cluster 3 represents the pre-adulthood group (62.7%); Cluster 2 and cluster 5 characterizes pre-reproductive (pre-adulthood and pre-adulthood) eels. The relationships between clusters are also shown by the presence of more than one growth stage in each cluster (Figure 2 and Table 5) indicating overlap in morphological characteristics among individuals of different stages in the population. In which, cluster 3 correlated with the remaining clusters through the presence of individual eels in almost all growth stages. Cluster 4 and cluster 1 lying on the same branch had the genetic coefficient of 3.0 with the contribution of the Marbled Eel in fingerling stage (28.2% and 67.9%, respectively). Cluster 2 and cluster 5 also showed a close relationship with genetic distance of 2.0 with the presence of individuals in the two stages of pre-adulthood and adulthood. These results can be explained by slow metamorphosis between the growth stages of Marbled Eel (Hagihara et al. 2012), growth rate, migration and changes in habitat such as water temperature, surface cycle, etc. moon, wind and rain (Sudo et al. 2017), and unseasonable sexual maturity of fish of *A. marmorata* during the silver eel stage (Kita and Tachihara 2020).

Thus, the results of analysing the morphological characteristics of this study have shown a high phenotypic diversity in the structure of the Marbled Eel population distributed in Thua Thien Hue related to four growth stages: juvenile, fingerlings, pre-adulthood and adulthood. During growth and development in freshwater bodies, Marbled Eel have a slow (incomplete) metamorphosis between developmental stages. These are meaningful data for the development of research plans and strategies for conservation and development of Eel fish stocks in Thua Thien Hue, Vietnam.

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Data availability statement

All data and materials are available in the manuscript.

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