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Morphometric and Genetic Variations of Freshwater Eels (*Anguilla* spp.) in Poso River, Central Sulawesi: Implications for Conservation Strategies

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Abstract: The freshwater eel, Anguillids, is a valuable nutrition and commodity fish found in various freshwater environments. However, the world's population of Anguillids is declining because of habitat degradation, pollution, and barriers to migration, all of which are prevalent threats in freshwater ecosystems such as the Poso River in Central Sulawesi. Establishing conservation areas is one of the efforts to protect eels and their habitats, which requires information on the anguillid's morphometrics and genetics, where high morphometric and genetic variations are indicators of adaptation or evolution of the species to survive environmental changes. Therefore, the study aims to assess the morphometric and genetic variations s in the Poso River, Central Sulawesi. Samples were collected in May 2021 and August 2023 along the Poso River. Different fishing gears were used depending on the location and the eel's phase of life. 150 eel samples were used for morphometric analysis, of which 38 were selected randomly for the genetic one. Genetic diversity analysis was performed using Cytochrome c Oxidase I (COI). The study identified three species: *A. bicolor*, *A. celebesensis*, and *A. marmorata*. The key characteristic distinguishing the three species was ADL/TL ratio. Most coefficients of variation of morphometric characters of each species were above 10%, indicating medium to high variation. A total of 11 haplotypes were identified, of which six belong to *A. marmorata* and five to *A. celebesensis*. Generally, haplotype diversity was low, ranging from 0.2923 to 0.9333, and nucleotide diversity ranged from 0.0005 to 0.0046. The low genetic diversity observed in this study is likely a result of the migratory nature of Anguillid eels. Morphometric and genetic variations can support restocking as a conservation strategy to bolster wild populations. However, comprehensive studies must be conducted to understand all aspects impacting Anguillid resources and establish conservation areas to protect their populations and habitats.

Keywords: Freshwater eel conservation, Anguillids, taxonomy validation, DNA barcoding, COI gene, genetic diversity

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1. Introduction

The freshwater eel, Anguillids, is a migratory fish species inhabiting various habitats from the ocean to the river ecosystem (Tsukamoto and Arai, 2001). Anguillids migrate and metamorphose as catadromous fish, beginning by spawning in the deep sea, where eggs hatch into larvae called leptocephalus. The larva floats towards the coast and estuary then transforms into an eel-like phase called glass

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eel. The river serves as a habitat for elvers and yellow eels for physical growth and gonad maturation, leading to the silver eel phase. Once the Anguillids eel reaches maturity, they migrate back to the ocean to spawn once in their lifetime (Kurogi *et al*., 2011).

Anguillids have high nutritional values, especially protein, fat, and vitamins A and E. Wijayanti *et al*. (2018) highlighted that the protein content of *A. bicolor* reached 17.51%, while Bote *et al*. (2024) mentioned that *A. anguilla* contains about 271.6 grams of protein per kilogram. As a high-value food commodity, the global demand for Anguillids continues to rise. The primary consumers are Japan, South Korea, China, parts of Southeast Asia and Europe, and the United States and Canada, with Japan leading the import market by bringing in 60,000 tons in 2002 (FAO, 2009). In 2012 – 2013, Japan's consumption was still the highest, estimated at 30-45% of global eel production (Shiraishi and Crook, 2015).

Despite their high economic value, the population of Anguillids is decreasing worldwide, particularly in subtropical regions. The juvenile abundance dropped by 99% for European and 80% for Japanese eels (Dekker, 2003). According to IUCN (Pike *et al*., 2020), 10 out of 20 species worldwide are endangered (EN) or critically endangered (CR). Indonesia is a tropical country and has nine different species/sub-species of freshwater eels, four of which are found in the Poso waters: *A. marmorata*, *A. celebesensis*, *A. interioris*, and *A. bicolor pacifica* (Sugeha *et al*., 2008; Fahmi *et al*., 2012). Among these species, *A. bicolor* is categorized as near threatened (NT), while the other three species are least concerned (LC) and data deficient (DD). Therefore, it is crucial to carry out further research to ensure their conservation status. Unfortunately, freshwater eel stocks in Poso waters have declined due to overfishing of the broodstock in Tentena (outlet of Lake Poso), not eco-friendly glass eel fishing at the estuary of the Poso River, and the construction of a dam for the Hydroelectric Power Plant in Sulewana, which has cut off the freshwater eels' migration path (Krismono and Kartamihardja, 2012).

Numerous studies have been conducted on freshwater eels in Poso waters, focusing on conservation, recruitment, and capture fisheries. Additionally, genetic studies have targeted genes such as D-loop, Cyt b, and 16S rRNA (Triyanto *et al*., 2008; Sugeha *et al*., 2008; Fahmi, 2015;). However, most of these studies have only taken samples from Lake Poso or the Poso River (estuary). More information on morphometric and genetic variation is needed, using mtDNA markers with COI target genes and wider sampling locations within the Poso River. DNA barcoding is the most commonly used and effective method for identifying fish species and validating taxonomy (Bhattacharya *et al*., 2015). The benefits include its ability to identify species when traditional morphological approaches fail, such as during the larvae phase, from partial specimens, or when dealing with damaged samples (Ward *et al*., 2009).

Morphometric and genetic information are crucial for fisheries management when creating conservation strategies. The information derived from genetics confirms taxonomy, which is a critical first step in species conservation (Fahmi, 2015). Moreover, morphometric and genetic analysis can help evaluate population structure and identify stocks for restocking and determining conservation zones to prevent genetic homogenization (Mojekwu and Anumudu, 2015 ; Pimentel *et al*., 2020;). Therefore, the study aims to assess the morphometric and genetic variations and their implications for eel conservation strategies in the Poso River, Central Sulawesi.

2. Materials and Methods

2.1 Location and Sampling

Samples were collected from three locations along the Poso River, Central Sulawesi (Figure 1), and conducted from May 2021 to August 2023 with varying times for each station. The sampling of eels in Poso 1 was carried out in May–June 2021, January–February and July–December 2022, and January–July 2023, while in Poso 2 and 3, it was only done in 2023, with June–July and May–August, respectively.

Different fishing gears were used to catch Anguillids depending on the sampling location and their phase of life. *Waya Masapi* was used to catch yellow eel in Poso 1 (outlets of Lake Poso), longline and folding traps were used for Poso 2 (middle part of the Poso River), and ATG

(*Gorong-gorong* fishing gear), a local fish trap, was used to catch for glass eel in the estuary, Poso 3 sampling site (Figure 2).

The Anguillids caught at each location were then randomly subsampled, resulting in 150 for morphometric analysis. The yellow eel was directly measured at the research site, while the glass eel was measured at the BRIN Laboratory in Cibinong West Java. Additionally, 38 samples, excluding *A. bicolor*, were randomly chosen and underwent a comprehensive genetic analysis at the BRIN Laboratory in Cibinong.

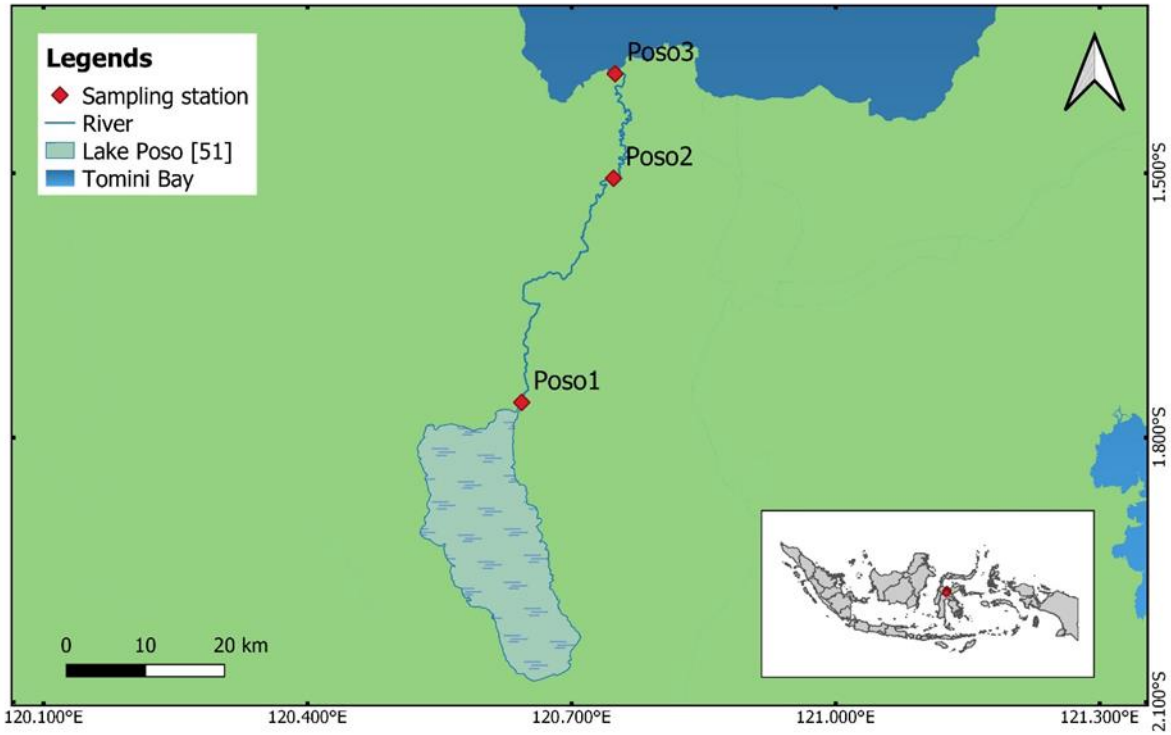


Figure 1. Map of Sampling locations in Poso River, Sulawesi Island, Indonesia

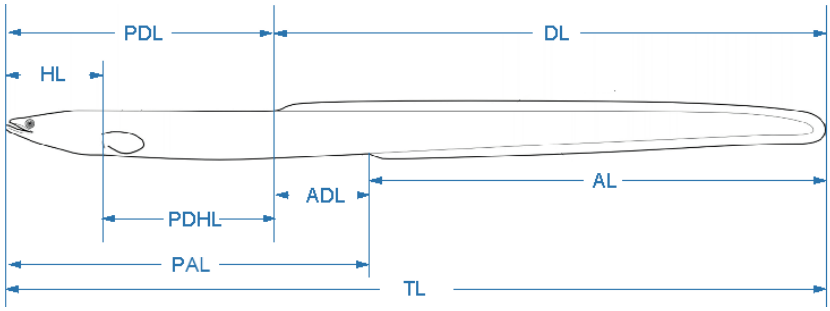
Figure 2. Fishing gears used for catching the Anguillids: (a) *Waya Masapi*/fence trap, (b) folding trap (modified fish trap), and (c) ATG (*Alat Tangkap Gorong-Gorong*)

The Anguillids caught at each location were then randomly subsampled, resulting in 150 for morphometric analysis. The yellow eel was directly measured at the research site, while the glass eel was measured at the BRIN Laboratory in Cibinong West Java. Additionally, 38 samples, excluding *A. bicolor*, were randomly chosen and underwent a comprehensive genetic analysis at the BRIN Laboratory in Cibinong.

2.2. Morphometric Analysis

Seven morphometric characteristics (Figure 2) were measured for the yellow eel using a ruler with 1 mm precision, while a macro microscope was operated for the glass eel. Based on Schindler and Schmidt (2006), measurement data was transformed using the following formula: Mtrans is the transformation result data, M is the measurement data, and TL is the Total Length).

$$Mtrans = \frac{(M \times 100)}{TL}$$



The freshwater eel morphometric characteristics (Silvergrip, 2009, modified). HL: Head Length, PDHL: Pre-Dorsal Head Length, PDL: Pre-Dorsal Length, DL: Dorsal Length, PAL: Pre-Anal Length, AL: Anal Length, and ADL: Ano-Dorsal Length.

The transformation data was then analyzed using the Kruskal-Wallis test to determine the effect of species differences on morphometrics. Further, Mann-Whitney U tests were conducted to identify the key characteristics distinguishing different species. Finally, discriminant analysis was applied to analyze the relationships between the different species based on morphometric characteristics. The entire morphometric analysis was conducted using SPSS 2016 (Shin *et al.*, 2022) for all the statistics tests and PAST 4.03 (Hammer *et al.*, 2001) software for running the discriminant analysis.

2.3. Genetic Analysis

2.3.1. Tissue Sampling

A total of 38 samples were collected for genetic analysis. Tissue samples were taken from either a yellow eel's pectoral fin or a glass eel by cutting approximately 1 cm with a sterile scissor, then preserved in a pro-analytic ethanol solution. Subsequently, all samples were continued for the DNA extraction.

2.3.2. DNA Extraction

The DNA extraction was performed using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan), following the manufacturer's protocol ver. 09.14.23. DNA concentration and purity were measured using Thermo Scientific NanoDrop Spectrophotometers based on Desjardins and Conklin (2010). The DNA was stored at -20°C for subsequent use.

2.3.3. PCR Amplification, Sequencing, and Analysis

The entire phase, including the primers selection, amplification, and visualization of PCR results, was carried out based on Ward *et al.* (2005) with modifications and optimization following the protocol of the product provider. Amplification of the COI gene on mtDNA using primary Fish F1 (5'TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC3') and Fish R1 (5'TAG-ACT-TCT-GG G-TGG-CCA-AAG-AAT-CA3'). A total of 25 µl of PCR reaction volumes were prepared by considering the volume ratio of each reagent according to the Thermo Scientific DreamTaq DNA Polymerase User Guide 2022. This consisted of 19.9 µl of Nuclease-free water, 2.5

µl of 10X PCR buffer, 0.5 µl of 10 mM dNTP, 0.5 µl of 10 pM each primer, 0.1 µl of 5 U/µ Taq DNA polymerase, and 1 µl DNA sample. The temperature was adjusted according to the following steps: initial denaturation of 2 minutes at 95°C, continued with 35 cycles denaturation of 30 seconds at 94°C, annealing of 30 seconds at 52°C, extension of 1 minute at 72°C, and the final extension of 10 minutes at 72°C was executed after those all cycles. The temperature was then held at 12°C. PCR products were visualized on 1.5% agarose gel by electrophoresis at 100 volts for approximately 30 minutes. The PCR product was sent for Sanger sequencing, with one part sent to 1st BASE Laboratories in Malaysia and another to the Center Laboratory of Sequencing BRIN using "E-Layanan Sains".

The DNA sequencing results were analyzed and modified using MEGA XI software version 11.0.13 (Kumar *et al*., 2008). In this analysis, additional sequences from GenBank were also used to confirm and compare intra-species and inter-species within the family and inter-family within the order. The accession numbers of these sequences include MW275927 and OR674041 for *A. marmorata*, OQ137029 for *A. celebesensis*, NC006536 for *A. borneensis*, and GU674219 for *Uroconger lepturus* (Family: Congridae).

The sequences were then compared with those in the NCBI (https://www.ncbi.nlm.nih.gov/) and BOLD (https/www.boldsystems.org/) databases by aligning them. The Kimura-2-parameter (K2P) model in MEGA XI software was used to estimate intra and interspecific genetic distances. The COI gene phylogenetic tree was constructed using the Neighbour Joining (NJ) method with 1000 bootstrap replications set on the Kimura-2-parameter model (K2P). In addition, DNASP 5.10 software was used to examine haplotype distribution and other genetic diversity analyses (Librado and Rozas, 2009).

3. Results and discussion

3.1. Morphometric

Morphometric analysis was conducted on three species of Anguillids, with sample sizes of 5 (*A. bicolor*), 34 (*A. celebesensis*), and 111 (*A. marmorata*). *Anguilla bicolor*, the least common among the three identified eel species, was found in limited numbers, with only five individuals discovered. This scarcity may be attributed to the brief sampling period at the mouth of the Poso River, which spanned only four months. Arai *et al*. (2001) revealed that *A. bicolor* in the Poigar River of North Sulawesi was only present during specific months due to variations in the duration of the leptocephalus metamorphosis phase and the age at recruitment of each species. The low number of *A. bicolor* individuals caught in Sulawesi waters, including in this study, suggests that the natural population of this species is limited compared to *A. marmorata* and *A. celebesensis* (Arai *et al*., 2001 and Sugeha *et al*., 2001).

The Total Length (TL) measured ranged from 36.22 to 1,315.00 mm. The Kruskal-Wallis test indicated a significant influence of species differences on morphometric characteristic variations ($p < 0.05$). Subsequently, the Mann-Whitney U test revealed that only one morphometric characteristic (ADL) differs significantly between the three species, as indicated in Table 1. *Anguilla bicolor* shares six morphometric characteristic similarities (HL, PDHL, PDL, DL, PAL, AL) with *A. celebesensis* and two similarities (PDL and DL) with *A. marmorata*. However, all seven morphometric characteristics in *A. celebesensis* differ relatively from *A. marmorata*.

It has been observed that there are many similarities between Anguillid species, which makes it difficult to distinguish them based on morphometric characteristics alone. Commonly, several Anguillid species have similar or overlapping morphometric measures. Sugeha and Suharti (2008) confirmed that distinguishing *A. celebesensis* and *A. interioris* can be challenging. Morphological analysis showed that all Anguillids were classified as *A. celebesensis*. However, the genetic analysis revealed that one sample was A. *interioris*.

Morphological similarities frequently appear in two or more species in the same habitats. The shape and size of a fish's body parts are closely linked to their environment. Environmental factors such as food can influence the Anguillid's size of fin and head in a habitat (Watanabe *et al*., 2009). By comparing the size morphometric characteristics of these three species, it can be

concluded that *A. bicolor* and *A. celebesensis* share similar habitats, while *A. marmorata* occupies a distinct habitat.

Seven morphometric characteristics describe the size of the dorsal, anal, and caudal fins. Fish fins generally play a vital role in regulating their stability while swimming. According to Chalchisa (2023), the shape and size of fins are related to the fish's behavior, especially movement. Additionally, the habitat or physical condition of the water, such as the boundary in the water, is also related to the fish's fin appearance. *Anguilla bicolor* and *A. celebesensis* have greater DL and AL to TL ratios than *A. marmorata*, indicating more active movements due to survival in challenging physical habitats.

Table 1. The Average (± SD) of transformed morphometric characteristic data for three Anguillid species in Poso River. Averages ± SD on the same line with different superscripts indicate significant differences ($p < 0.05$). All morphometric values are in per cent (%), except TL in millimetres (mm)

Characteristic Code	Species		
	A. bicolor	***A. celebesensis***	***A. marmorata***
HL	11.04 ± 1.97^{a}	11.86 ± 1.10^{a}	13.40 ± 1.71^{b}
PDHL	22.59 ± 11.42^{a}	15.99 ± 1.74^{a}	6.09 ± 6.50^{b}
PDL	41.56 ± 8.67^{ab}	43.30 ± 4.04^{a}	57.60 ± 5.50^{b}
DL	58.44 ± 8.67^{ab}	56.70 ± 5.04^{a}	42.40 ± 4.50^{b}
PAL	46.49 ± 12.34^{a}	52.14 ± 5.28^{a}	72.89 ± 7.65^{b}
AL	53.51 ± 12.34^{a}	47.86 ± 4.28^{a}	27.11 ± 2.65^{b}
ADL	1.86 ± 0.65^{a}	9.53 ± 9.29^{b}	17.84 ± 1.54^{c}
N	5	34	111
TL (min-max) *	48.37 - 910.00	37.52 - 1,110.00	36.22 - 1,315.00

According to the research conducted by Itakura and Wakiya (2020), *A. marmorata* tends to prefer riverbank habitats with vegetation and avoids waters with concrete substrates and sand. The study also found that the river's depth and velocity influence the Anguillid's size. Small-sized Anguillids (less than 24 cm) prefer riverine habitats with fast currents, while larger ones can be found in any depth and current. On the other hand, *A. bicolor* prefers marshy habitats and is commonly found in narrow and short rivers, as creeks with deeper rock-bottom waters and pools, but rarely in large rivers (Menon, 1999; Pethiyagoda, 1991; Arai and Kadir, 2017).

The morphological characteristic also includes the Anguillid's head because it is related to the size of some organs, such as the mouth. The size of the mouth consequently affects their feeding behavior and environment. Lammens and Visser (1989) reported that the breadth of the mouth in *A. anguilla* is adaptable to their environmental conditions, such as the size and availability of prey. They prefer an appropriate habitat based on their physical condition and function. Upon comparing the two groups of Anguillids, it is evident that the head size of *A. marmorata* is greater than that of *A. bicolor* and *A. celebesensis*. However, this does not necessarily imply that *A. marmorata* prey on larger animals than the other two species. *A. bicolor* and *A. marmorata* prey on relatively similar animals, with crabs and shrimps being their dominant prey (Sidqi *et al.*, 2018; Romanda *et al.*, 2019). Hence, further studies are required to confirm this, specifically regarding the size of the mouth breadth of each species.

The Discriminant Function Analysis (DFA) has identified two functions: Function 1 has an eigenvalue of 3.331 and explains 99.48% of all variances, while Function 2 has a 0.017 and 0.52%. Function 1 has two high-loading values, ADL and PDHL (0.967 and -0.316, respectively), while Function 2 has three high-loading values, AL, DL, and HL, as shown in Table 2. Function 1 significantly impacts the differences between the three species. It has an eigenvalue (EV) of 3.331, 99.5% of the variance, and a correlation coefficient 0.877. Among the morphometric characteristics within Function 1, ADL has the highest loading value of 0.967, significantly different from other characteristics. Therefore, ADL could be a key identification feature that distinguishes the three species.

Table 2. The eigenvalue, % variance, and DFA loading of morphometric characteristics in the Poso River. Characteristics with high loading are marked with an asterisk.

Function	**1**	**2**
Eigenvalue	3.331	0.017
Percentage Variance (%)	99.48	0.52
ADL	0.967*	-0.075
PDHL	-0.316*	-0.307
AL	-0.275	-0.782*
PAL	0.275	0.782*
DL[a]	-0.165	-0.601
PDL[a]	0.165	0.601
HL	0.253	0.400*

[a]) This variable was not used in the analysis.

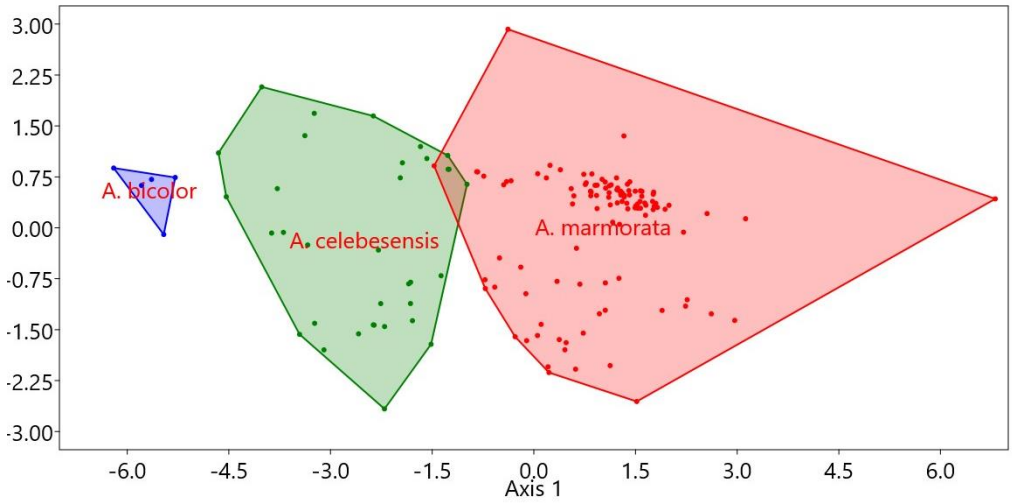


Figure 4. The Scatter Plot Function 1 and Function 2 of the three Anguillids morphometric characteristics. Different colors of the convex hulls represent each species: blue (*A. bicolor*), green (*A. celebesensis*), and red (*A. marmorata*).

According to Ege (1939), the range of AD/TL ratio in some Anguillid species is as follows: *A. bicolor* pacifica -6 – 3 %, *A. bicolor* bicolor: -3 – 4%, *A. celebesensis*: 6 – 12%, and *A. marmorata*: 12 – 20%. Some Anguillid samples analyzed in this study overlapped between species or were outliers. This variation can be caused by an individual's adaptation to their habitat, commonly called phenotypic plasticity. West-Ebenhard (2003) defines phenotypic plasticity as the ability of a genotype to produce more than one morphology, physiology, or behavior in response to environmental conditions. Different habitats will cause individual morphological differences, even within one species.

The DFA scatter plot shows that the three Anguillids species are separated into distinct groups, slightly overlapping *A. celebesensis* and *A. marmorata* (Figure 4). *Anguilla bicolor* is a distinct group, with its unique AD/TL ratio not overlapping with other species. This ratio is the most significant contributor to the composition of Function 1, as displayed by axis 1 on the graph. In contrast, *A. celebesensis* and *A. marmorata* share an AD/TL ratio of 0.12, with *A. celebesensis* at the upper limit and *A. marmorata* at the lower limit. Watanabe (2003) notes that *A. celebesensis* has an AD/TL ratio of 0.06–0.12, while *A. marmorata* has a ratio of 0.12–0.20. Generally, the ADL can separate these three groups. However, the grouping or species identification will be more precise when considering other morphological organs, such as tooth bands and vertebrae (Silvergrip, 2009).

Besides their measurable morphometric characteristics, skin appearance can be used to differentiate between Anguillid species. By direct observation, Anguillids can be divided into plain and patterned groups. Among the three species, *A. bicolor* can be distinguished from *A. marmorata* and *A. celebesensis* by their skin. *Anguilla bicolor* has plain skin with darker or black on the dorsal side, while the ventral side is lighter or white. On the other hand, *A. celebesensis* and *A. marmorata* have the patterned skin. Although the pattern is almost the same, it is still relatively easy to differentiate them morphologically by the ratio of ADL and TL. Nonetheless, validation with genetic analysis is necessary due to the high similarity of morphometric and overlapping key characteristics in some species, as conducted in this study.

Table 3 The coefficient of variation for three eel species in the Poso River.

Species	Coefficient of Variance (%)						
	HL/TL	DHL/TL	PDL/TL	DL/TL	PAL/TL	AL/TL	ADL/TL
A. bicolor	17.87	50.57	20.87	14.84	26.54	23.06	35.03
A. celebesensis	9.28	67.14	43.98	33.59	36.97	40.28	24.01
A. marmorata	12.75	123.18	37.32	50.70	25.59	68.81	14.24

Morphometric variation in each eel species can be seen from the coefficient of variance (Table 3); < 10% means low variation, 10-30% medium, and > 30% means high variation (Sokal and Rohlf, 2012). Almost all morphometric parameters in the three species showed moderate to high variation; only the HL/TL ratio of *A. celebesensis* showed low variation. As explained in the discussion earlier, head size is related to the size of other organs in the head, such as the mouth. In this case, it is presumed that the prey size of *A. celebesensis* in all sampling locations is relatively the same despite the differences in habitat.

Anguillid are considered carnivorous. *Anguilla bicolor* feeds on fish, worms, crabs, and shrimp (Sidqi *et al.*, 2018); the same was found in *A. marmorata* (Hartanto *et al.*, 2015). The feeding habits of fish may change, influenced by age, availability, and density of food sources in the water. Eels feed on invertebrates when small and become fish eaters when more significant (Rupasinghe and Attygalle, 2006). In this study, the identified samples of *A. celebesensis* were dominated by glass eel, which influenced the calculation results of the relatively small variation in the HL/TL ratio compared to *A. bicolor* and *A. marmorata*. Although there was high variation within each species, it did not lead to species differences that have been confirmed in subsequent molecular discussions.

3.1. Genetic

A genetic analysis was conducted on 38 samples presumed to be species of *A. celebesensis* and *A. marmorata* based on their morphometric characteristics. Samples of *A. bicolor* were excluded from the analysis because this species can be easily identified based on its skin pattern.

Table 4. Species validation using BLAST and BOLD

No.	N	Species	Similarity (%)	
			BLAST	BOLD
1	32	*A. marmorata*	98.28–100	99.52–100
2	6	*A. celebesensis*	98.93–99.85	94.18–100

Validation of species through BLAST and BOLD databases revealed similarity values ranging from 94.18% to 100%, confirming the validity of both species (Table 3). Bhattacharjee *et al.* (2012) classified the similarity range between the query and the database sequence into three groups: 97%–100% (significant), 92%–96% (moderate), and ≤91% (insignificant).

According to the BLAST and BOLD databases, *A. marmorata* had the lowest similarity percentages of 98.28% and 99.52%, respectively, indicating significant similarity. *Anguilla celebesensis* also showed a significant similarity of 98.93% in the BLAST database. However, in the BOLD database, *A.*

celebesensis had the lowest similarity of 94.18%, indicating moderate similarity (Table 4). Overall, these species were validated as such. The lower similarity percentages in BOLD were due to the need for more sufficient data compared to BLAST. In some cases, such as *A. celebesensis*, BOLD did not have more sequence variations than BLAST. The BOLD database was pre-curated, and then sequences were uploaded. On BLAST, anyone could upload the result of a species sequence without curation (Meiklejohn *et al.*, 2019).

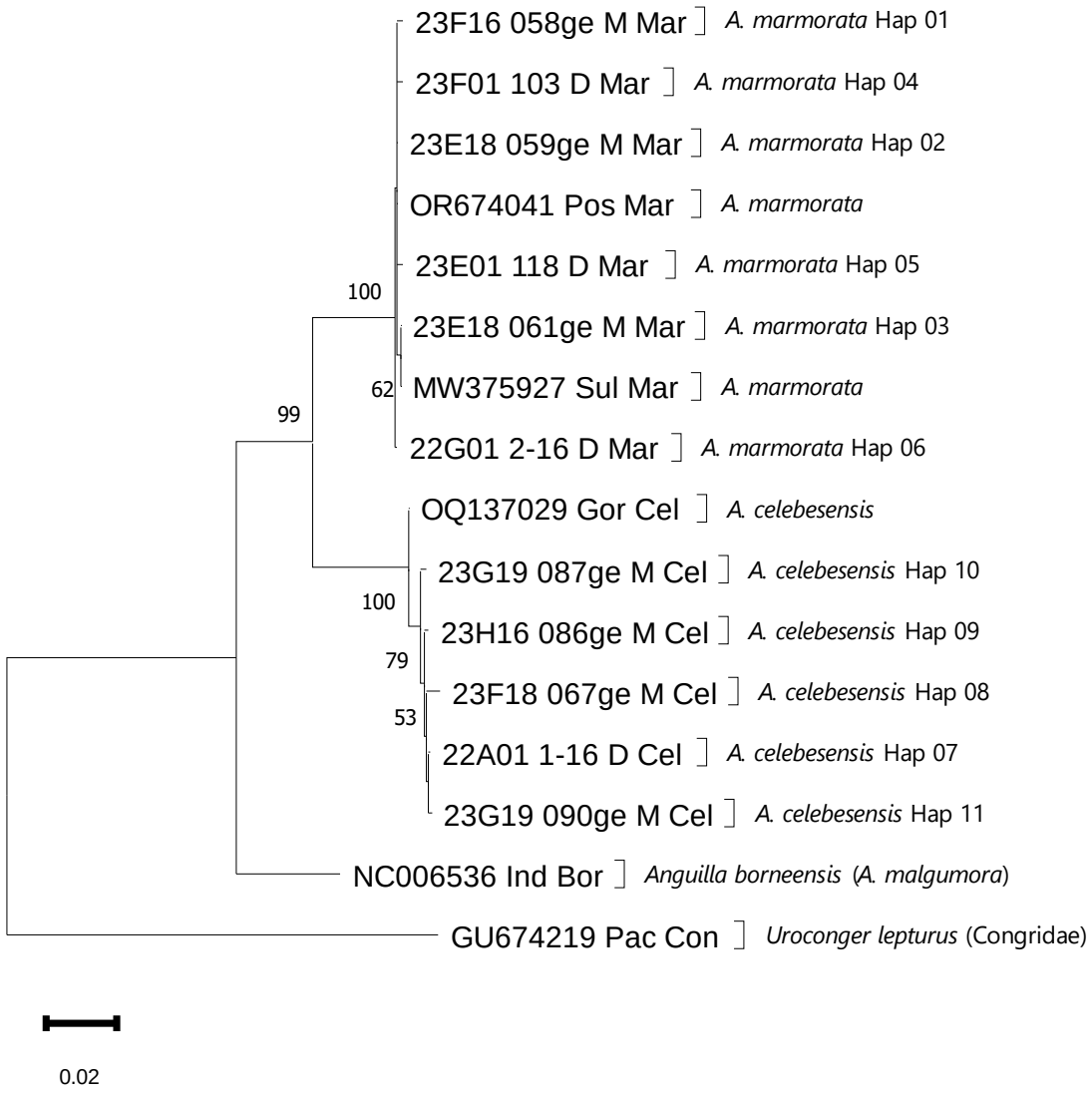


Figure 5. The phylogenetic tree grouped by haplotype in the Poso River. The branch number shows the NJ bootstrap's confidence level (1000 replications).

The reconstruction of the phylogenetic trees illustrated that the Anguillids samples were divided into two clades or species: *A. marmorata* and *A. celebesensis*, which were further grouped into six and five clusters, respectively (Figure 5). Sequences from the BLAST-NCBI database were used to confirm both species. Accession numbers MW275927 and OR674041 correspond to *A. marmorata*, and accession number OQ137029 corresponds to *A. celebesensis*. In addition, the accession number NC006536 for *A. borneensis* is used as a comparison of another species in the same family (Anguillidae), and the accession number GU674219 for the *Uroconger lepturus* of another family (Congridae) in the same order Anguilliformes.

The phylogenetic tree is constructed using the haploid of each species to simplify the appearance. The 0.01 scale represents a genetic change of 1 per 100 nucleotide sites in Figure 5. The length of the horizontal line on the branch indicates the degree of change, with longer lines representing greater changes and shorter lines indicating less change. The percentage value of each node reflects the large-scale support of the node. This trust value depends on the ratio of samples in each clade; the larger the sample size, the greater the trust value in the nodes. *Anguilla celebesensis* showed a lower percentage than *A. marmorata* because the sample size analyzed was smaller, with 6 and 32 in total samples, respectively.

Table 5 presents the results of a detailed genetic distance analysis on 11 haplotypes and several sequences from GenBank as comparators. The genetic distance between *A. marmorata* from GenBank and haplotypes 01–06 (*A. marmorata*) ranges from 0 to 0.0032, while the genetic distance between *A. celebesensis* from GenBank and haplotypes 07–11 (*A. celebesensis*) ranges from 0.0048 to 0.0081. These ranges, with less than 3% genetic distances, provide clear evidence that each haplotype group represents the same species as the sequences obtained from GenBank (Aoyama *et al*., 2000).

Conversely, the genetic distance between *A. marmorata* and *A. celebesensis* haplotypes is more distinct, ranging from 0.0555 to 0.0628. The analysis results between the two species' haplotypes and *A. borneensis* show a minimum genetic distance ranging from 0.0730 to 0.0840. These genetic distances, exceeding the 3% threshold, are crucial in establishing them as distinct species. As Watanabe *et al*. (2009) demonstrated, the genetic distance threshold between 2–3% in the COI gene is a significant marker for differentiating *A. rostrata* and *A. anguilla* species.

The highest genetic distance is shown between *Uroconger lepturus* (Family: Congridae) and haplotypes of *A. marmorata* and *A. celebesensis*, with minimum values of 0.2334 and 0.2360, respectively. The minimum ranges effectively prove that these two haplotypes belong to different families than *Uroconger lepturus*. For mtDNA markers, such as the COI gene, with an average genetic distance of 15.46%, species from different families can already be distinguished (Ward *et al*., 2005).

When comparing intraspecies, the *A. celebesensis* haplotype shows a higher genetic distance, ranging from 0.0016 to 0.0064, compared to *A. marmorata* haplotype, which ranges from 0.0016 to 0.0032 (Table 5). The genetic distance measures genetic differences between species or populations within a species (Nei, 1987). The value of genetic distance is represented by an index ranging from 0 to 1. A value closer to 0 means that the genetic distance between two populations is smaller, indicating that both populations have similar genetic diversity. On the other hand, a value closer to 1 signifies that the genetic distance between the two populations is greater. In this study, the genetic difference between *A. celebesensis* and *A. marmorata* populations is low (the maximum is 0.0628), indicating that the two populations have relatively low genetic differences. Watanabe *et al*. (2008) found that the genetic distance between *A. celebesensis* and *A. marmorata*, collected from different geographical locations, was 0.042, which is remarkably low, indicating almost no genetic difference despite the distant locations of collection sites such as Madagascar, Japan, Sulawesi, and Tahiti.

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Table 5 Genetic distances by haplotype species of Anguillid from Poso River

Genetic Distance per Haplotype	Bor	Cel	Mar	Mar	Hap01	Hap02	Hap03	Hap04	Hap05	Hap06	Hap07	Hap08	Hap09	Hap10	Hap11	Uro
A. borneensis																
A. celebesensis	0.0823															
MW375927_ *A. marmorata*	0.0730	0.0538														
OR674041_ *A. marmorata*	0.0749	0.0521	0.0016													
Hap01_ *A. marmorata*	0.0767	0.0538	0.0032	0.0016												
Hap02_ *A. marmorata*	0.0749	0.0521	0.0016	0.0000	0.0016											
Hap03_ *A. marmorata*	0.0730	0.0538	0.0000	0.0016	0.0032	0.0016										
Hap04_ *A. marmorata*	0.0767	0.0538	0.0032	0.0016	0.0032	0.0016	0.0032									
Hap05_ *A. marmorata*	0.0767	0.0503	0.0032	0.0016	0.0032	0.0016	0.0032	0.0032								
Hap06_ *A. marmorata*	0.0767	0.0503	0.0032	0.0016	0.0032	0.0016	0.0032	0.0032	0.0032							
Hap07_ *A. celebesensis*	0.0841	0.0081	0.0592	0.0574	0.0592	0.0574	0.0592	0.0592	0.0556	0.0556						
Hap08_ *A. celebesensis*	0.0879	0.0081	0.0628	0.0610	0.0628	0.0610	0.0628	0.0628	0.0592	0.0592	0.0064					
Hap09_ *A. celebesensis*	0.0841	0.0048	0.0592	0.0574	0.0592	0.0574	0.0592	0.0592	0.0556	0.0556	0.0032	0.0064				
Hap10_ *A. celebesensis*	0.0840	0.0048	0.0591	0.0573	0.0591	0.0573	0.0591	0.0591	0.0555	0.0555	0.0064	0.0064	0.0032			
Hap11_ *A. celebesensis*	0.0860	0.0064	0.0610	0.0592	0.0610	0.0592	0.0610	0.0610	0.0574	0.0574	0.0016	0.0048	0.0016	0.0048		
Uroconger lepturus	0.2173	0.2358	0.2334	0.2358	0.2381	0.2358	0.2334	0.2381	0.2381	0.2334	0.2452	0.2452	0.2405	0.2360	0.2428	

More similar species have a lower genetic distance, which is indicated by a value close to 0. Compared to *A. celebesensis*, the intraspecies of *A. marmorata* are lower, with a maximum genetic distance of 0.0032 compared to 0.0064 for *A. celebesensis*. Starting with the same minimum genetic distance of 0.0016, the genetic diversity of *A. celebesensis* is greater than that of *A. marmorata*. The low genetic distance within each population in our research underscores the significant genetic similarities individuals share within each species. In an ecological context, this low genetic distance is a crucial indicator of the well-connected nature of each species' population despite the diverse and complex habitats from which individuals originate (Sadler *et al.*, 2023). This connectivity results from the unique catadromous behavior of Anguillids, which migrate along the Poso River from the sea in Tomini Bay to Lake Poso upstream.

All sample sequences of the COI gene have been amplified with a base length of 625 bp. This amplification has identified 11 haplotypes, with six belonging to *A. marmorata* and five to *A. celebesensis* (Table 6).

Table 6. The Anguillids haplotype from the Poso River.

Species	Haplo-type	N	Sample Code
A. marmorata	1	1	23F16_058ge_M_Mar
A. marmorata	2	27	23E18_059ge; 23G19_071ge; 23G20_076; 23G20_071; 23G20_070; 23G20_069; 23G20_068; 23G20_067; 23G20_066; 23G20_065; 23G20_060; 23G20_059; 23F26_032; 23F16_004; 23G01_106; 21F01_PS7G; 21F01_PS1; 21E01_PS2; 21E01_PS3G; 21E01_PS4G; 21F01_PS5; 21F01_PS6; 22G01_7-16; 22H01_8-18; 22J01_11-20; 23A01_A0123; 23A01_B0123
A. marmorata	3	1	23E18_061ge_M_Mar
A. marmorata	4	1	23F01_103_D_Mar
A. marmorata	5	1	23E01_118_D_Mar
A. marmorata	6	1	22G01_2-16_D_Mar
A. celebesensis	7	1	22A01_1-16_D_Cel
A. celebesensis	8	2	23F18_067ge; 23G19_089ge
A. celebesensis	9	1	23H16_086ge_M_Cel
A. celebesensis	10	1	23G19_087ge_M_Cel
A. celebesensis	11	1	23G19_090ge_M_Cel

Table 7. Genetic Diversity Analysis

Population	n	Hn	Hd	π
A. celebesensis	6	5	0.93333	0.00459
A. marmorata	32	6	0.29234	0.0005
Total	38	11	0.49929	0.01569

n: Sample; Hn: Haplotype; Hd: Haplotype diversity; π (phi): nucleotide diversity

Further genetic diversity analysis showed that *A. celebesensis* and *A. marmorata* populations have haplotype diversities of 0.933 and 0.29234, respectively, with a total of 0.499 (Table 7). Additionally, the nucleotide diversity (π) in *A. celebesensis* and *A. marmorata* is 0.0046 and 0.0005, respectively, with a maximum of 0.01569.

The result above reveals that the populations of *A. celebesensis* and *A. marmorata* have different levels of haplotype diversity. *Anguilla celebesensis* has a haplotype diversity (Hd) of 0.933, which is high according to Nei's (1987) classification of 0.8 – 1.0. On the other hand, *A. marmorata* has a lower haplotype diversity of 0.29234 and is classified as the lowest haplotype diversity category (0.1 – 0.4). The sample size analyzed impacts the level of haplotype diversity. *Anguilla marmorata* shows lower haplotype diversity because it has a larger sample size with a relatively similar

number of haplotypes compared to *A. celebesensis*, which has a smaller sample size.

Haplotype diversity is important for the population's survival and adaptation to environmental changes. The number of haplotypes is one of the factors influencing genetic diversity. Low genetic diversity raises the risk of extinction because it restricts the potential of species to adapt to environmental changes. Organisms that tend to settle have a lower genetic structure than active or migratory organisms (Hellmair and Kinziger, 2014).

3.2. Morphometric and genetic analyses as fundamental conservation principles

Accurate identification of fish species is a fundamental step in fisheries conservation. Both morphometric and genetic analyses are essential for this initial task, completing each other with their respective advantages and limitations. Genetic methods can validate morphometric analyses, especially for species that exhibit morphometric similarities. Morphometric methods, on the other hand, play a critical role in identifying new species, especially for species that still need to be available in gene banks.

The regulation of the Government of The Republic of Indonesia (Peraturan Pemerintah) No. 6/2007 provides a comprehensive guide to fish resource conservation, encompassing ecosystem, species, and genetic levels. As the smallest unit, genetic conservation is a key principle in fish conservation. Heyden *et al*. (2015) emphasize the importance of conservation efforts for populations with unique genetic ancestry or low genetic diversity. The present study on *A. marmorata* and *A. celebesensis*, which reveals a low genetic diversity, raises concerns about the *potential* impact on these species. This underscores the urgent need for conservation management in the Poso River for Anguillid species.

Furthermore, environmental conditions have been observed to reduce current genetic diversity levels. Dam-building and habitat changes can disrupt the connection between the upstream (Lake Poso) and downstream (Poso River estuary), forming new species. Some species may evolve due to this disconnection, which can lead to adaptations (such as physiological, morphological, or other changes) to inhabit specific environments better (Heyden *et al*., 2015).

Two species of Anguillids in the Poso River, *A. marmorata* and *A. celebesensis*, have been verified morphometrically and genetically. *Anguilla marmorata* was more commonly sampled in this study than *A. celebesensis*, suggesting that the latter has a relatively smaller population. Fahmi *et al*. (2012) noted that *A. celebesensis* has a limited distribution, found only from the Northern to the central parts of Sulawesi waters, thus classifying it as an endemic species. Small biota populations tend to have low genetic variation due to inbreeding, which can reduce population fitness (Meffe, 1986; Frankham *et al*., 2002). Consequently, it is recommended that these species be protected or caught in limited numbers to ensure their sustainability. Conversely, *A. marmorata* has a broader distribution and a larger population than *A. celebesensis*. However, catching *A. marmorata* must be cautiously approached as it shares the same habitat and appears similar to the other species, *A. celebesensis* and *A. interioris,* which have similar skin patterns (Fahmi, 2015).

Population enhancement and habitat protection, including the population itself, can be viable approaches to addressing the challenges faced by Anguillids in the Poso River. Population enhancement through restocking requires the species to have genetic traits similar to those found in nature to avoid introducing genetic characteristics (Laikre *et al*., 2010).

On the other hand, the morphometric variation in the Poso River underscores the need for Anguillid conservation. The high morphometric variation indicates the diverse habitat in the Poso River and the need for habitat protection through the identification of conservation areas. Both the morphometric and genetic variation studies can contribute to this. However, it is crucial to understand that fisheries management is not a standalone task but a complex, interdisciplinary field that requires further consideration before determining the conservation area (Abell *et al*., 2007).

4. Conclusion

This study has successfully identified three species of freshwater Anguillid in the Poso River using morphological analysis for *Anguilla bicolor* and a combination of morphological and genetic analysis for *A. celebesensis* and A. marmorata. While the three species exhibit similar morphometric characteristics, the Ano-Dorsal (AD) length emerged as a key differentiating feature. Additionally, the genetic analysis revealed low genetic variation within *A. celebesensis* and *A. marmorata* population in the Poso River.

Identifying these species and determining key morphometric differences are significant for enhancing our understanding of species diversity and aiding in the accurate classification of Anguillid eels. This is particularly critical for conservation strategies, as accurate species identification can inform targeted conservation efforts and policies to preserve genetic diversity. Some conservation efforts that can be applied based on morpho-genetic aspects include restocking and identifying conservation areas. By advancing our knowledge of species differentiation and genetic diversity, this study lays the groundwork for more effective conservation strategies. It contributes to the broader scientific understanding of freshwater Anguillid eel and their ecological significance.

Future research should aim to include comprehensive genetic analyses for all identified species, including *A. bicolor*, with consistent sample size ratios to ensure fair comparison. Expanding the geographical scope of studies to include regions with significant geographical boundaries could provide deeper insights into morphometric and genetic variations. Employing other targeted genes in genetic analyses could further refine species differentiation and contribute to our broader understanding of Anguillid eel populations globally.

Data availability statement

The authors confirm that all necessary data have been included and accurately stated in this manuscript.

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Conflict of interests

The authors declare no conflict of interest in conducting and publishing this research.

Authors contributions

All authors in this paper are major contributors, with the authors' contributions following their respective competencies and roles. This research is part of a primary research (LPDP Rispro Invitasi) led by **T**. **OS, who** conceived and designed the study under the supervision of **MMK**, **RK**, and **SL**. **OS** and **T** conducted on-site sample collection and measurement. **MRW** conducted genetic analysis in the laboratory, including DNA extraction and PCR amplification. **OS** conducted data analysis and discussed to answer the research questions and obtain the conclusions with **RK** for statistical analysis, **SL** for genetic analysis, and **MMK** and **T** for conservation strategies in eel management. **OS** led the drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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