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Morphometrics and genetics variations of mullets (Pisces: Mugilidae) from Aceh waters, Indonesia

DERITA YULIANTO[1], I. INDRA[2], AGUNG S. BATUBARA[3], NUR FADLI[3], FIRMAN M. NUR[1], SYAMSUL RIZAL[3], MOHD. N. SITI-AZIZAH[4], ZAINAL A. MUCHLISIN[3,5,♥]

[1]Doctorate Program in Mathematics and Applied Sciences, Universitas Syiah Kuala. Jl. Tgk Chik Pante Kulu No. 5, Banda Aceh 23111, Aceh, Indonesia
[2]Faculty of Agriculture, Universitas Syiah Kuala. Jl. Tgk. Hasan Krueng Kalee No.3, Banda Aceh 23111, Aceh, Indonesia
[3]Faculty of Marine and Fisheries, Universitas Syiah Kuala. Jl. Tgk Syech Abdul Rauf, Banda Aceh 23111, Aceh, Indonesia
[4]Institute of Marine Biotechnology, Universiti Malaysia Terengganu. 21030 Kuala Terengganu, Terengganu, Malaysia
[5]Marine and Fisheries Research Center, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No.441, Kopelma Darussalam, Banda Aceh 23111, Aceh, Indonesia. Tel.: +62-651-7553205, Fax.: +62-651-7554229, ♥email: muchlisinza@unsyiah.ac.id, dejuli12@gmail.com

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Abstract. *Yulianto D, Batubara IIAS, Nur FM, Rizal S, Siti-Azizah MN, Muchlisin ZA. 2020. Morphometrics and genetics variations of mullets (Pisces: Mugilidae) from Aceh waters, Indonesia. Biodiversitas 21: 3422-3430.* Mullets are one of the commercial groups of coastal fish living in Aceh waters, in Indonesia. Presently, there is limited information on the bioecology, especially on the genetics and morphometrics of these fishes. Therefore, the objective of this study is to examine the morphology and genetic variations of *Liza macrolepis*, *Liza tade*, and *Moolgarda engeli* mullets. This study was conducted from January 2018 to July 2019 in four locations along the northern and eastern coasts of Aceh. Data were obtained by measuring the morphometrics of a total of 180 samples of the species in equal ratios. The data were analyzed using the ANOVA and Discriminate function analysis (DFA). The ANOVA test showed that at least 75% of characters are not significantly different among the mullets. Meanwhile, the discriminant function analysis produces the two functions with Eigenvalue of 0.627 and 0.107 with 85.5% and 17.2% total variants, respectively. Function 1 discriminates the mullet samples into two groups; the first was *L. tade*, and the second belonged to *L. macrolepis* and *M. engeli*. In addition, a total of 18 samples of mullets consisting of 8 samples of the *L. macrolepis*, 2 samples of the *L tade*, and 8 samples of the *M. engeli* were successfully amplified from the 5' region of the mitochondrial cytochrome oxidase subunit I (COX1) gene using a pair of primers (Fish F1 and Fish R1). Furthermore, a total of 7 haplotype sequences were produced from the ingroup where *L. tade* has one haplotype, *L. macrolepis* and *M. engeli* had three haplotypes, respectively. The genetic distance analysis showed that the interspecific distance was 0.38% and intraspecific was 8.2%. Therefore, the COI gene successfully discriminated against the mullet into three valid species.

Keywords: *COX1* gene, Discriminant Function Analysis, *Liza macrolepis, Liza tade, Moolgarda engeli*

INTRODUCTION

Mullets (Mugilidae) are coastal and diadromous marine fishes, commonly known to migrate from freshwater to seawater and vice versa (Beare et al. 2005; Arai and Chino, 2012)). Approximately 21 genera and 69 species of these fishes have been described worldwide (Thomson 1997; González-Castro and Ghasemzadeh 2016)). According to Turan et al. (2011), mullets are distributed in tropical, subtropical, and temperate regions. They belong to the euryhaline group of fishes that tolerate a wide range of salinity (Nelson 2007; Salvarina et al. 2018)).

Several species of mullets are commercially essential in the fisheries industry; for instance, *Mugil cephalus* is one of the most important marine fishes in Taiwan ((Whitfield et al. 2012). According to Tzanatos et al. (2005), mullets contribute 2.3% of the total production of marine fisheries in Yunani and they are also one of the main targets of coastal fisheries in several countries such as Brazil, Turkey, and Indonesia (Garbin et al. 2014; Solomon et al. 2018), Muchlisin et al. 2014) and Wahyudewantoro and Haryono (2013)). Unfortunately, overfishing and overexploitation have led to the depletion of its population in several countries (Aydin and Karadurmuş 2013; Garbin et al. 2014; Arslan and İşmen 2015; Gündoğdu and Baylan 2016; Yildiz and Karakulak 2016; Çiloğlu and Akgümüş 2019). A similar phenomenon was also reported by local fishermen in Aceh, Indonesia. This was shown in the decreasing number of catches and the smaller sizes of fish caught (interviews conducted on the local fishermen at Aceh, Indonesia).

However, several studies have reported that diverse species of mullets are found in Indonesian waters, although this number is not certain. For example, Katili (2011) reported that 6 species were discovered in Northern Celebes, namely *Liza permata, L. vaigiensis, L. macrolepis, Mugil chepalus, Valamugil connensius*, and *Oedaledhilus labiosus*. *Chelon subviridis* was recorded in Donan River, Central Java (Prastyo et al. 2017), while *M. cephalus* was discovered in Aloo estuary, Surabaya (Rahmatin et al. 2005). In Aceh Province, there are 9 species, namely *L. melanopterus, V. cunnecius, V. speigleri, M. cephalus*, ~~and~~ *Valamugil sp., Crenimugil crenilabis, L. macrolepis, Moolgarda engeli*, and *L. tade* (Muchlisin and Azizah 2009; Muchlisin et al. 2015; Yulianto et al. 2020). In addition, Kottelat et al. (1993)

stated that a total of 16 species of mullets were also discovered in Indonesian waters.

Generally, there are limited studies on morphometric and the genetics of mullets in Indonesia. It was recently discovered that only 3 studies have been conducted on mullets in certain places, namely Java, Sulawesi (Celebes), and Sumatra, one from each of the locations (Rahmatin et al. 2005; Katili 2011; Muchlisin 2014; Batubara et al. 2018). Morphometrics and genetics data are crucial because they validate the taxonomic status and relationship among species (Jayasankar et al. 2004; Kaupinis and Bukelskis 2010; Liu et al. 2010; Samy 2011; Durand et al. 2012; Rahman and Khan 2013). These methods are frequently combined in order to obtain a better analysis of the variability and the recognition of discrete groups (Muchlisin et al. 2012).

The information on morphometric and genetic relationship is important to generate an effective conservative plan because species with higher genetic distance behave differently with diverse characteristics, and a different species or population has different behavior characteristics, and therefore, it needs to be managed accordingly (Bergmuller and Taborsky 2010; Muchlisin et al. 2014). The objective of this study is to examine the morphology and genetic variation of mullet species, namely *Liza macrolepis*, *Liza tade*, and *Moolgarda engeli* harvested in Aceh waters, Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted at the Indonesian Fisheries Management Area 571 (*Wilayah Pengelolaan Perikanan, WPP-571*) located between the northern and eastern coast of Aceh and also bordered by the Malacca Strait, from January 2018 to July 2019. Morphometric and genetic analyses were carried out in the Laboratory of Ichthyology, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Indonesia, and in the School of Biology, Universiti Sains Malaysia, Malaysia, respectively. (Figure 1).

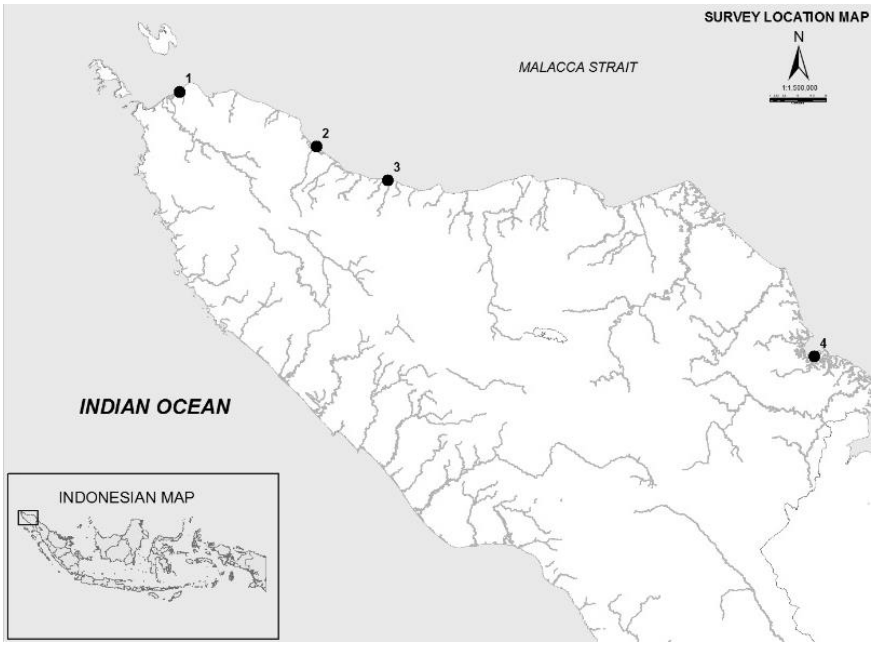

Figure 1. The map of Aceh Province showing sampling locations, (1) Aceh Besar, (2) Pidie, (3) Pidie Jaya, (4) Langsa City.

Table 1. The description of sampling locations of mullets in Aceh waters

No.	Location	Descriptions
1	Lambada Lhok, Aceh Besar District	Deforestation of mangroves with dominant species such as api-api *Avicennia* sp. and nipah *Nypa fruticans*. The salinity ranges between 20 ppt - 30 ppt. There are fishing ports and settlements in this area.
2	Tanjung Harapan Village, Sigli City, Pidie District	These areas consist of coastal aquaculture ponds, settlements, and muddy sediment. Salinity ranges between 25-30 ppt.
3	Muara Krueng Meuredu, Pidie Jaya District	The river mouth of Meuredue consists of mangrove plants, namely hipah *Nyfa fruticans* and api-api *Avicennia* sp. The water is clear and the salinity ranges from 17-25 ppt
4	Alur Teupin Arimaya, Gampong Alue Beurawe, Langsa City	A small and short river canal, aquaculture ponds and mangrove forests (*Rhizopora*) with salinity that ranges between 15-20 ppt.

Table 2. The description and code of the measured truss morphometric characters

Code	Characters	Descriptions
A	2-3	The distance from the lower jaw to the back of the head (nape)
B	3-4	The distance from the back of the head to the origin of the first dorsal fin base
C	4-5	The distance from the origin of first dorsal fin base to the origin of the second dorsal fin base
D	5-6	The distance from the origin of second dorsal fin base to the end
E	6-7	Distance from the end of the second dorsal fin to the upper base of the caudal fin
F	7-8	The distance from the upper base of caudal fin to the lower base
G	8-9	The distance from the upper base of the caudal fin to the origin of anal fin base
H	9-10	The distance from the origin of anal base to the end
I	10-11	The distance from the origin of pelvic fin base to the anal
J	11-12	The distance from the lower opening of operculum to the origin of pelvic fin base
K	12-1	The distance from the lower opening of operculum base to the lower jaw
L	1-2	The distance from the upper to the lower jaw
M	3-12	The distance from the end of head to the lower opening of operculum
N	4-12	The distance from the origin of first dorsal base to the lower opening of operculum
O	3-11	The distance from the end of head to the origin of pelvic fin base
P	4-11	The distance from the first dorsal fin base to the origin of pelvic fin
Q	5-11	The distance from the origin of second dorsal fin base to the origin of pelvic fin base
R	4-10	The distance from the origin of first dorsal fin base to the origin of anal
S	5-10	The distance from the origin of the second dorsal fin base to the origin of anal fin
T	6-10	The distance from the second dorsal fin to the origin of anal fin base
U	5-9	The distance from the base of the second dorsal fin to the anal fin
V	6-9	The distance from the base of second dorsal fin to the end of anal fin
W	6-8	The distance from the end of second dorsal fin to the lower tip of caudal fin base
X	7-9	The distance from the origin of upper caudal fin to the base of anal fin

Procedures

Sampling procedure

Sampling was conducted at four estuaries within the Fisheries Management Area 571 along the northern coast of Aceh, namely (1) Estuary of Lambada Lhok, Aceh Besar District, (2) Estuary of Tanjung Harapan Village, Pidie District, (3) Estuary of Meuredue River, Pidie Jaya District, (4) Estuary of Alue Beurawe, Langsa City, as shown in Figure 1. The descriptions of these locations are shown in Table 1. The samples were caught using casting nets and gillnets with mesh sizes of 2.0 and 2.5 inches respectively. In addition, the gillnets were set up in the waters for 3 hours (6 AM to 9 AM) and monitored within an interval of 30 minutes. The sampled fishes were anesthetized using clove oil and temporarily preserved in an icebox (4 °C) which was transported to the laboratory for further analysis.

Truss networks morphometric characters measurement Sub-procedures-2

The truss morphometric analysis was conducted on a total of 180 fish consisting of *Liza macrolepis*, *Liza tade*, and *Moolgarda engeli* species (60 samples each species). Additionally, a total of 12 homologous landmarks were determined along the outline of the fish and this led to 24 truss characters as shown in Figure 2 and Table 2. These landmarks were measured using digital calipers (Senator Polycal PC730F-150-0-150, errors 0.01 mm). However, this measurement was conducted on the left side body of the fish (González-Castro et al. 2012; Muchlisin 2013).

Truss morphometric data analysis

The data was transformed based on Palma and Andrade (2002) as follows: Mtrans = Log M - β (Log TL - Log TLmean), where M is the original measurement, Mtrans is transformed measurement, TL is total length, β is within-group slope regression of the Log M against Log TL, and TL mean is overall mean of total length. The transformed data were subjected to univariate (Analysis of Variant, ANOVA) and multivariate analysis (Discriminant Function Analysis, DFA) using SPSS software ver.20.0.

Sample collection for genetic analysis

A total of 10 samples from each of the species were randomly selected. The samples were individually photographed. Furthermore, approximately 1 cm of pectoral fin was cut out using a sterile scissor and preserved in TNES-urea buffer, while the other body parts were preserved in 10% formalin.

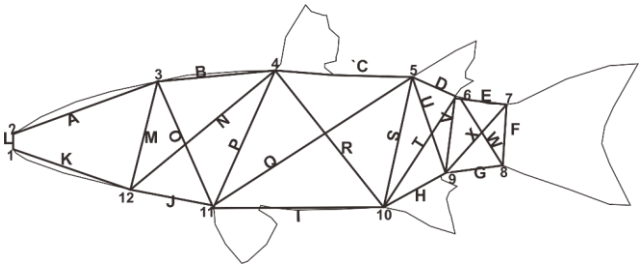

Figure 2. The characters of truss networks morphometric of mullets measured in this study.

DNA extraction and electrophoresis

The DNA was extracted using Aqua Genomic DNA in accordance with the producer's protocol. Approximately 50 µL sample of TNES-urea was taken and put into a sterile tube, subsequently mixed with 100µL of Aqua Genomic DNA and homogenized at room temperature for 30 minutes. Furthermore, 15µL of isopropanol was added and vortexed for 30 seconds, with the sample incubated at 60oC for 10 minutes. It was revortexed for 30 seconds and then centrifuged at 12,000 rpm for 5 minutes. The supernatant was removed and placed in a new sterile tube. Also, 300 µL of 100% isopropanol was added in the tube and re-vortexed for 30 seconds. The mixture was centrifuged at 12,000 rpm for another 2.5 minutes. The supernatant was discharged, and the pellet was washed twice using 70% ethanol, and dried at room temperature for 30 minutes. After that, 60 µL of distilled water was added into the tube and the sample was kept at -20 ℃ for electrophoresis (Muchlisin et al. 2013). The electrophoresis was conducted using 0.8 agaroses,100 voltage and the process lasted for 45 minutes. The gel was stained with ethidium bromide and viewed under a gel documentation machine (Gene Flas, Syngene Bio-Imaging). The quality of the extracted DNA was evaluated using a spectrophotometer (Unico SQ-4802).

PCR Amplification

Approximately 650-bp were amplified from genes in 5' regions of the mitochondrial cytochrome oxidase subunit I using the primer pairs in accordance with the study conducted by Ward et al. (2005)

FishF1 5'TCAACCAACCACAAAGACATTGGCAC3'
FishR1 5'TAGACTTCTGGGTGGCCAAAGAATCA3'

The 25 µl PCR reaction contained 16.65 µl of deionized water, 2.5 µl of 10X PCR buffer, 2.0 µl of $MgCl_2$ (25 mM), 0.5 µl of each primer (0.01 mM), 1.0 µl of mixed DNTP (0.05 mM), 0.25 µl of *Taq* polymerase, and 2.0 µl of DNA template. Amplifications were carried out using a Mastercycler® Eppendorf gradient thermal cycler (Brinkmann Instruments, Inc). The thermal regime consisted of an initial step with duration of 2 minutes at 94ºC followed by 30 cycles which lasted for 45 seconds at 94ºC, 45 seconds at 54ºC, 1 minute at 72ºC, 8 minutes at 72ºC and 4ºC. After the amplification process, the PCR products were carried out on 1.2% of agarose gel for 45 minutes and viewed under the GENEFLASH® Syngene Bio Imaging with the clearest products selected for purification (Ward et al. 2005; Muchlisin et al. 2012). The purification process was performed using a PCR Clean-up System purification kit (Promega), while the product was electrophoresed with 1.2% agarose gel and the clear product sent to a service provider for sequencing.

DNA analysis

According to Clustal W, multiple sequences were edited and aligned using the MEGA 4.0 program (Tamura et al. 2007). The nucleotide divergence among them was estimated with the Neighbour-Joining (NJ) method based on the Kimura 2 parameter. Confidence limits were assessed using the bootstrap procedure (Felsenstein 1985) and 1000 pseudoreplicates for NJ. The DnaSP Version 6.12.03 software was used to process the haplotypes (Rozas et al. 2003).

RESULTS AND DISCUSSION

One-way ANOVA of the morphometric data

A total of 180 fish samples belonging to the three species of mullets (60 fish each species) were measured to determine their truss morphometric characters (Table 3). The ANOVA test showed that at least 75% of characters are not significantly different among the mullets ($P>0.05$), indicate higher similarity among species. Generally, *L. tade* is different from the other two species with a significant influence on 3 characters, namely; the distance from the base of the second dorsal fin to the origin of anal fin base (character T), the distance from the base of the second dorsal fin to the base of the anal fin (character U), and the distance from the origin of the upper caudal fin to the base of the anal-fin (character X). These characters are significantly lower compared to the two other mullets as shown in Table 3. However, almost all characters in the *M. engeli*, and *L. macrolepis* were not significantly different, except the distance from the upper jaw to the lower jaw (character L) which was lower in the *L.macrolepis* than in *M. engeli*.

Discriminant function analysis of the morphometric data

The discriminant function analysis (DFA) produces the higher Eigenvalue of two functions by 0.627 and 0.107 with 85.4% and 14.7% total variants respectively, as shown in Table 4. Function 1 consists of two characters, namely the distance from the base of the first dorsal fin to the origin of the pelvic (character P), and the distance from the lower opening of operculum to the origin (character J). However, function 2 consist of 5 characters, namely the distance from the second dorsal fin to the end of the anal fin(character U), the distance from the upper base of the caudal fin to the lower base (character F), the distance from the end of the second dorsal fin to the lower tip of caudal fin base (character W), the distance from the origin of the anal base to its end (character H) and the distance from the origin of pelvic fin base to the origin of anal fin base (the character I) had a higher loading. Function 1 characterized the samples into two different groups; the first group was *L. tade* while the second belongs to *L. macrolepis* and *M. engeli*. However, some samples have overlapping characters as shown in Figure 3.

Genetic data analysis

A total of 18 species of mullets consisting of 8 samples of *L. macrolepis*, 2 samples of *L. tade*, and 8 of *M. engeli* were successfully amplificated with a pair of primers, FishF1 and FishR1 at 650 bp. The BLAST to NCBI database showed that the sequences had a higher similarity (98-100%) with 0% eigenvalue (Table 5). A total of 7 haplotypes were produced from the sequences. *L. tade* shared a similar haplotype (haplotype No. 1) with two

samples from Alue Beurawe, Langsa City (Code AR_02 and AR_04), *L. macrolepis* had 3 haplotypes (haplotype No. 2 to No. 4). Haplotype No. 2 shared by 6 samples, 5 from Lambada Lhok and 1 from Alue Beurawe, while haplotype No. 3 and 4 were shared by samples from Lambada Lhok. *Moolgarda engeli* had 3 haplotypes. Subsequently, 4 samples from Tanjung Harapan Village and 2 from Muara Kreung Meureudu shared haplotype No. 5. In addition haplotype No. 6 and 7 were shared by samples from each of the locations as shown in Table 6.

Table 3. Average±SD of truss morphometric characters of the three species mullets harvested from Aceh waters, Indonesia.

Characters	*Liza macrolepis* N = 60	*Liza tade* N = 60	*Moolgarda engeli* N = 60
A	1.213±0.061[a]	1.201±0.055[a]	1.211±0.074[a]
B	1.627±0.061[a]	1.611±0.061[a]	1.618±0.055[a]
C	1.457±0.087[a]	1.431±0.071[a]	1.456±0.066[a]
D	1.054±0.108[a]	1.109±0.071[b]	1.069±0.150[ab]
E	1.181±0.103[a]	1.182±0.073[a]	1.191±0.095[a]
F	1.129±0.109[ab]	1.107±0.091[a]	1.157±0.083[b]
G	1.207±0.091[a]	1.205±0.081[a]	1.21±0.072[a]
H	1.114±0.085[a]	1.152±0.076[b]	1.134±0.093[ab]
I	1.602±0.091[a]	1.609±0.056[a]	1.611±0.076[a]
J	1.434±0.093[a]	1.419±0.080[a]	1.432±0.068[a]
K	1.259±0.102[a]	1.275±0.082[a]	1.258±0.088[a]
L	0.757±0.080[a]	0.779±0.117[ab]	0.796±0.072[b]
M	1.299±0.076[a]	1.299±0.073[a]	1.309±0.051[a]
N	1.688±0.106[a]	1.694±0.070[a]	1.689±0.074[a]
O	1. 43±0.073a[b]	1.529±0.068[a]	1.557±0.067[b]
P	1.541±0.076[b]	1.510±0.072[a]	1.539±0.076[b]
Q	1.728±0.085[b]	1.689±0.133[a]	1.722±0.073[ab]
R	1.584±0.077[ab]	1.568±0.073[a]	1.596±0.064[b]
S	1.445±0.088[a]	1.433±0.082[a]	1.458±0.067[a]
T	1.452±0.083[b]	1.421±0.057[a]	1.465±0.064[b]
U	1.354±0.100[b]	1.321±0.065[a]	1.378±0.064[b]
V	1.244±0.091[a]	1.242±0.106[a]	1.261±0.089[a]
W	1.316±0.088[a]	1.341±0.092[a]	1.341±0.062[a]
X	1.385±0.076[ab]	1.362±0.058[a]	1.391±0.064[b]

Note: The values with different superscripts and in the same row are significantly different (P<0.05), n= total sample

Table 4. Eigenvalues and total variant of the truss morphometric data of the three species of mullet from Aceh waters

Function	1	2
Eigenvalue	**0.627[a]**	**0.107[a]**
% of Variance	**85.4**	**14.6**
Cumulative %	**85.4**	**100**
Canonical Correlation	**0.621**	**0.31**
P	.241*	.001
J	.098*	-.040
U	.332	.441*
F	.214	.405*
W	-.102	.364*
G[a]	.010	.332*
X[a]	.164	.324*
D[a]	-.070	.299*
R[a]	.108	.295*
S[a]	.129	.287*
T[a]	.227	.281*
H	-.210	.270*
M[a]	.008	.241*
V[a]	.069	.235*
C[a]	.112	.232*
E[a]	.015	.206*
O[a]	.095	.196*
Q[a]	.094	.187*
A[a]	.124	.156*
B[a]	.033	.148*
I	-.025	.146*
N[a]	.022	.128*
L[a]	.044	.074*
K[a]	-.052	.054*

Note: Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. Variables ordered by absolute size of correlation within function. *. Largest absolute correlation between each variable and any discriminant function. a. This variable was not used in the analysis

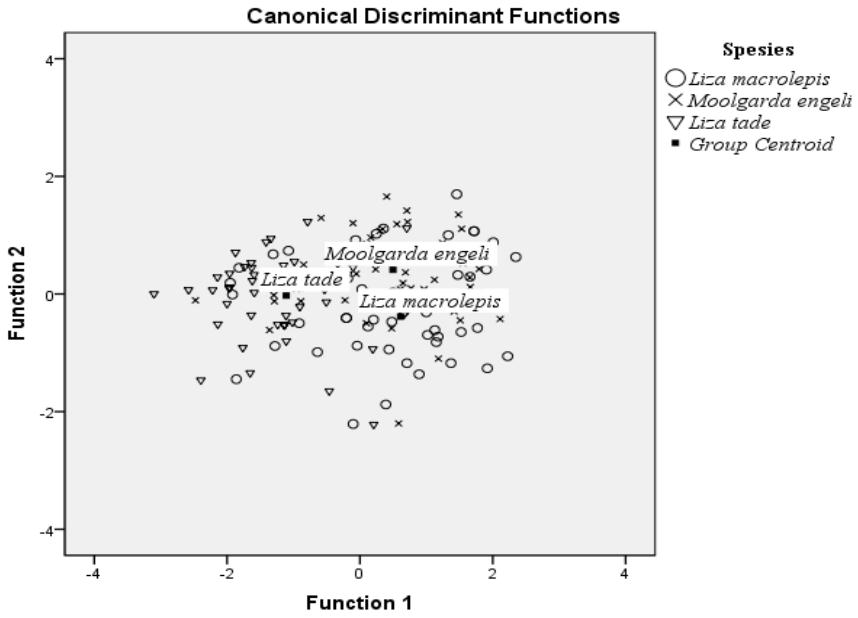

Figure 3. The scatter plot of Function 1 vs Function 2 of the truss morphometric characters produced by Discriminant Function Analysis

According to Table 7, the mean genetic distance analysis showed that intraspecific variation between species was 8.2%, while the interspecific variation among individuals of the same species was 1% in *L. macrolepis*, 0.13% in *M. engeli*. Furthermore, it was discovered that intraspecific variations of *L. tade* samples were 0% as shown in Table 8. The genetic tree constructed by Neighbour-Joining analysis Kimura 2-parameter model showed that the samples were clustered into three clades. The first belonged to *L. macrolepis*, which consists of 8 samples, 7 from Lambada Lhok, Aceh Besar, and 1 from Alue Beurawe, Kota Langsa with bootstrap value of 100% (at 1000x bootstraps). Meanwhile, the second and third clades belong to *L. tade* from Alue Beurawe, Kota Langsa with 98% bootstrap (at 1000x bootstrap) and consist of *M. engeli* from Pidie (5 samples) and Pidie Jaya (3 samples) with 100% bootstrap and 63% (at 1000x bootstrap) as shown in Figure 4.

According to the Analysis of Variant (ANOVA), *L. macrolepis* and *M. engeli* showed similar characteristics, although a certain character is significantly different, namely distance from the upper to the lower jaw (character L). However, *L. tade* and *L. macrolepis* showed differences in 7 characters namely D, H, O, P, Q, T, and U, which are mostly at the caudal part of the fish. In addition, *L. tade* and *M. engeli* also have 7 significantly different characters namely F, O, P, R, T, U, and X which are most representative of the middle part of the fish body, where *M. engeli* had the greater values of these parts compared to *L. tade*. Therefore, morphologically *L. macrolepis* is more similar to *M. engeli* (96% similarity), than *L. tade* (71% similarity) this shows that it is not always described by taxonomic closeness. The Discriminate function analysis (DFA) was used to classify the 3 species of mullets into two different groups. *L. tade* is categorized in the first group, while the second group consists of *L. macrolepis* and *M. engeli*. Therefore, morphological characters do not successfully discriminate the three species. This was achieved by genetic data which distinctively separated them into 3 different clades. This finding suggested that *L. macrolepis, L. tade*, and *M. engeli* have higher morphological similarities, even though they are genetically different. Therefore, their genetics and morphological data are not synchronous. Similarly, Rahman and Khan (2013) reported an asynchronous relationship between genetic and morphology data of 10 species from Parangipettai Waters, Southeast Coast of India. However, data of agreement was reported by Rasbora group in Lake Laut Tawar, Indonesia (Muchlisin 2013) and genus Kuhlia (*Kuhlia xenura* and *K. sandvicensis*) from O'ahu and Hawaii Islands (McRae 2007).

Generally, the present study showed that most characteristics in the head and caudal sections have no significant differences. The characteristics of the head section are related to the feeding behavior, while the causal section is playing an important role in swimming and movement (Muchlisin 2019), it is related to an environmental condition such as tide, current, and viscosity. Previous studies reported that they feed on algae and diatom (Luther 1964; Blaber 1976; Blay 1995; Dankwa et al 2005; Fatema et al. 2013; Muchlisin et al. 2014; Mondal et al. 2016; Gammanpila et al 2016). In addition, the characteristics of the caudal section play an important role in movement. It is suspected that environmental conditions, particularly currents, are similar in all the sampling locations; therefore its morphology is significantly not different from other species. The sampling locations are situated in the coastal part of the Malacca Strait, and the speed of the current ranges from 0.4 - 0.58 m/s while its salinity ranges from 20-30 ppt throughout the year (Setiawan et al. 2018; Rizal et al. 2010; Rizal et al. 2012; Muchlisin et al. 2014).

Table 5. The description of NCBI BLAST of the 18 sequences of the mullet samples

Seq.	Species	E-value	Ident.	Accession No.	Origin & sample code	Query length
1	*Liza tade.*	0	100%	KC970393.1	*Liza tade* AR 02	635
2	*Liza macrolepis*	0	98%	KJ202168.1	*Liza macrolepis* AR 03	624
3	*Liza tade*	0	100%	KC970393.1	*Liza tade* AR 04	628
4	*Liza macrolepis*	0	99%	KJ202168.1	*Liza macrolepis* LM 01	638
5	*Liza macrolepis*	0	99%	JQ060413.1	*Liza macrolepis* LM 02	613
6	*Liza macrolepis*	0	98%	KJ202168.1	*Liza macrolepis* LM 03	634
7	*Liza macrolepis*	0	99%	JQ060413.1	*Liza macrolepis* LM 01	616
8	*Liza macrolepis*	0	99%	JQ060413.1	*Liza macrolepis* LM 02	598
9	*Liza macrolepis*	0	98%	KJ202168.1	*Liza macrolepis* LM 03	635
10	*Liza macrolepis*	0	98%	KJ202168.1	*Liza macrolepis* LM 02	624
11	*Moolgarda engeli*	0	99%	JQ431912.1	*Moolgarda engeli* PD 01	629
12	*Moolgarda engeli*	0	99%	JQ431912.1	*Moolgarda engeli* PD 02	629
13	*Moolgarda engeli*	0	98%	JQ431912.1	*Moolgarda engeli* PD 01	636
14	*Moolgarda engeli*	0	99%	JQ431912.1	*Moolgarda engeli* PD 02	630
15	*Moolgarda engeli*	0	98%	JQ431912.1	*Moolgarda engeli* PD 03	637
16	*Moolgarda engeli*	0	99%	MH085787.1	*Moolgarda engeli* PJ 02	635
17	*Moolgarda engeli*	0	99%	MH085787.1	*Moolgarda engeli* PJ 03	612
18	*Moolgarda engeli*	0	98%	JQ431912.1	*Moolgarda engeli* PJ 05	637

Note: LM = Lambada Lhok, Aceh Besar; PD = Tanjung Harapan Village, Pidie; PJ = Muara Krueng Meureudu, Pidie Jaya and AR = Alue Beurawe, Langsa City

According to this study, mullet species had high morphological similarity (above 75%); however, they are genetically different. This is because they have distinct ancestors (Durand 2016), while the high similarity in morphology is due to the fact that they live in the same environment and have similar feeding habits. According to Frédérich et al. (2012) and Kaupinis and Bukelskis (2010), environmental factors have a strong effect on morphological variations of fish (Shuai et al. 2018).

In conclusion, the Truss morphometric character analysis shows that mullets are highly similar and this led to the difficulty in discriminating them. Two groups were successfully used to describe the three species analyzed, with *L. macrolepis* and *M. engeli* categorized together while *L. tade* was grouped separately. In accordance with the genetic analysis, they were successfully distinguished into three different clades, which means they are valid species.

Table 6. Haplotype number and frequencies, specimen I.D and contributing morph

Haplotype	Number of seq.	Specimen I.D	Contributing morph	Location
1	2	AR_02 AR_04	*L. tade*	Alue Beurawe
2	6	AR_03, LM_03, LM_011, LM_021 LM_031, LM_022	*L. macrolepis*	Alue Beurawe, Lambada Lhok
3	1	LM_01	*L. macrolepis*	Lambada Lhok
4	1	LM_02	*L. macrolepis*	Lambada Lhok
5	6	PD_01, PD_02, PD_011, PD_021, PJ_05, PJ_02	*M. engeli*	Tanjung Harapan Village and Muara Kreung Meuredu
6	1	PD_031	*M. engeli*	Tanjung Harapan Village
7	1	PJ_03	*M. engeli*	Muara Kreung Meureudu

Note: LM = Lambada Lhok, Aceh Besar; PD = Tanjung Harapan Village, Pidie; PJ = Muara Krueng Meureudu, Pidie Jaya and AR = Alue Beurawe, Langsa City.

Table 7. Inter-specific and intra-specific variation of COI gene among three species of mullets from Aceh waters, Indonesia.

Species	Inter-specific mean (%)	Intra-specific mean (%)	Intra-specific min. (%)	Intra-specific max. (%)
Liza macrolepis	1	6.2	1.9	10.6
Liza tade	0.0	1.8	1.5	2
Moolgarda engeli	0.13	16.7	16.2	17,2
Mean	0.38	8.2	6.5	9.9

Table 8. The genetic distance among mullets samples generated by Neighbor-Joining (NJ) based on Kimura-2 Paramaters

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*L_tade*_AR_02																		
2	*L_macrolepis*_AR_03	0.1																	
3	*L_tade*_AR_04	0.0	0.1																
4	*L_macrolepis*_LM_01	0.1	0.0	0.1															
5	*L_macrolepis*_LM_02	0.1	0.0	0.1	0.0														
6	*L_macrolepis*_LM_03	0.1	0.0	0.1	0.0	0.0													
7	*L_macrolepis*_LM_011	0.1	0.0	0.1	0.0	0.0	0.0												
8	*L_macrolepis*_LM_021	0.1	0.0	0.1	0.0	0.0	0.0	0.0											
9	*L_macrolepis*_LM_031	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0,0										
10	*L_macrolepis*_LM_022	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0									
11	M_*engeli*_PD_01	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2								
12	*M_engeli*_PD_02	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.0							
13	M_*engeli*_PD_011	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0						
14	*M_engeli*_PD_021	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0					
15	*M_engeli*_PD_031	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0				
16	*M_engeli*_PJ_05	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0			
17	*M_engeli*_PJ_02	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0		
18	*M_engeli*_PJ_03	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

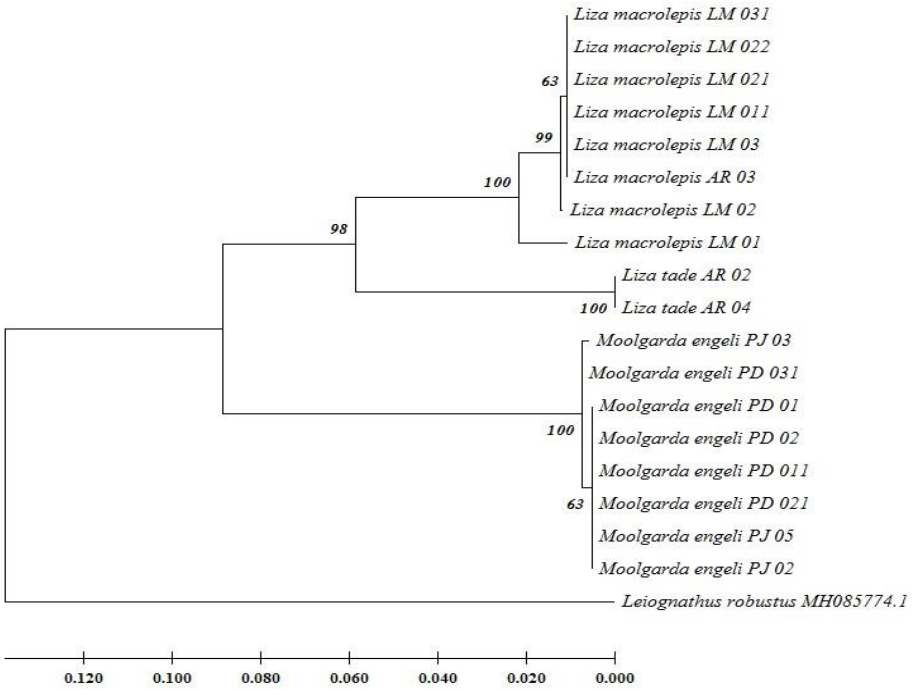

Figure 4. Phylogenetic tree of mullet samples from Aceh water using NJ method. LM = Lambada Lhok, Aceh Besar; PD = Tanjung Harapan Village, Pidie; PJ = Muara Krueng Meureudu, Pidie Jaya and AR = Alue Beurawe, Langsa City. *Leiognathus robustus* is an outgroup retrieved from Genbank (Acces Code: MH085774.1)

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