

## MORPHOLOGICAL CHARACTERISTICS AND GENETIC DIVERSITY OF FISH MOOLGARDA CUNNESIUS (VALENCIENNES, 1836) IN TAM GIANG LAGOON, VIETNAM

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### ABSTRACT

*Moolgarda cunnesius*, belongs to the Mugilidae family, is an important fish species in view of its immense contribution to the need of Vietnam in terms of nutrition, economic growth and development. The results of morphometric and genetic analysis of *M. cunnesius* in this study contributed the documents for identifications and the genetic diversity, which are the basic for further researches about *M. cunnesius* in breeding, species conservation and management at the Tam Giang Cau Hai Wetland Reserve. Research results showed that this species distributed in the North has a size ranging from 101.7 to 160.7 mm, with an average of 137 mm, corresponding to a weight of 10.6 - 35.6 g, with an average of 25.2 g, the fish distributed in the South has a size ranging from 112.4 - 172.7 mm with an average of 144 mm, corresponding to a weight of 10.6 - 35.6 g, an average of 28.3 g. Using CO1 gene DNA barcoding to study the genetic diversity of *M. cunnesius* in two regions of the North and the South of Tam Giang lagoon, the samples were amplified and sequenced the CO1 gene region. Amplified and sequenced 10 samples of CO1 gene of *M. cunnesius* sample in Tam Giang lagoon with the size of 650 bp. The average ratio of four types of nucleotides is A: 23.38%; T: 32.62%; G:18.15%; C: 25.85%. The rate of difference in CO1 sequences of the 2 samples is low compared to previous studies. The similarity coefficient of *M. cunnesius* in the two areas of Tam Giang lagoon is very high (0.9953).

**Keywords:** Genetic diversity, longarm mullet, morphological characteristics, *Moolgarda cunnesius*, Tam Giang lagoon.

### INTRODUCTION

*Moolgarda cunnesius* (Valenciennes, 1836) belongs to the family Mugilidae, order Mugiliformes (Thomson, 1984), inhabiting in marine, freshwater and brackish water and distributed in Indo-West Pacific (Froese and Pauly, 2021), which is one of the commercial species in Tam Giang lagoon, Vietnam (Vu, 2009). Recently, Qwabe and Cyrus, 2020 stated that *M. cunnesius* inhabited in the Mfolozi-Msunduzi estuary, South Africa, where the substrata with high component of fine sand was preferred. In Vietnam, Truong (1991) reported that the mullet living in Cua Be waters uses mainly floating plants and animals as food sources. Nguyen (2010) when researching on longarm mullet (*Mugil kelaartii* Gunther, 1861), found that it was caught in O Loan lagoon, Phu Yen province. The total production of longarm muller *M. cunnesius* exploiting in Thua Thien Hue in 2015 was 120.86 tons, in which, Phu Vang is the district with the largest total exploitation output (47.94 tons) (Dang and Vo, 2017). Nguyen and Nguyen (2012) revealed 177 species belonging to 129 genus of 73 families in 18 orders which were recorded in Tam Giang – Cau Hai lagoon, Vietnam, in which Mugilidae have 5 species including *Mugil cephalus* (Lin.,1758), *Valamugil cunnesius* (Val., 1836), *Moolgarda pedaraki* (Val., 1836), *Liza*

*melinoptera* (Val., 1836) and *Chelon haematocheilus* (Temm. & Schlegel, 1845). Nevertheless, while studies on *M. cunnesius* have concentrated on reporting the occurrence of the species and the species composition in Tam Giang lagoon, the data on morphologic characteristic as well as genetic diversity of this species in Tam Giang lagoon is limited. Therefore, the documents of morphological and molecular analyses of *M. cunnesius* are essential for accurate identification. According to Hebert *et al.* (2003a), DNA barcode basing on the use of gene sequences in the mitochondrial gene cytochrome c oxidase I (COI) can serve as the global bioidentification systems for animals. The species recognition is carried effectively through COI analysis (Hebert *et al.*, 2003b). Also, using DNA barcodes to identify species is used more and more by the scientists in the studies on the ecological system and evolution species, which is the vital tool to quantify species diversity (Kress, 2015). Chew *et al.* (2018) suggested that the integration of both approaches including morphological identification and molecular assessment is an important step to determine accurate identification. Durand and Borsa (2015) presented that their study used COI barcoding for identifying species in the family Mugilidae and determined molecular diagnosis for 24 of the species as well as for 25 putative cryptic species. In present study, the data will provide about morphologic perspectives and genetic diversity of assessment of longarm mullet, which is the scientific basic for future studies as well as for breeding and farming and conservation of this species.

## Materials and methods

### Fish material

The specimens of *M. cunnesius* were collected in two areas north and south of Tam Giang lagoon (water area of Dien Hai commune, Quang Loi commune, Huong Phong commune, Hai Duong commune) with 53 samples from September 2020 to June 2021, in which the area north was 31 samples while the South was 24 samples (Figure 1). The sample collection was repeated in the sampling locations monthly for 10 months (around 5 individuals per month) by directly fishing with local people, or by ordering samples from fishermen in the study area. Collected samples were cut pectoral fin samples and stored in eppendorf tubes with 96% alcohol and stored at -20°C until analysis.

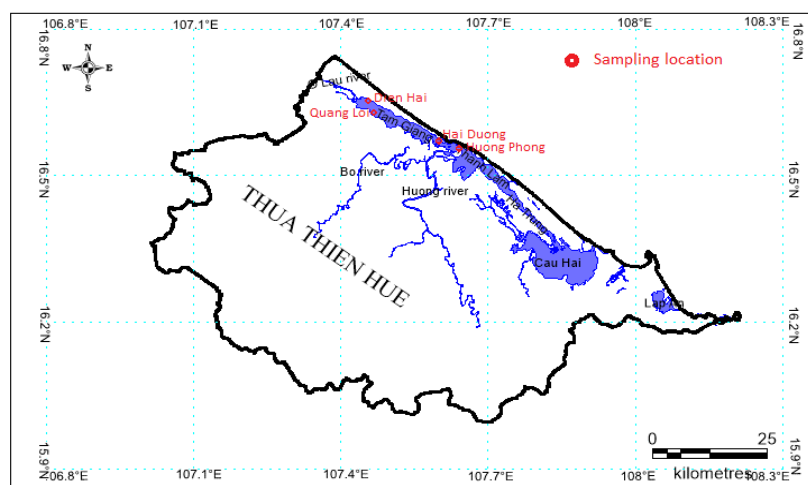


Figure 1. Map of sampling location of *M. cunnesius*

### **Morphological study**

Samples were treated as soon as the fish was fresh and fixed with 40% formol solution; Photograph and convert to 4% formol solution. Descriptive observations of the external morphological characteristics of fish according to the fish research guidelines of Pravdin (1973), Thomson (1954) and Nguyen (2005). The total of 31 morphologic measurements were used in this study for each specimen including Standard length (SL, mm), Body length, Fork length, Head length, Body height at dorsal fin base1, Body height at pelvic fin base, Tail stalk length, Tail stalk height, Dorsal fin length 1, Dorsal fin length 2, Pectoral fin length, Pelvic fin length, Tail fin length, Anal fin length, Dorsal fin height 1, Dorsal fin height 2, Pectoral fin height, Pelvic fin height, Tail fin height, Anal fin height, Head length behind eyes, Muzzle length, Length of snout to dorsal fin 1, Muzzle length to dorsal fin 2, Length of snout to ventral fins, Length of snout to anal fin, Head Width, Head Height, Distance between eyes, Eye Diameter, Mouth width.

Five Meristic charaters counted: the number of spines and first dorsal fin rays (D1); the number of rays and spines of second dorsal fin (D2); the number of rays and spines of pectoral fin (P), the number of rays and spines of ventral fin (V); the number of rays and caudal fin spines (C), the number of rays and spines of anal fin (A).

### **DNA extraction, PCR amplification and decoding**

Total DNA was extracted from the pectoral fins of each individual fish using the “GeneJET Genomic DNA Purification Kit DNA Extraction Kit” according to the manufacturer's instructions. The 650 bp mitochondrial COI was amplified with the primer pairs Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al.*, 2005). PCR reactions were performed with a total volume of 60 µl consisting of: 2 µl of total DNA (~50ng), 30 µl of Go Taq (2X), 3 µl of each primer (10pmol/µl) and 25 µl of Kit distilled water. The PCR machine (ESCO-AERIS-BG096) was used according to the following temperature program: DNA denaturation: 95°C/2 min; (2). Amplification: 35 cycles: (94°C/30 min; 54°C/30 min; 72°C/1 min); final 72°C/10 min. PCR products were electrophoresed on a 1% agarose gel and the DNA bands were visualized under a UV irradiator. Results were recorded using the Gel analysis software (UN-SCAN-IT gel version 6.1).

### **Diversity genetic analysis**

Sequences were initially aligned using the BioEdit version 7.2.5 sequence editing program. Search and compare research sequences with similar sequences on Genbank using BLAST program. Building phylogenetic tree by Test Maximum Likelihood method using MEGA version X software with bootstrap value repeated 1000 times for the each sample.

### **Data analysis**

The data were processed by MS Excel v. 365 and SPSS(IBM v. 20) programming. A one-way analysis of variance (ANOVA) was used to compare the mean of the morphological parameters between water areas with the significance level  $p < 0,05$ .

### **Results and discussion**

#### ***Morphological characteristics of the M. cunnesius in Tam Giang lagoon***

#### ***Morphological characteristics***

When analyzing 31 indicators, 5 counts of 53 samples of *M. cunnesius* combined with identification documents, the samples were identified as *M. cunnesius* (Valenciennes, 1836) with the main morphological features: Head relatively short, the top of the head is flat; The muzzle is slightly broad but short; Eyes round and medium large; The eye fat membrane is especially thick, covering the whole eye except for the pupil; The dorsal fin has 2, the first dorsal fin consists of stiff spines, the beginning of the first dorsal fin is located near the tip of the pectoral fin; The origin of the second dorsal fin is behind the origin of the anal fin; Pectoral fin long, beyond first dorsal fin starting point, pectoral fin tip black dotted, pectoral fin base with axillary scale. Research results show that this species in the area north has a size ranging from 78.9 - 126.6 mm, corresponding to a weight of 10.6 - 35.6 g. In particular, the variation in size as well as mass of this species distributed in the area south has a difference, in detail, the size ranges from 90.3 - 138.8 mm, the mass fluctuates in the range of 13.4 - 47.7 g; the number of spines and rays of first dorsal fin  $D1 = 4.0$ ; the number of spines and rays of second dorsal fin  $D2 = 1 - 2, 7 - 8$ ; the number of spines and rays of pectoral fin  $P = 1 - 3, 12 - 15$ ; the number of spines and rays of ventral fin  $V = 1, 5 - 6$ ; the number of spines and rays of caudal fin  $C = 0, 14 - 17$ ; the number of spines and rays of anal fin  $A = 1 - 3, 9 - 10$ .

The head length measured in the study was 25.9% of the SL for the northern fish and 26.1% of the SL for the southern fish. However, when analyzing ANOVA, there was no significant difference with  $p > 0.05$ . Head width of leaf mullet in 2 regions is 60.2% compared to HL and 59.8% compared to HL, respectively and has no statistical significance ( $p > 0.05$ ); similarly, the measured head height is 79.6% compared to HL and 78 % compared to HL and has no statistical significance ( $p > 0.05$ ).



**Figure 2. Morphology of *M. cunnesius* in Tam Giang lagoon, Vietnam**

The results of differences in size, mass and tip length between the two distribution regions are only preliminary data. Within the framework of our study, the difference is due to the first reason that the age of fish in the two regions is different; the second is due to the characteristics of the water environment and different nutritional regimes.

The length of the ventral fins of *M. cunnesius* was 12 % SL for fish from the northern region and larger than that of southern fish at 9.8 % SL. When analyzing ANOVA, this difference was statistically significant with  $p < 0.05$ . This showed that the length of the pelvic fins of fish in the North is different from the length of the pelvic fins of fish living in the South. The length of the caudal fin in the northern region with 27 % SL was higher than that in the southern region (25.4% SL) with a statistically significant difference with  $p < 0.05$ . The length of snout to dorsal fin 2 of *M. cunnesius* in the northern region was 73% higher than that in the southern region with 72.2% SL and this difference was statistically significant ( $p < 0.05$ ).

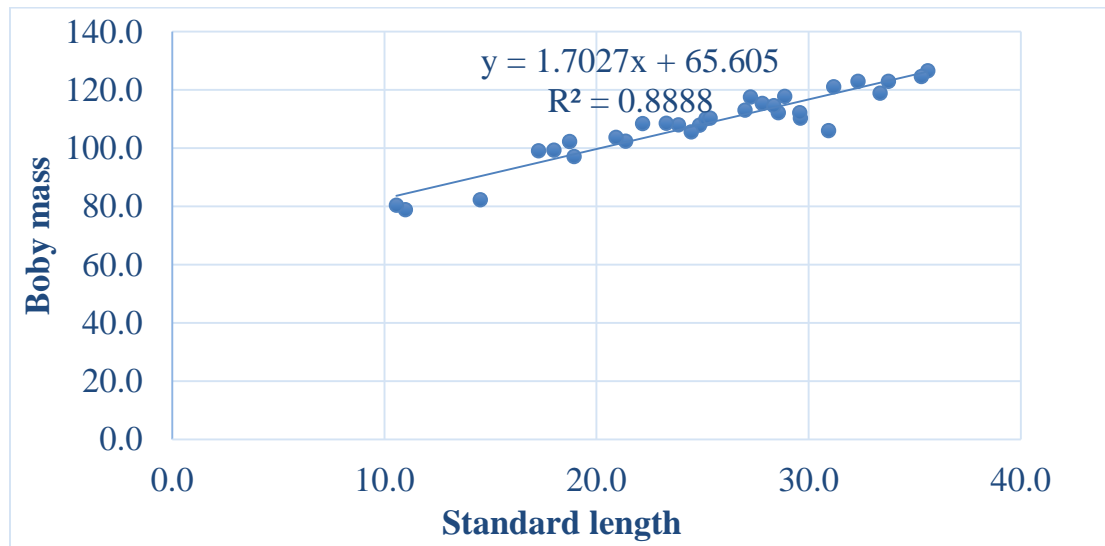
**Table 1. Morphological measurements (mm) of mullet species *M. cunnesius* distributed in Tam Giang lagoon, Vietnam. Means sharing a different letter in the same row differ statistically significantly  $p < 0.05$**

Targets	Area north		Area south	
	min-max	M ± SD	min-max	M ± SD
Standard length (SL, mm)	78.9 – 126.6	108.4 ± 12a	90.3 – 138.8	114.6 ± 12.8a
Body length	101.7 - 160.7	137 ± 14.8a	112.4 – 172.7	144 ± 15.7a
Fork length	13.2 – 148.4	112.2 ± 45a	106.1 – 161.9	134.7 ± 14.7b
Head length	20.4 – 27.8	25.9 ± 1.3a	24 – 28.2	26.1 ± 1.1a
Body height at dorsal fin base1	17.1 – 29.3	24.9 ± 1.9a	23 – 27.4	24.7 ± 1.3a
Body height at pelvic fin base	17.1 – 26.8	23.4 ± 1.5a	21.7 – 26.1	23.4 ± 1.2a
Tail stalk length	11.6 – 21.1	17.4 ± 2a	15.3 – 20.9	17.6 ± 1.3a
Tail stalk height	10.8 – 17.9	11.7 ± 1.3a	10.6 – 12.1	11.3 ± 0.4a
Dorsal fin length 1	9.1 – 14.1	11.4 ± 1.1a	10 – 12.7	11.5 ± 0.7a
Dorsal fin length 2	11.5 - 14	12.5 ± 0.5a	11.4 – 13.8	12.5 ± 0.6a
Pectoral fin length	6.5 - 30	10.8 ± 7.4a	6.5 – 29	7.3 ± 0.7a
Pelvic fin length	8 – 18.5	12 ± 3.5a	8.4 – 12.2	9.8 ± 0.9b
Tail fin length	24.8 – 32.5	27 ± 1.6a	21.9 – 29.4	25.4 ± 1.7b
Anal fin length	13.7 – 20.8	16.5 ± 1.3a	14.1 – 17.7	16.1 ± 0.8a
Dorsal fin height 1	9.3 - 19	11.5 ± 1.9a	9.9 – 13.1	11.3 ± 0.9a
Dorsal fin height 2	9 – 19.8	12.6 ± 1.7a	10.4 – 13.7	12.3 ± 0.8a
Pectoral fin height	21.6 – 26.5	20.6 ± 6.2a	19.6 – 24.6	22.3 ± 1.3a
Pelvic fin height	7.4 – 35.4	14.5 ± 4.4a	13.6 – 16	14.5 ± 0.5a
Tail fin height	20.8 – 36.1	26.4 ± 3.8a	21.1 – 32.2	27.5 ± 3.3a
Anal fin height	6.9 – 18.8	15.6 ± 1.9a	14.1 – 17.7	15.9 ± 0.9a
Head length behind eyes	7.8 – 16.7	13.5 ± 1.3a	10.9 – 15	13.5 ± 0.9a
Muzzle length	6.5 – 52.7	8.8 ± 8.2a	6.4 – 8.4	7.2 ± 0.5a
Length of snout to dorsal fin 1	39.5 – 53.7	49.5 ± 2.3a	46.6 – 52.4	49.6 ± 1.3a
Muzzle length to dorsal fin 2	69.5 – 75.4	73 ± 1.5a	70 – 76.1	72.2 ± 1.5b
Length of snout to ventral fins	35.6 – 41.5	38.7 ± 1.3a	36.4 - 41	38.1 ± 1.2a

Length of snout to anal fin	66.8 – 74.7	69.5 ± 1.7a	67.3 – 71.2	66.1 ± 12.6a
Head Width	54 – 84.4	60.2 ± 5a	55.4 – 68.5	59.8 ± 2.8a
Head Height	59.9 – 114.1	79.6 ± 7.9a	58.7 – 86.7	78 ± 5.1a
Distance between eyes	39.7 – 65.2	45.3 ± 4.6a	38.9 – 81.2	46 ± 7.9a
Eye Diameter	25.1 – 42.3	27.4 ± 3.1a	23.7 – 26.2	37.1 ± 48.1a
Mouth width	26.3 – 51.5	31.7 ± 4.4a	27.7 – 35.6	32.3 ± 2.1a

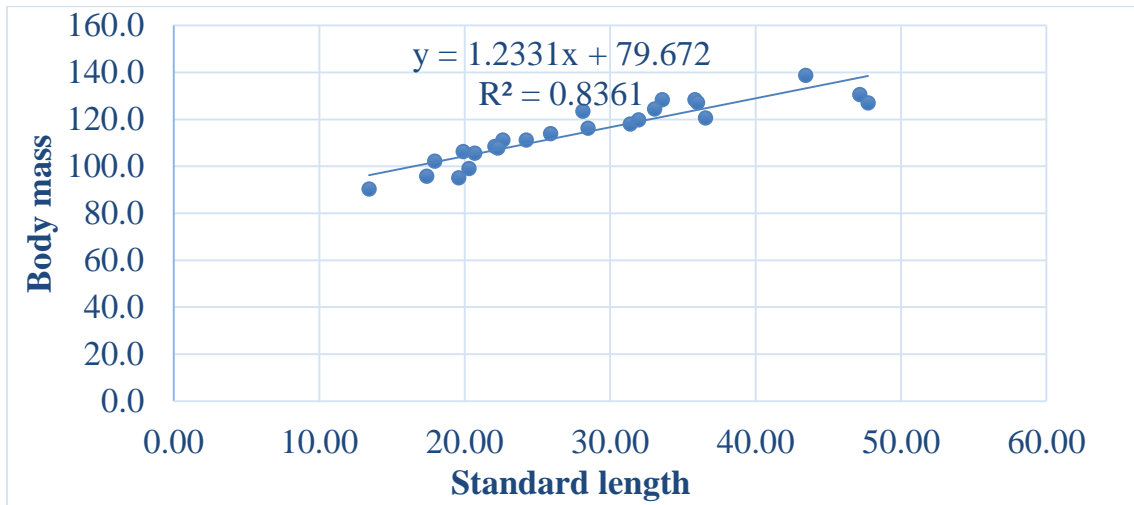
*Relationship between standard length and body weight*

The relationship between standard length and body weight is the criterion to evaluate the growth and development of *M. cunnesius* (Figures 3 and 4). The linear equation between standard dimension and body mass in the two areas shows that the relationship is positive (all R > 0.8, linear upward).



**Figure 3. Relationship between standard length and body mass of *M. cunnesius* in the North area**

Specifically, the area north region has a linear equation  $y = 1.7027x + 65.605$  ( $R^2 = 0.8888$ ), the area south has a linear equation  $y = 1.2171x + 4.5103$  ( $R^2 = 0.9832$ ). From the above analysis results, it shows that the relationship between standard length and body mass of most of the analyzed individuals in the two areas are individuals with large length often having large sizes and vice versa.



**Figure 4. Relationship between standard length and body mass of *M. cunnesius* in the South area**

The relationship between the standard length and body weight of northern and south fish of Tam Giang Lagoon shows that the Northern fish has a more close relationship. This difference is due to the first reason that the age of fish in the two areas is different; the second is due to the characteristics of the water environment and different nutritional regimes. However, this is just a preliminary data, to get accurate results and confirm the difference, it is necessary to study on a larger number of samples and more repetitions.

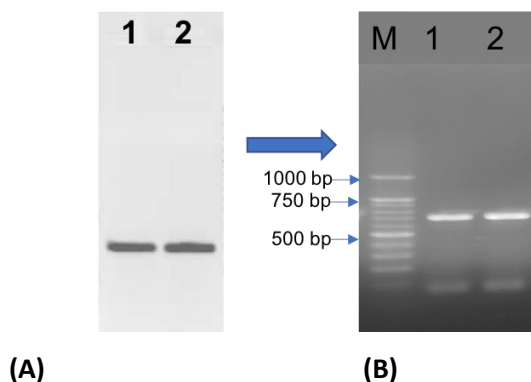
*Genetic diversity of Moolgarda cunnesius in Tam Giang lagoon, Vietnam*

**Total DNA extraction**

The total DNA extraction was extracted with good quality, which is tested on 1% gel electrophoresis, the electrophoresis image gave a fairly clear dark band (Figure 5). Regarding the quality of the total DNA, further studies can be carried out.

**PCR performance product**

Using 2 pairs of FishF1 & FishR1 primers to amplify the samples by PCR reaction. The results of PCR product analysis on 1% agarose gel showed that specific DNA fragments with size of about 650 - 750 bp were amplified (Figure 5 B & 6).



**Figure 5. The total DNA electrophoresis of *M. cunnesius* (A); Results of PCR product electrophoresis (B). (M: marker, 1, 2: sample symbol of *M. cunnesius*)**

The results of the analysis of the CO1 gene sequence of *Moolgarda cunnesius* in Tam Giang lagoon, Vietnam

After sequencing and editing with MEGA X software, Blast all samples were conducted to search and compare the study sequences with similar sequences on Genbank. As a result, the TM fish sample has a higher similarity than the DH, these two samples have a high similarity with *M. cunnesius* (Valenciennes, 1836). It means that this species is high haplotype diversity but low nucleotide diversity (Table 2).

When conducting genetic diversity analysis, all CO1 mitochondrial DNA gene sequences of the 2 samples were checked by comparing with the corresponding CO1 gene sequences on Genbank. The results show that the samples in this study belong to the species of longarm mullet *M. cunnesius* with more than 99.5% similarity, which is similar to the results of Tran et al., 2021 (Table 2).

**Table 2. The similarity of the Mooc\_017 gene sequence in the study with the 10 COI sequences of *M. cunnesius* on the GeneBank**

STT	Species	Gene	Code	Gene size (bp)	Query Cover (%)	Proportion (%) of homologous nucleotides
1	<i>Moolgarda cunnesius</i>	CO1	MW336954.1	704	98%	99.53%
2	<i>Moolgarda cunnesius</i>	CO1	MF628290.1	655	95%	99.84%
3	<i>Moolgarda cunnesius</i>	CO1	JQ045777.1	649	95%	99.68%
4	<i>Moolgardacunnesius</i>	CO1	KT231793.1	611	94%	99.84%
5	<i>Moolgarda cunnesius</i>	CO1	KX834271.1	588	90%	99.83%
6	<i>Moolgarda cunnesius</i>	CO1	EU595339.1	652	95%	99.84%
7	<i>Moolgarda cunnesius</i>	CO1	FJ238048.1	652	95%	99.68%
8	<i>Moolgarda perusii</i>	CO1	KY315413.1	655	95%	99.84%
9	<i>Moolgarda perusii</i>	CO1	LC484868.1	655	95%	99.68%
10	<i>Moolgarda perusii</i>	CO1	LC484868.1	655	95%	99.68%

After processing, the CO1 gene fragment is 650 bp in size. The results showed that the average rate of four types of nucleotides in was A: 23.38%; T: 32.62%; G:18.15%; C: 25.85%. This rate is quite similar to the results of studies on 143 bony fish species in Australia (Ward et al., 2005).

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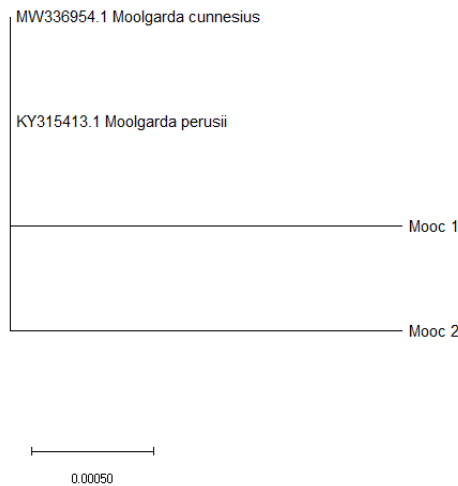
                10      20      30      40      50
MW336954.1: M. cunnesius  ....|....|....|....|....|....|....|....|....|....|
Mooc_017                GGTTCGGAAGTGCCTAAGCCTTCTTATCCGAGCAGAACTCAGCCAACCTG
Mooc_018                .....

                60      70      80      90     100
MW336954.1: M. cunnesius  ....|....|....|....|....|....|....|....|....|....|
Mooc_017                GGGCCCTTCTTGGGGACGATCAGATTTACAATGTGATTGTTACGGCACAT
Mooc_018                .....

                110     120     130     140     150
MW336954.1: M. cunnesius  ....|....|....|....|....|....|....|....|....|....|
Mooc_017                GCTTTCGTAATAATTTCTTTATAGTGATGCCAATTATGATCGGTGGGTT
Mooc_018                .....
    
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The results of comparing the two samples analyzed with the sequence MW336954.1 (Tran et al., 2021) have differences at 4 positions: 385, 402, 631, 634, showing that the similarity between the studied sample and the sequence MW336954.1 is very high (Figure 7).



**Figure 7. Relatives of the fish *Moolgarda cunnesius* in Tam Giang lagoon with 2 species of the genus *Moolgarda***

Genetic distance of leaf mullet in two waters of Tam Giang lagoon calculated by Kimura 2-parameter ratio is shown in Table 3.

**Table 3. Genetic distance of *M. cunnesius***

	MW336954.1 <i>M.cunnesius</i>	KY315413.1 <i>M.perusii</i>	Mooc_018	Mooc_017
MW336954.1_ <i>M. cunnesius</i>	***	0.0016	0.0016	0.0016
KY315413.1_ <i>M. perusii</i>		***	0.0016	0.0016
Mooc_018			***	0.0033
Mooc_017				***

Mooc\_017 and Mooc\_018 = *Moolgarda cunnesius*

The genetic distance between the two populations of mullet in Tam Giang lagoon is low (0.0033). This result is quite similar to the study of Tran et al. (2021) (0,0057). When building the phylogenetic tree of 2 samples of longarm mullet with 2 CO1 gene sequences of 2 species of the genus *Moolgarda* (sequences from Genbank). The results show that all fish samples belong to the same clade as *M. cunnesius* with 100% bootstrap value.

**Conclusion**

The *M. cunnesius* (Valenciennes, 1836) was distributed in the northern and southern areas of Tam Giang lagoon, Vietnam. This group of fish distributed in the north ranges in size from 101.7 to 160.7 mm, on average reaching 137 mm, corresponding to a weight of 10.6 - 35.6 g, an average of 25.2 g, fish distributed in the south range in size from 112.4 - 172.7 mm average 144 mm corresponding to the weight from 10.6 - 35.6 g, average 28.3 g. Extracting 10 total DNA samples of leaf mullet in the

two regions of the North and the South, 10 samples were amplified and sequenced the CO1 gene region. Amplified and sequenced 2 samples of CO1 gene of leaf mullet sample in Tam Giang lagoon with the size of 650. The average ratio of four types of nucleotides is A: 23.38%; T: 32.62%; G: 18.15%; C: 25.85%. The rate of difference in CO1 sequences of the 2 samples is low compared to previous studies. The similarity coefficient of the mullet in the two Tam Giang lagoon waters is very high (0.9953).

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## References

1. Dang, D.T., Vo. V.P., 2017. Distribution and exploitation situated of Longarm Mullet–*Moolgarda cunnesius* (Valenciennes, 1836) in Tam Giang – Cau Hai, Thua Thien Hue. *VNU Journal of Science: Natural Sciences and Technology*, 33(2S):295-301.
2. Dang, D.T., Vo, V.Q., Tran, Q.D., 2021. Polymorphic analysis of COI gene of longarm mullet *Moolgarda cunnesius* (Valenciennes, 1836) in Thua Thien Hue, Vietnam. *Stem Cell*, 12(2):1-9
3. Durand, J.D., Borsa P., 2015. Mitochondrial phylogeny of grey mullets (Acanthopterygii: Mugilidae) suggests high proportion of cryptic species. *C. R. Biologies*, 338:266–277
4. Froese, R. and D. Pauly. Editors. (2021). FishBase. *Moolgarda cunnesius* (Valenciennes, 1836). Accessed through: World Register of Marine Species at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=309761> on 2021-06-25
5. Hebert P.D.N., Cywinska A., Ball S.L., de waard J.R., 2003a. Biological identifications through DNA Barcodes. *Proc. R. Soc. Lond. B. Biol. Sci.*, 207:313–321.
6. Hebert, P.D.N., Sujeevan R., de Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B.*, 270:96-99.
7. Kress, W.J., García-Robledo, C., Uriarte, M., Erickson, D.L., 2015. DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution*, 30(1):25-35.
8. Nguyen, M.L., Pham, T.T., Nguyen, V.Q., Pham, V.C., Dao, H.L., Dinh, V.N., Dam, T.L., 2018. DNA barcoding application of mitochondrial CO1 gene to early identify fish species of family Gobiidae in VietNam. *Journal of Marine Science and Technology*, 18(4):36 – 62.
9. Nguyen, P.L., 2010. *Fish fauna and biological characteristics of some economic fish species in O Loan lagoon, Phu Yen province*. Doctoral thesis, University of Education, Hue University, Vietnam.
10. Nguyen, V.H., 2005. Three orders of bony fish, *Vietnamese freshwater fish* (volume III). Hanoi Agricultural Publishing House, pp. 1-250.
11. Nguyen, V.H., Nguyen, H.D., 2012. Research on fish species composition in Tam Giang - Cau Hai lagoon, Thua Thien - Hue province. *Journal of Biology*, 34(1):20-30.
12. Nwakanma, C., Ude G., Unachukwu, M.N., 2015. The use of DNA barcoding in identification of genetic diversity of fish in Ugwu-Omu Nike river in Enugu. *Nig J. Biotech.*, 29:27-33.
13. Pravdin I.F., 1973. *A Guide to Fish Research* (Vietnamese translation by Pham Thi Minh Giang). Science and Technology Publishing House, Hanoi, pp. 1-150.
14. Qwabe, W., Cyrus, D.P., 2020. Diet of the mullets *Planilizamacrolepis* and *Moolgardacunnesius* in the Mfolozi-Msunduzi Estuary, KwaZulu-Natal, South Africa, *African Journal of Aquatic Science*, 2020: 1–10
15. *Journal of Aquatic Science*, 2020: 1–10

16. Thapliyal, M., Sati, B.K., Kumar, R., Chandra, T., Thapliyal, A., 2013. DNA barcoding of fishes from River Song, Dehradun, Uttarakhand, using mitochondrial *cytochrome-c oxidase-I* gene. *Environment Conservation Journal*, 14(3):113-121.
17. Thomson J.M., 1984. *Mugilidae*. In Fischer W., Bianchi G. (eds). FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing area 51). FAO, Rome, volume 3.
18. Thomson J.M., 1997. The Mugilidae of the world. *Memoirs of the Queensland Museum*, 41(3): pp.457-562.
19. Truong, S.K., 1991. Nutritional characteristics of mullet *Mugil kelaartii* living in Cua Be waters. *Journal of biology*, 10:24-27.
20. Wang, L., Wu, Z., Liu, M., Liu, W., Zhao, W., Liu, H., You F., 2018. DNA barcoding of marine fish species from Rongcheng Bay. *China. PeerJ.*, 6:e5013: 1-19.
21. Ward, R.D., Zemplak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Phil. Trans. R. Soc. B.*, 360:1847–1857.
22. Vu, T. T., 2009. *The estuarine Ecosystems of Vietnam*. Viet Nam Education Publishing House, 327 (In Vietnamese).
23. Yen, D.T., 2014. Sequence comparison of DNA barcoding genes between new phenotype and wild strains of climbing perch (*Anabas testudineus* Bloch, 1792). *Journal of Science*, Can Tho University, 30:29-36.