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Research Article

Morphological Characteristics and Genetic Diversity of Fish *Moolgarda cunnesius* (Valenciennes, 1836) in Tam Giang Lagoon, Vietnam

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Authors' Contributions
TN and GTHP collected samples. LLTH, TN and GTHP processed the samples. TN and GVT planned the experiments. GTHP and TN performed experiments.TN and LLTH wrote the manuscript. GVT presented the idea, analyzed data and took the lead in writing the manuscript.

Keywords
Genetic diversity, Longarm mullet, Morphological characteristics, *Moolgarda cunnesius*, Tam Giang lagoon

Abstract | *Moolgarda cunnesius*, belongs to the Mugilidae family, is an important fish species in view of its immense contribution to the need of this country 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 identification and the genetic diversity, which are the basis for further research 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. *CO*1 gene DNA barcoding was used to study the genetic diversity of *M.cunnesius* in two regions of the North and the South of Tam Giang lagoon. Amplified and sequenced 10 samples of *CO*1 gene of *M. cunnesius* sample in Tam Giang lagoon were recorded 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 *CO*1 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).

Novelty Statement | The paper presented detailed morphological features and genetic diversity of *Moolgarda cunnesius* (Valenciennes, 1836) in Tam Giang Lagoon, Vietnam, which is the first study in this area to serve as a scientific basis for breeding and farming and conservation of this species in the future.

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Introduction

Moolgarda cunnesius (Valenciennes, 1836) is the member of 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

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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 18 orders with 73 families including 177 species 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 and 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. However, up to now, there have been no studies on the morphology and genetic polymorphism of *Moolgarda cunnesius* in Tam Giang lagoon, Thua Thien Hue province. According to Hebert *et al.* (2003a), DNA barcode basing on the use of gene sequences in the mtDNA cytochrome c oxidase 1 (*CO*1) can apply as the universal bioidentification systems for creatures. The species recognition is carried effectively through *CO*I 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). Aaron *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 *CO*1 barcoding for specifing species in Mugilidae family and determing molecular detection for 24 species and for 25 cryptic creatures. 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 speciments 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 locals, or by buying fish samples from fishermen in the area. The pectoral fin samples were cut off and put in eppendorf tubes with 96% alcohol, then they were stored at -86°C for further analysis.

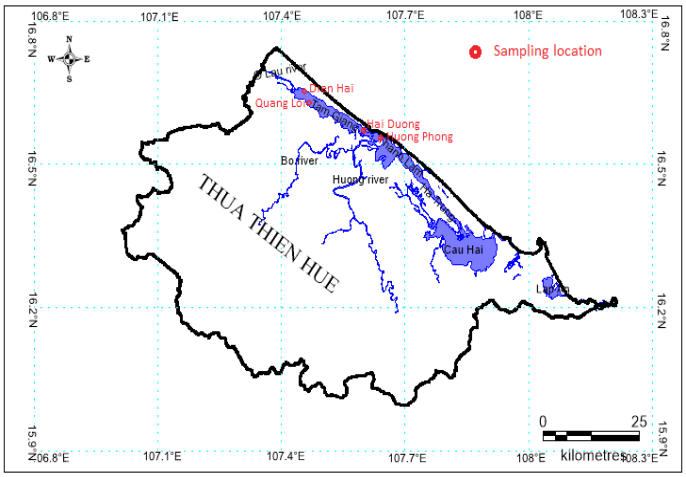

Figure 1: Map of sampling location of *M. cunnesius*.

Morphological study

Fish samples were conducted to study as soon as the fish was caught 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 (1984) 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 base 1, 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

GeneJET Genomic DNA Purification Kit DNA Extraction Kit was applied to extract the total DNA of each pectoral fin following the producer's procedure. The 650 bp DNA fragment (*CO*1) was amplified by PCR reaction with the using of universal primers: Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al.*, 2005). The total reaction volume of 50 µl was composed of: 2 µl of total DNA (~50ng), 25 µl of GoTaq (2X), 2.5 µl of each primer (10pmol/µl) and 18 µl of Kit free-RNA/DNA water. PCR was carried out in a thermal

cycler (ESCO-AERIS-BG096) following the thermal process: (1) DNA denaturation: 94˚C/5 min; (2) 35 cycles: (94˚C/ 1 minute; 54˚C/ 30 seconds; 72˚C/ 55 seconds); (3) final 72˚C/ 5 min. The amplified product was examined by electrophoresing on a 1% agarose gel; the amplification bands were taken in the gel documentation system. Gel analysis software (UN-SCAN-IT gel version 6.1) was applied to analyze the gel images.

Diversity genetic analysis

BioEdit software v.7.2.5 was conducted to edit and arrange sequence. 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 beginning of the anal fin base; Pectoral fin long, beyond first dorsal fin starting point, pectoral fin tip black dotted, pectoral fin base with axillary scale (Figure 2). 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 andrays of first dorsal fin D1 = 4.0; the number of spines andrays of second dorsal fin D2 = 1 – 2,7 – 8; the number of spines and rays and of pectoral fin P = 1 – 3, 12 – 15; the number of spinesand rays of ventral fin V = 1, 5 – 6; the number of spines and rays ofcaudal fin C = 0,14 – 17; the number of spines and rays of anal fin A = 1 – 3, 9 – 10.

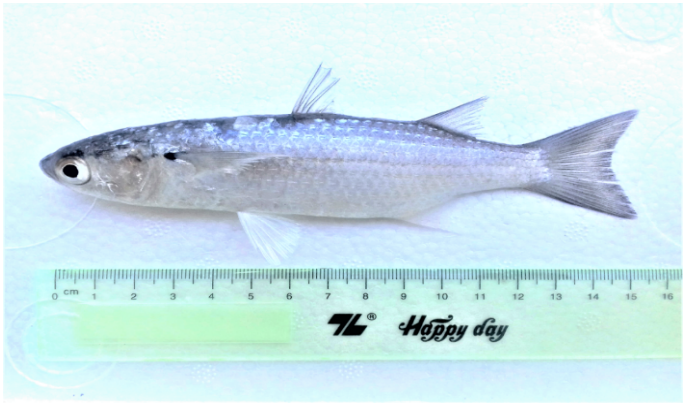

Figure 2: Morphology of *M. cunnesius* in Tam Giang lagoon, Vietnam.

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) (Table 1).

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 dissimilarity 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 reached statistical significance (p<0.05) (Table 1).

The length-weight relationship (LWR) 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).

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 LWR of most of the analyzed individuals in the two areas are individuals with large length often having large sizes and vice versa. Neves et al. (2020) studied about 5 species in Mugil from the Tropical Southwestern Atlantic that suggested morphological features were extremely preserved because of resemblances in the environment that species inhabited and their biographical features. The integrative taxonomy (including DNA barcoding) plays a vital role in conserving and sustainably using the resources of nature.

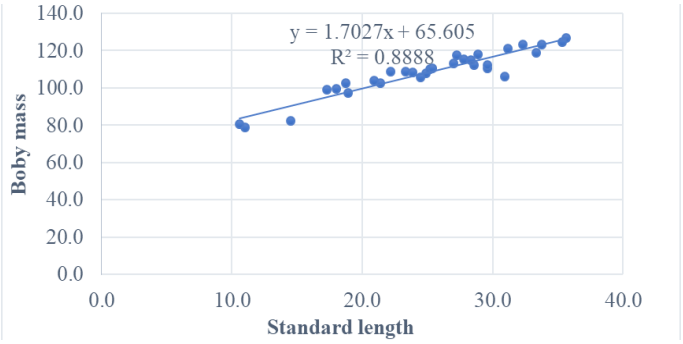

Figure 3: Relationship between standard length and body mass of *M. cunnesius* in the North area.

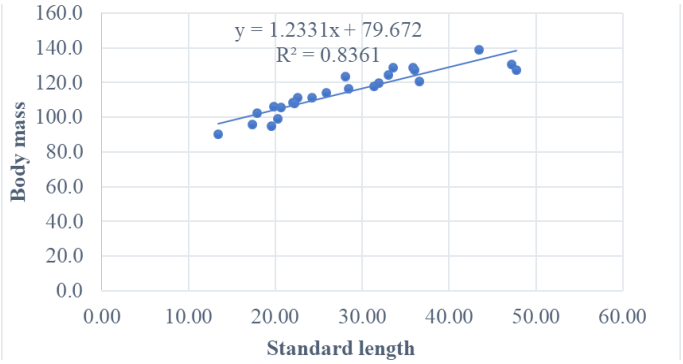

Figure 4: Relationship between standard length and body mass of *M. cunnesius* in the South area.

The correlation 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 polymorphism of Moolgarda cunnesius in Tam Giang lagoon, Vietnam

Total DNA extraction

The total DNA was extracted with high 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.

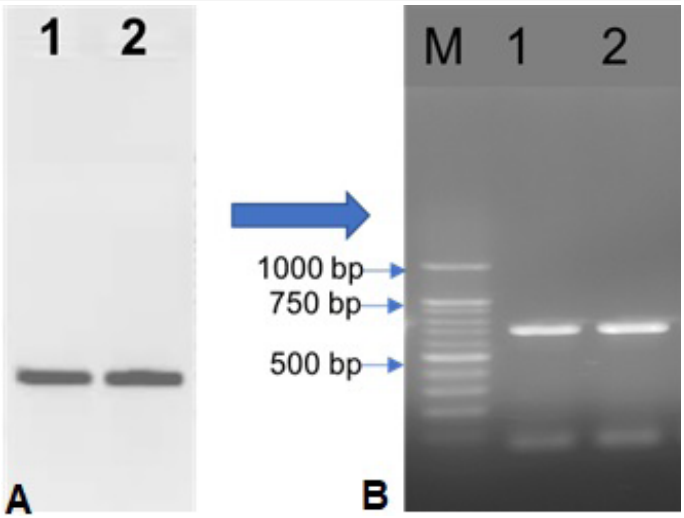

Figure 5: The total DNA electrophoresis of *M. cunnesius* (A) and Results of PCR product electrophoresis (B). M, marker; 1, 2, sample symbol of *M. cunnesius*.

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

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

                                110       120       130       140       150
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        GCTTTCGTAATAATTTTCTTTATAGTGATGCCAATTATGATCGGTGGGTT
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                160       170       180       190       200
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        TGGAAATTGACTTATCCCATTAATGATTGGGGCACCAGATATAGCATTCC
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                210       220       230       240       250
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        CCCGAATAAATAACATAAGCTTCTGGCTTCTTCCCCCTTCATTTCTTCTC
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                260       270       280       290       300
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        CTCCTGGCATCCTCTGCAGTAGAGGCTGGAGCCGGTACAGGATGAACTGT
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                310       320       330       340       350
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        TTACCCGCCTCTCGCCAGCAACCTAGCACATGCTGGAGCATCCGTTGACC
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                360       370       380       390       400
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        TTACTATCTTTTCCCTTCATCTGGCAGGGGTTTCCTCAATTTTAGGTGCT
Mooc_017                        ..................................................
Mooc_018                        ..................................T...............

                                410       420       430       440       450
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        ATTAATTTTATTACAACTATTATTAATATAAAACCTCCTGCTATCTCTCA
Mooc_017                        ...........C......................................
Mooc_018                        ..................................................

                                460       470       480       490       500
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        GTACCAAACCCCTCTATTTGTATGAGCAGTTCTTATTACAGCTGTCCTTC
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                510       520       530       540       550
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        TTCTTCTTTCTTTACCAGTTCTCGCTGCTGGGATTACTATGCTCCTAACA
Mooc_017                        ..................................................
Mooc_018                        ..................................................

                                560       570       580       590       600
                                ....|....|....|....|....|....|....|....|....|....|
MW336954.1: M. cunnesius        GATCGAAACTTAAATACCTCTTTCTTCGATCCTGCAGGGGGAGGAGATCC
Mooc_017                        ..................................................
Mooc_018                        ..................................................
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Figure 6: Comparison of the homologous sequences of the two fish samples with the sequence MW336954.1 *Moolgarda cunnesius* (Genbank).

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 base 1	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

PCR performance product

The results of PCR product analysis on 1% agarose gel showed that specific DNA fragments with size of about 650-750 bp were amplified (Figures 5B and 6).

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

After sequencing and editing, all sequences were conducted to search and compare with matched sequences on Genbank. As a result, these two samples have a high similarity with *M. cunnesius* (Valenciennes, 1836).

When conducting genetic diversity analysis, all *CO*1 mitochondrial DNA gene sequences of the 2 samples were checked by comparing with the corresponding *CO*1 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 (Dang *et al.*, 2021, Table 2).

After processing, the *CO*1 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).

Table 2: The similarity of the Mooc_017 gene sequence in the study with the 10 *CO*I sequences of *M. cunnesius* on the GeneBank.

No.	Species	Gene	Code	Gene size (bp)	Query cover (%)	Proportion (%) of homologous nucleotides
1	*Moolgarda cunnesius*	*CO*1	MW336954.1	704	98%	99.53%
2	*Moolgarda cunnesius*	*CO*1	MF628290.1	655	95%	99.84%
3	*Moolgarda cunnesius*	*CO*1	JQ045777.1	649	95%	99.68%
4	*Moolgardacunnesius*	*CO*1	KT231793.1	611	94%	99.84%
5	*Moolgarda cunnesius*	*CO*1	KX834271.1	588	90%	99.83%
6	*Moolgarda cunnesius*	*CO*1	EU595339.1	652	95%	99.84%
7	*Moolgarda cunnesius*	*CO*1	FJ238048.1	652	95%	99.68%
8	*Moolgarda perusii*	*CO*1	KY315413.1	655	95%	99.84%
9	*Moolgarda perusii*	*CO*1	LC484868.1	655	95%	99.68%
10	*Moolgarda perusii*	*CO*1	LC484868.1	655	95%	99.68%

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

	MW336954.1 *M. cunnesius*	**KY315413.1 *M. perusii***	**Mooc_018**	**Mooc_017**
MW336954.1_*M. cunnesius*	***	0.0016	0.0016	0.0016
KY315413.1_*M. perusii*		***	0.0016	0.0016
Mooc_018			***	0.0033
Mooc_017				***

Mooc_017 and Mooc_018 = *Moolgarda cunnesius.*

The results of comparing the two samples analyzed with the sequence MW336954.1 (Dang *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).

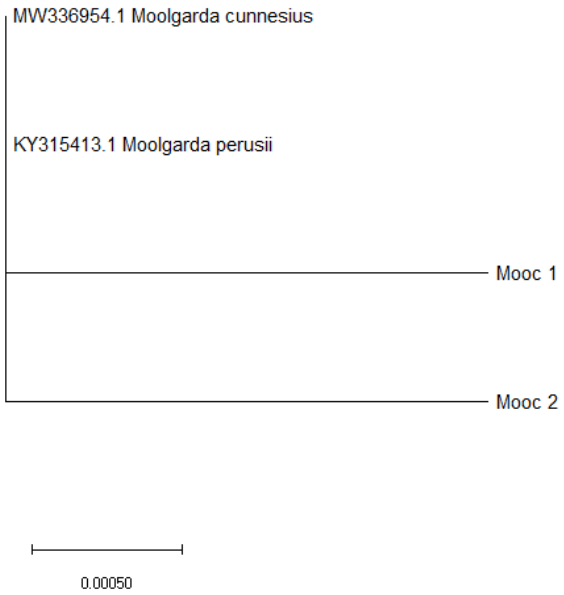

Figure 7: Relatives of the fish *Moolgarda cunnesius* in Tam Giang lagoon with 2 species of the genus Moolgarda.

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

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 Dang *et al.* (2021) (0.0057). When building the phylogenetic tree of 2 samples of longarm mullet with 2 *CO*1 gene of 2 species of the *Moolgarda* genus (downloaded from Genbank). The results show that all specimen are in the same branch as *M. cunnesius* with 100% bootstrap value.

Conclusions and Recommendations

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 specimens were amplified and sequenced the *CO*1 gene. Amplified and sequenced 2 samples of *CO*1 gene of 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 *CO*1 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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Conflict of interest

The authors have declared no conflict of interest.

References

Aaron, C.C.Y., Aziz, A.H., Tafzil Meriam, S.A.K., Seah, Y.G. and Nur-Asma, A., 2018. Morphological an molecular identification of mullet species (Mugilidae) from Setiu Wetland, Terengganu, Malaysia. *AACL Bioflux,* **11**(2): 429-438.

Dang, D.T., Vo, V.Q., and Tran, Q.D., 2021. Polymorphic analysis of *CO*I gene of longarm mullet *Moolgarda cunnesius* (Valenciennes, 1836) in Thua Thien Hue, Vietnam. *Stem Cell,* **12**: 1-9.

Dang, D.T., and Vo. V.P., 2017. Distribution and exploitation situated of Longarm Mullet–*Moolgarda cunnesius* (Valenciennes, 1836) in Tam Giang – Cau Hai, Thua Thien Hue. *VNU J. Sci. Natl. Sci. Technol.*, **33**(**2S**): 295-301.

Durand, J.D., and Borsa P., 2015. Mitochondrial phylogeny of grey mullets (Acanthopterygii: Mugilidae) suggests high proportion of cryptic species. *C. R. Biol.,* **338**: 266–277. https://doi.org/10.1016/j.crvi.2015.01.007

Froese, R. and Pauly, D., 2021. FishBase. *Moolgarda cunnesius* (Valenciennes, 1836). Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetailsandid=309761 on 2021-06-25

Hebert, P.D.N., Cywinska, A., Ball S.L. and de Waard, J.R., 2003a. Biological identifications through DNA Barcodes. *Proc. R. Soc. Lond. B. Biol. Sci.*, **207**: 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P.D.N., Sujeevan R., and 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. https://doi.org/10.1098/rsbl.2003.0025

Kress, W.J., García-Robledo, C., Uriarte, M., and Erickson, D.L., 2015. DNA barcodes for ecology, evolution, and conservation. *Trends Ecol. Evol.*, **30**: 25-35. https://doi.org/10.1016/j.tree.2014.10.008

Neves, J.M.M., Perez, A., Fabré, N.N., Pereira, R.J. and Mott, T., 2020. Integrative taxonomy reveals extreme morphological conservatism in sympatric Mugil species from the Tropical Southwestern Atlantic. *J. Zoolog. Syst. Evol. Res.*, **59**(1): 163–178. https://doi.org/10.1111/jzs.12421

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.

Nguyen, V.H., 2005. *Three orders of bony fish, Vietnamese freshwater fish* (volume III). Hanoi Agricultural Publishing House, pp. 1-250.

Nguyen, V.H., and Nguyen, H.D., 2012. Research on fish species compositionin Tam Giang - Cau Hai lagoon, Thua Thien - Hue province. *J. Biol.*, **34**: 20-30. https://doi.org/10.15625/0866-7160/v34n1.666

Nwakanma, C., Ude G., and 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. https://doi.org/10.4314/njb.v29i1.4

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.

Qwabe, W., and Cyrus, D.P., 2020. Diet of the mullets *Planiliza macrolepis* and *Moolgarda cunnesius* in the Mfolozi-Msunduzi Estuary, KwaZulu-Natal, South Africa, *Afr. J. Aquat. Sci.*, **2020**: 1–10.

Thomson, J.M., 1984. *Mugilidae*. In: *FAO species identification sheets for fishery purposes* (eds. W. Fischer and G. Bianchi). Westerm Indian Ocean (Fishing area 51). FAO, Rome, volume 3.

Truong, S.K., 1991. Nutritional characteristics of mullet *Mugil kelaartii* living in Cua Be waters. *J. Boil.*, **10**: 24-27.

Vu, T.T., 2009. *The estuanne ecosystems of Vietnam*. Vietnam Education Publishing House, 327 (In Vielnamese).

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., and Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B.,* **360**: 1847–1857. https://doi.org/10.1098/rstb.2005.1716