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Polymorphic analysis of *CO*I gene of longarm mullet *Moolgarda cunnesius* (Valenciennes, 1836) in Thua Thien Hue, Vietnam

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Abstract: Longarm mullets *Moolgarda cunnesius* (Valenciennes, 1836) belong to the family Mugilidae. They are found in fresh, brackish and salt water and distribute in Indo-West Pacific. In this study, mitochondrial cytochrome oxidase subunit I gene (*CO*I) were employed to investigate the genetic diversity of longarm mullets. *M. cunnesius* individuals were collected from Tam Giang lagoon (n=10) and Thua Thien Hue sea coast (n=9) were examined. Nineteen *COI* gene fragment sequences were deposited in GenBank database with accession numbers MW336937-MW336955. Among 19 sequences of *CO*I gene fragment, 11 distinct haplotypes were defined. Although the population haplotype diversity (Hd) was generally high (0.833 and 0.756), the nucleotide diversity (π) was relatively low (0.00276 and 0.00265) among the two populations. Additionally, the genetic distances ranged from 0-0.99% within the populations and the genetic distance between the two populations was 0.27%. Our study showed the population genetic diversity and structure of longarm mullets *M. cunnesius* in Thua Thien Hue, Vietnam.
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1. Introduction

Longarm mullets *Moolgarda cunnesius* (Valenciennes, 1836) (synonym: *Valamugil cunnesius* (Valenciennes, 1836)) (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=1111463), belong to the family Mugilidae. They are found in fresh, brackish and salt water and their distribution is Indo-West Pacific: South to South Africa.

DNA barcoding is a method of rapid, accurate, and automated species identifications by using short, standardized gene regions as internal species tags (Hebert et al., 2003; 2005). The most commonly used barcode region for animals, including fishes is a portion of the cytochrome c oxidase I (*CO*I) gene, found in mitochondrial DNA.

Recently, the use *CO*I gene to identify, analyze the phylogenetic relationships in many fish species has been carried out by researchers in the world such as *Anguilla eels* in Thua Thien Hue, Vietnam (Huyen et al., 2020a, 2020b); fish species from freshwater in Enugu and Anambra States of Nigeria (Ude et al., 2020); *Epinephelus coioides* in Quang Nam sea, Vietnam (Vi et al., 2019); *Sewellia spp*. in Thua Thien Hue, Vietnam (Dieu et al., 2019); *Diplodus sargus* and *Diplodus vulgaris* in the Egyptian Mediterranean Sea (Abbas et al., 2018); *Oxyeleotris heterodon* at Putali Gulf Sentani Lake (Abinawanto et al., 2018); freshwater fishes of Indo-Myanmar biodiversity hotspot (Barman et al., 2018); the genus *Capoeta* (Actinopterygii: Cyprinidae) from Anatolia (Bektas et al., 2018); fish species in the Taiwan strait (Bingpeng et al., 2018); marine fish species from Rongcheng Bay, China (Wang et al., 2018); *Siganus guttatus* in Quang Nam-Da Nang sea, Vietnam (Vi et al., 2018); *Helostoma temminckii* in the Mekong Delta (Yen et al., 2018); *Puntius ticto* from Halali Reservoir (Garg et al., 2017); nomei fish (Synodontidae: *Harpadon* sp.) in Tarakan Island, Indonesia (Nugroho et al., 2017); sharks in Guyana coastal markets (Kolmann et al., 2017); pufferfish species (Tetraodontidae) in Turkish Marine Waters (Turan et al., 2017); the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin (Van Ginneken et al., 2017); *Pangasius krempfi*, *P. mekongensis*, and *P. elongates* from Can Tho, Soc Tang, Tra Vinh, and Ca Mau, Vietnam (Yen et al., 2016); fishes of the Itapecuru Basin in Maranhão (Nascimento et al., 2016); Cyprinidae fish in the midstream of the Yangtze River (Shen et al., 2016); fishes in the upper reaches of the Salween

River, Nujiang River (Cheng et al., 2015); fishes in Ugwu-Omu Nike river in Enugu (Nwakanma et al., 2015); *Butis butis* and *Butis humeralis* from Tra Vinh and Soc Trang, Vietnam (Thao et al., 2015); freshwater leeches in Lake Gusinoe (Eastern Siberia, Russia) (Kaygorodova et al., 2014); *Anabas testudineus* from Hau Giang, Ca Mau, and Dong Thap, Vietnam (Yen et al., 2014); fishes from River Song, Dehradun, Uttarakhand (Thapliyal et al., 2013); freshwater fish species in Turkey (Keskin et al., 2013); billfishes from a variety of sources (Hanner et al., 2011); Canadian freshwater fishes (Hubert et al., 2008); Australia's fish species (Ward et al., 2005); carangid fishes from Kakinada coast, India (Persis et al., 2009), etc.

Concerning mullet fishes, the *COI* sequences of some Mugilidae fishes were analyzed (*Moolgarda perusii* (Aaron et al., 2018); *Mugil cephalus, Liza ramada, Liza aurata, Chelon labrosus, Chelon caeruleum sp. nov., and Liza carinata (*Deef, 2018*); Chelon labrosus*, *Liza aurata*, *Liza haematocheila*, *Chelon macrolepis*, *Liza ramado*, *Liza saliens, Chelon subviridis*, *Mugil cephalus*, *Moolgarda cunnesius* (Polyakova et al., 2013); *Mugil hospes* (Nirchio et al., 2018); *Mugil curema* (Nirchio et al., 2017); 55 currently recognized Mugilidae species (Durand et al., 2012)).

Our previous studies showed distribution and some gonad cell-tissue characteristics of the longarm mullets in Tam Giang lagoon, Thua Thien Hue (Tue and Phu, 2017; 2021). The aim of this study is to identify, isolate, purify, sequence and analyze of mitochondrial *CO*I gene fragment of *M. cunnesius* collected in Thua Thien Hue, Vietnam, for which no data are available at present.

2. Material and Methods

Sample collection

A total of nineteen individual longarm mullets *M. cunnesius* (Valenciennes, 1836) were captured from Tam Giang lagoon (n=10) and Thua Thien Hue sea coast (n=9), Vietnam (Figure 1 and Figure 2) were used for this study. Specimens were stored in the laboratory at the Department of Genetics, Faculty of Biology, University of Education, Hue University, Vietnam and stored in 98% alcohol, -20°C until further utilized.

Table 1. Specimens for this study with locality and voucher code

Locality	Number of specimens (n)	Voucher code
Tam Giang lagoon	10	T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10.
Thua Thien Hue sea coast	9	B1, B2, B3, B4, B5, B6, B7, B8 and B9.

Genomic DNA extraction, amplification, and sequencing

Genomic DNA was isolated from the stored muscle tissues using phenol-chloroform protocol (Sambrook and Russel, 2001). The quantity and quality of extracted DNA were estimated by measuring its absorbance value at 260 nm and determining the ratio of absorbance values at 260 nm and 280 nm, respectively. Genomic DNA was stored at -20°C until analysis.

Figure 1. Longarm mullet *M.cunnesius* (Valenciennes, 1836)

The fragment of *CO*I gene approximately 704 bp length located in the mitochondrial genome was amplified using the primer pair was designed by Ward et al. (2005).

FishF1 - 5'TCAACCAACCACAAAGACATTGGCAC3'

FishR2 - 5'ACTTCAGGGTGACCGAAGAATCAGAA3'

The polymerase chain reaction (PCR) was performed in a total volume of 60 µL, including 150 ng DNA, 50 pg of each primer, 20 µL 2× Go Taq® Green Master Mix (M7502, Promega, USA), and distilled water to the final reaction volume. PCR amplification was performed with denaturation for 10 min at 95°C; 30 cycles of 95°C for 1 min, annealing temperature 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were visualized on 0.8% agarose gels. The resulted mitochondrial *CO*I gene fragments were purified using Wizard®SV Gel and PCR CleanUp System (Promega), according to the manufacturer recommendations. *CO*I gene fragment sequencing was performed by First BASE Laboratories Sdn Bhd (Selangor, Malaysia).

Data analysis

All *CO*I gene fragment sequences were deposited in GenBank with accession numbers MW336937-MW336955. BLAST (Basic Local Alignment Search Tool) software was used for similarity searching of the *CO*I sequences in GenBank (http://blast.stva.ncbi.nlm.nih.gov/). For phylogenetic analyses, a total of 30 sequences were obtained from GenBank (Table 2). The sequences generated in the forward and reverse directions were edited and aligned in BioEdit version 7.0 (Hall, 1999).

The haplotype number (Nh), haplotype diversity (h), nucleotide diversity (π), number of polymorphic sites (S), number of mutations (η), and average number nucleotides differences (k) were calculated using DnaSP v6.12 (Rozas et al., 2017).

In addition, Geneious Prime 2020 sofware was used to calculate genetic distances and to construct a Maximum likelihood phylogenetic tree. The confidence level of the phylogenetic trees was tested with 1000 replications.

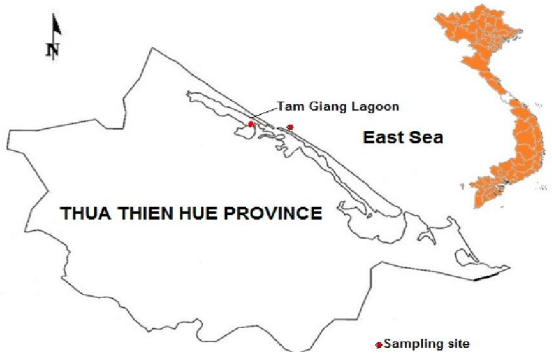

Figure 2. Map of sampling site for specimen's longarm mullet *M. cunnesius* in this study

Table 2. Voucher number, GenBank accession number, sampling location and reference of Mugilidae individual *CO*I sequences used in this study

No.	Species	Voucher number	GenBank accession numbers	Sampling location	Reference
1	*Moolgarda cunnesius*	K03-1	MF628290.1	Fujian, China	Xu et al., 2017
2	*Moolgarda perusii*	N3-4	KY315410.1	Fujian, China	Xu et al., 2017
3	*Moolgarda perusii*	M4-3	KY315388.1	Fujian, China	Xu et al., 2017
4	*Moolgarda cunnesius*	Vellar2	JQ045777.1	Tamil Nadu, India	Rahman et al., 2012
5	*Valamugil cunnesius*	Z711253	EU595340.1	South China Sea	Zhang et al., 2011
6	*Moolgarda cunnesius*	275	JQ060496.1	Australia	Durand et al., 2012
7	*Moolgarda cunnesius*	276	JQ060497.1	Yunlin, Taiwan	Durand et al., 2012
8	*Moolgarda cunnesius*	276b	JQ060499.1	Do Son Town, Vietnam	Durand et al., 2012
9	*Moolgarda cunnesius*	278	JQ060498.1	South Africa	Durand et al., 2012
10	*Moolgarda perussi*	264	JQ060504.1	New Caledonia	Durand et al., 2012
11	*Moolgarda perussi*	274	JQ060505.1	Taisi, Taiwan	Durand et al., 2012
12	*Moolgarda engeli*	198	JQ060500.1	Mariana Islands	Durand et al., 2012
13	*Moolgarda engeli*	200	JQ060501.1	Hawaii	Durand et al., 2012
14	*Moolgarda engeli*	201	JQ060502.1	French Polynesia	Durand et al., 2012
15	*Moolgarda engeli*	203	JQ060503.1	French Polynesia	Durand et al., 2012
16	*Moolgarda engeli*	204	JQ060506.1	New Caledonia	Durand et al., 2012
17	*Moolgarda engeli*	205	JQ060507.1	Philippines	Durand et al., 2012
18	*Moolgarda engeli*	206	JQ060508.1	Taiwan	Durand et al., 2012
19	*Moolgarda engeli*	207	JQ060509.1	West Papua	Durand et al., 2012
20	*Moolgarda seheli*	210	JQ060510.1	Mariana Island	Durand et al., 2012
21	*Moolgarda seheli*	234	JQ060516.1	Australia	Durand et al., 2012
22	*Moolgarda* sp.	JDD-2011a212	JQ060518.1	La Réunion	Durand et al., 2012
23	*Moolgarda* sp.	JDD-2011b 221	JQ060519.1	Sri Lanka	Durand et al., 2012
24	*Moolgarda* sp.	JDD-2011b 222	JQ060520.1	La Réunion	Durand et al., 2012
25	*Moolgarda* sp.	JDD-2011a 215	JQ060522.1	Oman	Durand et al., 2012
26	*Valamugil sp.*	JDD-2011a 209	JQ060631.1	-	Durand et al., 2012
27	*Valamugil sp.*	JDD-2011a 213	JQ060632.1	-	Durand et al., 2012
28	*Valamugil sp.*	JDD-2011b 221a	JQ060634.1	-	Durand et al., 2012
29	*Valamugil sp.*	JDD-2011a 214	JQ060633.1	-	Durand et al., 2012
30	*Mugil bananensis*	289	JQ060524.1	Ivory Coast	Durand et al., 2012

3. Results and Discussion

Genetic variation

The 704 bp partial *CO*I length fragment of mtDNA *CO*I gene was obtained from 19 individuals of longarm mullets *M. cunnesius*. The result of alignment on 19 sequences *CO*I gene fragment of longarm mullets *M. cunnesius* Thua Thien Hue, Vietnam showed 15 substitutions of nucleotide bases (position: 28, 261, 273, 339, 366, 408, 426, 429, 516, 539, 552, 579, 591, 604, and 639). The substitution of these nucleotide bases consisted of 10 transitions, 2 tranversions and no insertion and deletion. Besides that, a total of 11 haplotypes were defined among the 19 individuals. The Thua Thien Hue sea coast population exhibited six haplotypes (H1, H2, H3, H4, H5, and H6), and the Tam Giang population exhibited six haplotypes (H1, H7, H8, H9, H10, and H11). H1 was shared by the two populations (Table 3). This was higher compared to the haplotypes of the orange-spotted grouper *E. coioides* (Hamilton, 1882) (Vi et al., 2009), but lower compared to the haplotypes of Indian salmon, *Eleutheronema tetradactylum* (Thirumaraiselvi et al., 2011). However, the difference between haplotype numbers could be due to the differences in sample sources, numbers and the length of *CO*I gene sequences (Ma et al., 2011).

Table 3. Variable sites among 11 *CO*I haplotypes of longarm mullets *M. cunnesius* collected from Thua Thien Hue, Vietnam (n=19)

Halotype	Nucleotide substitution position														
	28	261	273	339	366	408	426	429	516	539	552	579	591	604	639
H1 (B1, B3, B4, B7, T1, T2, T4, T7, T8, T9)	C	C	C	A	G	C	T	T	A	T	T	T	G	C	T
H2 (B2)											C				
H3 (B5)					A								A		
H4 (B6)				G				C							
H5 (B8)						T									
H6 (B9)			T	G					G						
H7 (T3)	T									A					
H8 (T5)				G			G					C		T	C
H9 (T6)				G											
H10 (T7)		T													
H11 (T10)				G											

Note: All haplotypes are compared with H1

Genetic diversity

Haplotype diversity (Hd) and standard deviation at Tam Giang lagoon and Thua Thien Hue sea coast were 0.756±0.01678 and 0.833±0.01600, respectively. Meanwhile, the mean nucleotide diversity (π) and standard deviation were 0.00265±0.00100 (Tam Giang lagoon) and 0.00276±0.00074 (Thua Thien Hue sea coast) (Table 4). It was found that the longarm mullets *M. cunnesius* exhibited high haplotype diversity but low nucleotide diversity.

The association of high haplotype diversity and low nucleotide diversity is common in pelagic marine fishes (Liu et al., 2015). Our result was similar to the results for *Eleutheronema tetradactylum* from South Asian (Thirumaraiselvi et al., 2015), *Terapon jarbua* populations from the Taiwanese waters (Liu et al., 2015).

Genetic identity and genetic distance

Comparison of *CO*I gene fragments indicated that 19 specimens of *M. cunnesius* homologize 99.01-100% and the genetic distances were 0-0.99%. Analysis of *CO*I sequences of ten specimens from Tam Giang lagoon showed identity of 99,43-100% and genetic distances were 0-0.57%; and nine specimens from Thua Thien Hue coast showed identity of 99.01-100% and genetic distances were 0-0.99% (Table 5).

The values of genetic distance and genetic identity between two populations of longarm mullets *M. cunnesius* collected in Thua Thien Hue, Vietnam base on *CO*I gene fragments were calculated and given in Table 6. The outcome indicates that the value of genetic identity between two populations was high;

99.73% (the value of genetic distance was only 0.27%).

Based on the sequence of *CO*I gene segments of *M. cunnesius* individuals in Thua Thien Hue, Vietnam and the reference sequences taken from GenBank (Table 2), phylogenetic tree were built using Geneious Prime 2020 sofware (Figure 3). The phylogenetic tree showed that *M. cunnesius* from Thua Thien Hue sea coast and Tam Giang lagoon samples were clustered in one group with *M. cunnesius* from China, India, Taiwan, South China Sea, and Do Son Town, Vietnam from the GenBank.

The observations generated using *CO*I gene fragments revealed the high polymorphic levels and the genetic relationship of two populations of longarm mullets *M. cunnesius* collected in Thua Thien Hue, Vietnam.

Table 4. Genetic diversity of longarm mullets *M. cunnesius* populations based on *CO*I sequence

Population	Sample size (n)	Nh	Hd±SD	π±SD	S	η	k
Thua Thien Hue sea coast	9	6	0.833±0.01600	0.00276±0.00074	8	8	1.944
Tam Giang lagoon	10	6	0.756±0.01678	0,00265±0.00100	8	8	1.867
Total	19	11	0.784±0.00952	0.00267±0.00069	15	15	1.883

Table 5. Genetic identity between *CO*I gene fragments of longarm mullets *M. cunnesius* collected in Thua Thien Hue, Vietnam

	B1	B2	B3	B4	B5	B6	B7	B8	B9	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
B1	***	99.86	100	100	99.72	99.72	100	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	100	100	99.86
B2	99.86	***	99.86	99.86	99.57	99.57	99.86	99.72	99.43	99.86	99.86	99.57	99.86	99.15	99.72	99.72	99.86	99.86	99.72
B3	100	99.86	***	100	99.72	99.72	100	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	100	100	99.86
B4	100	99.86	100	***	99.72	99.72	100	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	100	100	99.86
B5	99.72	99.57	99.72	99.72	***	99.43	99.72	99.57	99.29	99.72	99.72	99.43	99.72	99.01	99.57	99.57	99.72	99.72	99.57
B6	99.72	99.57	99.72	99.72	99.43	***	99.72	99.57	99.57	99.72	99.72	99.43	99.72	99.29	99.86	99.57	99.72	99.72	99.86
B7	100	99.86	100	100	99.72	99.72	***	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	100	100	99.86
B8	99.86	99.72	99.86	99.86	99.57	99.57	99.86	***	99.43	99.86	99.86	99.57	99.86	99.15	99.72	99.72	99.86	99.86	99.72
B9	99.57	99.43	99.57	99.57	99.29	99.57	99.57	99.43	***	99.57	99.57	99.29	99.57	99.15	99.72	99.43	99.57	99.57	99.72
T1	100	99.86	100	100	99.72	99.72	100	99.86	99.57	***	100	99.72	100	99.29	99.86	99.86	100	100	99.86
T2	100	99.86	100	100	99.72	99.72	100	99.86	99.57	100	***	99.72	100	99.29	99.86	99.86	100	100	99.86
T3	99.72	99.57	99.72	99.72	99.43	99.43	99.72	99.57	99.29	99.72	99.72	***	99.72	99.01	99.57	99.57	99.72	99.72	99.57
T4	100	99.86	100	100	99.72	99.72	100	99.86	99.57	100	100	99.72	***	99.29	99.86	99.86	100	100	99.86
T5	99.29	99.15	99.29	99.29	99.01	99.29	99.29	99.15	99.15	99.29	99.29	99.01	99.29	***	99.43	99.15	99.29	99.29	99.43
T6	99.86	99.72	99.86	99.86	99.57	99.86	99.86	99.72	99.72	99.86	99.86	99.57	99.86	99.43	***	99.72	99.86	99.86	100
T7	99.86	99.72	99.86	99.86	99.57	99.57	99.86	99.72	99.43	99.86	99.86	99.57	99.86	99.15	99.72	***	99.86	99.86	99.72
T8	100	99.86	100	100	99.72	99.72	100	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	***	100	99.86
T9	100	99.86	100	100	99.72	99.72	100	99.86	99.57	100	100	99.72	100	99.29	99.86	99.86	100	***	99.86
T10	99.86	99.72	99.86	99.86	99.57	99.86	99.86	99.72	99.72	99.86	99.86	99.57	99.86	99.43	100	99.72	99.86	99.86	***

Table 6. Genetic identity between two populations of longarm mullets *M. cunnesius* in Thua Thien Hue, Vietnam base on *CO*I gene fragments

Population	Thua Thien Hue sea coast	Tam Giang lagoon
Thua Thien Hue sea coast	***	
Tam Giang lagoon	99.73	***

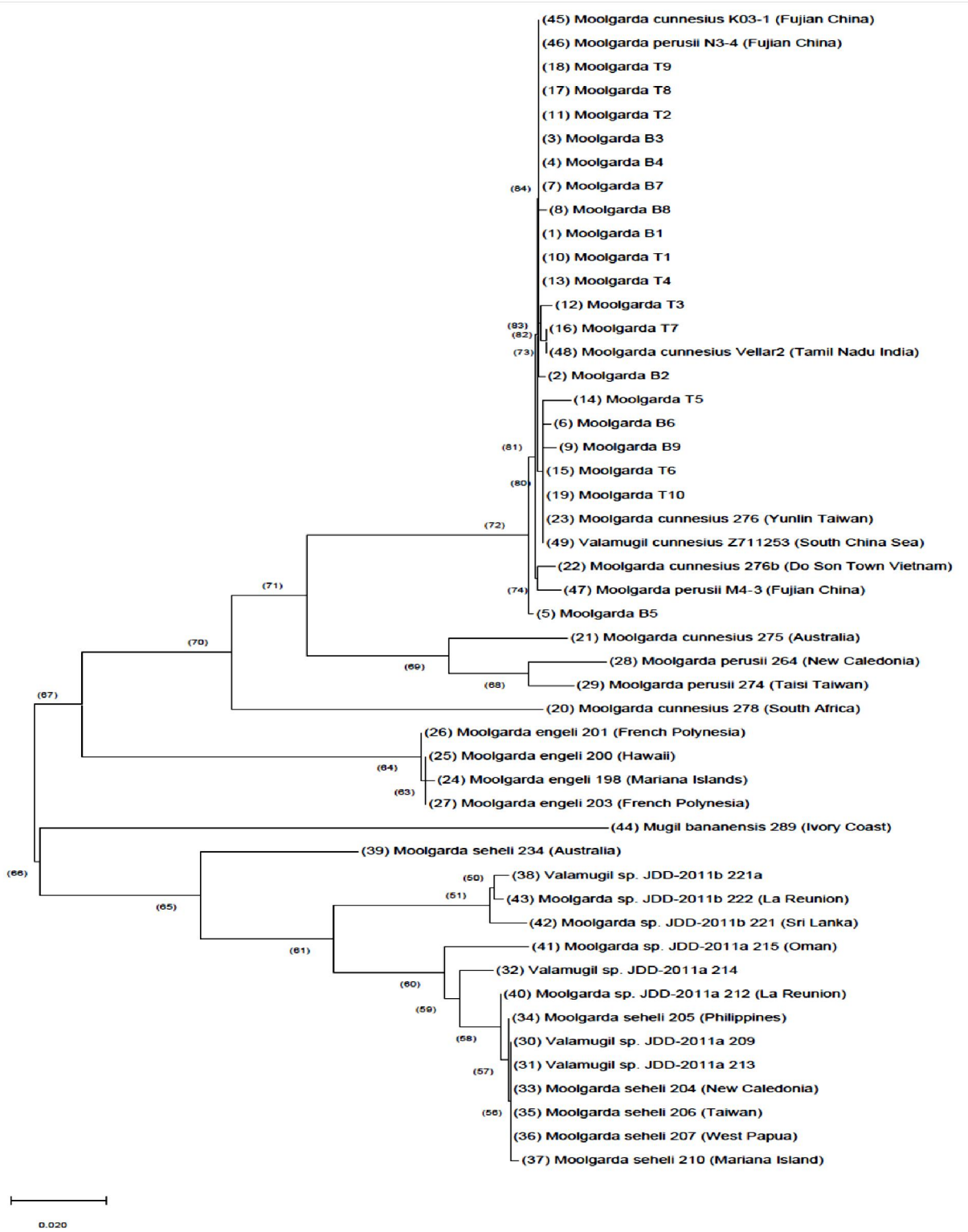

Figure 3. Phylogenetic tree of longarm mullets *M. cunnesius* collected from Thua Thien Hue, Vietnam base on the *CO*I fragment sequence. The confidence level was set to 1,000 replications. The value at each node is the bootstrap probability value. Sister groups and out-group were named, followed with the GenBank accession numbers, respectively. The specimens were labeled with abbreviations *Moolgarda* B and *Moolgarda* T.

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References

[1]Aaron CCY, Abdul Aziz AH, Siti Tafzil Meriam SAK. Giat Seah Y, Asma AN, Morphological and molecular identification of mullet species (Mugilidae) from Setiu Wetland, Terengganu, Malaysia. AACL Bioflux 2018;11(2):429-438.

[2]Abbas EM, Megahed ET, Hemeda SA and ElNahas AF. DNA barcoding and molecular population structure of two species from genus *Diplodus* based on *CO*I gene in the Egyptian Mediterranean Sea. International Journal of Fisheries and Aquatic Studies 2018;6(1):1-8.

[3]Abinawanto A, Sriyani ED. DNA barcoding to identify the genetic diversity of gabus sentani fish (*Oxyeleotris heterodon*, Weber 1907) at Putali Gulf Sentani Lake. AIP Conference Proceedings 2023:020118-1-4. doi: https://doi.org/10.1063/1.5064115.

[4] Barman AS, Singh M, Singh SK, Saha H, Singh YJ, Laishram M, Pandey PK. DNA Barcoding of freshwater fishes of Indo-Myanmar biodiversity hotspot. Scientific Report 2018;8:8579:1-12. doi: 10.1038/s41598-018-26976-3.

[5]Bektas Y, Aksu I, Kaya C, Turan D. DNA barcoding of the genus *Capoeta* (Actinopterygii: Cyprinidae) from Anatolia. Turk. J. Fish. & Aquat. Sci. 2018;19(9):739-752. doi: http://doi.org/10.4194/1303-2712-v19_9_03.

[6] Bingpeng X, Heshan L, Zhilan Z, Chunguang W, Yanguo W, Jianjun W. DNA barcoding for identification of fish species in the Taiwan strait, PLoS ONE 2018;13(6):e0198109. doi: https://doi.org/10.1371/journal.pone.0198109.

[7]Chen W, Ma X, Shen Y, Mao Y, He S. The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. Scientific Report 2015;5:17437:1-12. doi: 10.1038/srep17437.

[8]Deef LEM. A new species of mullet, *Chelon caeruleum* (Family: Mugilidae), with description of its genetic relationship to some Mugilids. Croatian Journal of Fisheries 2018;76: 107-114. doi: 10.2478/cjf-2018-0014.

[9]Dieu V, Viet TV, Thao PDD. Employing morphological characteristics and DNA barcoding for species identification of hillstream loach (*Sewellia spp*.) distributed in Thua Thien Hue. Journal of Sciences, Hue University 2019;128(3C):1-12 (in Vietnamese).

[10]Durand JD, Shen KN, Chen WJ, Jamandre BW, Blel H, Diop K, Nirchio M, de Leon FJG, Whiteld AK, Chang CW et al. Systematics of the grey mullets (Teleostei : Mugiliformes : Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Molecular Phylogenetics and Evolution 2012;64:73-92.

[11]Garg RK, Dubey N, Batav N, Pandey P, Singh RK. Mitochondrial *CO*I gene sequence analyses of *Puntius ticto* and compared with seven species of genus *Pun*tius of family Cyprinidae: A finding for phylogenetic positioning and DNA barcoding as model study for Cryptic species. J. Proteomics Bioinform 2017;10:214-221. doi: 10.4172/jpb.1000445.

[12]Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp. Ser. 1999;41:95-98.

[13]Hanner R, Floyd R, Bernard A, Colette BB, Shivji M. DNA barcoding of billfishes. Mitochondrial DNA 2011;22(S1):27-36. doi: 10.3109/19401736.2011.596833.

[14]Hebert PDN, Gregory TR. The Promise of DNA Barcoding for Taxonomy. Systematic Biology 2005;54(5):852-859. doi: 10.1080/10635150500354886.

[15]Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burridge M, Watkinson D, Dumont P, Curry A, Bentzen P, Zhang J, April J, Bernatchez L. Identifying Canadian Freshwater Fishes through DNA Barcodes. PLoS ONE 2008;3(6):e2490. doi: 10.1371/journal.pone.0002490.

[16]Huyen KT, Nghia VD, Ngoc TN, Dan TV, Phu VV, Dung TQ, Linh NQ. Using DNA barcodes based on mitochondrial COI and 16S rRNA genes to identify *Anguilla* eels in Thua Thien Hue province, Vietnam. Genetics and Molecular Research 2020;19(4):gmr18772. doi: http://dx.doi.org/10.4238/gmr18772.

[17]Huyen KT, Linh NQ. Phylogenetic analysis of *Anguilla marmorata* population in THua Thien Hue, Vietnam based on the cytochrome C oxidase I (COI) gene fragments. ABM Express 2020; 10:122. doi: http://doi.org/10.1186/s13568-020-01059-7.

[18]Kaygorodova IA, Mandzyak N, Petryaeva F, Pronin NM. Genetic Diversity of Freshwater Leeches in Lake Gusinoe (Eastern Siberia, Russia). The Scientific World Journal 2014:1-11. doi: http://dx.doi.org/10.1155/2014/619127.

[19]Keskin E, Agdamar S, Tarkan AS. DNA barcoding common non-native freshwater fish species in Turkey: Low genetic diversity but high population

structuring. Mitochondrial DNA 2013;24(3):276-287. doi: 10.3109/19401736.2012.748041.

[20]Kolmann MA, Elbassiouny AA, Liverpool EA, Lovejoy NR. DNA barcoding reveals the diversity of sharks in Guyana coastal markets. Neotropical Ichthyology 2017;15(4): e170097.

[21]Liu SYV, Huang IH, Liu MY, Lin HD, Wang FY, Liao TY. Genetic stock structure of *Terapon jarbua* in Taiwanese waters. Marine and Coastal Fisheries 2015;7:464-473. doi: https://doi.org/10.1080/19425120.2015.1074966.

[22]Ma HY, Ma CY, Ma LB. Population genetic diversity of mud crab (*Scylla paramamosain*) in Hainan Island of China based on mitochondrial DNA. Biochemical Systematics and Ecology 2011;39:434-440.

[23]Nascimento MHS, Almeida MS, Veira MNS, Filho DL, Lima RC, Barros MC, Fraga EC. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão. Brazil Genet. Mol. Res. 2016;15(3):1-11. doi: http://dx.doi.org/10.4238/gmr.15038476.

[24]Nirchio M, Paim FG, Milana V, Rossi AR and Oliveira C. Identification of a new mullet species complex based on an integrative molecular and cytogenetic investigation of *Mugil hospes* (Mugilidae: Mugiliformes). Front. Genet. 2018;9(17):1-9. doi: 10.3389/fgene.2018.00017.

[25]Nirchio M, Oliveira C, Siccha-Ramirez ZR, de Sene VF, Sola L, Milana V, Rossi AR. The *Mugil curema* species complex (Pisces, Mugilidae): a new karyotype for the Pacific white mullet mitochondrial lineage. Comparative Cytogenetics 2017;11(2):225-237. doi: https://doi.org/10.3897/CompCytogen.v11i2.1159.

[26]Nugroho ED, Nawir D, Amin M, Lestari U. DNA barcoding of nomei fish (Synodontidae: *Harpadon* sp.) in Tarakan Island, Indonesia. AACL Bioflux 2017;10(6):1466-1474.

[27]Nwakanma C, Ude G, Unachukwu MN. The use of DNA barcoding in identification of genetic diversity of fish in Ugwu-Omu Nike river in Enugu. Nig J. Biotech 2015;29:27-33. doi: http://dx.doi.org/10.4314/njb.v29i1.4.

[28]Persis M, Chandra Sekhar Reddy A, Rao LM, Khedkar GD, Ravinder K, Nasruddin K. *CO*I (cytochrome oxidase-I) sequence based studies of Carangid fishes from Kakinada coast, India. Mol. Biol. Rep. 2009;36:1733-1740. doi: 10.1007/s11033-008-9375-4.

[29]Polyakova N, Boutin A, Brykov V. Barcoding and phylogenetic inferences in nine Mugilid species (Pisces, Mugiliformes). Anim. Syst. Evol. Divers. 2013;29(4):272-278. doi: http://dx.doi.org/10.5635/ASED.2013.29.4.272.

[30]Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 2017;34: 3299-3302.

[31]Sambrook J and Russell DW. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.2001.

[32]Shen Y, Guan L, Wang D, Gan X. DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. Ecology and Evolution 2016:1-13.

[33]Thao NP, Yen DT. Comparing morphological characteristics and DNA barcoding of two goby species *Butis butis* and *Butis humeralis*. Journal of Science, Can Tho University 2015;40(2): 23-30 (in Vietnamese).

[34]Thapliyal M, Sati BK, Kumar R, Chandra T, Thapliyal A. DNA barcoding of fishes from River Song, Dehradun, Uttarakhand, using mitochondrial *cytochrome-c oxidase*-I gene. Environment Conservation Journal 2013;14(3):113-121.

[35]Thirumaraiselvi R, Thangaraj M. Genetic diversity analysis of Indian salmon, *Eleutheronema tetradactylum* from South Asian based on mitochondrial COI gene sequence. Not. Sci. Biol. 2015;7(4):417-422. doi: 10.15835/nsb.7.4.9668.

[36]Tue DD, Phu VV. Some gonad cell-tissue characteristics of longarm mullets *Moolgarda cunnesius* (Valenciennes, 1836) in Thua Thien Hue's coastal areas. Journal of Science, Hue University 2021;130(1A):41-49 (in Vietnamese). doi: https://doi.org/10.26459/hueunijns.v130i1A.5938.

[37]Tue DD, Phu VV. Characteristic distribution and exploitation situation of longarm mullet-*Moolgarda cunnesius* (Valenciennes, 1836) in Tam Giang-Cau Hai Lagoon, Thua Thien Hue. Vietnam Journal of Natural Science and Technology 2017;33(2S):295-301 (in Vietnamese).

[38]Turan C, Gürlek M, Ergüden D, Uyan A, Karan S, Doğdu SA. Assessing DNA Barcodes for Identification of Pufferfish Species (Tetraodontidae) in Turkish Marine Waters. Natural and Engineering Sciences 2017;2(3):55-66.

[39]Ude GN, Igwe DO, Brown C, Jackson M, Bangura A, Ozokonkwo□Alor O, Ihearahu OC, Chosen O, Okoro M, Ene C, Chieze V, Unachukwu M, Onyia C, Acquaah G, Ogbonna J, Das A. DNA barcoding for identification of fish species from freshwater in Enugu and Anambra States of Nigeria. Conservation Genetics Resources 2020;12:643-658.

[40]Van Ginneken M, Decru E, Verheyen E, Snoeks J. Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin,

Africa. European Journal of Taxonomy 2017;310:1-32. doi: https://doi.org/10.5852/ejt.2017.310.

[41]Vi NTT, Tuan VS, Long NV. Genetic diversity of the orange-spotted spinefoot (*Siganus guttatus*) population in Quang Nam-Da Nang sea based on the DNA analysis of the genetic region cytochrome oxidase I in mitochondria. Journal of Science and Technology, Da Nang University 2018;9(130):92-95 (in Vietnamese).

[42]Vi NTT, Binh DT, Oanh TT. Genetic diversity of the orange-spotted grouper *E. coioides* (Hamilton, 1822) population in Quang Nam sea based on the DNA analysis of cytochrome oxidase I in DNA in mitochondria the genetic region. Journal of Science and Technology, Da Nang University 2019;17(11):44-47 (in Vietnamese).

[43]Wang L, Wu Z, Liu M, Liu W, Zhao W, Liu H, You F. DNA barcoding of marine fish species from Rongcheng Bay, China. PeerJ. 2018;6:e5013:1-19. doi: 10.7717/peerj.5013.

[44]Ward RD, Zemlak TS, Innes BH, Last BR, Hebert PDN. DNA Barcoding Australia's fish pecies. Phil. Trans. R. Soc. 2005;B(360):1847-1857.

[45]Yen DT. 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 2014;30:29-36 (Vietnamese).

[46]Yen DT, Kiet N, Nen BS, Thuong NV, Loan NB, Dinh TD. DNA barcodes and morphology of *Pangasius krempfi*, *P. mekongensis and P. elongates*. Journal of Biotechnology 2016;14(1):29-37 (in Vietnamese).

[47]Yen DT, Thao NP, Ut TV, Dinh TD. Genetic diversity of kissing gourami *(Helostoma temminckii)* in the Mekong Delta. Journal of Science, Can Tho University 2018;54(7B):86-93 (in Vietnamese). doi: 10.22144/ctu.jvn.2018.144.

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