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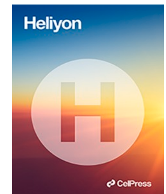

Research article

Phylogenics of the genus *Glossogobius* in the Mekong Delta based on the mitochondrial cytochrome *b* (*cytb*) gene

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ABSTRACT

The *Glossogobius* species play an essential role in food supply and are distributed widely from the marine to freshwater, especially in the Mekong Delta, Vietnam (VMD). Some of their morphometrics and meristics are observed to vary with species and sampling sites. Therefore, the present study aims to verify if their mitochondrial Cytochrome *b* (*Cytb*) gene, one of the popular gene sequences used in fish phylogenic variation assessment, varies with species and sampling sites in the VMD. The *Cytb* gene size was 1300 bp for GcytbH/GcytbL primer pair and 1045 bp for GluMuq1-F/Mixcyto937-2R. The genetic distances within and among these three fish species groups were 0–11%. The *Cytb* gene sequences' similarity between this study and the NCBI database was 85.84–100%. The *Glossogobius* specimens were observed to disperse in small branches of the phylogenetic tree with a low K2P value, suggesting that the *Cytb* genetic diversity may be low among species.

1. Introduction

The Mekong Delta is one of the most vast and fertile deltas in Southeast Asia and represents Vietnam's most significant food production, aquaculture, and fishing region. The area's coastline is more than 700 km, with about 360,000 km^2 within the exclusive economic zone bordering the East Sea and the Gulf of Thailand, creating favorable conditions for agriculture and fisheries development [1]. These natural conditions have developed the fish system of this area, in which the goby species of the family Gobiidae are pretty diverse [2–5].

The use of genetic structure and genetic relationships to accurately distinguish fish species is a fundamental method to serve the study of species diversity and the protection of aquatic resources, especially those of endangered fish species that have not previously been identified. In recent years, molecular biological methods have been applied to identify fish species due to the limitations of morphological methods. DNA barcoding helps rapidly identify species by examining a short specific target gene and can identify cryptic species that were not previously recognizable by traditional identification methods [6]. Currently, the classification and

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identification of species belonging to the order Gobiiformes are facing many difficulties due to their small body size and morphological similarities. This order is one of the most significant species compositions in the bony fish class, in which the number of families and genera of this order has not yet been unified [7]. Among the exploited gobiids, the species of the Gobiidae family play an important role because some species have a higher economic value than others, contributing to the ecosystem's biodiversity due to a large number of fish species [3,7,8].

According to Eschmeyer et al. [9], the family Gobiidae has many species worldwide, with about 2836 being mainly found in tropical and subtropical countries. The Indo-Pacific gobiid genus *Glossogobius* presently includes 28 described species and some undescribed species. Most are bottom-dwelling, riparian carnivores, limited to freshwater as adults. Larvae drifting into the sea are thought to be common, consistent with the wide distribution of some species [10]. In the Mekong Delta, Vietnam, *Glossogobius* is a genus of the Gobiidae family characterized by a wide range of habitats, from coastal to riverine regions. *Glosogobius giuris*, *G. aureus*, and *G. sparsipapillus* are the most common species belonging to this genus [11]. An elongated body, narrow tail, and flat head characterize these fishes. The muzzle is long and pointed. The lower jaw is longer than the upper jaw. Their mouth is wide, and the incision is slightly oblique, extending posteriorly beyond the anterior edge of the eye [12]. According to Nguyen [13], these fishes have high nutritional value when used as food for human consumption. With a wide distribution and high value, many morphological studies of these fishes have been carried out. Previous studies have shown that *G. giuris, G. aureus,* and *G. sparsipapillus* have regional morphological variation [14–16]. These studies suggest that environmental changes can alter the morphological value of these fish. However, a more solid basis for this conclusion is needed. The *Cytb* gene sequencing is commonly used to determine genetic relationships between species [17–19]. For example, the phylogeny of four species of goby *Gymnogobius* Gill, 1863 is studied by Harada et al. [19]. Similarly, Iwata et al. [20] identify the genetic relationship of gobies at the family level using the *Cytb* gene. Hence, in this study, the *Cytb* gene sequence is used to analyze the genetic relationships among species of the three species in the genus *Glossogobius*.

2. Materials and methods

2.1. Fish collection and analysis

Cai Rang - Can Tho (CR), Long Phu - Soc Trang (LP), Hoa Binh - Bac Lieu (HB), and Dam Doi - Ca Mau (DD) were the sites selected to collect fish samples used in this study (Fig. 1). A total of 12 fish samples (3 species × 4 sampling sites) representing three species of

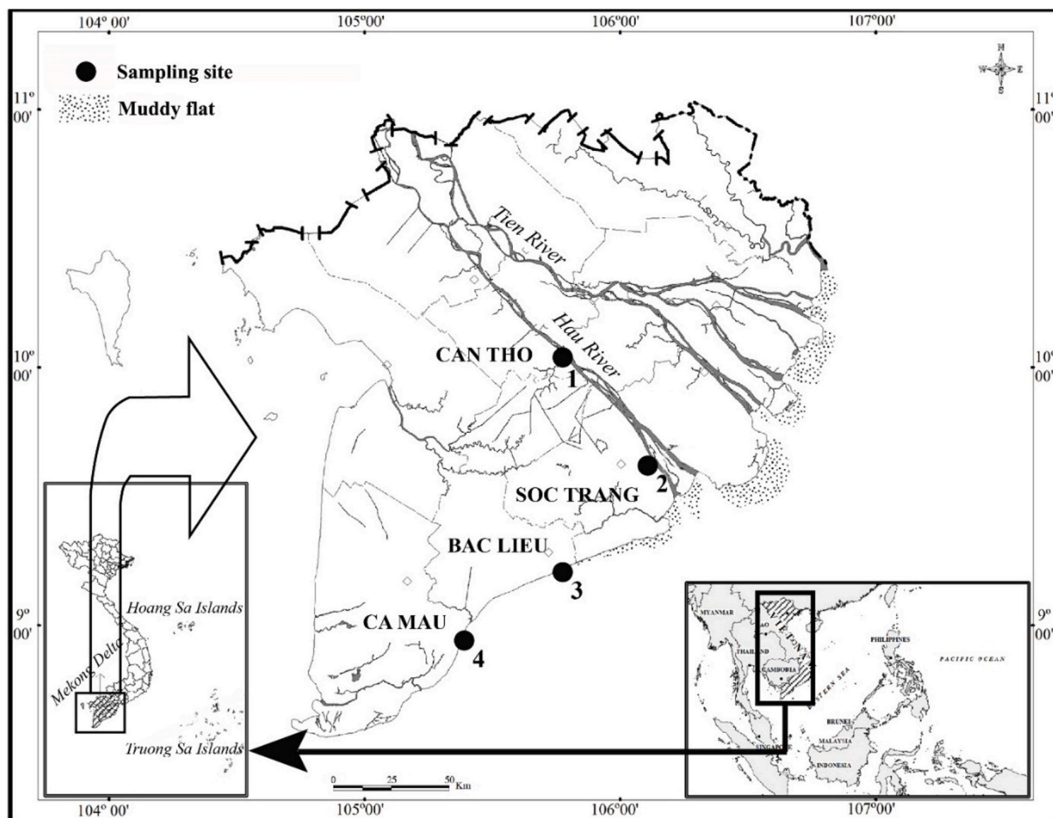

Fig. 1. The sampling map modified from Fig. 1 of Dinh (2018) (●: Collection sites; 1: Cai Rang - Can Tho; 2: Long Phu - Soc Trang; 3: Hoa Binh - Bac Lieu; 4: Dam Doi - Ca Mau).

Glossogobius were collected. The phenomenon of semi-diurnal tides with an amplitude of ~1.2 m occurred at coastal study points. The average temperature in the four areas was ~27 °C, pH was ~8, and the salinity ranged from ~0‰ (CR) to ~23‰ (DD) [21]. Fish samples were collected from January to June 2021 using trawl nets (1.5 cm mesh aperture in the cod-end). At each study site, fish specimens were collected 2–3 h after releasing the trawl nets (at high tide). The morphological characteristics described by Tran et al. [2] were used to identify the fishes of the genus *Glossogobius* (Table 1). The M222 was the chemical used to anesthetize fish before fixing it in 5% formalin. The use of fish in this research was approved by the Scientific Committee of the School of Education, Can Tho University, under the Animal Welfare Assessment number BQ2020-01/KSP. All experiments were conducted according to established animal welfare guidelines. After classification, *Glossogobius*' dorsal fins were cut and stored in 70% ethanol for DNA extraction.

2.2. DNA extraction

Approximately 100 mg of fish mesh from preserved samples in alcohol was used for DNA extraction. The fish's total DNA was extracted using the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam) with the procedure of the manufacturer's recommendations. The obtained DNA was stored at −20 °C. The OD values were measured to determine the concentration and purity of the DNA solution after extraction on a Nanodrop (Denovix).

2.3. Amplification of Cyt b gene by PCR

In this study, two primer pairs, GcytbH/GcytbL [22] and GluMuq1-F/Mixcyto937-2R [23], were used to amplify the *Cytb* gene segment with the following sequence:

GcytbH (forward primer): 5′ GACTTGAAAAACCACCGTTG 3’

GcytbL (reverse primer): 5′ CTCCGATCTCCGGATTACAAGAC 3’

GluMuq1-F (forward primer): 5′-GGCTTGAAAAACCACCGTTG-3′

Mixcyto937-2R (reverse primer): 5′-GGGCGGAATGTTAGGCTTCG-3′

The PCR reaction was performed with a total volume of 50 μl, including 40 μl DEPC water; 5 μl PCR Buffer 10X (10X); 1.5 μl of reverse primer and forward primer (10 p.m./μl); 1 tube EZ Mix (Phu Sa); and 2 μl template DNA.

The PCR reactions were performed with the following thermal cycling conditions: initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C (GcytbH/GcytbL)/57 °C (GluMuq1-F/Mixcyto937-2R) for 30 s and elongation at 72 °C for 45 s; followed by a final extension at 72 °C for 5 min and store at 25 °C for 2 min.

The PCR products were then electrophoresed on 2% agarose gel in 1X TBE buffer at 110V for 40 min to check for successful amplification. All successfully amplified samples were then purified using the PCR Purification Kit (Jena Bioscience) with the procedure recommended by the manufacturer.

2.4. DNA sequencing

PCR products were sent for sequencing to PhuSa Biochem LTD, Can Tho City, Vietnam, according to the method of Sanger et al. [24] on ABI 3500 (Thermofisher). Sequencing results were obtained as.ab1 file (Appendix). Bidirectional sequencing was applied to minimize the probability of sequence errors.

2.5. Data analysis

Each chromatogram was manually screened and aligned in Bioedit software. Sequences were checked for deletion, insertion, and stop codon before being registered in the NCBI with the accession number (Table 2). Nucleotide composition was analyzed using Bioedit v.7.2 software [25]. These sequences were referenced to homologous sequences on the NCBI Gene Bank database using the BLAST tool (BLAST, http://blast.ncbi.nlm.nih.gov/).

The "DNA flow and genetic difference" tool of the DNASP v.5 software was used to estimate the genetic diversity of species of the genus *Glossogobius*. The values of nucleotide diversity (π) and haplotype (h) in the entire distribution area and each locality were used to calculate the genetic diversity of the studied species. Nucleotide diversity was defined as the mean weighted sequence divergence between haplotypes [26]. Haplotype diversity varies from 0 to 1, which measures individuals' frequency and the number of haplotypes [27].

Table 1
The identification key to species of *Glossogobius* genus from the Mekong Delta.

Couplet	Character	Species
1a	Cheek with five uniserial rows of sensory-papillae	*Glossogobius sparsipapillus*
1b	Cheek with five multiserial rows of sensory papillae.	2
2a	Behind the eye, sensory-papillae rows are branchless. On the cheek, each sensory-papillae rows have one sensory papilla. 32–42 scales along the body.	*Glossogobius aureus*
2b	Behind the eye, sensory-papillae rows branch. The 3rd and 4th sensory-papillae rows are three minor rows arranged close together. The idle row has lots of giant papillae. 30–32 scales along the body.	*Glossogobius giuris*

Table 2
Nucleotide percentage (%) of *Cytb* gene of three *Glossogobius* species.

Species	Sites	Accession number	% A	% C	% G	% T	%AT	%GC
Glossogobius aureus	Cai Rang - Can Tho	ON932583	25.60	31.86	15.53	27.01	52.61	47.39
	Long Phu - Soc Trang	ON932584	25.57	31.92	15.52	26.98	52.55	47.44
	Hoa Binh - Bac Lieu	ON932585	26.75	29.91	14.56	28.77	55.52	44.47
	Dam Doi - Ca Mau	ON932586	26.26	29.96	14.98	28.81	55.07	44.94
Glossogobius giuris	Cai Rang - Can Tho	ON932587	25.65	30.60	15.52	28.23	53.83	46.12
	Long Phu - Soc Trang	ON932588	25.62	30.65	15.55	28.19	53.81	46.20
	Hoa Binh - Bac Lieu	ON932589	25.98	30.43	15.27	28.32	54.30	45.70
	Dam Doi - Ca Mau	ON932590	25.78	30.44	15.56	28.22	54.00	46.00
Glossogobius sparsipapillus	Cai Rang - Can Tho	ON932591	26.60	29.76	14.84	28.71	55.31	44.60
	Long Phu - Soc Trang	ON932592	26.59	29.77	14.84	28.80	55.39	44.61
	Hoa Binh - Bac Lieu	ON932593	26.62	29.86	14.80	28.72	55.34	44.66
	Dam Doi - Ca Mau	ON932594	26.53	29.81	14.82	28.84	55.37	44.63

The "Align by ClustalW" tool in the MEGA X software was used to align the sequences of *G. aureus* and *G. giuris*, and *G. sparsipapillus* [28]. In addition, this software was used to calculate genetic distances between species by the tool "Compute pairwise distances" using the Kimura 2-parameter model. The "Maximum likelihood" (ML) tree of K2P spaces with a bootstrapped value of 1000 times was constructed to supply a pictorial representation of the differentiation pattern between species.

3. Results

3.1. Gene sequencing

The *Cytb* segments of *G. aureus* and *G. sparsipapillius* were amplified successfully with primer GcytbH/GcytbL with product size ~1300 bp (Fig. 2a) but unsuccessfully against *G. giuris*. Afterward, the GluMuq1-F/Mixcyto937-2R primer was applied and successfully amplified the *Cytb* segment of *G. giuris* (Fig. 2b). The size of the product amplifying the *Cytb* gene was quite large (>1000 bp). The PCR product showed clear bands.

The results showed that the percentage of nucleotides of *G. aureus*, *G. giuris*, and *G. sparsipapillus* were similar. The percentage of nucleotide C was the highest, followed closely by T and A, whereas this value of G was the lowest (Table 2); the % AT of all three species was higher than the % GC.

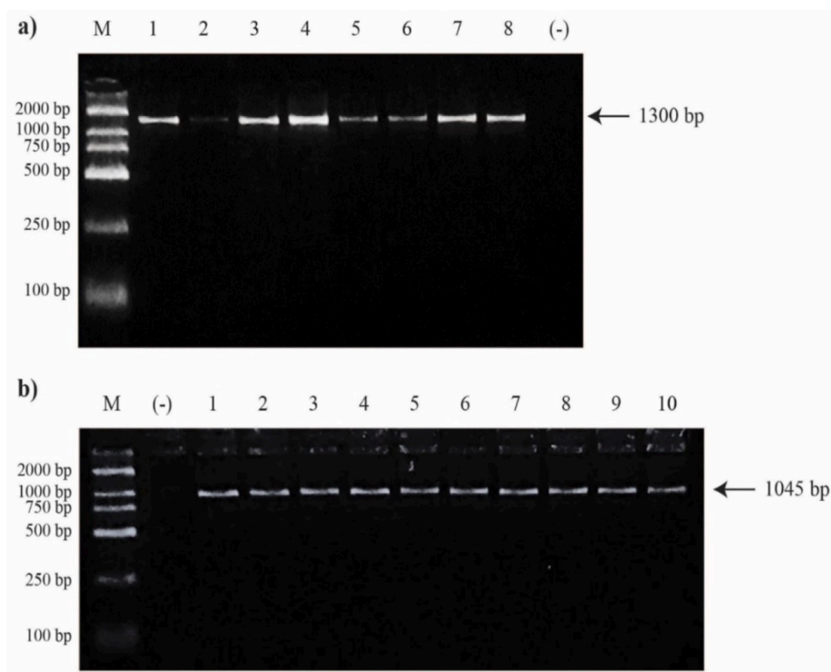

Fig. 2. PCR amplification results of *Cytb* gene migrated in 2% agarose electrophoresis of *Glossogobius aureus* and *G. sparsipapillus* with primer GcytbH/GcytbL (a; M: marker visualized from DL2000 ladder, lanes 1–4: *G. aureus*, lanes 5–8: *G. sparsipapillus*, (−): negative control) and of *G. giuris* with Primer GluMuq1-F/Mixcyto937-2R (b; M: marker visualized from DL2000 ladder, (−): negative control, lanes 1–10: *G. giuris*).

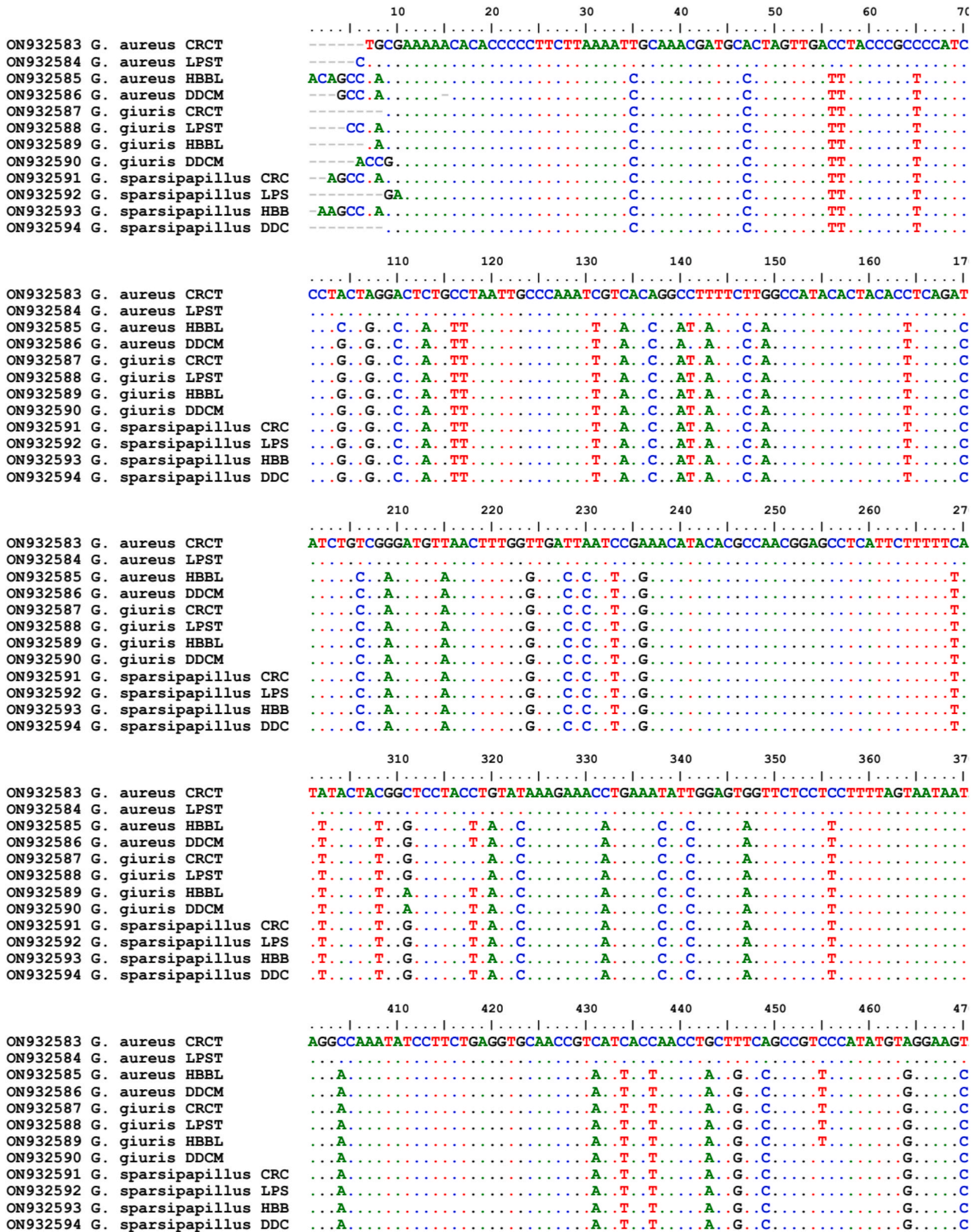

Fig. 3. The alignment of the partial sequence of the *Cytb* gene from three *Glossogobius* species (sequences were aligned using BioEdit, and dots indicated sequence identity).

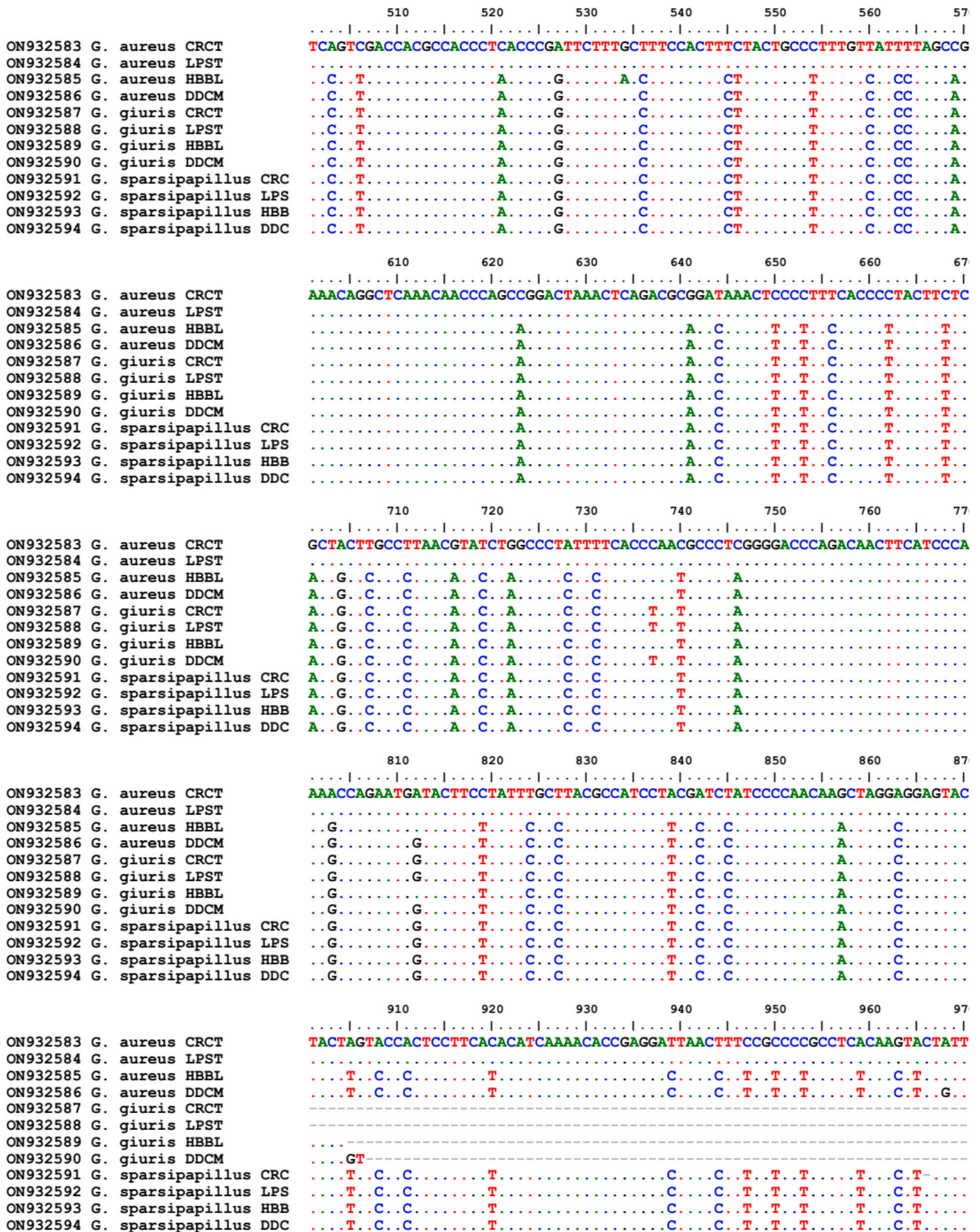

Fig. 3. (*continued*).

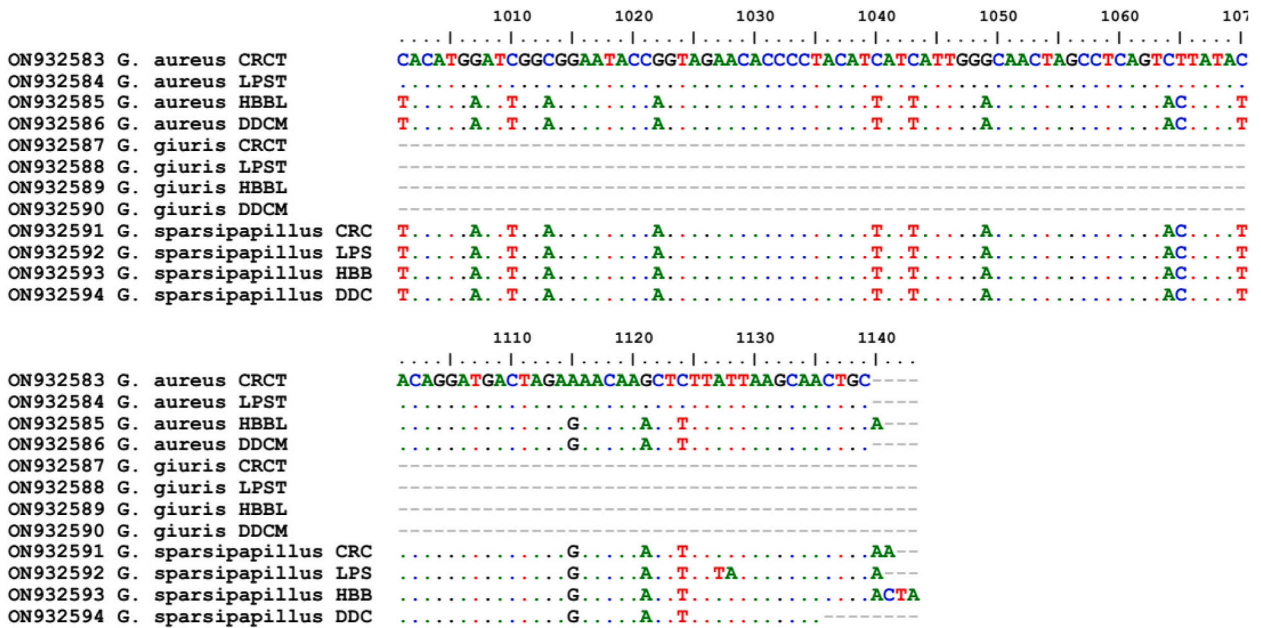

Fig. 3. (*continued*).

The results of comparing the *Cytb* gene sequences of *G. aureus*, *G. giuris*, and *G. sparsipapillus* by the “Align by ClustalW” revealed the variable nucleotides were 132; 8 and 2, respectively (Fig. 3). The *Cytb* gene sequences of three *Glossogobius* species were compared with homologous sequences on the Genbank database using the BLAST tool on NCBI presented in Table 3. In general, the *Cytb* gene sequences of the species *G. spasipapillus* with *G. giuris* from Europe showed the highest similarity of 86.03%. The *Cytb* gene of *G. sparsipapillus* species was similar to that of *G. giuris,* as the *Cytb* gene sequence of this species was not recorded on the NCBI Gene Bank. Four *G. giuris* and two *G. aureus* samples from LP and CR exhibited a low identity value of 86.02–86.35 compared to *G. giuris* control, whereas *G. aureus* from CR and LP and *G. aureus* from the Philippines were identical with 100% similarity.

3.2. Genetic differentiation

The genetic differentiation of *Glossogobius* spp. based on the 12 Cytb gene sequences by DnaSP v.5 software was presented in Table 4. This Table revealed nine haplotypes in 12 individuals of the *Glossogobius* genus, the haplotype diversity (Hd) = 0.94, nucleotide diversity (π) = 0.047, the number of segregating sites (S) = 136, and the average number of differences (K) = 42.09. Comparing results among *Glossogobius* spp. indicated that the highest genetic differentiation was found for *G. giuris* (4 haplotypes, $Hd = 1.00$, $\pi = 0.005$; $S = 8$; $K = 4.43$), followed by *G. aureus* (3 haplotypes, $Hd = 0.83$, $\pi = 0.099$; $S = 132$; $K = 87.67$), and the least genetic differentiation was recorded for *G. sparipapillus* (2 haplotypes, $Hd = 0.50$, $\pi = 0.001$; $S = 2$; $K = 1.00$).

Table 3
The similarity of the *Cytb* gene sequence of three *Glossogobius* species in the study with species on Gene Bank.

No.	Morphology method	DNA barcoding method					
		Species	Accession number	GS (bp)	QC (%)	I (%)	Site
1	*G. aureus* – CR	*G. aureus*	MN306491	744	64	100	Philippines
2	*G. aureus* – LP	*G. aureus*	MN306491	744	64	100	Philippines
3	*G. aureus* – HB	*G. giuris*	KF415566	1135	99	86.13	Europe
4	*G. aureus* – DD	*G. giuris*	KF415566	1135	99	86.02	Europe
5	*G. giuris* – CR	*G. giuris*	KF415566	1135	99	86.28	Europe
6	*G. giuris* – LP	*G. giuris*	KF415566	1135	99	86.35	Europe
7	*G. giuris* – HB	*G. giuris*	KF415566	1135	100	86.22	Europe
8	*G. giuris* – DD	*G. giuris*	KF415566	1135	99	85.84	Europe
9	*G. sparsipapillus* – CR	*G. giuris*	KF415566	1135	99	85.94	Europe
10	*G. sparsipapillus* – LP	*G. giuris*	KF415566	1135	98	85.87	Europe
11	*G. sparsipapillus* – HB	*G. giuris*	KF415566	1135	98	86.03	Europe
12	*G. sparsipapillus* – DD	*G. giuris*	KF415566	1135	99	85.96	Europe

(GS: gene size in bp; QC: query cover; I: identity; CR: Cai Rang - Can Tho, LP: Long Phu - Soc Trang, HB: Hoa Binh - Bac Lieu, DD: Dam Doi - Ca Mau).

Table 4
Genetic differentiation among three populations of *Glossogobius* species.

Populations	Sample size	Genetic differentiation				
		Number of segregating sites (*S*)	Number of haplotypes (*h*)	Haplotype diversity (*Hd*)	Average number of differences (*K*)	Nucleotide diversity (π)
Glossgobius aureus	4	132	3	0.83	87.67	0.099
Glossogobius giuris	4	8	4	1.00	4.33	0.005
Glossogobius sparsipapillus	4	2	2	0.50	1.00	0001
Total	12	136	9	0.94	42.09	0.047

3.3. Genetic distance and phylogenetic tree analysis

The *Cytb* gene sequence of *G. aureus* exhibited significant variation due to the intraspecific genetic distance of up to 11% (Table 5). Meanwhile, the *Cytb* gene sequences of *G. giuris* and *G. sparsipapillius* were barely different, with 0% intraspecific genetic distance. The interspecific genetic distance of *G. aureus*-*G. giuis* was equivalent to *G. aureus*-*G. sparsipapillus,* about 9% higher than the genetic distance between *G. giuis* and *G. sparsipapillus,* only 0%.

The phylogenetic tree of twelve *Cytb* gene sequences was performed using the Maximum Likelihood method (Fig. 4). Accordingly, the genetic tree was divided into three groups with a reliable bootstrap coefficient as high as 100%. Group I consisted of individuals belonging to three species, *G. aureus*, *G. giuris,* and *G. sparsipapillus,* together with in-group control *G. giuris*-Europe. Group II showed the separation of two specimens, *G. aureus*-CR and *G. aureus*-LP, from group I. This subgrouping might be due to the genetic difference between these two samples with other samples as high as 9%. The *G. spasipapillus* had a smaller body size than the other two species in the genus, while *G. giuris* and *G. aureus* showed more morphological similarities. The common feature of Group I and Group II was that the *Glossogobius* samples did not separate into groups according to the sampling location. For example, in group I, *G. spasipapillus* samples collected at HB, DD, and CR were genetically identical. Or in group II, *G. aureus* collected at CR was genetically similar to *G. aureus* collected at LP. Group III consisted of three out-group controls consisting of *A. viridipunctatus*, *B. butis*, *P. freycineti*, indicating the reliability of the genetic tree. Thus, the three species, *G. aureus*, *G. giuris*, and *G. sparsipapillus,* in the present study were closely related genetically to each other based on the *Cytb* gene.

4. Discussion

The relatively low similarity showed that the *Cytb* gene sequence had significant genetic variability. Meyer [29] noted that the *Cytb* gene sequence tended to differ within the same species, mainly in the second codon position. Genetic characteristics of three fish species might be used to identify fish populations or for studies that understand the genetic relationships between closely related species. The *Cytb* gene sequence was used in fish to address many phylogenetic questions, ranging from closely related species relationships to more profound phylogenetic questions. Therefore, the *Cytb* gene sequence was considered an appropriate marker or marker to study differences in genetic relationships and phylogenetics [17].

Grant & Bowen [30] noted that the haplotype diversity in bony fish was more than 80%. Several studies also reported haplotype diversity in fish within this range [31,32]. The study's haplotype diversity ranged from 50% to 100% for the three analyzed species. Although the study sample consisted of 12, the results obtained from this study were similar to the above studies. Nucleotide diversity was 9.90%, 0.50%, and 0.10% for *G. aureus*, *G. giuris,* and *G. sparsipapillus,* respectively. The calculated nucleotide diversity values for the *Cytb* gene varied by species and generally agreed with those observed in other fish species [17].

Apostolidis et al. [33] emphasized that the *Cytb* gene contained a high percentage of A + T nucleotide (53–57%). The current study showed that the percentage of A + T of all three *Glossogobius* species was 52.55–55.52%, indicating an average level of genetic diversity for *Glossogobius* species. In previous studies, the *Cytb* gene had high C, A, and T nucleotide content but a low G [34,35]. Compared with the species in this study, the *Cytb* gene was also rich in C percentage (29–32%) and low in G percentage (14–16%), which was consistent with previous reports for *Acipenser gueldenstaedtii, A. stellatus*, and the *H. huso* [17], and Actinopterygian fish [36].

According to Hoese & Allen [37], species of the genus *Glossogobius* (*G. giuris*, *G. aureus*, and *G. sparsipapillus*) displayed nearly similar external features (depressed head, cylindrical body, two distinct dorsal fins, pelvic fins fused, mouth large 10–15% SL, tongue bifurcated, snout long and pointed, mandibular protrusion, gill slit, at least six longitudinal papillae lines across the cheek, 27–30 vertebrae). In previous studies, external morphological characteristics played an important role when it was necessary to identify and learn about a fish species' ecology, evolution, or phylogenetic relationships. But it was challenging to do with species of the order

Table 5
Percentage of Kimura 2-parameter genetic distances based on the *Cytb* sequence of *Glossogobius* genus (italic values are intraspecies distances).

Species	*Glossgobius aureus*	*Glossgobius giuris*	*Glossgobius sparsipapillus*
Glossgobius aureus	*11%*		
Glossgobius giuris	9%	*0%*	
Glossgobius sparsipapillus	9%	0%	*0%*

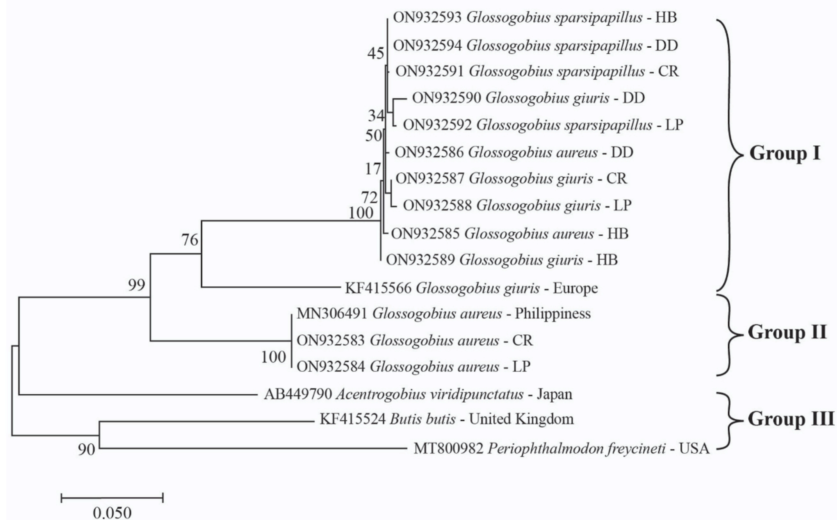

Fig. 4. Molecular phylogenetic analysis by Maximum Likelihood method using the Kimura 2-parameter model, with 1000 times bootstrap. Branch lengths correspond to the mean number of nucleotide substitutions per site. The scale bar indicates substitutions per site (CR: Cai Rang - Can Tho, LP: Long Phu - Soc Trang, HB: Hoa Binh - Bac Lieu, DD: Dam Doi - Ca Mau).

Gobiidae, especially the genus *Glossogobius*, since they exhibited similar physical characteristics and were difficult to distinguish. Due to the sensory perception of the external morphology, many studies did not agree on the phylogenetic tree of these fishes.

The *Cytb* gene sequence analysis of the *Glossogobius* species revealed some fundamental differences when compared with sequences of the same species obtained from the NCBI. Only the *G. aureus* at CR and LP was 100% similar to the *G. aureus* in Philippines. The *Cytb* gene data in the GenBank of *Glossogobius* species were reference sequences, sometimes imprecise, leading to difficulty in species identification; therefore, further research on this genus was needed.

This study built a phylogenetic tree based on *Cytb* genes of three species of *Glossogobius* genus at four study sites. Despite the differences in morphological features, in the present study, *G. giuris* and *G. sparsipapillus* were genetically similar, with a low genetic distance of 0%. Essentially, the genetic distance indicated the barcoding value of gene sequences for species identification and could be used for evolutionary genetics comparison [38]. According to Linh et al. [39], the DNA barcoding method showed efficiency, and high accuracy, which solved taxonomic ambiguities in many cases. For example, wild and square-headed perches differed in head shape and morphological parameters, but their *COI* gene was similar [40,41]. Another example was *Oncorhynchus mykiss* having two different names (rainbow salmon and steelhead salmon) with different morphologies and life cycles, yet they were the same species [42,43]. Therefore, *G. giuris* and *G. sparsipapillus* were distinct species even though their *Cytb* genes were similar.

According to Zemlak et al. [44], the threshold of conspecific genetic distance is less than 3.5%; if the genetic divergence is larger than 3.5%, they are deemed distinct species. So, according to Zemlak's criteria, *G. aureus* exhibited a marked difference from *G. giuris* and *G. sparsipapillus,* because their genetic distances were greater than 3.5% (9%). Several species were morphologically similar yet different species. For example, in Africa, seven different fish species called "Acará" were based on the *COI* and *12S* rRNA gene sequences [45]. Thus, species identification by morphological methods should be combined with DNA barcoding methods, even in some cases, which may require using two or more different genes. Differences in genetics and morphology between *Glossogobius* species were insignificant due to their close history. The present results were still ambiguous, so it was necessary to continue to study more molecular markers in the nucleus of these species. This result again demonstrated that combining morphology and DNA barcoding in fish taxonomy was necessary, especially in the genus *Glossogobius*.

5. Conclusion

The genetic relationships between the three species in the genus *Glossogobius* (*G. aureus*, *G. giuris*, and *G. sparsipapillus*) were close and not affected by environmental factors at different sampling sites. The *Cytb* gene sequences of three *Glossogobius* species in the study were 80–100% similar to the reference species on NCBI. The results of this study have provided a database of the *Cytb* gene and the phylogeny of the *Glossogobius* genus in the Mekong Delta, thereby helping to propose strategies to conserve the species' biodiversity.

Author contribution statement

Gieo Hoang Phan, Tran Thi Huyen Lam: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Quang Minh Dinh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data;

Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ton Huu Duc Nguyen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Ethical clearance

The use of fish in the present study is assessed and approved by the Council for Science and Education, School of Education, Can Tho University (Animal Welfare Assessment number: BQ2020-01/KSP).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] T. Le, M.T. Nguyen, V.P. Nguyen, D.C. Nguyen, X.H. Pham, T.S. Nguyen, V.C. Hoang, P.L. Hoang, H. Le, N.C. Dao, Provinces and City in the Mekong delta, in: T. Le (Ed.), Geography of Provinces and Cities in Vietnam I, Ha Noi, Vietnam Education Publishing House, 2006, pp. 49–94.
[2] D.D. Tran, K. Shibukawa, T.P. Nguyen, P.H. Ha, X.L. Tran, V.H. Mai, K. Utsugi, Fishes of Mekong Delta, Vietnam, Can Tho, Can Tho University Publisher, 2013.
[3] A.T. Diep, Q.M. Dinh, D.D. Tran, Species composition of gobiidae distributed in the coastal areas, Soc Trang Province, VNU Journal of Sciences: Natural Sciences and Technology 30 (3) (2014) 68–76.
[4] D.D. Tran, V.T. Nguyen, H.T.M. To, T.T. Nguyen, Q.M. Dinh, Species composition and biodiversity index of gobiid assemblage in estuarine areas of the Mekong Delta, Vietnam, Egyptian Journal of Aquatic Biology and Fisheries 24 (7) (2020) 931–941.
[5] K.N. Le, N.H. Son, T.N.H. Nguyen, H.A. Le, V.D. Tran, T.D. Nguyen, D.D. Tran, P.H. Ha, T.M.H. To, T.V. Nguyen, T.T. Vo, T.T. Nguyen, Q.M. Dinh, Fish species composition in Hau river basin at Hau Giang Province, VNU Journal of Science: Natural Sciences and Technology 34 (1) (2018) 90–104.
[6] P.D. Hebert, A. Cywinska, S.L. Ball, J.R. Dewaard, Biological identifications through DNA barcodes, Proceedings of the Royal Society of London Series B: Biological Sciences 270 (1512) (2003) 313–321.
[7] C.E. Thacker, Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei), Mol. Phylogenet. Evol. 26 (3) (2003) 354–368.
[8] D.D. Tran, B.P.C. Le, Q.M. Dinh, N.V. Duong, T.T. Nguyen, Fish species composition variability in Cu Lao Dung, Soc Trang, Vietnam, AACL Bioflux 14 (4) (2021) 1865–1876.
[9] W.N. Eschmeyer, R. Fricke, R.v.d. Laan, Catalog of Fishes: Classification, 2019. http://www.calacademy.org/scientists/catalog-of-fishes-classification/.
[10] D.F. Hoese, G.R. Allen, A review of the amphidromous species of the *Glossogobius celebius* complex, with description of three new species/Revue des especes amphidromes du complexe *Glossogobius celebius*, avec la description de trois especes nouvelles, Cybium, International Journal of Ichthyology 35 (4) (2011) 269–285.
[11] R. Froese, D. Pauly, FishBase, 2022. www.fishbase.org.
[12] V.H. Nguyen, Freshwater Fish of Viet Nam vol. 3, Agriculture Publishing House, Ha Noi, 2005.
[13] N.T. Nguyen, Fauna of Vietnam - Gobioidei vol. 5, Science and Technics Publishing House, Ha Noi, 2000.
[14] T.H.D. Nguyen, Q.M. Dinh, Morphometric and meristic variations in *Glossogobius giuris* distributed in different locations in the Mekong Delta, TNU Journal of Science and Technology 226 (10) (2021) 31–38.
[15] T.H.D. Nguyen, H.T.T. Nguyen, T.C. Tran, Y.T.N. Nguyen, Q.M. Dinh, Morphometric and meristic variations of *Glossogobius sparsipapillus* along the coastline in the Mekong delta, Vietnam, International Journal of Zoology and Animal Biology 3 (1) (2020) 1–9.
[16] G.H. Phan, Q.M. Dinh, N.T. Truong, T.H.D. Nguyen, Variation in morphometric characteristics of *Glossogobius aureus* distributed from can Tho to Ca Mau, Vietnam Agricultural Science Journal 19 (7) (2021) 863–874.
[17] Y. Çiftci, O. Eroğlu, Ş. Firidin, Mitochondrial cytochrome b sequence variation in three sturgeon species (*A. stellatus* Pallas, 1771, *A. gueldenstaedtii* Brandt, 1833, H. huso Linnaeus, 1758) from the Black sea Coasts of Turkey, Turk. J. Fish. Aquat. Sci. 13 (2) (2013) 291–303.
[18] M.N. Dawson, K.D. Louie, M. Barlow, D.K. Jacobs, C.C. Swift, Comparative phylogeography of sympatric sister species, Clevelandia ios and *Eucyclogobius newberryi* (Teleostei, gobiidae), across the California Transition zone, Mol. Ecol. 11 (6) (2002) 1065–1075.
[19] S. Harada, S.-R. Jeon, I. Kinoshita, M. Tanaka, M. Nishida, Phylogenetic relationships of four species of floating gobies (*Gymnogobius*) as inferred from partial mitochondrial cytochrome b gene sequences, Ichthyol. Res. 49 (4) (2002) 324–332.
[20] A. Iwata, T. Kobayashi, K. Ikeo, T. Imanishi, H. Ono, Y. Umehara, C. Hamamatsu, K. Sugiyama, Y. Ikeda, K. Sakamoto, Evolutionary aspects of gobioid fishes based upon a phylogenetic analysis of mitochondrial cytochrome b genes, Gene 259 (1–2) (2000) 5–15.
[21] D.D. Tran, T.V. Nguyen, T.M.H. To, T.T. Nguyen, M.Q. Dinh, Species composition and biodiversity index of gobiid assemblage in estuarine areas of the Mekong Delta, Vietnam, Egyptian Journal of Aquatic Biology and Fisheries 24 (7) (2020) 931–941.
[22] Y.-M. Ju, J.-H. Wu, P.-H. Kuo, K.-C. Hsu, W.-K. Wang, F.-J. Lin, H.-D. Lin, Mitochondrial genetic diversity of Rhinogobius giurinus (Teleostei: gobiidae) in East Asia, Biochem. Systemat. Ecol. 69 (2016) 60–66.

[23] J.-D. Durand, K.-N. Shen, W.-J. Chen, B.W. Jamandre, H. Blel, K. Diop, M. Nirchio, F.G. De León, A.K. Whitfield, C.-W. Chang, Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy, Mol. Phylogenet. Evol. 64 (1) (2012) 73–92.
[24] F. Sanger, S. Nicklen, A.R. Coulson, DNA sequencing with chain-terminating inhibitors, Proc. Natl. Acad. Sci. USA 74 (12) (1977) 5463–5467.
[25] T. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, in: Nucleic Acids Symposium Series, 1999, pp. 95–98.
[26] M. Nei, W.-H. Li, Mathematical model for studying genetic variation in terms of restriction endonucleases, Proc. Natl. Acad. Sci. USA 76 (10) (1979) 5269–5273.
[27] M. Nei, F. Tajima, DNA polymorphism detectable by restriction endonucleases, Genetics 97 (1) (1981) 145–163.
[28] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874.
[29] A. Meyer, Evolution of mitochondrial DNA in fishes, in: P.W. Mochachka, T.P. Mommsen (Eds.), Biochemistry and Molecular Biology of Fishes, Elsevier Press Amsterdam, New York, 1993, pp. 1–38.
[30] W.S. Grant, B.W. Bowen, Shallow population Histories in Deep evolutionary Lineages of marine fishes: Insights from Sardines and Anchovies and Lessons for conservation, J. Hered. 89 (1998) 415–426.
[31] V. Birstein, P. Doukakis, Molecular analysis of *Acipenser sturio* L., 1758 and *Acipenser oxyrinchus Mitchill*, 1815: a review, Boletin-Instituto Espanol De Oceanografia 16 (1/4) (2000) 61–74.
[32] J. Stabile, J. Waldman, F. Parauka, I. Wirgin, Stock structure and homing fidelity of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) based on RFLP and sequence analysis of mitochondrial DNA, Genetics 144 (1996) 767–775.
[33] A. Apostolidis, C. Triantaphyllidis, A. Kouvatsi, P. Economidis, Mitochondrial DNA sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout) populations, Mol. Ecol. 6 (6) (1997) 531–542.
[34] P. Cantatore, M. Roberti, G. Pesole, A. Ludovico, F. Milella, M. Gadaletal, C. Saccone, Evolutionary analysis of cytochrome b sequences in some Perciformes: evidence for a slower rate of evolution than in mammals, J. Mol. Evol. 39 (6) (1994) 589–597.
[35] A. Almodóvar, A. Machordom, J. Suárez, Preliminary results from characterization of the Iberian Peninsula sturgeon based on analysis of the mtDNA cytochrome b, Boletin-Instituto Espanol de Oceanografia 16 (1/4) (2000) 17–28.
[36] G.C. Johns, J.C. Avise, A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene, Mol. Biol. Evol. 15 (11) (1998) 1481–1490.
[37] D.F. Hoese, G.R. Allen, Description of three new species of *Glossogobius* from Australia and New Guinea, Zootaxa 1981 (1) (2009) 1–14.
[38] Y.P. Kartavtsev, S.N. Sharina, T. Goto, O.A. Rutenko, V.V. Zemnukhov, A.A. Semenchenko, D.L. Pitruk, N. Hanzawa, Molecular phylogenetics of pricklebacks and other percoid fishes from the Sea of Japan, Aquat. Biol. 8 (1) (2009) 95–103.
[39] N.M. Linh, N. Van Quan, P. Van Chien, D.H. Ly, D. Van Nhan, D.T. Len, DNA barcoding application of mitochondrial COI gene to identify some fish of family Gobiidae in Vietnam Vietnam, J. Mar. Sci. Technol. 18 (4) (2018) 443–451.
[40] D.T. Yen, T.N. Trinh, Morphological comparison between new phenotype and wild strains of climbing perch (*Anabas testudineus*), Can Tho University Journal of Science 29 (2013) 86–95.
[41] D.T. Yen, 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 (2014) 29–36.
[42] M.F. Docker, D.D. Heath, Genetic comparison between sympatric anadromous steelhead and freshwater resident rainbow trout in British Columbia, Canada, Conserv. Genet. 4 (2003) 227–231.
[43] D.E. Pearse, S.A. Hayes, M.H. Bond, C.V. Hanson, E.C. Anderson, R.B. Macfarlane, J.C. Garza, Over the falls? Rapid evolution of ecotypic differentiation in steelhead/rainbow trout (*Oncorhynchus mykiss*), J. Hered. 100 (5) (2009) 515–525.
[44] T.S. Zemlak, R.D. Ward, A.D. Connell, B.H. Holmes, P.D. Hebert, DNA barcoding reveals overlooked marine fishes, Molecular Ecology Resources 9 (2009) 237–242.
[45] A. Ardura, A.R. Linde, J.C. Moreira, E. Garcia-Vazquez, DNA barcoding for conservation and management of Amazonian commercial fish, Biol. Conserv. 143 (6) (2010) 1438–1443.