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Genetic Characteristics of *Periophthalmus chrysospilos* through *COI* Gene Analysis in Coastal Provinces of the Mekong Delta

Ton Huu Duc Nguyen[1], Gieo Hoang Phan[2,*] and Quang Minh Dinh[1]

[1]*Faculty of Biology Education, School of Education, Can Tho University, Can Tho 900000, Vietnam*
[2]*Faculty of Agriculture and Rural Development, Kien Giang University, Kien Giang 920000, Vietnam*

([*]Corresponding author's e-mail: phgieo@vnkgu.edu.vn)

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Abstract

Periophthalmus chrysospilos is an amphibious gobiid species inhabiting intertidal mudflats and mangrove ecosystems across Southeast Asia, with the Mekong Delta as a critical habitat. This study investigates the genetic diversity of *P. chrysospilos* populations across 4 coastal provinces of the Mekong Delta - Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau - through mitochondrial cytochrome c oxidase subunit I (*COI*) gene sequence analysis. Genomic DNA was extracted, amplified, and sequenced to assess nucleotide composition, genetic distances, and phylogenetic relationships. The analyzed *COI* gene sequences were 645 base pairs in length. The results demonstrated a high degree of sequence similarity (99.21 - 99.84 %) between *P. chrysospilos* populations in the Mekong Delta and those from Singapore. Nucleotide composition analysis revealed a higher proportion of adenine-thymine (AT) content than guanine-cytosine (GC). Furthermore, genetic distances among samples were minimal (0.00 - 0.03), suggesting low levels of genetic differentiation. Phylogenetic analysis clustered all sampled individuals into a single clade, confirming their classification as a single species. These findings indicate limited genetic divergence among populations and highlight the necessity for further investigations into environmental and anthropogenic factors affecting genetic structure. This study contributes to understanding *P. chrysospilos* genetic diversity and provides insights relevant to conservation and aquaculture management strategies.

Keywords: *COI* gene sequence, Conservation genetics, Genetic diversity, Mekong Delta, Mudskipper, *Periophthalmus chrysospilos*, Phylogenetics

Introduction

Periophthalmus chrysospilos, belonging to the family Oxudercidae, inhabits coastal mudflats and mangrove swamps in Southeast Asia [1], with the Mekong Delta (VMD) being one of its key distribution areas [2,3]. This species is ecologically unique and attracts scientific interest because it can thrive in a semi-terrestrial environment, where it can respire through the skin and buccal mucosa [4]. It plays a crucial role in wetland ecosystems by participating in food webs, facilitating nutrient cycling, and maintaining ecological balance [5,6]. Beyond its ecological significance, *P. chrysospilos* holds potential economic value for sustainable aquaculture. Although not yet widely exploited, some studies suggest that this species could become a valuable local resource, contributing to the livelihoods of coastal communities [7]. However, natural populations of *P. chrysospilos* are increasingly threatened by habitat degradation due to unsustainable resource extraction, water pollution, and climate change [8]. In this context, genetic studies, particularly those focusing on genetic diversity, are essential for understanding the species' adaptability and population sustainability in natural habitats. Insights from genetic research aid in conserving wild populations and support the development of efficient aquaculture strategies, especially as coastal ecosystems face mounting environmental pressures.

The *COI* gene (Cytochrome c oxidase subunit I) is a part of mitochondrial DNA and is considered one of the most widely used genetic markers in studies of biodiversity and evolution. It encodes a protein that plays a crucial role in the electron transport chain and is directly involved in cellular respiration [9]. With its high conservation across species but enough variation to distinguish between species or even populations, the *COI* gene has become an ideal choice for DNA barcoding to identify species and investigate genetic diversity [10,11]. The use of the *COI* gene in population studies of animals, mainly fish, has proven remarkably effective for species identification, as demonstrated in species from the Gobidae family [12], *Pangasius* genus [13], *Channa* genus [14], *Anguilla* genus [15], *Papus* genus [16], etc. This gene enables scientists to accurately identify species, even from small or incomplete biological samples [17]. In studies of mudskippers, including *P. chrysospilos*, the *COI* gene provides significant insight into phylogenetic relationships and genetic traits. Studying the *COI* gene can offer scientific evidence of species adaptation and evolutionary potential, especially in ecologically distinctive regions like the VMD, where fish populations live under rapidly changing environmental conditions. The application of the *COI* gene in research on this fish species not only helps determine the genetic diversity of the species in coastal provinces such as Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau but also clarifies the role of environmental factors in shaping and maintaining this diversity.

Recent studies on *P. chrysospilos* primarily focus on its ecological and biological characteristics, including population structure [18], reproduction [19], growth [20], burrow architecture [3], otolith structure [21], etc. These studies have elucidated the ecological significance of this species within mangrove and coastal mudflat ecosystems. Additionally, some research has addressed its tolerance to extreme environmental conditions, such as hypoxic mudflats and high temperatures [4,22]. However, genetic data, particularly regarding genetic diversity, remains limited for this species. In genetic studies, the cytochrome c oxidase subunit I (*COI*) gene has been widely applied to analyze genetic characteristics in various goby species (Gobiidae), including research on taxonomy, population structure, and geographic differentiation [17]. Nevertheless, studies focusing on *P. chrysospilos* are still scarce, especially concerning populations in the VMD, where environmental fluctuations may significantly influence the species' genetic diversity. Therefore, this study aims to analyze the genetic diversity of *P. chrysospilos* based on *COI* gene sequences from 4 coastal provinces in the Mekong Delta: Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau. Additionally, the research investigates genetic differentiation among these regions, thereby clarifying the environmental factors that shape the species' genetic characteristics. This study contributes to a deeper understanding of the genetic makeup of *P. chrysospilos*, a species uniquely adapted to Southeast Asian mangrove ecosystems. By applying *COI* gene analysis, the research provides insights into population structure and genetic differentiation and expands the scientific foundation for understanding the role of environmental factors in the evolution and adaptation of this species. Furthermore, the findings enhance the genetic diversity database of the Gobiidae family, a crucial group for conservation and ecosystem management.

Materials and methods

Sample collection and processing

DNA was extracted from 4 fish specimens collected from 4 provinces: Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau (3 fish specimens × 4 sampling sites; **Figure 1**). All specimens were accurately identified to the species level based on the description provided by Tran *et al.* [2]. Environmental parameters such as temperature, pH, and salinity were referenced by Dinh and Nguyen [3], as the sampling environments were similar. All sampling procedures were approved by the Scientific Council of the Faculty of Education, Can Tho University (approval code BQ2021-05/KSP) following an assessment of animal welfare. Since DNA extraction was conducted in the laboratory, the specimens were preserved in 90 % ethanol and stored at −20 °C.

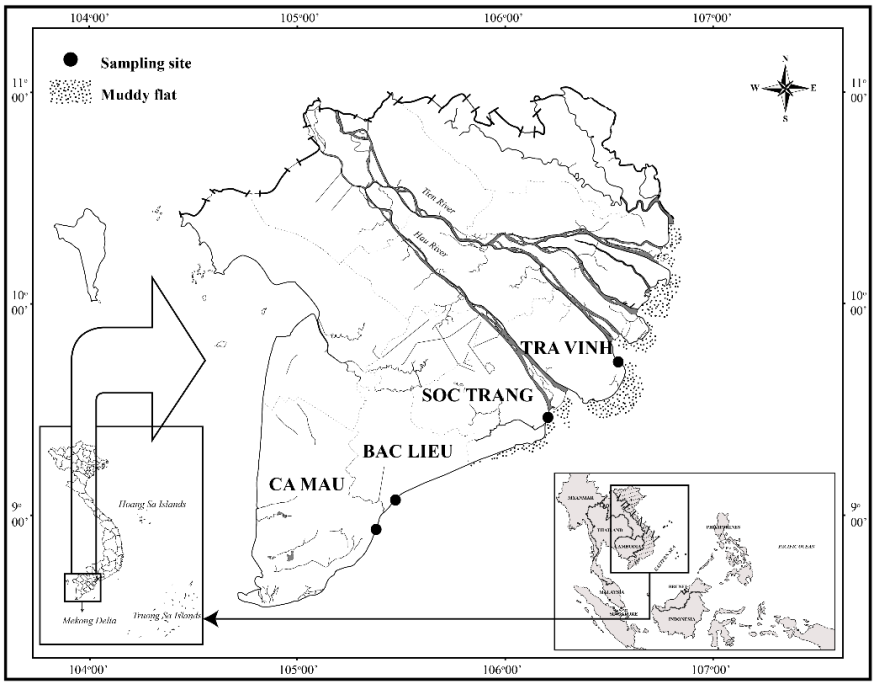

Figure 1 Map showing sampling sites [23].

DNA amplification and sequencing

Total DNA was extracted from fish fins according to the manufacturer's instructions using the TopPURE® Genomic DNA Extraction Kit (ABT, Vietnam). The primer pair *FishF1*/*FishR1*, as described by Ward *et al.* [17], was used to amplify approximately 630 base pairs (bp) of the cytochrome oxidase I (*COI*) subunit.

Polymerase Chain Reaction (PCR) was conducted in a 50 µL reaction volume containing 40 µL of DEPC water, 5 µL of 10X PCR Buffer, 1.5 µL of each primer (10 pmol/µL), 1 tube of EZ Mix (Phu Sa), and 2 µL of genomic DNA. The thermal cycling conditions were as follows: An initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 45 s; followed by a final extension at 72 °C for 5 min and a hold at 25 °C for 2 min. The method assessed PCR product quality using electrophoresis on a 1.5 % TBE agarose gel run at 110 V for 40 min, with the DL2000 marker (Phu Sa company, Vietnam) to estimate product size.

The amplified products were purified with a Purification Kit (Jena Bioscience) as per the manufacturer's protocol. Using the method outlined by Sanger *et al.* [24], the purified PCR products were sequenced on an ABI 3500 (ThermoFisher) at PhuSa Biochem LTD in Can Tho City, Vietnam. All sequences were submitted to GenBank for assignment of the accession number after editing. Information on the sequences after registration is shown in **Table 1**.

Table 1 List of locality information and GenBank's accession numbers.

Samples	Sites	Length (bp)	Accession number
Periophthalmus chrysospilos-TV	Duyen Hai, Tra Vinh	645	OP764034
Periophthalmus chrysospilos-ST	Tran De, Soc Trang	645	OP764035
Periophthalmus chrysospilos-BL	Dong Hai-Bac Lieu	645	OP764036
Periophthalmus chrysospilos-CM	Dam Doi, Ca Mau	645	OP764037

Data analysis

The sequences were meticulously analyzed using FinchTV 1.4.0 software (http://www.geospiza.com). Interfering bases at both ends were trimmed and edited before further analysis. The ClustalW function in MEGA X, following Kumar, *et al.* [25], was employed

to align the *COI* sequences. Genetic distances within and between groups were estimated using the *K2P* model. The optimal nucleotide substitution models were identified using the "Find Best DNA Model" tool in MEGA X, with the model showing the lowest Bayesian Information Criterion (BIC) score selected for phylogenetic analysis [26]. Analyzed phylogenetic relationships among *P. chrysospilos* individuals using the Maximum Likelihood method based on the Neighbor-Joining model, with 1,000 bootstrap replicates, in MEGA X.

Results and discussion

The sequence and nucleotide percentage of the *COI* gene

Four DNA samples collected from Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau showed clear, bright bands without any secondary bands when analyzed using 1 % agarose gel electrophoresis. The results of the quality assessment were presented in **Figure 2**.

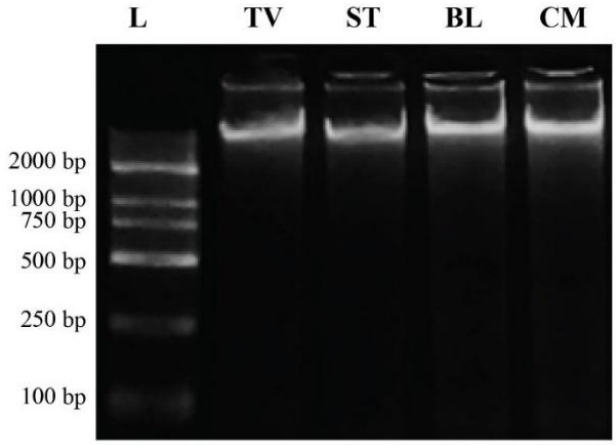

Figure 2 Total DNA after electrophoresis. (L: DL2000 DNA marker; TV: Tra Vinh; ST: Soc Trang; BL: Bac Lieu; CM: Ca Mau).

In addition, the total DNA samples were measured for optical density (OD) at 260 and 280 nm wavelengths to assess DNA concentration and the presence of contaminants. The results in **Table 2** showed that the OD260/OD280 ratios for the samples in this study ranged from 1.94 to 2.05, indicating that the extracted DNA was high purity and suitable for PCR analysis.

Table 2 OD measurement results in total DNA samples.

Samples	Conc (ng/µl)	A260/A280
Periophthalmus chrysospilos-TV	251.9	2.05
Periophthalmus chrysospilos-ST	461.5	1.97
Periophthalmus chrysospilos-BL	249.0	1.96
Periophthalmus chrysospilos-CM	611.6	1.94

This study subjected the high-quality total DNA samples to PCR amplification to target the *COI* gene region. The target gene region was successfully amplified using the primers *FishF1* and *FishR1*, producing a product of approximately 645 bp in all 4 samples. **Figure 3** showed the PCR products on a 1.5 % agarose gel after electrophoresis, with clear, bright bands and no secondary bands. Therefore, these samples were selected for sequencing to support further analyses.

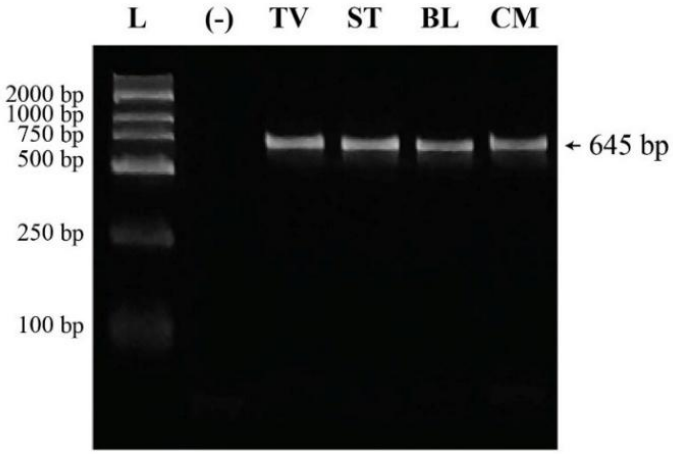

Figure 3 PCR products after electrophoresis (L: DL2000 DNA marker; (-): negative control; TV: Tra Vinh; ST: Soc Trang; BL: Bac Lieu; CM: Ca Mau).

The sequencing results showed that, in all 4 sequences, the highest percentage of nucleotides was observed for C and T, each accounting for approximately 29 %, followed by A at around 23 %, and the lowest for G at about 19 % (**Table 3**). When examined by nucleotide pairs, the percentage of AT pairs was higher than that of GC pairs in all sequences. Overall, there were no significant differences in the percentage of each nucleotide type across the samples from the 4 sampling sites.

Table 3 Nucleotide percentage of samples.

No.	Samples	Access code	Size (bp)	% A	% C	% G	% T	% AT	% GC
1	Tra Vinh	OP764034	645	22.95	29.15	18.91	28.99	51.94	48.06
2	Soc Trang	OP764035	645	23.10	29.15	18.76	28.99	52.09	47.91
3	Bac Lieu	OP764036	645	22.95	29.15	18.91	28.99	51.94	48.06
4	Ca Mau	OP764037	645	23.10	29.15	18.76	28.99	52.09	47.91

The BLAST results from the NCBI gene database **Table 4**. It revealed a high degree of similarity between the *COI* gene sequences of *P. chrysospilos* and those of *P. chrysospilos* from Singapore. Specifically, the coverage of the samples was 98 %, with similarity percentages ranging from 99.21 to 99.84 %. These results suggested that the samples collected in the VMD and Singapore may belong to the same species.

Table 4 *COI* gene sequence homology of *P. chrysospilos* samples with species on GenBank.

No.	Samples	DNA barcoding method					
		Species	Access code	Gene size (bp)	Query Cover (%)	Percent identity (%)	Site
1	Tra Vinh - OP764034	*P. chrysospilos*	MN690438.1	675	98	99.84	Singapore
2	Soc Trang - OP764035	*P. chrysospilos*	MN690438.1	675	98	99.68	Singapore
3	Bac Lieu - OP764036	*P. chrysospilos*	MN690438.1	675	98	99.84	Singapore
4	Ca Mau - OP764037	*P. chrysospilos*	MN690438.1	675	98	99.21	Singapore

Genetic distance

The K2P genetic distance analysis revealed the genetic differentiation among the samples from the 4 sites: Tra Vinh (TV), Soc Trang (ST), Bac Lieu (BL), and Ca Mau (CM). Specifically, the genetic distance between TV and BL was 0.000, indicating no genetic

difference between these sites. The distance between TV and ST was 0.002, suggesting a slight difference. Similarly, BL also showed a distance of 0.002 compared to ST and CM, indicating high similarity among these samples. Meanwhile, the distance between CM and ST was 0.003, the largest among the recorded values. However, overall, the genetic distances between the samples were relatively low, suggesting they could belong to the same species.

Table 4 Percent Kimura 2-parameter genetic distances.

Samples	TV	ST	BL	CM
TV				
ST	0.002			
BL	0.000	0.002		
CM	0.002	0.003	0.002	

Phylogenetic analysis

The phylogenetic tree was based on the *COI* gene sequences, revealing that the *P. chrysospilos* samples from VMD formed a monophyletic group with 2 main clades. The sample from ST(OP764035) separated earliest, while the remaining samples (TV, BL and CM) formed a subclade with high bootstrap support (89). This subclade was further divided into 2 smaller groups: 1 group consisting of TV (OP764034) and BL (OP764036) with a bootstrap value of 61, and the other group being CM (OP764037). Additionally, the *P. chrysospilos* sample from Singapore (MN690438.1) was placed as an outgroup relative to the VMD group, indicating a genetic difference between the populations in these 2 regions. Furthermore, the *P. modestus* sample from Malaysia (KX223930) was used as an outgroup, clearly separating from the *P. chrysospilos* group, confirming species-level differences. These results are consistent with the earlier genetic distance matrix, where TV and BL showed the highest similarity (genetic distance of 0.000) and were placed together in the phylogenetic tree. Meanwhile, the CM sample exhibited greater separation, especially compared to ST, aligning with the highest genetic distance (0.003) recorded between these 2 sites. These results suggest genetic differentiation between *P. chrysospilos* populations in Vietnam, potentially influenced by geographical or ecological factors affecting gene flow between the regions.

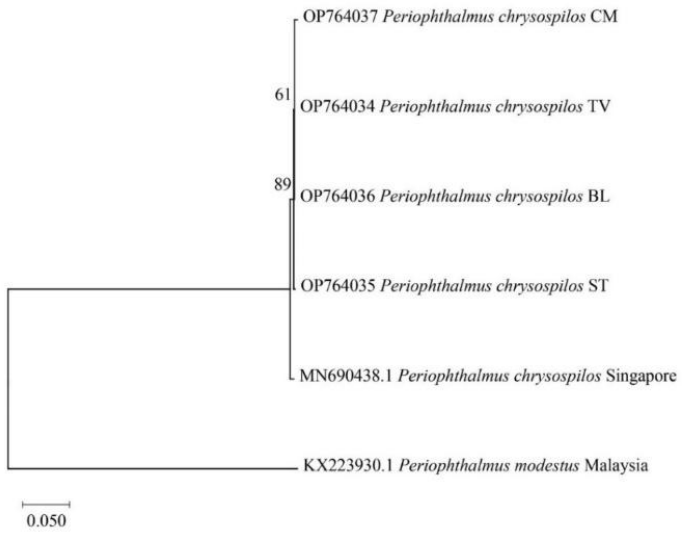

Figure 4 Maximum Likelihood tree based on *COI* sequence using the Neighbor-Joining model with the bootstrap test (1,000 replicates). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (TV: Tra Vinh, ST: Soc Trang, BL: Bac Lieu, CM: Ca Mau).

Discussion

The nucleotide composition data of the *COI* gene in *P. chrysospilos* also indicated a high degree of conservation across samples, with the percentages of C (29 %) and T (28 - 29 %) being higher than those of A (23 %) and G (18 - 19 %). Furthermore, the nucleotide percentages in this species showed minimal variation between individuals from all 4 sampling sites, suggesting that the findings of this study are consistent with previous research on the *COI* gene, which exhibits high conservation within species and low variability [27]. Due to this characteristic, the *COI* gene has been widely used for species identification in various fish species [28-30]. The GC content is an essential indicator for describing nucleotide composition and is related to genome size [31]. This study found that the AT nucleotide pair percentage was higher than that of GC in all samples, aligning with the general characteristics of species within the Oxudercidae family [22]. This result has been reported in several studies conducted in Australia [17], Canada [32], Cuba [33], and various fish species in Taiwan [27].

The analysis of the *COI* gene sequences of *P. chrysospilos* collected from VMD, including Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau, revealed a low level of genetic diversity. This genetic homogeneity may be attributed to the ecological characteristics and distribution of the species. *P. chrysospilos* inhabit mangrove forests and coastal mudflat ecosystems, where the environmental conditions are similar, and there is little geographic isolation between the sampling sites [3]. Consequently, the species' ability to move between regions could reduce genetic differentiation and maintain population homogeneity. Compared to previous studies, the genetic diversity of *P. chrysospilos* in the VMD is lower than in other goby species in the region. For example, a study on *P. novemradiatus* in Malaysia found higher intra-species genetic distances (0.002 - 0.012) [34], indicating more substantial differentiation between populations. Similarly, studies on *Pangasius krempfi*, *P. mekongensis*, and *P. elongatus* in the Mekong River recorded higher genetic diversity (K2P = 0.009 - 0.012), likely due to the Mekong River's flow's influence, creating ecological isolation between populations [13]. Compared to other *P. chrysospilos* populations from different regions, the BLAST results of this study indicated a high similarity (99.21 - 99.84 %) between the *COI* sequences of samples from the Mekong Delta and those from Singapore. This suggests that the populations in these 2 regions may share a common origin or have had genetic exchanges in their evolutionary history. Several factors could influence the low genetic diversity of *P. chrysospilos* in the Mekong Delta, including strong gene flow between regions, the absence of significant geographic barriers, and the species' widespread distribution. Without significant geographical barriers, such as rivers or mountain ranges, individuals can freely move and interbreed between coastal areas, leading to high genetic homogeneity [12]. Additionally, environmental stability may limit selective pressures, resulting in minimal genetic differentiation between populations [5]. Although low genetic diversity helps maintain population stability, it also challenges conserving the species. Genetic homogeneity may reduce the adaptability of *P. chrysospilos* to environmental changes [35], such as salinity, temperature, or water pollution [8]. Therefore, to conserve and manage *P. chrysospilos* populations, strategies for protecting natural habitats, especially mangrove forests and coastal mudflats, are necessary. Expanding research to include more areas, including neighboring coastal regions, will provide a more accurate assessment of the species' genetic differentiation across a broader range.

The habitat plays a crucial role in shaping aquatic species' adaptation and genetic differentiation [36,37], especially for those living in coastal and mangrove ecosystems. In this study, the genetic analysis of *P. chrysospilos* revealed high genetic similarity among the samples from VMD in the phylogenetic tree, which may reflect the strong gene flow between areas with similar ecological conditions. However, the slight differences observed between the populations in Soc Trang (ST) and the other sites and the genetic differentiation from the Singapore population suggest that environmental factors may have influenced the species' adaptation over time. The coastal waters of the VMD display significant fluctuations in salinity and temperature due to tidal regimes, seasonal saltwater intrusion, and river flow from the Mekong [3]. *P. chrysospilos* is a species known for its ability to adapt to brackish and saline waters [38]. However, strong salinity fluctuations could exert

selective pressure, leading to minor genetic differences between populations living under different environmental conditions. Previous studies have shown that ecological pressures can lead to geographic genetic differentiation in some fish species. For instance, Shen *et al.* [39] found that populations of *Mugil cephalus* living in areas with different salinity levels exhibited significant genetic differences, even though the geographic distance between populations was insignificant. Similarly, studies on *P. modestus* have shown genetic differences between populations living in environments with more significant salinity fluctuation than those in more stable environments [22]. Additionally, water pollution from aquaculture and industrial activities can reduce population size, increase genetic drift, and affect adaptation [35]. Furthermore, habitat fragmentation due to mangrove deforestation and coastal infrastructure development can limit movement and mating between populations, leading to a decline in genetic diversity over time [36]. Although clear genetic differentiation has not yet been observed, if these environmental impacts continue, they may negatively affect the species' ability to adapt and long-term survival. Therefore, habitat conservation, genetic diversity monitoring, and expanded research will help maintain the future stability and sustainable development of *P. chrysospilos*. This suggested that environmental variability, especially in salinity, may be a key factor driving the genetic differentiation of *P. chrysospilos* populations in the Mekong Delta and potentially between populations in other regions. Adapting this species to such fluctuating conditions highlights its resilience but also underscores the importance of considering environmental factors in the conservation and management of aquatic species.

An important factor influencing genetic diversity is the level of connectivity between populations. As *P. chrysospilos* is a species living in coastal and mangrove ecosystems, its ability to move between areas is not restricted by significant geographical barriers. This facilitates continuous gene flow between populations, reducing genetic differentiation across regions [12]. However, when compared with populations from Singapore, the higher genetic differentiation could reflect geographic isolation and environmental differences between the 2 regions. Previous studies have also shown that isolation between populations living in different ecosystems can lead to significant genetic differences. For example, the study by Ward *et al.* [17] on goby species in Australia showed that populations living in distinct ecosystems exhibited notable genetic differentiation. In summary, this study's results suggest that strong gene flow helps maintain genetic stability among *P. chrysospilos* populations in the Mekong Delta; selective pressures from local environmental factors may gradually create minor genetic differences between populations living in different ecological conditions. These findings highlight the interplay between gene flow and environmental pressures in shaping the genetic structure of populations, emphasizing the importance of understanding both genetic connectivity and local ecological factors in conservation and management strategies for aquatic species.

With the low genetic diversity observed in *Periophthalmus chrysospilos*, the conservation of this species requires intervention measures to maintain and enhance genetic diversity while considering the role of environmental factors in shaping genetic variation. Firstly, habitat protection strategies such as mangrove forest restoration and minimizing the impact of coastal development activities should be implemented to maintain ecological corridors that connect populations and promote gene flow. Regular genetic monitoring using techniques like microsatellites or SNPs also helps assess genetic diversity loss and identify timely intervention measures [40,41]. Furthermore, environmental factors play a significant role in shaping the species' genetic variation. Conditions such as salinity fluctuations, temperature, and water pollution can exert selective pressures on populations, leading to changes in allele frequencies across generations. Further research into the adaptability of *P. chrysospilos* to these environmental changes will provide valuable information for conservation efforts. At the same time, developing sustainable aquaculture programs with appropriate breeding selection processes can help maintain and enhance genetic diversity without depleting the natural gene pool. Finally, collaboration between scientists, regulatory bodies, and local communities is key to ensuring the long-term sustainability of *P. chrysospilos* [42,43].

The findings from the genetic diversity study of *P. chrysospilos* provide valuable insights for developing

conservation strategies and sustainable management of this species. While the genetic homogeneity across populations in the VMD contributes to the stability of the population, it also reduces the species' ability to adapt to environmental changes. Climate change, salinity intrusion, and environmental pollution can significantly affect populations, especially if they lack genetic diversity to cope with new conditions [8]. Some studies have indicated that fish species with low genetic diversity are more vulnerable to environmental impacts. For instance, Zhu *et al.* [14], in their study on *Channa* species (*Channa argus*, *Channa maculata*, *Channa asiatica*, and *Channa striata*), found that populations with higher genetic diversity showed better resilience when environmental conditions changed. This suggests that maintaining genetic diversity in *P. chrysospilos* populations could enhance the species' resilience to environmental changes in their habitats. Expanding research to include more areas will provide a more accurate assessment of the genetic differentiation of this species across a broader range. This would contribute to the development of more effective conservation strategies based on a comprehensive understanding of the species' genetic structure in Southeast Asia. Conservation efforts can help ensure the long-term survival and adaptability of *P. chrysospilos* by focusing on maintaining genetic diversity of this species in the face of ongoing environmental challenges.

Conclusions

This study provides valuable insights into the genetic diversity of *P. chrysospilos* populations in the coastal provinces of the Mekong Delta using *COI* gene analysis. The results indicate a low level of genetic differentiation among populations from Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau, suggesting high genetic homogeneity. Despite the overall genetic similarity, the Soc Trang population exhibited slight variations compared to other sampling sites. The phylogenetic analysis also revealed a close genetic relationship between *P. chrysospilos* populations in Vietnam and Singapore, highlighting a potential shared evolutionary history. The findings emphasize the importance of conserving *P. chrysospilos* populations in the Mekong Delta, particularly in the face of environmental changes and habitat degradation. Further research incorporating a larger sample size and additional genetic markers would provide a more comprehensive understanding of the species' genetic structure and adaptive potential. This knowledge will contribute to developing effective conservation and management strategies for *P. chrysospilos* and other mangrove-associated species.

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References

[1] RVD Laan, WN Eschmeyer and R Fricke. Family-group names of recent fishes. *Zootaxa* 2014; **3882(1)**, 1-230.

[2] DD Tran, K Shibukawa, TP Nguyen, PH Ha, XL Tran, VH Mai and K Utsugi. *Fishes of Mekong Delta, Vietnam*. Can Tho University Publisher, Can Tho, Vietnam, 2013.

[3] QM Dinh and THD Nguyen. Burrow behaviour, structure and utilization of the amphibious mudskipper *Periophthalmus chrysospilos* Bleeker, 1853 in the Mekong Delta. *Saudi Journal of Biological Sciences* 2023; **30(2)**, 103525.

[4] DA Clayton. Mudskippers. *Oceanography and Marine Biology: An Annual Review* 1993; **31**, 507-577.

[5] G Polgar and G Crosa. Multivariate characterisation of the habitats of 7 species of Malayan mudskippers (Gobiidae: Oxudercinae). *Marine Biology* 2009; **156(7)**, 1475-1486.

[6] EO Murdy. A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae, Oxudercinae). *Australian Museum Journal* 1989; **11**, 1-93.

[7] M Kottelat. *The fishes of the inland waters of Southeast Asia: A catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries (Raffles Bulletin of Zoology)*. Raffles Bulletin of Zoology, Singapore, 2013.

[8] Intergovernmental Panel on Climate Change. *Climate change 2021: The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. Intergovernmental Panel on Climate Change, Geneva, Switzerland, 2021.

[9] PDN Hebert, A Cywinska, SL Ball and JR Dewaard. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 2003; **270(1512)**, 313-321.

[10] C Saccone, C De Giorgi, C Gissi, G Pesole and A Reyes. Evolutionary genomics in Metazoa: The mitochondrial DNA as a model system. *Gene* 1999; **238(1)**, 195-209.

[11] V Sachithanandam, PM Mohan, N Muruganandam, I Chaaithanya, P Dhivya and R Baskaran. DNA barcoding, phylogenetic study of *Epinephelus* spp. from Andaman coastal region, India. *Indian Journal of Geo-Marine Sciences* 2012; **42(3)**, 203-211.

[12] NM Linh, NV Quan, PV Chien, DH Ly, DV Nhan and DT Len. DNA barcoding application of mitochondrial COI gene to identify some fish of family Gobiidae in Vietnam. *Vietnam Journal of Marine Science and Technology* 2018; **18(4)**, 443-451.

[13] TY Duong, K Nguyen, SN Bui, VT Nguyen, BL Nguyen and DD Tran. DNA barcodes and morphological characteristics of *Pangasius krempfi*, *P. mekongensis* and *P. elongatus*. *Vietnam Journal of Biotechnology* 2016; **14(1)**, 29-37.

[14] SR Zhu, JJ Fu, Q Wang and JL Li. Identification of *Channa* species using the partial cytochrome c oxidase subunit I (*COI*) gene as a DNA barcoding marker. *Biochemical Systematics and Ecology* 2013; **51**, 117-122.

[15] T Arai and H Taha. Contrasting patterns of genetic population structure in tropical freshwater eels of genus *Anguilla* in the Indo-Pacific. *Heliyon* 2021; **7(5)**, e07097.

[16] C Zhang, H Liu, X Huang, Z Yuan, S Zhang, S Xu, J Liu, Y Wang, D Wang and J Hu. Comparative analysis of the systematics and evolution of the *Pampus* genus of fish (Perciformes: Stromateidae) based on osteology, population genetics and complete mitogenomes. *Animals* 2024; **14(5)**, 814.

[17] RD Ward, TS Zemlak, BH Innes, PR Last and PD Hebert. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2005; **360(1462)**, 1847-1857.

[18] QM Dinh, THD Nguyen, TTK Nguyen, TTH Lam, NT Truong and DD Tran. Population biological traits of *Periophthalmus chrysospilos* Bleeker, 1853 in the Vietnamese Mekong Delta. *PeerJ* 2022; **10**, e13289.

[19] QM Dinh, THD Nguyen, TTH Lam, NT Truong, TTK Nguyen and Z Jaafar. Reproduction ecology of an emerging fishery resource, the amphibious mudskipper *Periophthalmus chrysospilos*, in the Mekong Delta. *Ecology and Evolution* 2022; **12(1)**, e8507.

[20] QM Dinh, THD Nguyen, NT Truong, LT Tran and TTK Nguyen. Morphometrics, growth pattern and condition factor of *Periophthalmus chrysospilos* Bleeker, 1853 (Gobiiformes: Oxudercidae) living in the Mekong Delta. *The Egyptian Journal of Aquatic Research* 2022; **48(2)**, 157-161.

[21] TTH Lam, QM Dinh and THD Nguyen. Otolith morphometry and its role in determining the growth of *Periophthalmus chrysospilos* distributed in some coastal provinces in the Mekong Delta. *Veterinary Integrative Sciences* 2024; **22(1)**, 299-313.

[22] G Polgar, A Sacchetti and P Galli. Differentiation and adaptive radiation of amphibious gobies (Gobiidae: Oxudercinae) in semi-terrestrial habitats. *Journal of Fish Biology* 2010; **77(7)**, 1645-1664.

[23] QM Dinh. Aspects of reproductive biology of the red goby *Trypauchen vagina* (Gobiidae) from the Mekong Delta. *Journal of Applied Ichthyology* 2018; **34(1)**, 103-110.

[24] F Sanger, S Nicklen and AR Coulson. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National academy of Sciences* 1977; **74(12)**, 5463-5467.

[25] S Kumar, G Stecher and K Tamura. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 2016; **33(7)**, 1870-1874.

[26] D Posada and TR Buckley. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 2004; **53(5)**, 793-808.

[27] X Bingpeng, L Heshan, Z Zhilan, W Chunguang, W Yanguo and W Jianjun. DNA barcoding for identification of fish species in the Taiwan Strait. *PLoS One* 2018; **13(6)**, e0198109.

[28] JB Zhang and R Hanner. DNA barcoding is a useful tool for the identification of marine fishes from Japan. *Biochemical Systematics and Ecology* 2011; **39(1)**, 31-42.

[29] M Chakraborty and SK Ghosh. An assessment of the DNA barcodes of Indian freshwater fishes. *Gene* 2014; **537(1)**, 20-28.

[30] A Karahan, J Douek, G Paz, N Stern, AE Kideys, L Shaish, M Goren and B Rinkevich. Employing DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern Mediterranean, a hot-spot area for biological invasion. *Journal for Nature Conservation* 2017; **36**, 1-9.

[31] XQ Li and D Du. Variation, evolution, and correlation analysis of C + G content and genome or chromosome size in different kingdoms and phyla. *PLoS One* 2014; **9(2)**, e88339.

[32] N Hubert, R Hanner, E Holm, NE Mandrak, E Taylor, M Burridge, D Watkinson, P Dumont, A Curry and P Bentzen. Identifying Canadian freshwater fishes through DNA barcodes. *PLoS One* 2008; **3(6)**, e2490.

[33] A Lara, JLPD León, R Rodríguez, D Casane, G Côté, L Bernatchez and E García-Machado. DNA barcoding of Cuban freshwater fishes: Evidence for cryptic species and taxonomic conflicts. *Molecular Ecology Resources* 2010; **10(3)**, 421-430.

[34] MP Tan, HM Gan, MH Nabilsyafiq, AG Mazlan, TNAM Jaafar, MN Siti Azizah, M Danish-Daniel and YY Sung. Genetic diversity of the Pearse's mudskipper *Periophthalmus novemradiatus* (Perciformes: Gobiidae) and characterization of its complete mitochondrial genome. *Thalassas: An International Journal of Marine Sciences* 2020; **36**, 103-113.

[35] JG Prunier, M Chevalier, A Raffard, G Loot, N Poulet and S Blanchet. Genetic erosion reduces biomass temporal stability in wild fish populations. *Nature Communications* 2023; **14(1)**, 4362.

[36] AC Bockelmann, TBH Reusch, R Bijlsma and JP Bakker. Habitat differentiation vs. isolation-by-distance: The genetic population structure of Elymus athericus in European salt marshes. *Molecular Ecology* 2003; **12(2)**, 505-515.

[37] AS Martinez, JR Willoughby and MR Christie. Genetic diversity in fishes is influenced by habitat type and life-history variation. *Ecology and Evolution* 2018; **8(23)**, 12022-12031.

[38] G Polgar. Species-area relationship and potential role as a biomonitor of mangrove communities of Malayan mudskippers. *Wetlands Ecology and Management* 2009; **17(2)**, 157-164.

[39] KN Shen, BW Jamandre, CC Hsu, WN Tzeng and JD Durand. Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet *Mugil cephalus*. *BMC Evolutionary Biology* 2011; **11(83)**, 1-17.

[40] G Tesfaye, M Curto, P Meulenbroek, GK Englmaier, PD Tibihika, E Alemayehu, A Getahun and H Meimberg. Genetic diversity of Nile tilapia (*Oreochromis niloticus*) populations in Ethiopia: Insights from nuclear DNA microsatellites and implications for conservation. *BMC Ecology and Evolution* 2021; **21**, 1-14.

[41] W Zhu, J Fu, M Luo, L Wang, P Wang, Q Liu and Z Dong. Genetic diversity and population structure of bighead carp (*Hypophthalmichthys nobilis*) from the middle and lower reaches of the Yangtze River revealed using microsatellite markers. *Aquaculture Reports* 2022; **27**, 101377.

[42] CH Faunce and JE Serafy. Mangroves as fish habitat: 50 years of field studies. *Marine Ecology Progress Series* 2006; **318**, 1-18.

[43] PL Munday, RR Warner, K Monro, JM Pandolfi and DJ Marshall. Predicting evolutionary responses to climate change in the sea. *Ecology Letters* 2013; **16(12)**, 1488-1500.