Population Genetic Structure of the So-Iny Mullet (*Planiliza haematocheilus*) along the Coast of Thailand

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ABSTRACT

The so-iny mullet (*Planiliza haematocheilus*) is a coastal marine fish harvested commercially in Thailand. Over the past ten years, the volume of so-iny mullet catches has decreased. As a result, genetic information is required to create an effective sustainable management plan. Our study examined the genetic structure of the so-iny mullet population along the coast of Thailand. One hundred sixty-four samples were caught from nine fishing sites along the Thai coast: southern Thailand (Nakhon Si Thammarat, Pattani, Satun, Krabi, and Phang Nga), western upper Gulf of Thailand (Samut Songkram and Petchburi), and eastern upper Gulf of Thailand (Trat and Rayong). Genetic variation was examined in the partial nucleotide sequence of the control region in mitochondrial DNA (mtDNA *CR*) (550–556 base pairs), and 44 haplotypes were found. Unique haplotypes were observed in all of the populations studied; in some populations there were several, implying that there is a large female effective population size. Population genetic structure analysis revealed differences among samples from southern Thailand, the western upper Gulf of Thailand, and the eastern upper Gulf of Thailand. In conclusion, we suggest that migration behavior as well as geographical distance between so-iny mullet habitats shaped the genetic structure of the populations in Thailand. Our findings can help guide so-iny mullet management in Thailand.

Keywords: Genetic diversity, Mitochondrial DNA, Mullet, Thailand

INTRODUCTION

The so-iny mullet (*Planiliza haematocheilus*) is distributed widely in estuarine and coastal waters of tropical and temperate areas of the Indo-Pacific (Chen *et al*., 2018; Zhao *et al*., 2021). In Thailand, so-iny mullet is a popular food fish and is a main ingredient in many Thai dishes. In the last ten years, so-iny mullet catches have decreased in volume (e.g., from 8,000 t in 2009 [Fishery Statistics Analysis and Research Group, 2011] to 2,500 t in 2019 [Fishery Statistics Analysis and Research Group, 2021]). Such reduction may reflect the decline in abundance of so-iny mullet and hence may result in loss of genetic diversity. Therefore, fishery management measures should be applied to maintain genetic diversity. Such manipulation requires genetic information on so-iny mullet, such as population structure as well as genetic variation within populations (Ward, 2000). However, this type of information has never before been reported for so-iny mullet in Thailand.

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Population structure refers to the presence of a systematic difference in allele frequencies between subpopulations. Genetic structure changes are caused by factors affecting gene frequencies or genetic diversity patterns. There are several factors affecting genetic differences in marine populations, such as geographic barriers and current, which prevent subpopulations from mating (Johannesson *et al*., 2018). In addition, larval duration and dispersal ability of larvae also determine population structure (Weersing and Toonen, 2009). The genetic diversity among and within populations indicates survival ability of a species, whereby high genetic diversity enhances adaptability of organisms (Orr and Unckless, 2014). Information on population structure would be beneficial in designing appropriate management and maintaining the sustainable exploitation of marine animals (Wellmann and Bennewitz, 2019). In Thailand, so-iny mullet lives along the coast, both in the Gulf of Thailand and the Andaman Sea (Wittayanon, 1993). The Thai-Malay Peninsula separates these two seas. Therefore, we hypothesized that geographic barriers as well as current patterns are factors that have caused genetic differences in so-iny mullet living in these two areas. Population differentiation has been observed in other marine animals living in these two areas, such as the oceanic paddle crab (*Varuna literata*) (Suppapan *et al*., 2017), the hard clam (*Meretrix lyrata*) (Suppapan *et al*., 2021), the blue swimming crab (*Portunus pelagicus*) (Khamnamtong *et al*., 2021), and the ornate threadfin bream (*Nemipterus hexodon*) (Supmee *et al*., 2021).

Over the last decade, nucleotide sequences in mitochondrial DNA have been used to analyze population structure in marine animals (Marini *et al*., 2021). Due to its high mutation rate, it is appropriate for studies of genetic variability. In addition, studies of maternal heredity do not require as many samples as other types of genetic markers, and the lack of recombination makes it possible to study the patterns directly (Zarei and Alipanah, 2014). In our study, the nucleotide sequence in the control region (mtDNA *CR*) was used to analyze the genetic structure of so-iny mullet because of its high mutation rate, e.g., 5–10 times higher than those of other parts of mitochondrial DNA, and 25–100 times higher than those of nuclear genes (Bronstein *et al*., 2018). The use of mtDNA *CR* nucleotide sequences has been previously reported in population genetic studies in many marine species. Examples include silver pomfret (*Pampus argenteus*) (Sun *et al*., 2013), yellowfin tuna (*Thunnus albacares*) (Kunal *et al*., 2013), blackspot seabream (*Pagellus bogaraveo*) (Robalo *et al*., 2021), Chinese silver pomfret (*Pampus chinensis*) (Sun *et al*., 2021), and blue swimming crab (*Portunus pelagicus*) (Lu *et al*., 2022). The objective of this study was to analyze the genetic diversity and population structure of so-iny mullet in Thailand by analyzing the nucleotide sequences from the control region in the mitochondrial DNA. The knowledge gained from this study can be used to benefit research and policy efforts in Thailand, such as the development of sustainable so-iny mullet management and conservation policies.

MATERIALS AND METHODS

Sample collection

In this study, 164 individuals of so-iny mullet were caught from fishing grounds in the Gulf of Thailand and the Andaman Sea, including the provinces of Trat (TR), Rayong (RY), Samut Songkram (SM), Petchburi (PB), Nakhon Si Thammarat (NS), Pattani (PT), Satun (ST), Krabi (KB), and Phang Nga (PN) (Figure 1, Table 1). The whole bodies of fish samples were packed in ice and transported to the laboratory at Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, where they were stored at -20 °C until DNA extraction.

DNA extraction, PCR amplification and nucleotide sequencing

Muscle tissue taken from each individual was used for DNA extraction using genomic DNA Extraction Mini Kit (FAVORGEN Biotech Corp., Taiwan), following the protocol recommended by the manufacturer. The primer pair was designed to amplify the target DNA in the mtDNA *CR* based on a complete mtDNA genome sequence of the redeye mullet *Liza hematocheil* obtained from GenBank (Accession number NC_024531) using

the Primer 3 program (Untergasser *et al*., 2012). After that, primers (PH_CR_H1: 5' GAC ATT TTC CGA AGG GGT CC 3' and PH_CR_L1: 5' TGG CAA TAG CCT AGA TGA CAC C 3') were used to amplify the target DNA by polymerase chain reaction (PCR). DNA amplification was carried out in a thermocycler (Major Cycler, CYCLER, Taiwan). The total reaction mixture was 50 µL and consisted of 26.5 µL of nuclease - free water, 5 µL of 10X *Taq* buffer, 5 µL of 25 mM $MgCl_2$, 4 µL of 2 mM dNTP mix, 2 µL of 10 mM concentrations of each primer, 0.5 µL of 2.5 units *Taq* DNA polymerase (Thermo Scientific, USA), and 5 µL of 50–100 ng template DNA. PCR was performed with the following profile: initial denaturation for 4 min at 94 °C, followed by 35 cycles consisting of 40 s at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were examined by agarose gel electrophoresis. Then, the PCR products with correct size (800 bp) were purified using a DNA product purification kit (Tiangen BioTech, China) and sent to the 1st BASE Laboratory (Selangor, Malaysia) for direct sequencing.

Data analysis and genetic diversity

Nucleotide sequences were validated against the GenBank database. The nucleotide sequences were aligned using the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/) and edited. Multiple sequences were aligned using ClustalW version 1.83 (Thompson *et al*., 1994). Genetic diversity standard indices were examined using DnaSP version 6.00 (Rozas *et al*., 2017). The Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) statistics were estimated to test the deviation from a neutral population using ARLEQUIN version 3.5 (Excoffier and Lischer, 2010) based on 10,000 replicates.

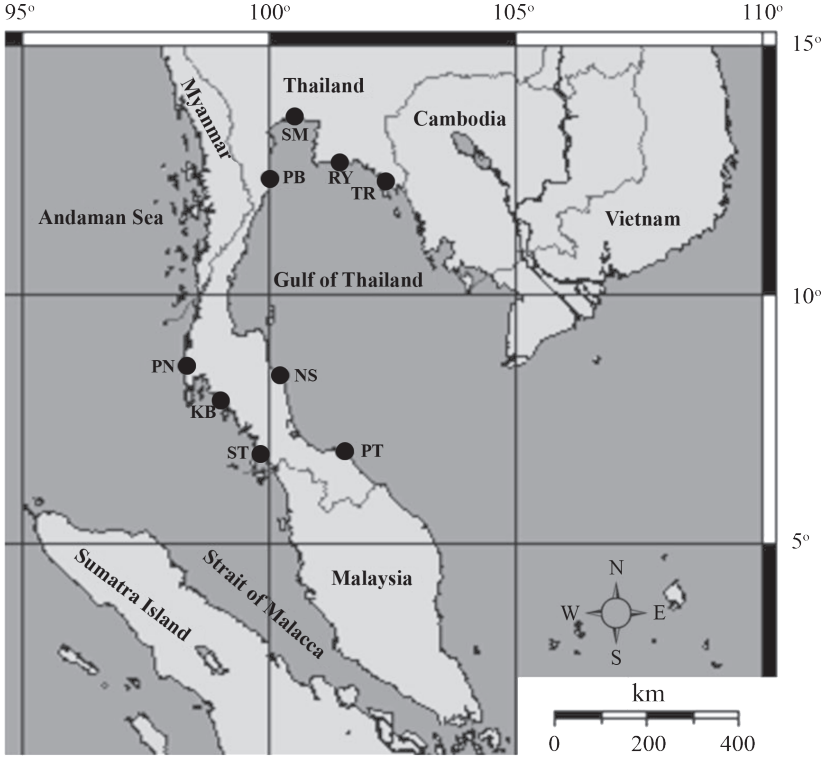

Figure 1. So-iny mullet collection localities along the coast of Thailand.
Note: TR = Trat; RY = Rayong; SM = Samut Songkram; PB = Petchburi; NS = Nakhon Si Thammarat; PT = Pattani; ST = Satun; KB = Krabi; PN = Phang Nga
(Source: Wikimedia commons contributors, 2021).

Population structure

The genetic structure of the so-iny mullet was studied by creating seven putative groupings. First, the dataset was divided into nine groups based on the sampling sites (single region), namely, Trat (TR), Rayong (RY), Samut Songkram (SM), Petchburi (PB), Nakhon Si Thammarat (NS), Pattani (PT), Satun (ST), Krabi (KB), and Phang Nga (PN). Second, the data were divided as the Gulf of Thailand population (TR, RY, SM, PB, NS, and PT samples) and the Andaman Sea population (ST, KB, and PN samples). Third, the data were divided as the lower Gulf of Thailand (NS and PT samples), the upper Gulf of Thailand (TR, RY, SM, and PB samples), and the Andaman Sea (ST, KB, and PN samples). Fourth, the populations were defined as the lower Gulf of Thailand (NS and PT samples) and the Andaman Sea (ST, KB, and PN samples). Fifth, the populations were defined as the upper Gulf of Thailand (TR, RY, SM, and PB samples) and the Andaman Sea (ST, KB, and PN samples). Sixth, the data were separated into the lower Gulf of Thailand (NS and PT samples) and the upper Gulf of Thailand (TR, RY, SM, and PB samples). Seventh, the data were divided into the western upper Gulf of Thailand (SM and PB samples) and the eastern upper Gulf of Thailand (RY and TR samples). To estimate levels of genetic variation within and among putative populations, a hierarchical analysis of molecular variance (AMOVA) was analyzed with ARLEQUIN version 3.5 (Excoffier and Lischer, 2010). The Φ-statistics, namely Φ_{CT} (among groups), Φ_{SC} (among populations within groups), and Φ_{ST} (within populations) were examined at different hierarchical levels using 10,000 permutations ($p<0.05$). The pairwise FST was evaluated to compare genetic distances among populations using 10,000 permutations ($p<0.05$). The neighbor-joining phylogenetic tree was constructed based on the matrix of Kimura 2-parameter distances to examine the relationships among haplotypes as implemented in MEGA version 11 (Tamura *et al*., 2021). Bootstrapping with 1,000 replicates was employed to obtain relative support for tree topology. The minimum spanning network (MSN) was constructed based on the mean number of pairwise differences among haplotypes using ARLEQUIN version 3.5 (Excoffier and Lischer, 2010) and was drawn by hand.

RESULTS

Genetic diversity

The sequencing results revealed that the obtained PCR products comprised sequences ranging from 550–556 nucleotides. There were 546 aligned site positions, divided into 435 monomorphic sites and 111 polymorphic sites. The composition of the nucleotide sequence consisted of A (29.2%), T (30.5%), G (17.9%), and C (22.4%). The base contents of A+T and G+C were 59.7% and 40.3%, respectively. The nucleotide sequences of 44 haplotypes were found and deposited in GenBank with accession numbers ON858420–ON858463. The number of haplotypes shared among the sampling locations was 9, and within the sampling locations, there were 10 haplotypes. Twenty-five haplotypes were sampling location specific and hence called "unique haplotypes." The Pattani sample had the highest number of unique haplotypes (nine haplotypes), while unique haplotypes were not found in Krabi (Table 2). The haplotype diversity ranged from 0.125 to 0.949, with a total population value of 0.905±0.016. The nucleotide diversity ranged from 0 to 0.072, with a total population value of 0.064±0.003. The genetic diversity values are shown in Table 1. The Tajima's *D* and Fu's *Fs* values were negative in the total population, -2.527 ($p>0.05$) and -7.492 ($p>0.05$), respectively (Table 1).

Population structure

A hierarchical analysis of molecular variance (AMOVA) was performed based on the variation in mtDNA *CR* sequences. The first putative structure (single region) showed that the Φ-statistic was significant ($\Phi_{ST} = 0.782$, $p = 0.000$), indicating that there is genetic difference among the nine so-iny mullet populations. In the second putative structure (the Gulf of Thailand vs the Andaman Sea), a significant genetic difference between the two seas ($\Phi_{CT} = 0.461$, $p = 0.038$) was observed. In the third putative structure (the lower Gulf of Thailand vs the upper Gulf of Thailand vs the Andaman Sea) the Φ-statistic showed a significant difference ($\Phi_{CT} = 0.484$, $p = 0.016$), indicating genetic structure of the so-iny mullet populations among these areas.

The fourth putative structure (the lower Gulf of Thailand vs the Andaman Sea) exhibited no genetic significance between the two seas (Φ_{CT} = 0.247, p = 0.098). The fifth putative structure (the upper Gulf of Thailand vs the Andaman Sea) showed a significant genetic difference between the two seas (Φ_{CT} = 0.667, p = 0.031). The sixth putative structure (the lower Gulf of Thailand vs the upper Gulf of Thailand) showed a significant difference (Φ_{CT} = 0.131, p = 0.041), indicating a genetic structure in the so-iny mullet populations in the Gulf of Thailand (Table 3). The seventh putative structure (western upper Gulf of Thailand vs the eastern upper Gulf of Thailand) revealed a significant difference (Φ_{CT} = 0.629, p = 0.045), indicating a genetic structure of the so-iny mullet populations in the upper Gulf of Thailand (Table 3). Every pairwise F_{ST} of the populations indicated significant differences between populations, except for the comparisons between PN and RY; between SM and PB; between NS and the group of PT, ST, KB, and PN; between PT and the group of ST, KB, and PN; between ST and the group of KB and PN; and between KB and PN (Table 4).

Three distinct haplotype lineages were revealed in the neighbor-joining tree. Haplogroup I comprised 26 haplotypes of the Andaman Sea and the lower Gulf of Thailand. Haplogroup II was made up of 10 haplotypes that represented the vast majority of so-iny mullet in the western upper Gulf of Thailand. Haplogroup III consisted of 8 haplotypes from the eastern upper Gulf of Thailand (Figure 2). As shown in Figure 3, the haplotype network of the so-iny mullet was divided into three haplogroups. Haplogroup I consisted of 26 haplotypes from southern Thailand (ST, KB, PN, PT and NS), and haplotypes H03 and H10 were common haplotypes. Haplogroup II comprised 10 haplotypes from the western Gulf of Thailand (TR, RY, SM, PB, and NS), and haplotype H27 was a common haplotype. Haplogroup III was composed of 8 haplotypes from the eastern Gulf of Thailand (TR and RY), and haplotypes H40 and H41 were common haplotypes. The most common haplotype of the minimum spanning network was haplotype H27. Haplogroup I was connected with haplogroup II and separated by 50 mutation steps. Haplogroup III was connected with haplogroup I and separated by 66 mutation steps.

Table 1. Sampling sites, number of samples (n), genetic diversity indices, and neutrality test results of the so-iny mullet (*Planiliza haematocheilus*) estimated from mtDNA *CR* sequences.

Sampling sites	**n**	**No. Polymorphic sites**	**No. Haplotypes**	**Haplotype diversity (*h*) (mean±SD)**	**Nucleotide diversity (π) (mean±SD)**	**Tajima's *D***	**Fu's *Fs***
Trat (TR)	14	77	5	0.758±0.084	0.059±0.014	-1.526	-7.131
Rayong (RY)	13	78	10	0.949±0.051	0.072±0.007	-2.675	10.478
Samut Songkram (SM)	16	1	2	0.125±0.106	0	-1.162	-1.521
Petchburi (PB)	18	4	5	0.667±0.083	0.001±0	-0.673	-0.700
Nakhon Si Thammarat (NS)	15	57	4	0.467±0.148	0.025±0.012	-0.899	2.693
Pattani (PT)	17	14	12	0.941±0.043	0.004±0	-1.351	13.523
Satun (ST)	23	11	10	0.893±0.039	0.007±0	1.670	-0.795
Krabi (KB)	24	5	5	0.714±0.079	0.002±0	-0.341	-0.455
Phang Nga (PN)	24	4	5	0.721±0.058	0.001±0	-0.209	-0.914
Total	**164**	**111**	**44**	**0.905±0.016**	**0.064±0.003**	**-2.527**	**-7.492**

Table 2. The mtDNA CR haplotype distributions of the so-iny mullet (*Planiliza haematocheilus*) from nine sampling sites along the coast of Thailand.

Haplotype	TR	RY	SM	PB	NS	PT	ST	KB	PN	Total
H01	-	-	-	-	-	-	6	-	-	6
H02	-	-	-	-	-	2	1	-	-	3
H03	-	-	-	-	2	4	1	-	-	7
H04	-	-	-	-	-	-	2	-	-	2
H05	-	-	-	-	-	-	2	-	-	2
H06	-	-	-	-	-	-	3	-	-	3
H07	-	-	-	-	-	-	2	-	-	2
H08	-	-	-	-	-	-	1	-	-	1
H09	-	-	-	-	-	-	4	-	-	4
H10	-	-	-	-	-	-	1	12	8	21
H11	-	-	-	-	-	-	-	3	10	13
H12	-	-	-	-	-	-	-	2	-	2
H13	-	-	-	-	-	-	-	3	2	5
H14	-	-	-	-	-	-	-	4	-	4
H15	-	-	-	-	-	-	-	-	3	3
H16	-	-	-	-	-	-	-	-	1	1
H17	-	-	-	-	-	2	-	-	-	2
H18	-	-	-	-	-	1	-	-	-	1
H19	-	-	-	-	-	1	-	-	-	1
H20	-	-	-	-	-	1	-	-	-	1
H21	-	-	-	-	-	1	-	-	-	1
H22	-	-	-	-	-	1	-	-	-	1
H23	-	-	-	-	-	1	-	-	-	1
H24	-	-	-	-	-	1	-	-	-	1
H25	-	-	-	-	-	1	-	-	-	1
H26	-	-	-	-	-	1	-	-	-	1
H27	4	3	15	9	11	-	-	-	-	42
H28	-	-	-	-	1	-	-	-	-	1
H29	-	-	-	6	1	-	-	-	-	7
H30	-	-	-	1	-	-	-	-	-	1
H31	-	-	-	1	-	-	-	-	-	1
H32	-	-	-	1	-	-	-	-	-	1
H33	-	-	1	-	-	-	-	-	-	1
H34	-	1	-	-	-	-	-	-	-	1
H35	-	1	-	-	-	-	-	-	-	1
H36	-	1	-	-	-	-	-	-	-	1
H37	-	1	-	-	-	-	-	-	-	1
H38	-	1	-	-	-	-	-	-	-	1
H39	-	1	-	-	-	-	-	-	-	1
H40	2	2	-	-	-	-	-	-	-	4
H41	6	1	-	-	-	-	-	-	-	7
H42	-	1	-	-	-	-	-	-	-	1
H43	1	-	-	-	-	-	-	-	-	1
H44	1	-	-	-	-	-	-	-	-	1
Total	**14**	**13**	**16**	**18**	**15**	**17**	**23**	**24**	**24**	**164**

Note: TR = Trat; RY = Rayong; SM = Samut Songkram; PB = Petchburi; NS = Nakhon Si Thammarat; PT = Pattani; ST = Satun; KB = Krabi; PN = Phang Nga

Table 3. Hierarchical analysis of the molecular variance for so-iny mullet (*Planiliza haematocheilus*) sampled at nine sampling sites along the coast of Thailand, estimated from mtDNA *CR* sequences.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Φ-statistic
1) Single region (TR×RY×SM×PB×NS×PT×ST×KB×PN)					
Among populations	8	2219.193	15.088 Va	78.24	Φ_{ST} = 0.782*
Within populations	155	650.570	4.197 Vb	21.76	
Total	163	2869.762	19.285		
2) Gulf of Thailand×Andaman Sea					
Among groups	1	1084.155	11.184 Va	46.12	Φ_{CT} = 0.461*
Among populations within groups	7	1135.037	8.871 Vb	36.58	Φ_{SC} = 0.678*
Within populations	155	650.570	4.197 Vc	17.31	Φ_{ST} = 0.826*
Total	163	2869.762	24.252		
3) Lower Gulf of Thailand×upper Gulf of Thailand×Andaman Sea					
Among groups	2	1399.936	10.812 Va	48.47	Φ_{CT} = 0.484*
Among populations within groups	6	819.256	7.300 Vb	32.72	Φ_{SC} = 0.634*
Within populations	155	650.570	4.197 Vc	18.81	Φ_{ST} = 0.811*
Total	163	2869.762	22.317		
4) Lower Gulf of Thailand×Andaman Sea					
Among groups	1	222.118	2.554 Va	24.73	Φ_{CT} = 0.247
Among populations within groups	3	374.748	5.831Vb	56.44	Φ_{SC} = 0.749*
Within populations	98	190.619	1.945Vc	18.83	Φ_{ST} = 0.811*
Total	102	787.485	10.331		
5) Upper Gulf of Thailand×Andaman Sea					
Among groups	1	1343.513	18.895 Va	66.73	Φ_{CT} = 0.667*
Among populations within groups	5	499.697	5.152 Vb	18.20	Φ_{SC} = 0.546*
Within populations	125	533.691	4.269 Vc	15.08	Φ_{ST} = 0.849*
Total	131	2376.902	28.317		
6) Lower Gulf of Thailand×upper Gulf of Thailand					
Among groups	1	315.781	2.827 Va	13.18	Φ_{CT} = 0.131*
Among populations within groups	4	764.068	12.003 Vb	55.93	Φ_{SC} = 0.644*
Within populations	87	576.829	6.630 Vc	30.89	Φ_{ST} = 0.691*
Total	92	1656.667	21.461		
7) Western upper Gulf of Thailand×eastern upper Gulf of Thailand					
Among groups	1	408.678	13.290 Va	62.99	Φ_{CT} = 0.629*
Among populations within groups	2	17.308	0.059 Vb	0.28	Φ_{SC} = 0.007*
Within populations	57	441.687	7.748Vc	36.73	Φ_{ST} = 0.632*
Total	60	867.672	21.099		

Note: * indicates significant difference ($p<0.05$); TR = Trat; RY = Rayong; SM = Samut Songkram; PB = Petchburi; NS = Nakhon Si Thammarat; PT = Pattani; ST = Satun; KB = Krabi; PN = Phang Nga

Table 4. Pairwise FST values between all possible combinations of the so-iny mullet (*Planiliza haematocheilus*) populations from nine sites along the Thailand coast estimated from mtDNA *CR* sequences.

Population	TR	RY	SM	PB	NS	PT	ST	KB	PN
TR	-								
RY	-0.005	-							
SM	0.701*	0.522*	-						
PB	0.709*	0.534*	0.159	-					
NS	0.575*	0.381*	0.075*	0.081*	-				
PT	0.752*	0.694*	0.973*	0.967*	0.835	-			
ST	0.757*	0.702*	0.949*	0.945*	0.823	0.238	-		
KB	0.792*	0.741*	0.985*	0.980*	0.871	0.763	0.557	-	
PN	0.794*	0.744*	0.987*	0.982*	0.874	0.761	0.538	0.157	-

Note: * indicates significant difference ($p<0.05$); TR = Trat; RY = Rayong; SM = Samut Songkram; PB = Petchburi; NS = Nakhon Si Thammarat; PT = Pattani; ST = Satun; KB = Krabi; PN = Phang Nga

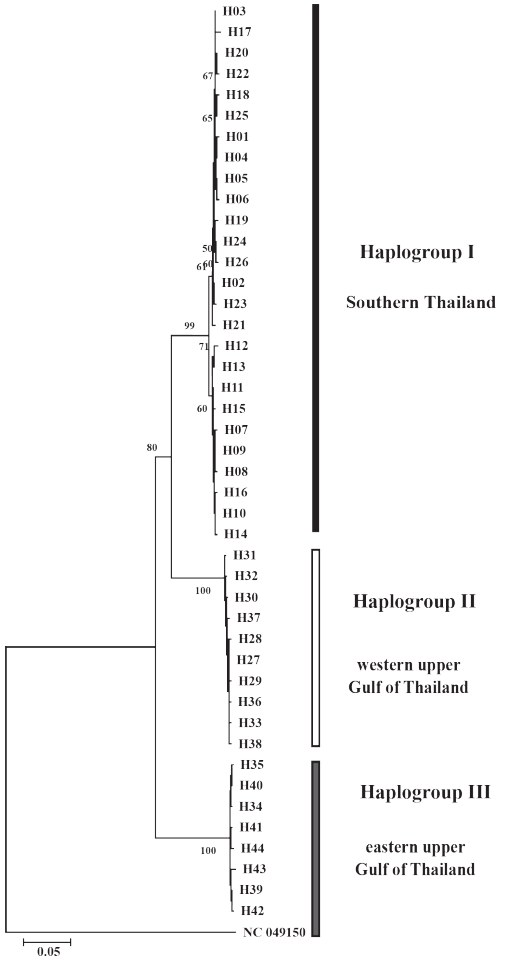

Figure 2. Neighbor-joining phylogenetic tree constructed from mtDNA *CR* sequences of so-iny mullet (*Planiliza haematocheilus*) with *Nemipterus hexodon* (accession number NC 049150) as an outgroup. Bootstrap values are represented by the numbers on the branches.

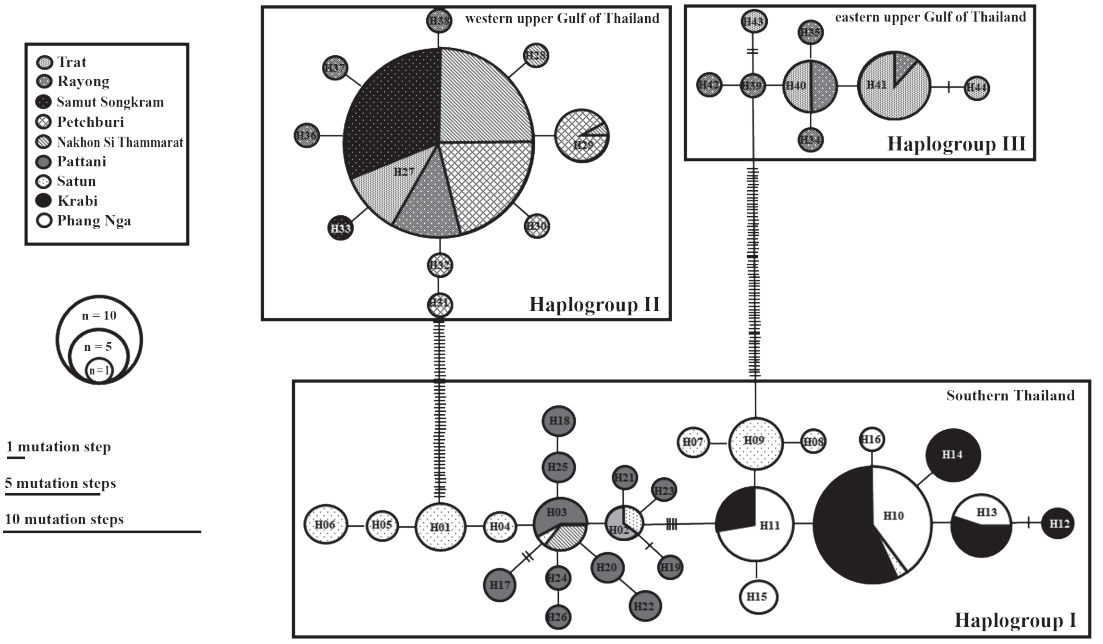

Figure 3. Minimum spanning network of the 44 haplotypes in mtDNA *CR* of the so-iny mullet (*Planiliza haematocheilus*). The sizes of the circles are proportional to the frequency of haplotypes. The vertical bars on the lines indicate the numbers of mutation steps separating two haplotypes. A lack of vertical bars on any line connecting haplotypes indicates that a single mutation step separates two haplotypes.

DISCUSSION

Genetic diversity

In this study, the (A+T) base contents of all 44 partial mtDNA *CR* haplotypes were higher than the (G+C) base contents, which is consistent with other marine species, such as the vesicomyid bivalve (*Calyptogena marissinica*) (Yang *et al*., 2019) and the varunid crab (*Helice wuana*) (Tang *et al*., 2018). Rich bases (A+T) are a common feature of animal mitochondrial genomes (Fourdrilis *et al*., 2018). Nucleotide diversity is a parameter used to quantify the degree of polymorphism within a population (Korunes and Samuk, 2021). In this study, the levels of genetic diversity of the populations in Rayong, Trat, and Nakhon Si Thammarat were higher than those of other populations. Interestingly, the so-iny mullet population from Samut Songkram had low genetic diversity, indicating a risk of genetic diversity loss. In the past ten years, the harvesting of mugil species in Samut Songkram has increased steadily (Fishery Statistics Analysis and Research Group, 2021). As a result, the population size of mugil species may be declining, resulting in decreased genetic diversity. Thus, management of the so-iny mullet population in this area should be focused on helping to determine breeding populations and appropriate conservation units. There were 25 unique haplotypes among 44 haplotypes identified in this study. The existence of several unique haplotypes in all sampling locations suggests that the female effective population size is large (Korstian *et al*., 2015). Numerous unique haplotypes reflect a large population size, which allows for the retention of numerous distinct haplotypes in females. The genetic diversity pattern of the so-iny mullet population revealed high haplotype diversity but low nucleotide diversity. A rapid accumulation of novel mutations in a population can result in this pattern (Ueda and Itino, 2017). This pattern has been described as a feature of genetic variation in marine species (Liu *et al*., 2015). High haplotype diversity but low nucleotide

diversity has been reported in several marine fish, including the crescent grunter (*Terapon jarbua*) (Chanthran *et al*., 2020), blue-spotted mudskipper (*Boleophthalmus boddarti*) (Theeranukul *et al*., 2021), and Pearse's mudskipper (*Periophthalmus novemradiatus*) (Tan *et al*., 2020). Tajima's *D* and Fu's *Fs* tests produced negative scores for most populations. A negative Tajima's *D* indicates higher than expected low-frequency polymorphisms. An excess of alleles, as would be anticipated from recent population growth or genetic hitchhiking of the so-iny mullet population in Thailand, is indicated by a negative Fu's *Fs* value (Ashfaq *et al*., 2014).

Population structure

In this study, the so-iny mullet population in Thailand was divided into three populations: southern Thailand (Nakhon Si Thammarat, Pattani, Satun, Krabi, and Phang Nga), western upper Gulf of Thailand (Samut Songkram and Petchburi), and eastern upper Gulf of Thailand (Trat and Rayong). Interestingly, genetic differentiation was not found between the populations of so-iny mullet inhabiting the coasts of the lower Gulf of Thailand and the Andaman Sea. Although these two regions are geographically separate, the two populations do not seem to be successfully prevented from sharing genes. By stepping-stone mechanisms along feeding grounds, the so-iny mullet could migrate through the Malacca Strait (Suppapan *et al*., 2017). However, the mullet populations in the Gulf of Thailand were divided into two populations, in the lower and upper Gulf of Thailand. This could be due to tide differences in the Gulf of Thailand, which could impede gene flow in the two so-iny mullet populations. The sea current flows counterclockwise in the upper Gulf of Thailand, while a large circle of water flows clockwise in the lower Gulf of Thailand (Saramul, 2017). Thus, we suggest that there is no geographical barrier in the Gulf of Thailand, though sea currents act as a barrier to gene flow in the so-iny mullet population (Panithanarak, 2017). Furthermore, the population of so-iny mullet in the upper Gulf of Thailand was distinct from that in the Andaman Sea. Whether as free-moving gametes, larvae, or adults, most marine species spend a part of their life cycle in open waters. In marine species with long dispersal capabilities (e.g., planktotrophic larvae), it is reasonable to expect high gene flow and little genetic structure (Cordero *et al*., 2014). For many marine species, genetic homogeneities across long distances are maintained by high larval dispersal, such as high levels of gene flow along the 3,000 km dispersal range of the sesarmid crab (*Neosarmatium meinerti*) (Ragionieri *et al*., 2010) and the porcelain fiddler crab (*Uca annulipes*) (Silva *et al*., 2010). Certain factors, such as geographic remoteness and larval behavior, may prevent the spread of genetic material between populations (Pascual *et al*., 2017). In this study, the so-iny mullet populations were unable to maintain gene flow between the upper Gulf of Thailand and the Andaman Sea populations, as larval behavior seems to support the structure separating the mullet in the upper Gulf of Thailand from those in the Andaman Sea. This result could be consistent with the behavior of the so-iny mullet, as in its last larval stage, this species typically returns to its parental environments (Santana *et al*., 2018). This larval behavior has the potential to disrupt gene flow in the so-iny mullet population across a large geographical distance between the Andaman Sea and the upper Gulf of Thailand. Interestingly, the so-iny mullet populations were genetically distinct within the upper Gulf of Thailand, between the west and east coasts of the upper Gulf of Thailand, where the populations are close and there are no geographical barriers. Groups of mullet spawn in the ocean and migrate to their original habitats (Salvarina *et al*., 2018). Therefore, the migration behavior of mullet is likely to be the cause of disrupted gene flow between the western and eastern populations of the upper Gulf of Thailand. A study of the genetic structure of mullet in Thailand found that, in addition to the genetic differences in mullet populations in each area, lack of geographical barriers did not promote gene flow; in contrast, sea currents did limit the gene flow of the so-iny mullet populations. In addition, the main factor influencing the genetic structure of mullet in Thailand is likely their migration behavior. This is consistent with the reports of population genetic structure caused by the migration behavior of another member of the mullet group in the Andaman Sea, the greenback mugil (*Liza subviridis*) (Supmee *et al*., 2017), as well as a mullet species (*Mugil cephalus*) found along the lower and upper seaboards of China (Liu *et al*., 2009).

Guidelines for the conservation of genetic diversity

Our findings revealed the distinctive population structure of Thai so-iny mullet populations. As a result, fisheries management should be planned based on genetic groups. In the case of healthy populations, a management plan to maintain genetic diversity should be conducted to control overfishing and prevent habitat destruction, such as by limiting fishing activity during the spawning season, controling coastal pollution, and not using illegal fishing gear. In the case of deteriorated populations, genetic diversity should be conserved by restocking with so-iny mullet from the original population to avoid altering the genetic backup of the population, which could lead to population integrity loss. Currently, climate change and waste pollution may also have an impact on so-iny mullet populations. As a result, changes in genetic diversity should be monitored regularly. However, when developing conservation guidelines to preserve genetic diversity, data from more variable molecular markers, such as microsatellites, should also be considered.

CONCLUSION

In our study, 164 mtDNA *CR* nucleotide sequences with a size of 550–556 base pairs were analyzed to determine the genetic structure of so-iny mullet along the coast of Thailand. The results of multiple genetic structure analyses indicated that the so-iny mullet were divided into three populations: southern Thailand (the lower Gulf of Thailand and the Andaman Sea), the western upper Gulf of Thailand, and the eastern upper Gulf of Thailand. Our study provides critical information for developing long-term management strategies to preserve the genetic diversity of Thai so-iny mullet populations.

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