Research article

Genetic diversity of blue-spotted mudskipper (*Boleophthalmus boddarti*) populations in Gulf of Thailand

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Article Info

Abstract

Environmental changes and the reduction of habitat can threaten populations of mudskippers, which have a distinct life cycle compared with other fish species. Genetic diversity and structure are crucial information for the conservation plan of this species. The genetic diversity was investigated on the blue-spotted mudskipper, *Boleophtalmus boddarti,* in the Gulf of Thailand. In total, 178 fish were collected from six locations in the Gulf. Based on the 320 bp sequences of the mitochondrial control region of the 55 haplotypes observed, the most common was in 88 fish from all locations. Total haplotype diversity and nucleotide diversity values (mean ± SD) were $h = 0.751 \pm 0.036$ and $\pi = 0.0069 \pm 0.0001$, respectively. There was a significant ($p = 0.011$) difference in π between inner and outer Gulf samples. Although the analysis of molecular variance suggested the absence of genetic structuring within the Gulf, two clear groups of haplotypes were evident in the median-joining network of haplotypes. Group I included haplotypes from all locations and group II was identified by haplotypes with an additional adenine at the 16078th position based on the mitochondrial genome sequence of *B. boddarti* (Accession no. KF87427). The results of the nonmetric multidimensional scaling and Bayesian assignment test were indicative of genetic divergence between the inner and outer Gulf, suggesting that despite the high potential for dispersal of planktonic larvae, water currents may act as a physical barrier to gene flow in the study area. The observed signals of population divergence between *B. boddarti* from the inner and outer Gulf of Thailand may account for the presence of this oceanographical barrier. Mismatch distributions, based on the observed number of differences among haplotype pairs, produced a unimodal distribution with a peak close to the y-axis, suggesting recent demographic expansion. The results could augment future study with baseline information on the maternal genetic variation and structure of the blue-spotted mudskipper, *B. boddarti*, populations in the Gulf of Thailand.

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Introduction

The blue-spotted mudskipper, *Boleophthalmus boddarti* (Pallas 1770) (Actynopterygii, Gobiidae), is a benthic fish species widely distributed in the Indo-West Pacific region (Murdy, 1989). It can be commonly found in shallow-water mangrove habitats and mudflat areas, being part of the group of fish commonly referred to as 'mudskippers' (Murdy, 1989). In the Gulf of Thailand, *B. boddarti* is a common fish, found along the coast, especially in mangrove and mudflat habitats (Murdy, 1989; Darumas, 1997). The Gulf of Thailand is bordered by Malaysia, Thailand, Cambodia and Vietnam, with the southern part of the Gulf connecting to the South China Sea. The surface area of the Gulf is approximately 35,000 km^2 and can be divided from the north to the south into the upper Gulf (or inner Gulf), the central Gulf and the lower Gulf (Sripoonpan and Saramul, 2021). In this study, the central and lower Gulf are referred to as the outer Gulf of Thailand.

Mudskippers are ecologically important elements of mangrove ecosystems (Clayton and Vaughan, 1988; Al-Khayat and Jones, 1999) and have been used as environmental bioindicators for coastal water (Ansari et al., 2014). Recently, the populations of many mudskipper species were threatened by the high demand of mudskipper fish for human consumption in many eastern Asian countries and their use as ingredient for traditional medicine, especially in India and China (Banerjee et al., 1997), determined a high degree of exploitation of natural populations as well as aquaculture farming (Polgar and Lim, 2011). The habitat loss due to the decline of mangrove forests that occurred in the 20th century (Luther and Greenberg, 2009; Polidoro et al., 2010) can also play an important role in the sustainability of their populations. The reduction of these areas was caused by anthropogenic activities, such as deforestation, pollution and coastal development (Polidoro et al., 2010). In recent decades, the Thai mangrove area underwent a reduction of approximately 50% (Huitric et al., 2002). Since mangrove forests are known to be the elective habitat for *B. boddarti*, their reduction could have effect on fish populations, particularly on the genetic structure of the species. Another factor that plays an important role in shaping the genetic structure of *B. boddarti* is its life cycle, with adults that rarely migrate after settling on suitable habitats (Murdy, 1989). This feature suggests that this species' potential for dispersal depends almost exclusively on the planktonic larval stage (Chen et al., 2015). Therefore, the loss of habitat and oceanographic factors could have stronger effects on the

diversity and structure of the species, compared with other marine fishes. The knowledge of a species' genetic structure and the level of population genetic variability is fundamental for the management of natural resources and to plan effective and long-term measures (Avise, 1994). Studies on the genetic diversity and structure of mudskipper populations have focused mostly on *Boleophthalmus pectinirostris* (Kanemori et al., 2006; Liu et al., 2009; Chen et al., 2015) and *Periophthalmus* spp. (Mukai and Sugimoto, 2006; He et al., 2015; Nabilsyafiq et al., 2019; Tan et al., 2020). For *B. boddarti*, relatively little information is available on its genetic diversity and structure. Only one study on genetic variation and diversity between *B. boddarti*, and *B. dussumeiri* from the Vellar estuary mangrove, India has been reported that random amplified polymorphic DNA genetic markers were used and showed that the value (mean ± SD) for genetic diversity in *B. boddarti* (H = 0.0116 ± 0.0066) was higher than that in *B. dussumeiri* (H = 0.0056 ± 0.0024) (Ramanadevi et al., 2013).

The aim of the current study was to investigate the genetic diversity of *B. boddarti* populations in the Gulf of Thailand using sequences of a portion of the mitochondrial control region and to discuss the factors affecting the genetic diversity and structure of the species in the Gulf of Thailand in terms of habitat loss and larval dispersal capability.

Materials and Methods

Ethics statements

Animal care and all experimental procedures were approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC), Chulalongkorn University, Bangkok, Thailand (Approval no.1423011).

Samples and sampling sites

The distinct characteristic of the species is the iridescent blue spots on their brown body skin, dorsal fins, especially on the latero-ventral origin of pectoral fins and identification was based on the Murdy (1989) taxonomic revision. Tissue samples (fin clips) of 178 blue-spotted mudskippers were collected from six locations in the Gulf of Thailand. Geographical samples were grouped into two main areas, namely the inner Gulf of Thailand [Chachoengsao (CH, *n* = 39), Samut Songkhram (SS, *n* = 25), and Phetchaburi (PB, *n* = 22)] and the outer Gulf of Thailand [Rayong (RY, *n* = 32), Nakhon Si Thammarat (NK, *n* = 36), and Pattani (PT, *n* = 24)] (Fig. 1). Fish were

captured by local fishers using traps or hand nets. Fin clips of approximately 2 mg were taken from the pectoral fin. Prior to taking the fin clip, each fish was shocked into unconsciousness by immersion in seawater mixed with ice 1:1 (weight per volume, w/v). The fin clips were preserved in absolute ethanol and kept at -20°C.

Extraction of genomic DNA and polymerase chain reaction

The extraction of genomic DNA from the fin clips was carried out using a modified salting out procedure (Miller et al., 1988). Successively, individual DNA pellets were re-suspended in 25 μL of Tris-EDTA (ethylenediaminetetraacetic acid) buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0).

The 20 μL polymerase chain reaction (PCR) solution consisted of 10 μL KAPA *Taq* ready mix (KAPABIOSYSTEM, USA), 7.4 μL distilled water, 0.4 pmol of both forward and reverse primers (B.boddNCR-F [5'-CAC GAA CCC ATT CAA ACA AG-3'] and B.boddNCR-R [5'-AGT TTA CGA GTT TAG GGG GG-3'], designed by the authors), and 10–20 ng template

DNA. The PCR reactions were carried out using a PTC-200 thermocycler (Bio-Rad, USA) under the following conditions: 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 58°C for 45 s and 72°C for 45 s and finally at 72°C for 7 min. Each PCR product was run through 1.0% (w/v) agarose gel electrophoresis, stained with ethidium bromide and visualized under an ultraviolet transilluminator.

DNA sequencing

The PCR products were purified using polyethylene glycol (PEG) precipitation (Paithankar and Prasad, 1991) and sequenced using the dideoxyterminal method (Sanger et al., 1977) with a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA). Sequencing reactions were carried out under the following condition: 96°C for 2 min, followed by 30 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 2 min and were finally cooled at 20°C for 20 min.

Data analysis

Sequences were aligned using ClustalX v.1.83 (Thompson et al., 1997) and corrected visually. The correct taxonomic assignment of fin clips to *B. boddarti* was assessed by comparing the obtained sequences with those available on GenBank, using the BLAST tool (https://ncbi.nlm.nih.gov/). Haplotype frequencies, haplotype diversity (*h*) and nucleotide diversity (π) were calculated using DnaSP v.5.10 (Librado and Rozas, 2009). Differences between the total haplotype and nucleotide diversity indices calculated for samples of the inner and outer Gulf were assessed based on a t-test on arcsine square-root-transformed values (Archie, 1985). Two-tailed t-tests were used because there was no expected direction of differences in the haplotype or nucleotide diversity values. The median-joining network of haplotypes was generated using NETWORK v. 5.0.0.1 (Bandelt et al., 1999). The pairwise genetic differentiation between populations was calculated based on the fixation index (F_{ST}), using Arlequin v. 3.5.1.2 (Excoffier and Lischer, 2010). Based on these estimates, sampling locations were ordinated in a bi-dimensional space using nonmetric multidimensional scaling because it may show patterns of genetic relatedness not evidenced by traditional cluster analyses, when neither dichotomic structure nor phylogenetic inference are required (Guiller et al., 1998).

Analysis of molecular variance (Excoffier et al., 1992) was used to examine the partitioning of genetic variance into: 1) within local samples, 2) among local samples within
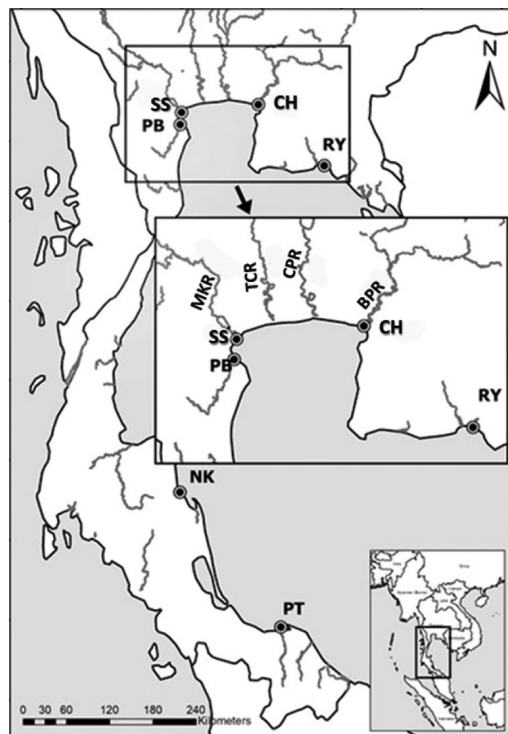

Fig. 1 Six sampling sites of *Boleophtalmus boddarti* in Gulf of Thailand, where inner Gulf sites are CH = Chachoengsao, SS = Samut Songkhram and PB = Phetchaburi, outer Gulf sites are RY = Rayong, NK = Nakhon Si Thammarat and PT = Pattani and four main rivers in the inner Gulf are BPR = Bang-Pakong River, CPR = Chao-Phraya River, TCR = Tha-Chin River and MKR = Mae-Klong River

groups and 3) between groups. It was hypothesized that the populations consisted of two groups according to the geography of the Gulf, namely the inner Gulf (CH, SS and PB) and the outer Gulf of Thailand (RY, NK and PT). The significance of Φ-statistics values was assessed based on permutation tests with 10,000 replicates, as implemented in Arlequin (Excoffier and Lischer, 2010).

BAPS v. 5.2 (Corander et al., 2003; Corander and Marttinen, 2006) was used to detect hidden population substructuring by clustering genetically-similar sampled individuals into groups, hereafter called 'haplogroups'. BAPS adopts a Bayesian approach with a stochastic optimization algorithm for analyzing models of population structure, which greatly improves the speed of the analysis compared to traditional MCMC-based algorithms (Corander and Marttinen, 2006). When testing for population clusters, five replicates were run for each value of k (the maximum number of haplogroups) up to k = 9. In addition, the number of reference individuals nri = 500 was used and the admixture analysis was repeated 500 times per individual.

Population history was inferred based on mismatch distribution analysis (Rogers and Harpending, 1992) of the total data set, as implemented in Arlequin. The data fit with the expected models of demographic (Rogers and Harpending, 1992) and spatial expansions (Excoffier, 2004) was evaluated based on a bootstrap approach (Schneider and Excoffier, 1999), using the sum of squared deviations between the observed and the expected mismatch distributions. In addition, the raggedness index (r; Harpending, 1994) and F_S statistics of Fu (1997) were computed to assess population expansion and tested with coalescent simulations with 10,000 replicates, as implemented in DnaSp.

Results

In total, 178 sequences of the mitochondrial control region, aligned and trimmed to 320 bp, corresponded to 55 haplotypes (GenBank accession numbers KY560833–KY561011). The most common haplotype (H1) was shared by all local samples, whereas 45 haplotypes (81.8%) were location-private. The insertion of an adenine at the 16078th base position, based on the mitochondrial genome sequence of *B. boddarti* (Accession no. KF87427), was observed in 14 haplotypes, of which 12 (H25, H29, H30, H32, H34, H37, H39, H40, H42, H44, H49, H50) were unique. The insertion of an adenine was found in individuals from RY, NK and PT, which were located in the outer Gulf of Thailand (Table 1).

Table 1 Haplotypes of partial mtDNA control region detected in *Boleophtalmus boddarti* from six locations within Gulf of Thailand, where sshaded cells show haplotypes with additional adenine at 16078th position of sequence

N = haplotype total number, n = sample size; CH = Chachoengsao; SS = Samut Songkhram; PB = Phetchaburi; RY = Rayong; NK = Nakhon Si Thammarat; PT = Pattani

Values (mean ± SD) for the haplotype diversity and nucleotide diversity ranged from $h = 0.540 \pm 0.117$ (for SS) to 0.830 ± 0.068 (for PT) and $\pi = 0.0029 \pm 0.0023$ (for SS) to 0.0094 ± 0.0056 (for RY), respectively. The total haplotype diversity and nucleotide diversity were $h = 0.751 \pm 0.036$ and $= 0.0069 \pm 0.0001$, respectively (Table 2). The total values of haplotype diversity did not significantly differ between the inner and outer Gulf of Thailand based on a t-test ($p = 0.538$), whereas those of nucleotide diversity did ($p = 0.011$). This suggested that the genetic diversity within each of the two areas was different at the nucleotide level. The occurrence of the insertion of an adenine at the 16078th position of the sequences in individuals of group II may have accounted for the difference between the two values of nucleotide diversity.

The analysis of molecular variance showed that there was no significant differentiation between samples from the inner (CH, SS, PB) and outer Gulf of Thailand (RY, NK, PT), ($\Phi_{CT} = 0.046$, $p > 0.05$). On the other hand, there were significant molecular variances for the 'within local samples' and 'among local samples within group' components (Table 3).

Nonmetric multidimensional scaling of pairwise F_{ST} values separated the inner and outer sampling locations of *B. boddarti* along the vertical axis (Fig. 2). The very low value of stress index (< 0.001) indicated that relative distances among samples on the plot accurately represented the levels of their genetic divergence.
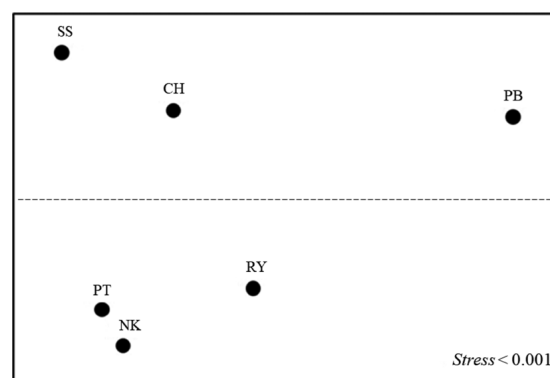

Fig. 2 Nonmetric multidimensional scaling plot of *Boleophtalmus boddarti* samples based on pairwise F_{ST} values, where dashed line indicates separation of inner Gulf samples (CH = Chachoengsao, SS = Samut Songkhram and PB = Phetchaburi) from outer Gulf samples (RY = Rayong, NK = Nakhon Si Thammarat and PT = Pattani) along vertical axis

Table 2 Estimates of genetic diversity in six *Boleophtalmus boddarti* locations in Gulf of Thailand

Sampling site	Code	n	Nh	h (± SD)	π (± SD)
Inner Gulf of Thailand					
Chachoengsao	CH	39	17	0.781±0.005	0.0045±0.0008
Samut Songkram	SS	25	7	0.540±0.117	0.0029±0.0023
Phetchaburi	PB	22	8	0.788±0.068	0.0049±0.0034
Total		86	27	0.732±0.052	0.0045±0.0006
Outer Gulf of Thailand					
Rayong	RY	32	16	0.811±0.071	0.0094±0.0056
Nakhon Si Thammarat	NK	36	12	0.656±0.088	0.0073±0.0046
Pattani	PT	24	10	0.830±0.068	0.0089±0.0054
Total		92	32	0.764±0.047	0.0086±0.0009
All locations		178	55	0.751±0.036	0.0069±0.0001

n = sample size; Nh = number of haplotypes; h = haplotype diversity; π = nucleotide diversity

Table 3 Analysis of molecular variance based on control region haplotypes among two groups of *Boleophtalmus boddarti* collected from inner Gulf and outer Gulf of Thailand

Source of variation	df	Variance component	Percentage of variation	Φ-statistic	p-value
Between groups	1	0.05400	4.64	$\Phi_{CT} = 0.046$	0.087
Among local samples within groups	4	0.04460	3.80	$\Phi_{SC} = 0.040$	0.004
Within local samples	172	1.06547	91.56	$\Phi_{ST} = 0.084$	< 0.001

df = degrees of freedom

The median-joining network of haplotypes could be split into two groups, based on the absence (group I) or insertion (group II) of an adenine at the 16078th position. In addition, group I had a clear star-phylogeny, with the most common haplotype (H1) connected by one or two mutations to other low-frequency or unique haplotypes (Fig. 3). In group II, there were 14 haplotypes, of which two were shared (H24 and H25) and 12 were unique haplotypes (H26, H30, H31, H33, H35, H38, H40, H41, H43, H45, H50, H51), as shown in Table 1. All haplotypes found in the group had the adenine insertion and were present only in the samples located in the outer Gulf of Thailand (Fig 3).

The Bayesian assignment test revealed the presence of two haplogroups (HGs) with maximum value of associated posterior probability ($PP = 1$). No instances of uncertain assignment were detected. HGs had different abundances in the six locations considered (Fig 4). HG1 was the most frequent, being represented in all regions, whereas HG2 was present in the three locations from the outer Gulf of Thailand (RY, NK, PT) and in a small portion for the PB location (Fig. 4).

The mismatch distribution deviated significantly from the model of Rogers and Harpending (1992) of recent demographic expansion; on the other hand, it fitted the curve relative to the model of spatial expansion (Fig. 5). Furthermore, the highly significant value of the Fu (1997) index was consistent with population expansion ($F_S = -71.683$, $p < 0.001$).
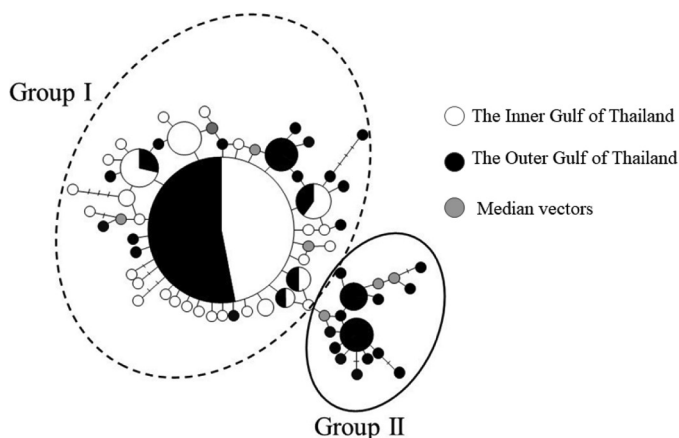

Fig. 4 Results of Bayesian assignment analysis for sequences of control region in 178 individuals of *Boleophtalmus boddarti*, where pie charts show pooled individuals in respective six locations (CH = Chachoengsao, SS = Samut Songkhram and PB = Phetchaburi, RY = Rayong, NK = Nakhon Si Thammarat and PT = Pattani), ggray shows proportion of haplogroup I and black shows proportion of haplogroup II

Fig. 3 Median joining network of partial control region haplotypes for *Boleophtalmus boddarti* in Gulf of Thailand, where haplotypes are represented as circles, area of each circle is proportional to the number of individuals exhibiting that haplotype, each line represents a mutational step, whereas transversal bars represent additional mutational steps, gray circles represent median vectors and the two groups are defined on the basis of the insertion of an adenine at 16078th position of sequences of group II
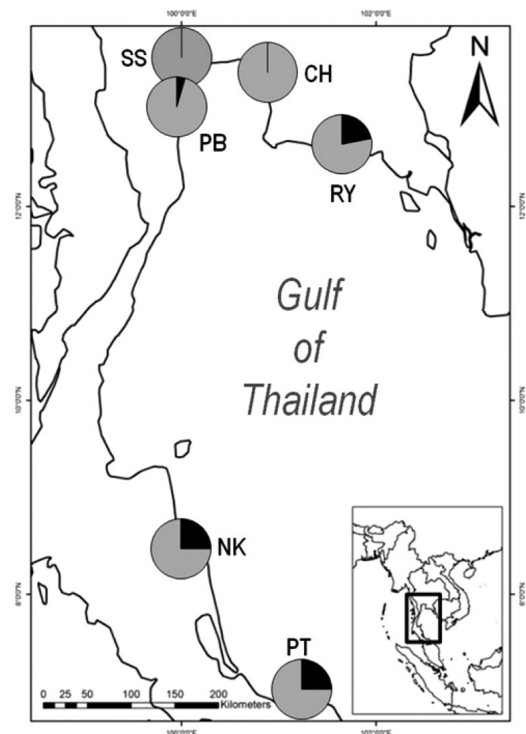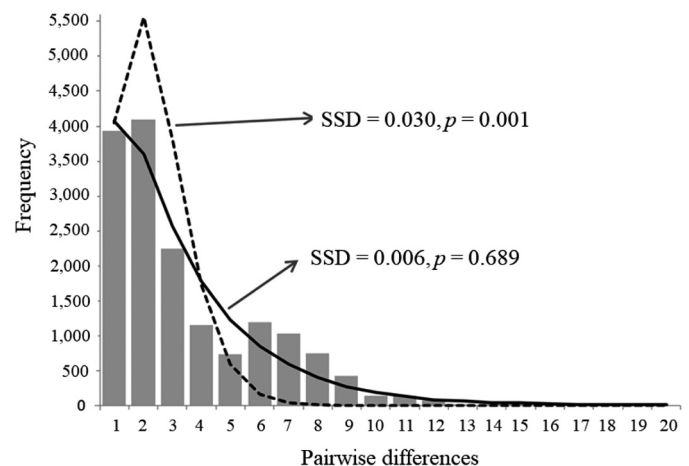

Fig. 5 Frequency distributions of number of pairwise nucleotide differences (mismatch) between CR haplotypes for total dataset for *Boleophtalmus boddarti*, where solid and dotted lines are theoretical distributions under assumptions of spatial and temporal demographic expansions, respectively, and SSD is sum of squared deviations

Discussion

The analysis of the 178 sequences of the mitochondrial control region in *B. boddarti* provided a contribution to the understanding of the species' genetic structure in the Gulf of Thailand. The high number of unique haplotypes within each sampling location suggested the occurrence of a degree of genetic diversity within that area, consistent with the results reported for the closely related species *B. pectinirostris*, through the analysis of cytochrome *b* (Kanemori et al., 2006)and the control region (Chen et al., 2015). In addition, the current results indicated that *B. boddarti* in the Gulf of Thailand was characterized by moderate-to-high levels of genetic variability, as shown by the haplotype diversity values. The total value of haplotype diversity ($h = 0.751 \pm 0.036$) was lower than that found in the congeneric *B. pectinirostris*, which had a mean ± SD value of $h = 0.965 \pm 0.006$ (Chen et al., 2015). However, it was slightly higher than that reported for *Periophthalmus novemradiatus* of $h = 0.613$ (Tan et al., 2020). The total values of nucleotide diversity in *B. boddarti* and *B. pectinirostris* were similar, being $\pi = 0.0069 \pm 0.0001$ and $\pi = 0.0064 \pm 0.0002$, respectively (Chen et al., 2015). These values were higher than that for *P. novemradiatus* of $\pi = 0.0020$ (Tan et al., 2020)

In the current investigation, the genetic diversity values of the *B. boddarti* samples from the inner Gulf of Thailand, namely Samut Songkhram (SS), Phetchaburi (PB) and Chachoengsao (CH), were slightly lower than those of the samples from the outer Gulf (Rayong (RY), Nakhon Si Thammarat (NK), Pattani (PT)). The lowest mean ± SD genetic diversity was in the SS population ($h = 0.540 \pm 0.117$ and $\pi = 0.0029 \pm 0.0023$) (Table 2). A decline in the population size due to the anthropogenic disturbance occurring in this area may have accounted for the low genetic diversity (Bank et al., 2013). However, other local factors such as pollution, habitat loss and restricted gene flow may have contributed to that result (Kanemori et al., 2006; Takegaki, 2008; Chen et al., 2015). Other investigations on marine animals have reported that some of the effects of water pollution are reductions in the genetic diversity and the effective population size, such as in the mussel *Mytilus galloprovincialis* (Ma et al., 2000), the barnacle *Balanus glandula* (Ma et al., 2000), the sandhopper, *Talitrus saltator* (Ungherese et al., 2010) and the crab *Pachygrapsus marmoratus* (Fratini et al., 2008). Anthropogenic impacts on the coastal environments within the inner Gulf of Thailand have been increasing over the past two decades, due to the growing development of industrialization and other economic activities (Wattayakorn, 2006). The high degree of anthropogenic

disturbance can heavily affect coastal environments and reduce intertidal habitats for marine species, especially mangrove areas (Cheevaporn and Menasveta, 2003).

The expectation of effective gene flow due to larval dispersal in *B. boddarti* was corroborated by the hierarchical analysis of genetic structure, which showed the absence of deep structuring in the Gulf of Thailand, despite the patchy distribution of mangrove habitats in the study area. The lack of genetic structuring was reported for other marine organisms in the Gulf of Thailand, such as ornate threadfin bream (Supmee et al., 2021), cobia (Phinchongsakuldit et al., 2013) and green mussel (Prakoon et al., 2010). These studies suggested that dispersal ability, water current and the lack of strong physical barrier in the area may have accounted for their findings. Adult mudskippers hardly migrate after settling on suitable habitats (Murdy, 1989; Hong et al., 2007); hence, the planktonic larvae play a major role in producing gene flow (Chen et al., 2015). However, the presence of group II in the haplotype network (Fig. 3) and the differences in proportions of individuals for the two haplogroups based on the Bayesian assignment analysis (Fig. 4) suggested the occurrence of genetic divergence between the inner and outer Gulf. The dispersal ability of fish larvae is not only dependent on the duration of their planktonic stage, because other factors such as the habitat type and water currents may have important effects (Shanks, 2009). For example, in red lionfish (*Pterois volitans*) and vagabond butterflyfish (*Chaetodon vagabundus*), despite their similar lengths of the planktonic larval stage (25–40 d and 29–48 d, respectively) their dispersal distances are different, being 48.5 km in the former and 100–500 m in the latter (Shanks, 2009). Although the length of the planktonic larval stage of *B. boddarti* is still unknown, if it is assumed to be similar to that of the congeneric *B. pectinirostris* (30–42 d, according to Zhang et al., 1989 and Chen et al., 2015), the dispersal distance of *B. boddarti* larvae should vary from 100 m to 48.5 km. Since no deep genetic structure of *B. boddarti* population was found in the current study, it could be argued that its larvae could have the ability to disperse throughout the area of the Gulf with the largest geographical distance between populations approximately 600 km. The ability of the mudskipper larvae to disperse through long distances was also suggested in *P. novemradiatus* (> 1,600 km) by Tan et al. (2020). Nevertheless, oceanographic characteristics can influence the dispersal ability of *B. boddarti*, as observed for many marine fish species (Giovannotti et al., 2009; McManus and Woodson, 2012). From this perspective, it is noteworthy that the surface waters of the inner Gulf of Thailand are strongly influenced by the

outflow of Bang-Pakong, Chao-Phraya, Tha-Chin and Mae-Klong Rivers (Fig. 1). In the Gulf, these four major rivers create complex gyre patterns (Sojisuporn et al., 2010). In particular, a year-round stable gyre is present within the inner Gulf. This gyre, which moves counterclockwise from April to September and clockwise from October to March, could determine a high degree of larval retention within the inner part of the Gulf; therefore reducing genetic connectivity between this area and the outer parts of the Gulf.

Haplotypes, having the adenine insertion at the 16078th position, were found in individuals from RY, NK and PT that are the three more external locations (Fig. 1 and Table 1). According to Zhang et al. (2016) who reported on the complete sequence of the mtDNA genome of *B. boddarti* from Malaysia, the sequence of the mitochondrial control region consists of an additional adenine at the same position (Accession No. KF874277). This observation suggested that most of the individuals containing the sequence with the additional adenine are located in the southern part of the Gulf and down to the Malay Peninsula. However, these haplotypes were also found on the samples from the easternmost sample (RY) in the current study. Therefore, the possibility of gene flow must be considered between RY and the southern populations of NK and PT. There are two possible ways for recruitment from the southern to the eastern populations. First, the currents in the Gulf of Thailand move from south to east in May to September (Sojisuporn et al., 2010), which includes the spawning season of mudskippers from August to October (Quang et al., 2016). Second, the planktonic larvae of the fish may be transported in ship ballast water, for vessels moving from the Songkla port (in the southern part of the Gulf) to the Map Ta Put and Laem Chabang ports (in the eastern part of the Gulf). Larval dispersal mediated by ballast water was observed in round goby *Neogobius melanostomus* (Gollasch et al., 2000; Hensler and Jude, 2007). The lack of haplotypes with the additional adenine base in the inner Gulf of Thailand suggested there were low levels of gene flow between individuals from the inner Gulf of Thailand and those outside the area. Surface water circulation in the inner Gulf could represent an oceanographical barrier to gene flow between the areas.

The pattern of high haplotype diversity and low nucleotide diversity observed in *B. boddarti* in the Gulf of Thailand suggested that the large number of closely-related haplotypes, which were separated by only a few mutations, might have accumulated by the rapid expansion of populations after the effect of population bottlenecking (Grant and Bowen, 1998) and low effective population size (Stepien, 1999).

The F_S statistics of Fu (1997) and the mismatch distribution suggested that recent spatial expansion had occurred. Habitat availability as a consequence of sea-level rise after the last glaciations may account for this result (Janko et al., 2007; Voris, 2000; Ho et al., 2015).

In conclusion, the current results did not show deep genetic structuring of the species in the study area. However, they revealed lower genetic diversity in the inner Gulf populations and highlighted the occurrence of genetic divergence between the inner and outer Gulf populations via the presence of unique haplotypes clustering as group II for the outer Gulf populations (Fig. 3) and the differences in proportions of individuals of the two haplogroups in the populations based on the Bayesian assignment analysis (Fig. 4). These results could provide baseline information for future study on the maternal genetic variation and structure of blue-spotted mudskipper, *Boleophtalmus boddarti* populations in the Gulf of Thailand. From a conservation perspective, the absence of pronounced genetic structuring of the blue-spotted mudskipper shown in the current study suggested that *B. boddarti* in the Gulf of Thailand can be managed as a single population. However, if the genetic structuring is not detected, further investigation is needed using other genetic markers, such as other genes in the Mt DNA or microsatellites, to consolidate the results or to improve understanding on the genetic structure of the species. Since the adults of *B. boddarti* rarely migrate, the preservation of its habitat must be of concern in terms of both habitat loss and pollution, especially in the inner Gulf of Thailand, where lower genetic diversity was identified and anthropogenic disturbance continues to increase rapidly.

Conflicts of Interest

The authors declare that there are no conflicts of interests.

Acknowledgements

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