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Research article

Mitochondrial variation of wild populations of two brackish ricefish (Teleostei: Beloniformes) in Thailand

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Abstract

Importance of the work: Both the Javanese ricefish (*Oryzias javanicus*) that is distributed in brackish water and the Indian ricefish (*O. dancena*) that inhabits brackish or freshwater are utilized as aquatic animal models in biological experiments. However, the population genetics of these two species in Thailand are unknown.
Objectives: To report on the genetic diversity of Javanese and Indian ricefish in Thailand.
Materials & Methods: The genetic diversity of Javanese and Indian ricefish were analyzed based on cytochrome c oxidase subunit 1 (*COI*, 750 bp in length).
Results: The Javanese ricefish populations were separated into two groups. Group 1 was composed of Javanese ricefish collected mainly from the Andaman Sea. Group 2 consisted of the fish populations in the Gulf of Thailand. Evidence of cryptic species was found in the Javanese ricefish group 2 (considered as *O.* cf. *javanicus*). The Indian ricefish were also distinguished into groups 1 and 2, where group 1 consisted mainly of Indian ricefish living in the Andaman Sea, while those inhabiting the Gulf of Thailand were phylogenetically placed in group 2. The demographics indicated that the population size was stable for both species, with genetic diversity also discussed.
Main finding: The genetic diversity among populations of brackish ricefish in Thailand may be is associated with the biogeographical regions of the Gulf of Thailand and the Andaman Sea.

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Introduction

Among nonmammalian vertebrates, teleost fish exhibit a great range of biodiversity in various aquatic environments (Volff, 2005; Ravi and Venkatesh, 2008; Ikenaga, 2013). Many studies have reported genetic diversity in fish which has improved the understanding of the relationships between their genetic variation and intraspecific geographical distributions, such as blue badis (*Badis badis*) (Mukhopadhyay and Bhattacharjee, 2014), Asian blue panchax (*Aplocheilus panchax*) (Beck et al., 2017), the long-whiskered catfish (*Steindachneridion scriptum*) (Paixão et al., 2018) and Nile tilapia (*Oreochromis niloticus*) (Tibihika et al., 2020). Fish of the genus *Oryzias* belonging to the family Adrianichthyidae in the order Beloniformes are distributed in East Asia, Southeast Asia and South Asia (Parenti, 2008). Recently, more than 30 species in this genus have been recognized (Parenti et al., 2013; Hilgers and Schwarzer, 2019). In Thailand, there are five species of ricefish: *O. minutillus*, *O. mekongensis*, *O. songkhramensis*, *O. javanicus* and *O. dancena* (Parenti, 2008; Termvidchakorna and Magtoon, 2008; Magtoon, 2010). Of these, *O. javanicus* and *O. dancena* mainly inhabit waters in mangrove areas or along the coasts of the Gulf of Thailand and the Andaman Sea (Termvidchakorna and Magtoon, 2008). *O. javanicus* (commonly known as Java medaka or Javanese ricefish) is found in brackish or marine water, while *O. dancena* (known as Indian medaka or Indian ricefish) lives in brackish or freshwater (Inoue and Takei, 2003; Yusof et al., 2011). Kunlapapuk et al. (2015) recorded Javanese ricefish from the Northwest Gulf of Thailand. Yusof et al. (2013) reported that Javanese ricefish were found only along the west coast of Peninsular Malaysia. For decades, both fishes have been utilized as animal models in various fields such as physiology, toxicology and developmental biology (Inoue and Takei, 2003; Imai et al., 2005; Kim et al., 2015; Maeng et al., 2019; Takehana et al., 2020). Takehana et al. (2003) assessed the molecular variation of Japanese ricefish throughout Japan and reported relationships between biogeography and ricefish populations. In Thailand, mitochondrial gene sequences have been reported for populations of Thai medaka or Thai ricefish *O. minutillus* (Ngamniyom et al., 2020). Population genetic studies of fish in genus *Oryzias* (Javanese and Indian ricefish) are crucial for understanding the association between teleosts and natural biogeography. However, little is known about the genetic variations and intraspecific population differentiation of both ricefish species in Thailand (Termvidchakorna and Magtoon, 2008; Magtoon, 2011).

Cytochrome c oxidase subunit I (*COI*) is a mitochondrial coding gene that is ~~frequently~~ used for DNA barcode identification or analyses of phylogenetics and population genetics in various animals, including teleost fish (Hebert et al., 2003; Razo-Mendivil et al., 2010; Shen et al., 2016; Xu et al., 2019). Jamaluddin et al. (2011) assessed the influence of geomorphological factors in populations of snakehead murrel (*Channa striata*) in Peninsular Malaysia using partial *COI* sequences. Furthermore, Delrieu-Trottin et al. (2018) revealed evidence of cryptic species in blackblotch blenny (*Cirripectes alboapicalis*) associated with zoogeography in the South Pacific Ocean inferred from COI sequences.

Therefore, the objective of this study was to investigate intraspecific population differentiation of Indian ricefish and Javanese ricefish in Thailand based on partial nucleotide sequences of mitochondrial *COI*.

Materials and Methods

Fish were captured using a scoop net with a handle in mangrove swamps, canals, drainage areas and estuaries along the coastal wetlands of the Gulf of Thailand and Andaman Sea (Fig. 1). There were 64 individual samples of adult fish

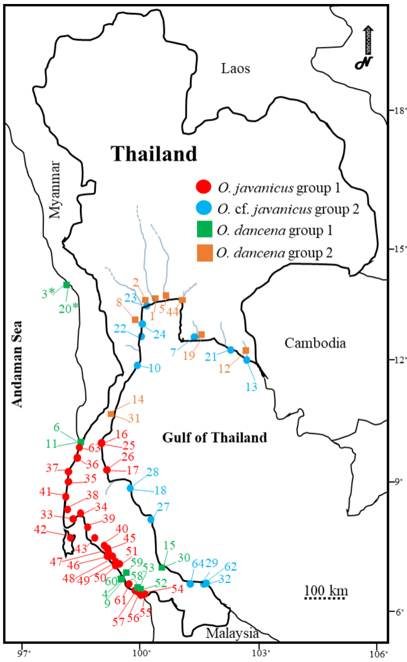

Fig. 1 Map of Thailand illustrating fish collection sites (64 samples from 64 localities) located in the Gulf of Thailand and Andaman Sea, where numbers (1–64) indicate locations of sampling, Javanese ricefish (*Oryzias javanicus*) are indicated by solid red dots and solid light blue dots and Indian ricefish (*O. dancena*) are indicated by solid green squares and solid brown squares, while green asterisks indicate specimens from Myanmar.

with a standard length greater than 28 mm and all were used for genetic analysis. It should be noted that all field sampling of fish was conducted in public areas that did not require any special permit. Fish were anaesthetized in a tricaine methanesulfonate solution (approximately 200 mg/L). They were identified as *O. javanicus* or *O. dancena* according to the taxonomic key of *Oryzias* species described by Roberts (1998), Parenti (2008) and Magtoon (2011). The caudal fin tissues were dissected from each fish using a lancet and transferred to absolute ethanol for the storage of materials at -20 °C prior to the experimental procedure. In addition, two specimens of *O. dancena* were purchased from local fisherman in Dawei, Myanmar and preserved in absolute ethanol.

Genomic DNA of *O. javanicus* and *O. dancena* was extracted from caudal fin tissue using an DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. The nucleic sequences of the forward and reverse primers used for *COI* amplification (approximately 950 bp) were 5′–CCYCAGGGCTGRTAAGAAGAGGA–3′ and 5′–CCGACAGTAAATATRTGAGGGCTC–3′. These COI primers were designed according to information from the GenBank database (Clark et al., 2016) for *O. javanicus* or *O. dancena* from the reports of Setiamarga et al. (2009). The thermal cycling conditions were: initial denaturation at 95 °C for 5 min; 34 cycles of denaturation for 30 sec at 94 °C, annealing for 35 sec at 55 °C, and extension for 1.5 min at 72 °C, with a final extension of 10 min at 72 °C. The PCR products were run on 1% (weight by volume) agarose gel stained with SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific, USA) and visualized under blue-light transillumination. The products were extracted from the gels using a QIAquick Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's instructions. Nucleotide sequencing was conducted using an automated ABI 3730xl DNA analyzer (Applied Biosystems, USA). The *COI* DNA sequences were deposited in GenBank database (accession numbers: MW246182-MW246245). The sequences of all fish samples were multiple-aligned and trimmed using the BioEdit ver. 7.2 (Tippmann, 2004). The number of *COI*s used for phylogenetic reconstruction was 750 bp in length.

Phylogenetic analysis was performed following maximum likelihood methods using the molecular evolutionary genetics analysis (MEGA) software version 6 (Tamura and Nei, 1993) with 1,000 bootstrapping replicates and a Tamura-Nei model with a gamma distribution with invariant sites (G+I). Bootstrapping support was > 80% for justification. *COI* sequences of *O. javanicus* (AB498067.1) and *O. dancena* (NC_012976.1) from Setiamarga et al. (2009) were added to the analysis. In addition, *COI* sequences of *O. haugiangensis* (MK777124, originating from Ninh Thuan, Vietnam; Thu et al., 2019) to distinguish *O. javanicus* and *O. haugiangensis* which are morphologically resemble (Roberts, 1998; Magtoon, 2011). Nucleotide sequences of *COI* of *Strongylura anastomella* (KP864662.1) and *Zenarchopterus buffonis* (MK600518.1) that were related to species in Beloniformes were included as outgroups. A minimum spanning network was also generated to support the phylogenetic trees (10,000 permutations) using the PopART version 1.7 software (Bandelt et al., 1999; Leigh and Bryant, 2015). Haplotype and nucleotide diversity (Nei, 1987; Grant and Bowen, 1998) were quantified. Neutrality tests based on Tajima's D and Fu's Fs were calculated (10,000 permutations) to verify the null hypothesis of neutrality with a stable population size (Tajima, 1989; Fu, 1997). The mismatch distribution (Rogers and Harpending, 1992) were demographically evaluated to examine an expansion model for Javanese and Indian ricefish based on the R_2 test (Ramos-Onsins and Rozas, 2002) and raggedness (r) values (Harpending et al., 1993) and for a goodness-of-fit test. Population genetic parameters described above were calculated using DnaSP version 5.10.01 (Librado and Rozas, 2009). In addition, the nitrogenous bases in the *COI* sequences were counted using Science Buddies (www.sciencebuddies.org) (Hess et al., 2011). Differences in nucleobase contents between groups of Java or Indian ricefish were analyzed using an independent t test (IBM SPSS Statistics 16; International Business Machines Corporation; Armonk, NY, USA). Results were considered significant if $p < 0.05$.

Ethics statements

Animal care and all experimental procedures were approved by the Animal Experiment Committee Srinakharinwirot University (Approval no. SWU-A-004-2563).

Results

The 64 fish specimens consisted of 42 Javanese ricefish and 22 Indian ricefish (numbers of samples 1 to 64 represented individual fish, as shown in the map in Fig. 1). Of the Javanese ricefish sample, 18 fish were collected from the Gulf of Thailand, and 24 were captured from the Andaman Sea. Of the Indian ricefish, 11 were sampled in the Gulf of Thailand, 9 and 2 were collected in the Andaman Sea of Thai and

Myanmar regions (Fig. 1). The phylogenetic trees based on the partial *COI* sequences showed that Javanese ricefish were clearly discriminated from Indian ricefish. The phylogram of the fish populations was determined using the maximum likelihood method (Fig. 2). The populations of Javanese ricefish were distinctly divided into two groups: *O. javanicus* groups 1 and 2 (> 84% bootstrapping values).

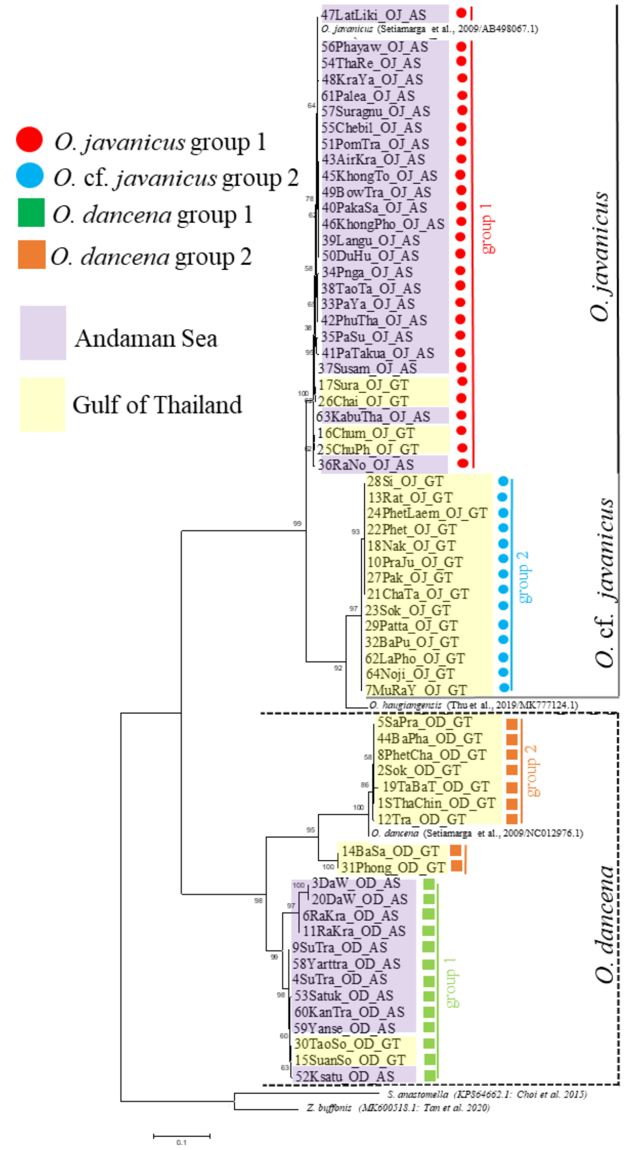

Fig. 2 Maximum likelihood-based phylogenetic tree (1,000 bootstrapping replicates with bootstrapping support > 80%) of Javanese and Indian ricefish populations in Gulf of Thailand and Andaman Sea based on mitochondrial *COI* gene sequences, where the solid red dots are *O. javanicus* goup 1 and the solid blue dots are *O. javanicus* goup 2. Numbers (1–64) indicate sample locations of fish corresponding to Fig. 1. Solid green squares are *O. dancena* group 1 and solid brown squares are *O. dancena* group 2, and *Strongylura anastomella* and *Zenarchopterus buffonis* were used as outgroups.

Javanese ricefish, group 1 was monophyletic and was closely related to group 2. Group 1 comprised fish from samples 16, 17, 25, 26, 33–43, 45–51, 54–57, 61 and 63. Almost all the Javanese ricefish came from the Andaman Sea of Thailand, with the exception of fish from samples 16, 17, 25 and 26, which were collected from the Gulf of Thailand.

Group 2 included Javanese ricefish from samples 7, 10, 13, 18, 21–24, 27–29, 32, 62 and 64. These fish specimens were captured from the Gulf of Thailand. The fish in group 2 all presented with morphological confirmation as *O. javanicus* but their genotypic characteristics were closely related to *O. haugiangensis*. Therefore, group 2 was considered as "*O.* cf. *javanicus* group 2" for cryptic Javanese ricefish.

Like Javanese ricefish, the Indian ricefish could also be separated into two monophyletic populations: *O. dancena* groups 1 and 2 (> 90% bootstrapping values) Group 1 of Indian ricefish was composed of specimens from 3, 4, 6, 9, 11, 15, 20, 30, 52, 53 and 58–60. All these fish were collected from Thai waters of the Andaman Sea while the samples from localities 3 and 20 were from Myanmar waters. In contrast, the Indian ricefish individuals from localities 15 and 30 were collected from the Gulf of Thailand.

Furthermore, the Indian fish from samples 1, 2, 5, 8, 12, 14, 19, 31 and 44 belonged to *O. dancena* group 2, which inhabited the Gulf of Thailand (Figs. 2 and 3). Additional *COI* sequences of Javanese ricefish and Indian ricefish previously reported by Setiamarga et al. (2009) were included in *O. javanicus* group 1 and *O. dancena* group 2, respectively. The results of the current study showed that *O. haugiangensis* (Thu et al., 2019), was more closely related to the Javanese ricefish than the Indian ricefish in this study. The pooled samples of Javanese ricefish were paraphyletic to a single taxon of *O. haugiangensis*. Javanese and Indian ricefish were clearly different from the outgroups *S. anastomella* and *Z. buffonis* phylogenetically (Fig. 2).

Likewise, the minimum spanning network revealed four groups of *O. javanicus* and *O. dancena*, with each species separated into groups 1 and 2. *O. javanicus* group 1 was located in the Andaman Sea, whereas group 2 mainly inhabited the Gulf of Thailand. In Indian ricefish, *O. dancena* group 1 consisted mainly of fish from the Andaman Sea, whereas group 2 consisted of samples from the Gulf of Thailand. The network represented 29 distinct *COI* haplotypes that consisted of 20 haplotypes of the Andaman Sea and 9 haplotypes of the Gulf of Thailand (Fig. 3) Notably, the numbers used to identify the fish samples in Fig. 3 were consistent with those used in Figs. 1 and 2.

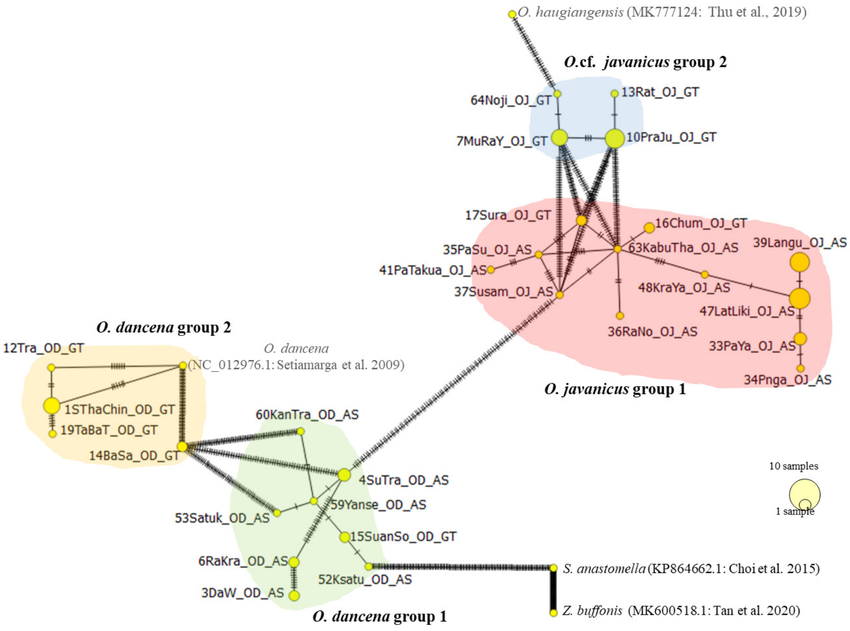

Fig. 3 Minimum spanning network illustrating groups of the Javanese and Indian ricefish populations collected from Gulf of Thailand and Andaman Sea, where the network was based on mitochondrial *COI* gene sequences with the *COI* sequences of *Oryzias dancena* (NC_012976.1, Setiamarga et al., 2009) and *O. haugiangensis* (MK777124, Thu et al., 2019) were added to this network, *Strongylura anastomella* and *Zenarchopterus buffonis* were used as the outgroups and circles with proportional sizes indicate each distinct haplotype.

Nucleotide polymorphism within the *COI* region was considered, excluding additional region sequences from the nucleotide database; the haplotype diversity of *O. javanicus* group 1 was 0.876 ± 0.04 (mean ± SD) from 12 haplotypes. The nucleotide diversity of group 1 was 0.0063 ± 0.001. For group 2, haplotype diversity was 0.659 ± 0.09, with four haplotypes, and the nucleotide diversity being 0.0031 ± 0.0004. In Indian ricefish, the haplotype diversity of *O. dancena* group 1 was 0.923 ± 0.05 from 8 haplotypes. The nucleotide diversity of group 1 was 0.021 ± 0.005. For group 2, the haplotype diversity was 0.694 ± 0.147 with five haplotypes and the nucleotide diversity was 0.039 ± 0.015.

According to neutrality tests, only *O. javanicus* group 1 showed negative values of Tajima's D test and Fu's Fs test, which were -0.618 and -2.341, respectively. These tests were not significant. In contrast, the Tajima's D and Fu's Fs values of *O.* cf. *javanicus* group 2 were 0.652 and 1.019, respectively. Positive values of both tests were also found for *O. dancena* groups 1 and 2. In *O. dancena* group 1, the values were 0.972 for Tajima's D test and 1.832 for Fu's Fs. In group 2, the corresponding values were 0.154 and 8.598. A significant *p* value was not obtained for any the neutrality test.

In the mismatch distribution, all groups of ricefish presented a multimodal distribution according to r values (Fig. 4). The *O. dancena* groups tended to show a bimodal distribution (Fig. 4). The r and R_2 statistic values for *O. javanicus* group 1 were 0.0376 and 0.0972. In group 2 of Javanese ricefish, the values were 0.2875 for r and 0.1730 for R_2. The r and R_2 values of the *O. dancena* group 1 were 0.0914 and 0.1922. In group 2 of Indian ricefish, the r value was 0.4005, and R_2 was 0.1817.

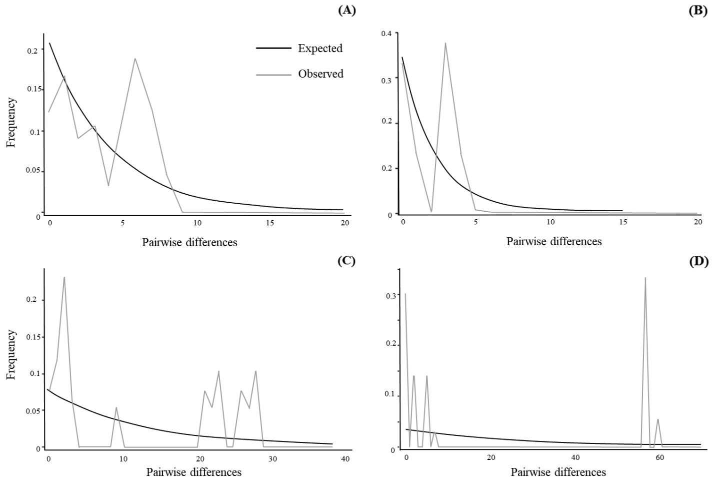

Fig. 4 Mismatch distributions of ricefish populations in Gulf of Thailand and Andaman Sea: (A) *Oryzias javanicus* group 1; (B) *O.* cf. *javanicus* group 2; (C) *O. dancena* group 1; (D) *O. dancena* group 2

In nucleobase contents, the nitrogenous base contents of Javanese ricefish were obviously separated from those of Indian ricefish. The numbers of adenine (A) and thymine (T) bases in Javanese ricefish were lower than those in Indian ricefish, but the guanine (G) and cytosine (C) contents were in the opposite direction. In Javanese ricefish, the contents of T and C were significantly different between *O. javanicus* groups 1 and 2 ($p < 0.01$). In contrast, the contents of A and G in *O. javanicus* group 1 were not different from those in group 2 ($p > 0.05$). In Indian medaka, the contents of A, G and C in group 1 overlapped with those in group 2 (Fig. 5A). Regarding the average number of nucleobases, the number of T bases was lower in *O. javanicus* group 1 than in group 2, whereas the number of C bases was higher in group 1 than in group 2. The average A and G contents seemed to be similar between both groups In Indian ricefish, the average T content was higher in group 1 than in group 2 (Fig. 5B). For the percentage of G/C, the *O. javanicus* group 1 differed from that in group 2. However, their percentages overlapped for groups 1 and 2 of Indian ricefish (Fig. 5C). In the average G and C contents, the value in *O. javanicus* group 1 was highly significantly ($p < 0.01$) higher than that in group 2. In Indian ricefish, the average G and C contents were higher in group 2 than in group 1 (Fig. 5D).

Discussion

The genetic populations of Javanese and Indian ricefish showed different distribution lineages between the Gulf of Thailand and the Andaman Sea of Thailand, with specimens of Indian ricefish collected from Myanmar also included in *O. dancena* group 1 Phylogenetic analysis revealed two different genetic groups of each species of ricefish inferred from *COI* polymorphism. These genetic relationships were consistent with the results of the minimum spanning network. In teleosts, genetic diversity among populations is affected by limitations to gene flow levels caused by environmental

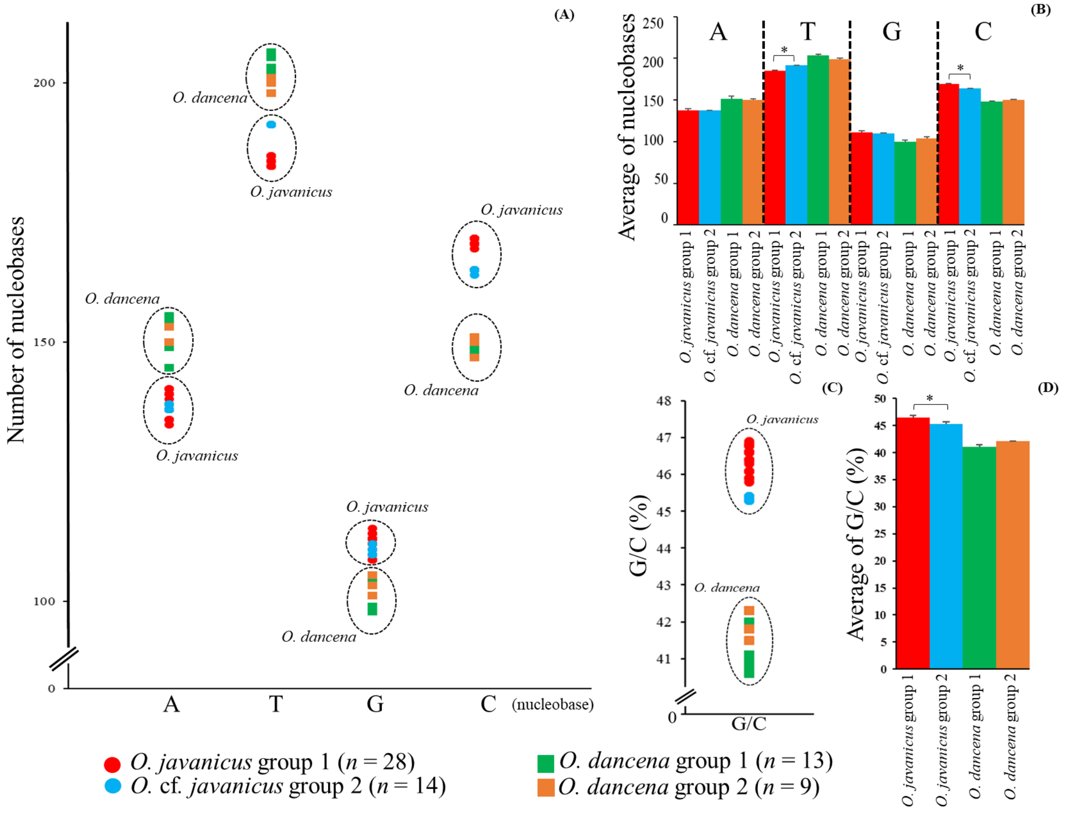

Fig. 5 Nucleobase contents of partial *COI* sequences of Javanese and Indian ricefish populations collected from Gulf of Thailand and Andaman Sea: (A) numbers of A, T, C and G; (B) average of nucleotides; (C) percentage of G/C content; (D) average percentage of G/C content solid red dots represent *Oryzias javanicus* group 1 and solid light blue dots represent *O.* cf. *javanicus* group 2. Solid green squares indicate *O. dancena* group 1 and solid brown squares indicate *O. dancena* group 2.

factors, such as biogeology, bioregionalisation, climate and the river system and stream density (Shelley et al., 2019, 2020). The distribution patterns of fish populations and communities influenced by environmental variables have been reported in a number of species such as longear sunfish (*Lepomis megalotis*), green sunfish (*L. cyanellus*), streaked prochilod (*Prochilodus lineatus*), fat sleeper (*Dormitator maculatus*) and blue panchax (*Aplocheilus panchax*) (Sivasundar et al., 2001; Husemann et al., 2012; Galván-Quesada et al., 2016; Beck et al., 2017). In wild Japanese ricefish, Takehana et al. (2003) reported genetic variation related to biogeography in Japan. In Thailand, Thai ricefish populations have been mainly separated into two groups based on the *COI* gene and displacement (D) loops (Ngamniyom et al., 2020). Recently, Ngamniyom (2020) reported that the population diversity of *O. mekongensis* and *O. songkhramensis* associated with river positions in northeastern Thailand. The Gulf of Thailand and the Andaman Sea of Thailand are physical barriers separated by the Thai-Malaysian Peninsula (Mahidol et al., 2007; Phinchongsakuldit et al., 2013; Sheldon et al., 2015; Lange et al, 2019), where the geographical pattern includes long mountain and hill ranges that were also taken into the account (Gupta, 2005; Wolf et al., 2020). These characteristics suggest that the genetic dispersal of Javanese and Indian ricefish populations is probably related to biogeography of Thailand. Furthermore, the natural barriers between the Gulf of Thailand and the Andaman Sea may be a main factor causing the existence of the groups of Javanese ricefish or Indian ricefish from a molecular genetics viewpoint.

The Javanese ricefish population showed incongruence with biogeography, with four specimens (from samples 16, 17, 25 and 26) from the Gulf of Thailand being included in the group with the fish population from the Andaman Sea. In southern Thailand, the Isthmus of Kra is the narrowest land area between the Gulf of Thailand and the Andaman Sea on the Thai-Malaysian Peninsula (de Bruyn et al., 2005; Parnell, 2013). In the current study, Javanese ricefish were collected near the Isthmus of Kra in the Gulf of Thailand. Therefore, four fish specimens from the Gulf of Thailand may be genetically related to the fish population from the Andaman Sea or alternatively, they were matriarchally originated from the Andaman population. The phylogenetic trees revealed a close relationship of Javanese ricefish in Thai waters with *O. haugiangensis* from southeastern Vietnam. This result corresponded with a report by Naruse (1996) indicating that Javanese ricefish from Jakarta, Indonesia were more closely related to *O. melastigma* than Javanese ricefish from Sulawesi and Singapore, based on genetic distance. Javanese ricefish have been shown to be more closely related to *O. haugiangensis* than Indian ricefish (Dyer and Chernoff, 1996; Parenti 2008). However, the morphologies of *O.* cf. *javanicus* were not different from those reported by Roberts (1998), Parenti (2008) and Magtoon (2011). For bony fish, cryptic species are found in molecular phylogenies, for example, blackblotch blenny (*Cirripectes alboapicalis*), Amazonian catfish (*Centromochlus existimatus*) and African long-fin tetra (*Bryconalestes longipinnis*) (Delrieu-Trottin et al., 2018; Cooke et al., 2012; Arroyave et al., 2019). However, it remains to be determined whether some individuals included in the current study truly represent cryptic species. More specimens of Javanese ricefish and other *Oryzias* spp. may be required to obtain molecular and morphological data allowing a precise phylogenetic relationship and cryptic species identification. Similar to Javanese ricefish, the relationship within the groups of the Indian ricefish population were associated with biogeography, but only two specimens (in localities 15 and 30) from the southern part of the Gulf of Thailand were grouped with the fish population of the Andaman Sea. Indian ricefish is commonly distributed in low-salinity water or freshwater along the Gulf of Thailand and the Malay Peninsula. Javanese ricefish populations have been recorded on the west coast of Peninsular Malaysia but not on the east coast (Yusof et al., 2013). It was difficult to find the Javanese ricefish from the Gulf of Thailand, when collecting samples in the current study. Many Javanese ricefish presented in the Andaman coast compared to the Gulf of Thailand. However, additional samples of Javanese or Indian ricefish at the macrogeographic distribution level should be analyzed to confirm results of the present study.

According to the genetic variation data, the haplotype diversity was significantly highest in *O. dancena* group 1. The distinct differences among samples may be expected (Wu and Fang, 2005). The number of haplotypes identified was in accord with the minimum spanning network analysis. In contrast, the nucleotide diversity was relatively low in *O.* cf. *javanicus* group 2. This may indicate a historically small effective population size of females (Wu and Fang, 2005). Almost all values obtained from the neutrality tests of the fish populations were positive and did not show significant differences. These results suggested the existence of a balancing selection in the examined populations. Only Javanese group 1, which showed negative values, may have undergone population expansion (Tajima, 1989; Fu, 1997). However, a significant negative value was not supported by either the D or Fs tests. The current results for Javanese group 1 were similar to findings reported for some populations of Jarbua terapon (*Terapon jarbua*) from Malaysian waters (Chanthran et al., 2020) and shortfin silverside (*Chirostoma humboltianum*) in Central México (García-Martínez et al., 2015). In addition, the results of the D and Fs tests for Javanese ricefish group 1 and the Indian ricefish groups were similar to those reported for crevalle jack (*Caranx hippos*) fish populations in the Colombian Caribbean (Caiafa-Hernández et al., 2018). Ramos-Onsins and Rozas (2002) suggested that Fu's Fs test is more powerful than Tajima's D test for assessing population growth with a larger sample size. Thus, the small sample sizes of ricefish evaluated in the current study might have been the cause for the lack of significant values. In the demographic investigation, multimodal peaks, including bimodal peaks, were displayed in the observed mismatch distributions. These results might indicate a constant population size (Rogers and Harpending, 1992).

In the nucleobase analysis, significant differences in T and C nitrogenous bases and the percentage of G and C bases were found in the Javanese ricefish groups. A partial *COI* region has been reported among various fish populations to reveal their genetic structure correlated with geographical characteristics and for use in molecular barcoding (Martins et al., 2003; Jamaluddin et al., 2011; Wang et al., 2015; Bingpeng et al., 2018). In the current study, the DNA polymorphism of the Javanese ricefish group may have been higher than that of the Indian ricefish group. The nitrogenous bases, T, C or G/C contents may be used as alternative barcodes for the further molecular analysis of the Javanese ricefish populations in Thailand.

In summary, the genetic dispersion of Java and Indian ricefish populations seems to be biogeographically related, with separation imposed by the Thai-Malaysian Peninsula. The fish populations were clearly separated into different groups between the Gulf of Thailand and the Andaman Sea of Thailand. These *Oryzias* spp. specimens showed genetic diversity related to the coastal ecology of Thailand. In addition, the current study provided preliminary data on the genetic variation, population structure and demography of Javanese and Indian ricefish in Thailand and representative samples from Myanmar. Although results from *COI* polymorphism indicated clear intraspecific biogeographic differences between different coastal regions of *O. javanicus* and *O. dancena* in Thai (and Myanmar) waters. Further studies focusing on nuclear DNA (microsatellites) may be useful in further understanding population subdivision of these species. MtDNA is transmitted maternally and therefore studies of *COI* polymorphism cannot evaluate the genetic contribution mediated by males (Birky et al., 1989).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Arroyave, J., Martinez, C.M., Stiassny M.L.J. 2019. DNA barcoding uncovers extensive cryptic diversity in the African long-fin tetra *Bryconalestes longipinnis* (Alestidae: Characiformes). J. Fish Biol. 95: 379–392. doi.org/10.1111/jfb.13987

Bandelt, H., Forster, P., Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16: 37–48. doi.org/10.1093/oxfordjournals.molbev.a026036

Beck, S.V., Carvalho, G.R., Barlow, A., et al. 2017. Plio-Pleistocene phylogeography of the Southeast Asian Blue Panchax killifish, *Aplocheilus panchax*. PLoS One 12: e0179557. doi.org/10.1371/journal.pone.0179557

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., Jianjun, W. 2018. DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One 13: e0198109. doi.org/10.1371/journal.pone.0198109

Birky Jr, C.W., Furest, P., Maruyama, T. 1989. Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121: 613–627.

Caiafa-Hernández, I., Narváez-Barandica, J., Acero-Pizarro, A. 2018. Genetic variation and genetic structure of *Caranx hippos* (Teleostei: Carangidae) in the Colombian Caribbean. Rev. Biol. Trop. 66: 122–135. dx.doi.org/10.15517/rbt.v66i1.25770

Chanthran, S.S.D., Lim, P.E., Li, Y., et al. 2020. Genetic diversity and population structure of *Terapon jarbua* (Forskål, 1775) (Teleostei, Terapontidae) in Malaysian waters. Zookeys 911: 139–160.

Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W. 2016. GenBank. Nucleic Acids Res. 44: D67–72. doi.org/10.1093/nar/gkv1276

Cooke, G.M., Chao, N.L., Beheregaray, L.B. 2012. Five cryptic species in the Amazonian catfish *Centromochlus existimatus* identified based on biogeographic predictions and genetic data. PLoS One 7: e48800. doi.org/10.1371/journal.pone.0048800

de Bruyn, M., Nugroho, E., Hossain, M.M., Wilson, J.C., Mather, P.B. 2005. Phylogeographic evidence for the existence of an ancient biogeographic barrier: The Isthmus of Kra Seaway. Heredity 94: 370–378. doi.org/10.1038/sj.hdy.6800613

Delrieu-Trottin, E., Liggins, L., Trnski, T., Williams, J.T., Neglia, V., Rapu-Edmunds, C., Planes, S., Saenz-Agudelo, P. 2018. Evidence of cryptic species in the blenniid *Cirripectes alboapicalis* species complex, with zoogeographic implications for the South Pacific. ZooKeys 810: 127–138. doi:10.3897/zookeys.810.28887

Dyer, B.S., Chernoff, B. 1996. Phylogenetic relationships among atheriniform fishes (Teleostei: Atherinomorpha). Zool. J. Linn. Soc. Lond. 117: 1–69. doi.org/10.1111/j.1096-3642.1996.tb02148.x

Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–25. doi.org/10.1093/genetics/147.2.915

Galván-Quesada, S., Doadrio, I., Alda, F., et al. 2016. Molecular phylogeny and biogeography of the amphidromous fish genus *Dormitator* Gill 1861 (Teleostei: Eleotridae). PLoS One 11: e0153538. doi.org/10.1371/journal.pone.0153538

García-Martínez, R.M., Mejía, G.H.O., García-De León, F.J., Barriga-Sosa, I.A. 2015. Extreme genetic divergence in the endemic fish *Chirostoma humboldtianum*: implications for its conservation. Hidrobiológica 25: 95–106.

Grant, W.S., Bowen, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. J. Hered. 89: 415–426. doi.org/10.1093/jhered/89.5.415

Gupta, A. 2005. The Physical Geography of Southeast Asia. Oxford University Press. Oxford, UK.

Harpending, H.C., Sherry, S.T., Rogers, A.R., Stoneking, M. 1993. The genetic structure of ancient human populations. Curr. Anthropol. 34: 483–496.

Hebert, P.D., Ratnasingham, S., de Waard, J.R. 2003. Barcoding animal life: Cytochrome c oxidase subunit divergences among closely related species. Proc. Biol. Sci. 270: S96–S99. doi: 10.1098/rsbl.2003.0025

Hess, K.L., Corda, C.J., Lanese, K.M. 2011. Science buddies: Advancing informal science education.Science332:550–551.doi:10.1126/science.1196978

Hilgers, L., Schwarzer, J. 2019. The untapped potential of medaka and its wild relatives. Elife 8: e46994. doi: 10.7554/eLife.46994

Husemann, M., Ray, J.W., King, R.S., Hooser, E.A., Danley, P.D. 2012. Comparative biogeography reveals differences in population genetic structure of five species of stream fishes. Biol. J. Linn. Soc. Lond. 107: 867–885. doi.org/10.1111/j.1095-8312.2012.01973.x

Ikenaga, T. 2013. Teleost fish. In: Manto M., Schmahmann J.D., Rossi F., Gruol D.L., Koibuchi, N. (Eds.). Handbook of the Cerebellum and Cerebellar Disorders. Springer. Dordrecht, the Netherlands, pp. 1463–1480.

Imai, S., Koyama, J., Fujii, K. 2005. Effects of 17β-estradiol on the reproduction of Java medaka (*Oryzias javanicus*), a new test fish species. Mar. Pollut. Bull. 51: 708–714. doi.org/10.1016/j.marpolbul.2005.02.018

Inoue, K., Takei, Y. 2003. Asian medaka fishes offer new models for studying mechanisms of seawater adaptation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 136: 635–465. doi.org/10.1016/S1096-4959(03)00204-5

Jamaluddin, J.A.F, Pau, T.M., Siti-Azizah, M.N. 2011. Genetic structure of the snakehead murrel, *Channa striata* (Channidae) based on the cytochrome c oxidase subunit I gene: Influence of historical and geomorphological factors. Genet. Mol. Biol. 34: 152–160. doi.org/10.1590/S1415-47572011000100026

Kim, B.M., Choi, B.S., Kim, H.S., Rhee, J.S., Au, D.W., Wu, R.S., Choi, I.Y., Lee, J.S. 2015. Transcriptome profiling of larvae of the marine medaka *Oryzias melastigma* by Illumina RNA-seq. Mar. Genomics. 3: 255–258. doi.org/10.1016/j.margen.2015.07.013

Kunlapapuk, K., Kulabtong, S., Saipattana, P. 2015. Updated checklist of freshwater and brackish fishes of Phetchaburi Basin, Northwest Gulf of Thailand Drainages. Biodivers. J. 6: 837–842.

Lange, I.D., Schoenig, E., Khokiattiwong, S. 2019. World Seas: An Environmental Evaluation, 2nd ed., Vol. 2. Academic Press. Cambridge, UK.

Leigh, J.W., Bryant, D. 2015. PopART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6: 1110–1116. doi.org/10.1111/2041-210X.12410

Librado, P., Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452. doi.org/10.1093/bioinformatics/btp187

Maeng, S., Yoon, S.W., Kim, E.J., Nam, Y.K., Sohn, Y.C. 2019. Transcriptional activity of an estrogen receptor β subtype in the medaka *Oryzias dancena*. Dev. Reprod. 23: 333–344.

Magtoon, W. 2010. *Oryzias songkhramensis*, a new species of ricefish (Beloniformes; Adrianichthyidae) from northeast Thailand and Central Laos. Trop. Nat. Hist. 10: 107–129.

Magtoon, W. 2011. First record of ricefish, *Oryzias haugiangensis* (Beloniformes: Adrianichthyidae: Oryziinae) from Southeast Thailand. Trop. Nat. Hist. 11: 9–20.

Mahidol, C., Na-Nakorn, U., Sukmanomon, S., Taniguchi, N., Nguyen, T.T. 2007. Mitochondrial DNA diversity of the Asian moon scallop, *Amusium pleuronectes* (Pectinidae), in Thailand. Mar. Biotechnol. 9: 352–359. doi.org/10.1007/s10126-006-6137-y

Martins, C., Wasko, A.P., Oliveira, C., Foresti, F. 2003. Mitochondrial DNA variation in wild populations of *Leporinus elongatus* from the Paraná River basin. Genet. Mol. Biol. 26: 33–38. doi.org/10.1590/S1415-47572003000100006

Mukhopadhyay, T., Bhattacharjee, S. 2014. Study of the genetic diversity of the ornamental fish *Badis badis* (Hamilton-Buchanan, 1822) in the Terai region of Sub-Himalayan West Bengal, India. Int. J. Biodivers. 2014: 791364. doi.org/10.1155/2014/791364

Naruse, K. 1996. Classification and phylogeny of fishes of the genus *Oryzias* and its relatives. Fish Biol. J. Medaka 8: 1–9.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press. New York, NY, USA.

Ngamniyom, A. 2020. Molecular assessment and transcriptome profiling of wild fish populations of *Oryzias mekongensis* and *O. songkhramensis* (Adrianichthyidae: Beloniformes) from Thailand. PLoS One 15: e0242382. doi.org/10.1371/journal.pone.0242382

Ngamniyom, A., Sriyapai, T., Sriyapai, P. 2020. Molecular analysis of population and *De Novo* transcriptome sequencing of Thai medaka, *Oryzias minutillus* (Teleostei: drianichthyidae). Heliyon 6: e03079. doi.org/10.1016/j.heliyon.2019.e03079

Paixão, R.V., Ribolli, J., Zaniboni-Filho, E. 2018. Genetic variation of the endangered neotropical catfish *Steindachneridion scriptum* (Siluriformes: Pimelodidae). Front. Genet. 9: 48. doi.org/10.3389/fgene.2018.00048

Parenti, L.R. 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). Zool. J. Linn. Soc. 154: 494–610. doi.org/10.1111/j.1096-3642.2008.00417.x

Parenti, L.R., Hadiaty, R.K., Lumbantobing, D., Herder, F. 2013. Two new ricefishes of the genus *Oryzias* (Atherinomorpha: Beloniformes: Adrianichthyidae) augment the endemic freshwater fish fauna of Southeastern Sulawesi, Indonesia. Copeia 2013: 403–414. doi.org/10.1643/CI-12-114

Parnell, J. 2013. The biogeography of the Isthmus of Kra region: A review. Nord. J. Bot. 31: 1–15. doi.org/10.1111/j.1756-1051.2012.00121.x

Phinchongsakuldit, J., Chaipakdee, P., Collins, J.F., Jaroensutasinee, M., Brookfield, J.F.Y. 2013. Population genetics of cobia (*Rachycentron canadum*) in the Gulf of Thailand and Andaman Sea: Fisheries management implications. Aquacult. Int. 21: 197–217. doi.org/10.1007/s10499-012-9545-1

Ramos-Onsins, S.E., Rozas, J. 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19: 2092–2100. doi.org/10.1093/oxfordjournals.molbev.a004034

Ravi, V., Venkatesh, B. 2008. Rapidly evolving fish genomes and teleost diversity. Curr. Opin. Genet. Dev. 18: 544–550. doi.org/10.1016/j.gde.2008.11.001

Razo-Mendivil, U., Vázquez-Domínguez, E., Rosas-Valdez, R., de León, G.P., Nadler, S.A. 2010. Phylogenetic analysis of nuclear and mitochondrial DNA reveals a complex of cryptic species in *Crassicutis cichlasomae* (Digenea: Apocreadiidae), a parasite of Middle-American cichlids. Int. J. Parasitol. 40: 471–486. doi.org/10.1016/j.ijpara.2009.10.004

Roberts, T.R. 1998. Systematic observations on tropical Asian medakas or rice fishes of the genus *Oryzias* with descriptions of four new species. Ichthyol. Res. 45: 213–224. doi.org/10.1007/BF02673919

Rogers, A.R., Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9: 552–569. doi.org/10.1093/oxfordjournals.molbev.a040727

Setiamarga, D.H., Miya, M., Yamanoue, Y., Azuma, Y., Inoue J.G., Ishiguro, N.B. 2009. Divergence time of the two regional medaka populations in Japan as a new time scale for comparative genomics of vertebrates. Biol. Lett. 5: 812–816. doi.org/10.1098/rsbl.2009.0419

Sheldon, F.H., Lim, H.C., Moyle, R.G. 2015. Return to the Malay Archipelago: The biogeography of Sundaic rainforest birds. J. Ornithol. 156: S91–S113. doi.org/10.1007/s10336-015-1188-3

Shelley, J.J., Dempster, T., Le Feuvre M.C., Unmack P.J., Adams M., Laffan S.W., Swearer S. 2019. A revision of the bioregionalisation of freshwater fish communities in the Australian Monsoonal Tropics. Ecol. Evol. 9: 4568–4588. doi.org/10.1002/ece3.5059

Shelley, J.J., Swearer, S., Dempster, T., Adams, M., Le Feuvre, M.C., Hammer, M.P., Unmack, P.J. 2020. Plio-Pleistocene sea-level changes drive speciation of freshwater fishes in north-western Australia. J. Biogeogr. 47: 1727–1738. doi.org/10.1111/jbi.13856

Shen, Y., Guan, L., Wang, D., Gan, X. 2016. DNA barcoding and evaluation of genetic diversity in Cyprinidae fish in the midstream of the Yangtze River. Ecol. Evol. 6: 2702–2713. doi.org/10.1002/ece3.2060

Sivasundar, A., Bermingham, E., Ortí, G. 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American rivers. Mol. Ecol. 10: 407–417. doi.org/10.1046/j.1365-294X.2001.01194.x

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585–595. doi.org/10.1093/genetics/123.3.585

Takehana, Y., Nagai, N., Matsuda, M., Tsuchiya, K., Sakaizumi, M. 2003. Geographic variation and diversity of the cytochrome b gene in Japanese wild populations of medaka, *Oryzias latipes*. Zoolog. Sci. 20: 1279–1291. doi.org/10.2108/zsj.20.1279

Takehana, Y., Zahm, M., Cabau, C., Klopp, C., Roques, C., Bouchez, O. 2020. Genome sequence of the Euryhaline Javafish Medaka, *Oryzias javanicus*: A small aquarium fish model for studies on adaptation to salinity. G3 Genes Genom. Genet. 10: 907–915. doi.org/10.1534/g3.119.400725

Tamura, K., Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512–526. doi.org/10.1093/oxfordjournals.molbev.a040023

Termvidchakorna, A., Magtoon, W. 2008. Development and identification of the ricefish *Oryzias* in Thailand. Science Asia 34: 416–423.

Thu, P.T., Huang, W.C., Chou, T.K., Van Quan, N., Van Chien, P., Li, F., Shao, K.T., Liao, T.Y. 2019. DNA barcoding of coastal ray-finned fishes in Vietnam. PLoS One 14: e0222631. doi.org/10.1371/journal.pone.0222631

Tibihika, P.D., Curto, M., Alemayehu, E., Waidbacher, H., Masembe, C., Akoll, P., Meimberg, H. 2020. Molecular genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus* L. 1758) in East African natural and stocked populations. BMC Evol. Biol. 20: 16. doi.org/10.1186/s12862-020-1583-0

Tippmann, H.F. 2004. Analysis for free: Comparing programs for sequence analysis. Brief Bioinform. 5: 82–87. doi.org/10.1093/bib/5.1.82

Volff, J. 2005. Genome evolution and biodiversity in teleost fish. Heredity 94: 280–294. doi.org/10.1038/sj.hdy.6800635

Wang, J., Xiao, J., Zeng, M., et al. 2015. Genomic variation in the hybrids of white crucian carp and red crucian carp: Evidence from ribosomal DNA. Sci. China Life Sci. 58: 590–601. doi.org/10.1007/s11427-015-4835-2

Wolf, A., Roberts, W.H.G, Ersek, V., Johnson, K.R., Griffiths, M.L. 2020. Rainwater isotopes in central Vietnam controlled by two oceanic moisture sources and rainout effects. Sci. Rep. 10: 16482. doi.org/10.1038/s41598-020-73508-z

Wu, H.L., Fang, S.G. 2005. Mitochondrial DNA genetic diversity of black muntjac (*Muntiacus crinifrons*), an endangered species endemic to China. Biochem. Genet. 43: 407–416. doi.org/10.1007/s10528-005-6779-x

Xu, Y., Zhang, S., Wang, H., Wang, M., Li, G. 2019. Mitochondrial gene sequence (COI) reveals the genetic structure and demographic history of *Lymantria dispar* (Lepidoptera: Erebidae: Lymantriinae) in and around China. Insects 10: 146. doi.org/10.3390/insects10050146

Yusof, S., Ismail, A., Koito, T., Kinoshita, M., Inoue, K. 2011. Occurrence of two closely related ricefishes, Javanese medaka (*Oryzias javanicus*) and Indian medaka (*O. dancena*) at sites with different salinity in Peninsular Malaysia. Environ. Biol. Fish. 93: 43–49. doi.org/10.1007/s10641-011-9888-x

Yusof, S., Ismail, A., Rahman, F. 2013. Distribution and localities of Java medaka fish (*Oryzias javanicus*) in Peninsular Malaysia. Malay. Nat. J. 65: 38–46.