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Ahmad Dwi Setyawan <smujo.id@gmail.com>
to me

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Thank you for submitting the manuscript, "Phylogeography of the blue panchax, Aplocheilus panchax (Hamilton, 1822), in Inc population" to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you process by logging in to the journal web site:

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Reply Forward

Reviewer B:

Overall, this study would make an interesting addition to previously published studies. The study system is particularly interesting given their current dist make sense. As such, I had to stop reading before finishing the manuscript.

English needs improving throughout document as lots of spelling errors and sentences that don't make sense.

I don't think the Results and Discussion should be merged as it currently is and strongly recommend the authors to use separate headings for these two

There are statements as to why different sequence lengths show how diverse this population is and how it might have a big ecological effect, but I don't evidence and examples.

I stopped reading at line 187 as I feel the authors need to first go through their manuscript to ensure that the English makes sense and accurately reflect getting lost in translation. There are a lot of big statements that I do not think are justified nor appropriate for this study.

22-26: very broad introduction to phylogeography that is also quite repetitive and simplistic.

29-31: Not sure if the entire classification is needed here as the reader can determine this from its binomial nomenclature.

36: "megadiverse organisms' evolution" doesn't really make sense…perhaps consider changing to something along the lines of "…have all contributed t

42: the previous study did not use the 'worlds' *A. panchax,* just a few populations distributed across SE Asia, but not all.

58-59: I'm not sure what the authors are trying to say here. Are they saying that all Indonesian waters are polluted?

63-66: would be good to have sample sizes for each station

Figure 1: quality needs to be improved as currently quite grainy

71: is that 20 samples for all sites together? Or each site? If it's all sites together, this sample size is quite small

Phylogeography of the blue panchax, *Aplocheilus panchax*, in Indonesia, with special focus on the Bangka Island population

Abstract. Previous study devided Blue panchax, *Aplocheilus panchax* into three different clades, namely West (W), Central (C), and East (E) clades. Blue panchax populations from Indonesia were belong to Central and East clades. However, that study did not include blue panchax samples from pits with harsh conditions in Bangka Island. Therefore, this study aimed to assess phylogeographic of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. The results indicate genetic distances ranged from 0.17% to 110.45%, and all the specimens from Bangka Island had a genetic distance < 0.05. There was also a clear genetic gap between the Bangka and other populations, with the values ranging between 1.42% and 103.87%. Furthermore, the seventy-nine sequences analyzed showed genetic variability of 0.22 for nucleotide diversity (π), 0.895 for Haplotype diversity (Hd), 68.028 for Fu's Fs statistic, 2.00 ($P < 0.02$) for Fu and Li's D' test statistic, and 2.365 ($P < 0.02$) for Fu and Li's test statistic. The Bangka population of *Aplocheilus panchax* established a distinct clade from the Western (W), Eastern (E), and Central (C) clades. Molecular data established that the population on Bangka Island is a novel clade for Indonesia and a global blue panchax phylogeographic.

Keywords: *Aplocheilus panchax*, abandoned tin mining pits, Bangka Island populations, new clade position

Running title: phylogeographic position of *Aplocheilus panchax*

INTRODUCTION

Phylogeography analyzes and understands organism diversity and biogeography (Bobo-Pinilla et al., 2021). Furthermore, it is a study of geographical distributions of closely related genetic lineages or geographic ordination of genotypes (Rius and Turon, 2020). Therefore, phylogeographic research is essential to evaluate the geographical distribution of these genetic lineages and their pattern, as well as elaborate ecology factors and organism biodiversity (Lone et al., 2021).

The blue panchax (*Aplocheilus panchax* Hamilton, 1882) is an endemic species to the Oriental region (Costa 2013; Costa 2016; Beck et al. 2017), widely distributed across the Indo-Malayan Islands, including Indonesia, the Indo-China region, and India (Dekar et al. 2018; Bolotov et al. 2020). *Aplocheilus panchax* is a fish from Genus *Aplocheilus*, Family Aplocheilidae, Suborder Aplocheiloidei, Order Cyprinodontiformes, and Class Actinopterygii (Parenti and Hartel 2011; Furness et al. 2015). Fishes belonging to Order Cyprinodontiformes are also known as *Aplocheiloid killifishes* or *livebearer*s (Pohl et al., 2015; Braganca et al., 2018).

Indonesia is one of the world's biodiversity hotspots with many different habitats and a highly complicated geological history (*Bruyn et al., 2014;* von Rintelen et al., 2017). Generally, Southeast Asia's complex climatic and geological history caused this region to have high biodiversity (Beck et al., 2017; Fortes et al., 2018). Biogeography, geology, climate, and ecology of Southeast Asia have led to megadiverse organisms' evolution. Additionally, the region is home to several endemic and ecologically well-adapted species (Hughes 2017; von Rintelen et al. 2017). The *Aplocheilus panchax* can be used to understand further how climatic changes and sea-level fluctuations have influenced the species' distribution within this region. Specifically, sea-level variations result from glacial cycles that continued throughout the Pleistocene, interfering with and restricting the network of all populations. These examples show proof of isolation in palaeodrainage basins (Beck et al., 2017).

Previous study placed worlds' *A. panchax* populations into three different clades; West (W), Central (C), and East (E) clades. The *A. pachax* populations from Indonesia were devided into Central and East clades. Central clade consisted of Pekanbaru, Jambi, and West Sumatera populations. East clade was formed by *A. pachax* populations from Java, Bali, Kalimantan, and Sulawesi islands (Beck et al. 2017). Nevertheless, the study by Beck et al. (2017) did not include *A. panchax* samples from Bangka Island. Bangka Island, the biggest tin producer in Indonesia, has unique waters like a lake or pit, known as kolong, formed and abandoned after tin mining activity. This water has low pH (acid waters), low

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nutrients, minimum dissolved oxygen (DO), and heavy metals. However, *Aplocheilus panchax*, locally known as ikan Kepala Timah, can live in this extreme habitat (Kuniawan et al. 2019, Kurniawan et al. 2020; Mustikasari et al. 2020a). The genus *Aplocheilus*, which includes *A. panchax*, was classified as an extremophile fish due to its ability to survive in harsh environmental circumstances (Riesch et al., 2015; Kurniawan and Mustikasari 2021). Mustikasari et al. (2020a, b) explored the presence and morphological variety of blue panchax (*A. panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of various ages.

This study aimed to assess phylogeographic of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. This is the first time the populations from Bangka Island have been genetically analyzed using the cytochrome oxidase I (COI) gene and compared to another on the global distribution of blue panchax. Furthermore, the presence and genetic profile of *A. panchax* can be a model of extremophile fish study. Therefore, it is used as a bioindicator for harsh habitats since the global distribution, ecological and genetic evolution with the Bangka Island population corresponds to others in Indonesian waters.

MATERIALS AND METHODS

Study area

The study was conducted in Pangkalpinang City and Bangka Regency of Bangka Belitung Archipelago Province, Indonesia. Fish samples were collected from abandoned tin mining lakes (pits) of different ages and the Limbung River. Station A and B (< 5 years old), Station C and D (5-15 years), Station E and F (15 - 25 years), Station G (25 - 50 years), Station H (50 - 100 years), Station I and J (> 100 years), and Limbung River Stream of Bangka Regency as Station K are shown in Figure 1 (Mustikasari et al. 2020a, b).

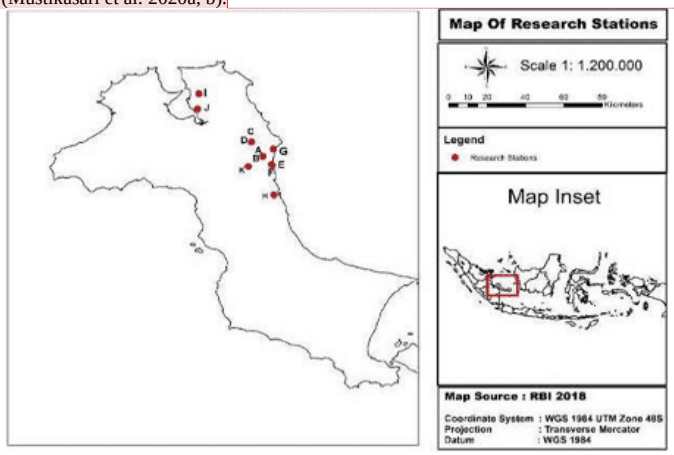

Figure 1. Map of research stations at Bangka Island, Indonesia (Mustikasari et al. 2020a, b)

Procedures

Samples preparation for molecular analysis

The twenty samples were collected at 09.00 am-1.00 pm from closed and open waters of abandoned tin mining lakes (pits) and waters of Limbung River Stream, Bangka Island, using nets with a mesh size of about 0.4 mm. Furthermore, this study utilized a 2.0 ml cryotube with ethanol absolute to preserve the sample for molecular analysis.

Molecular analysis

The genomic DNA extraction was analyzed with gSYNC™ DNA Extraction Kit (Geneaid, GS300). Nucleic acid (genomic DNA) concentration was measured using Nanodrop™ 2000/2000c spectrophotometers. Furthermore, molecular analysis was referred to Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika Science Indonesia, in 2021.

PCR amplification was conducted with (2x) MyTaq HS Red Mix (Bioline, BIO-25048) and KOD FX Neo (Toyobo, KFX-201). The components 1 x 25 µl PCR Master Mix were dd H2O 9.5 µl; MyTaq HS Red Mix, 2x 12.5 µl; 10 µM VF2_t1 0.5 µl; 10 µM Fish F2_t1 0.5 µl; 10 µM Fish R2_t1 0.5 µl; 10 µM Fish FR1d_t1 0.5 µl; and DNA Template 1 µl.

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Primer sequence of PCR amplification were VF2-t1 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAA GACATTGGCAC-3'; FR1d-t1 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3'; FishR2_t1 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3'; and FishF2_t1 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (Ivanova et al. 2007).

The predenaturation phase initiated the Polymerase Chain Reaction (PCR) cycling for 1 minute (95 °C). Subsequently, the actual PCR amplification was conducted for 35 cycles, denaturation process for 15 seconds (95 °C), annealing process for 15 minutes (50 °C), and extension process for 45 seconds (72 °C). The PCR products (1 µL) were assessed by electrophoresis with 1% TBE agarose with Marker 100bp DNA ladder (loaded 2 µL). Furthermore, the quality and length of the PCR products were analyzed by agarose gel electrophoresis. Bi-directional Sequencing conducted the sequencing step at 1st base Asia.

Data analysis

The contig sequences were obtained from reverse and forward sequences aligned with Program BioEdit. The phylogenetic study was carried out using MEGA XI (Tamura et al. 2021), utilizing the Maximum Parsimony (MP) statistical approach, and a bootstrap consensus tree was constructed using 1,000 replicates. The phylogenetic tree was constructed by comparing sequences of A. panchax from Bangka Island with some sequences of *A. panchax* from a previous study (Beck et al., 2017). The study conducted in India (Tamil Nadu and Kolkotta), Cambodia, Vietnam, Thailand (Krabi), Malaysia (Sungai Batu Pahat, Penang, Dungun), Singapore, and Indonesia (Aceh, Pekanbaru, Pulau Laut, West Sumatra, Jambi, Bogor, Surabaya, Banjarmasin, Bali, and Sulawesi) as shown in Figure 2 aim to investigate the position of *A. panchax* population from Bangka Island. Furthermore, it used a sequence of *Aplocheilus andamanicus* (Katwate et al. 2018). *Aplocheilus warneri* (accession number KJ844713.1) was used as outgroup species (Pohl et al. 2015) for this phylogenetic analysis. Sequences metadata from Beck et al. (2017) and Katwate et al. (2018) from NCBI (National Center for Biotechnology Information) were utilized. DnaSP v5 was used to assess the haplotype (Hd) and nucleotide diversity (π), as well as to perform Fu and Li's F and Tajima's D neutrality tests (Čekovská et al. 2020). A phylogenetic network was constructed according to the median-joining method within the software Network 10. The Kimura 2 Parameter (K2P) genetic distance was calculated in the MEGA XI (Tamura et al., 2021).

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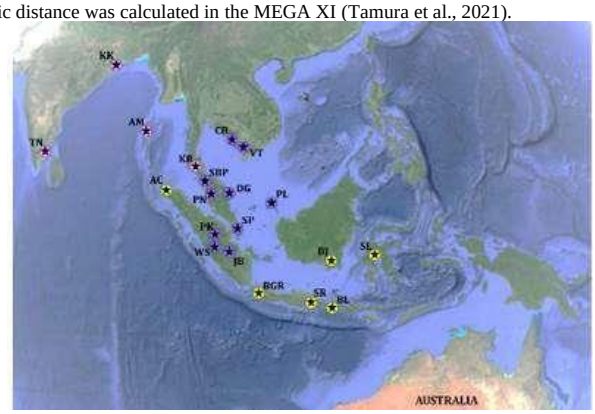

Figure 2. Sampling locations for *Aplocheilus panchax* over 20 areas, namely Tamil Nadu (TN), Kolkotta (KK), Andaman Island (AM) Cambodia (CB), Vietnam (VT), Krabi (KB), Sungai Batu Pahat (SBP), Aceh (AC), Penang (PN), Dungun (DG), Pulau Laut (PL), Singapore (SP), Pekanbaru (PK), West Sumatera (WS), Jambi (JB), Bogor (BG), Surabaya (SR), Banjarmasin (BJ), Bali (BL) and Sulawesi (SL). (Map was reconstructed from Beck et al. 2017 and Katwate et al. 2018).

RESULTS AND DISCUSSION

DNA quantity and quality

The successfulness of organisms identification methods based on genetic material, such as PCR, relies on the quantity and quality of nucleic acid, purification method, and PCR amplification process. Unfortunately, the low amount and quality of genetic material (DNA) and the appearance of inhibitors inhibit the PCR amplification efficiency (Chowdhury et al., 2016; Dwiyitno et al., 2018; Kuffel et al. 2021). The analysis of the quantity of sample' DNA by Nanodrop spectrophotometer resulted in DNA concentration (ratio $A_{260/280}$) for all samples between 1.65 and 1.99 (Table 1).

Table 1. Quantity of Sample DNA.

No	Sampel*	$A_{260/280}$	No	Sampel*	$A_{260/280}$
1	BK_A01	1.72	11	BK_F01	1.87
2	BK_A02	1.90	12	BK_F02	1.65
3	BK_B01	1.91	13	BK_G01	1.75
4	BK_B02	1.87	14	BK_G02	1.59
5	BK_C01	1.87	15	BK_G03	1.81
6	BK_C02	1.91	16	BK_G04	1.88
7	BK_D01	1.93	17	BK_K01	1.65
8	BK_D02	1.99	18	BK_K02	1.83
9	BK_E01	1.90	19	BK_K03	1.71
10	BK_E02	1.89	20	BK_K04	1.70

Note: *) sampel' volume (30 µl)

The measurement of DNA concentration with Nanodrop was conducted at a wavelength of 260 nm. In comparison, the protein was measured at a wavelength of 280 nm with pure DNA having an absorbance ratio of 260/280 between 1.6 and 2.0 (Setiaputri et al. 2020), 1.7-2.0 (Ruchi et al. 2018), or 1.8-20 (Pratomo et al. 2021). The absorbance value below the low absorbance limit indicates the presence of polysaccharides, phenol, and protein contamination. Meanwhile, above the absorbance 2.0 indicates the presence of RNA contamination during the DNA isolation process (Rosilawati et al. 2002; Farmawati et al. 2015; Rizko et al. 2020). The success of a purification extraction and the quantity of DNA is highly dependent on the isolation of the resulting DNA. Therefore, the isolation process using commercial kits is safer from processing errors that cause contamination. However, studies on several specimens with various treatments stated that not all commercial kits can harvest DNA in high concentrations (Hajibabaei et al., 2006; Setiaputri et al., 2020).

The product of PCR also showed that the COI gene length of *A. panchax* populations from Bangka Island was about 700 bp. Meanwhile, the COI gene length for *A. panchax* was around 621 bp in other studies with accession numbers KJ957593.1, KJ957617.1, and KJ957618.1 from Beck et al. (2017), and also accession number MG813789.1 (Sample *A. andamanicus*) from Katwate et al. (2018). The length showed that the COI gene from Bangka Island was the longest. The mitochondrial gene, namely COI, is commonly used for DNA barcoding, about 650 bp. Meanwhile, these gene sequences are also used for ecological and evolutionary studies (Yang et al. 2019). The COI gene length differences of *A. panchax* populations indicate a diversity of *A. panchax* around the Oriental region. It plays a vital role in a global study to collect information about biodiversity and be a key in phylogenetic and phylogeographic analyses (Buhay 2009).

Island biogeography investigates how the richness of the ecosystems and the complexity of the biodiversity may cause speciation. The adaptive radiation, speciation, climate cycles, and topographical complexity can shape island biodiversity (Dorey et al. 2020). Phylogenetic and phylogeographic analyses based on mitochondrial DNA (mt-DNA) are used in island biodiversity exploration. In addition, mt-DNA possesses some characteristics, including cell quantity, genome size, haploid, maternal inheritance, and extremely low probability of paternal leakage, mutation rate, and change mainly caused the mutation. These features make mt-DNA a useful and one of the most often used markers in molecular analysis. The marker has been frequently used to study genetic diversity, population organization, phylogeography, and organism evolution (Gupta et al. 2015).

The phylogenetic tree and phylogeographic of *A. panchax* population from Bangka Island

The phylogenetic tree indicated that the ancestral relationship between the *A. panchax* from the distribution sequences was well supported by COI gene sequence analysis based on well-supported clades (>90% bootstrap values). The phylogenetic tree analysis showed *A. panchax* widespread throughout the Oriental region, including Indonesia as a part of Southeast Asia countries. However, the population from Bangka has different sequences from others. Bangka Island individuals formed a distinct clade from those previously described by Beck et al. (2017) and Katwate et al. (2018). (Figure 3).

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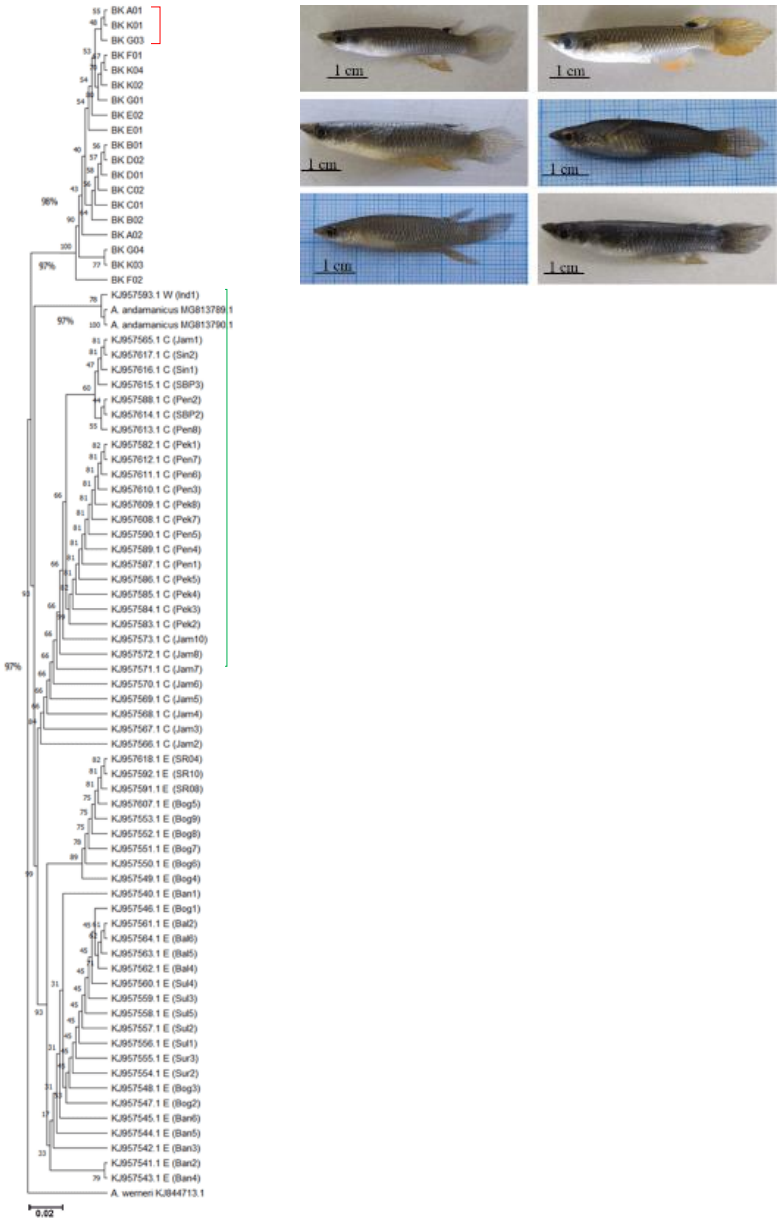

Figure 3. The phylogenetic position of *A. panchax* from Bangka Island is supported by Maximum Parsimony (MP) bootstrap values. A sequence of *A werneri* (KJ844713.1) was used as the outgroup. Sequences of topotypes of populations from Bangka Island are in red, and Beck et al. (2017) and Katwate et al. (2018) were *in the green.*

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Beck et al. (2017) revealed their research about Bayesian posterior probabilities that displayed for Western (W) clade, Eastern (E) clade, and Central (C) clade. We added a novel clade to previous phylogenetic tree (Beck et al. 2017; Katwate et al. 2018) by adding samples from Bangka Island (Figure 3). Therefore, the authors also recommended a new map reconstruction of global phylogeographic on blue panchax (*A. panchax*) gene distribution (Figure 4).

A previous study by Katwate et al. (2018) denied the presence of *A. panchax* in the Indo-Malay region. They provided morphological and molecular evidence and demonstrated that *A. andamanicus* and *A. armatus* distinct and valid killifish species in the Indo-Malay region. However, the present study supported Beck et al. (2017) by showing that killifish from Indonesia, especially Bangka populations, is *A. panchax*. The present result was supported by morphological data that killifish from Bangka Island is *A. panchax* (Mustikasari et al. 2020b).

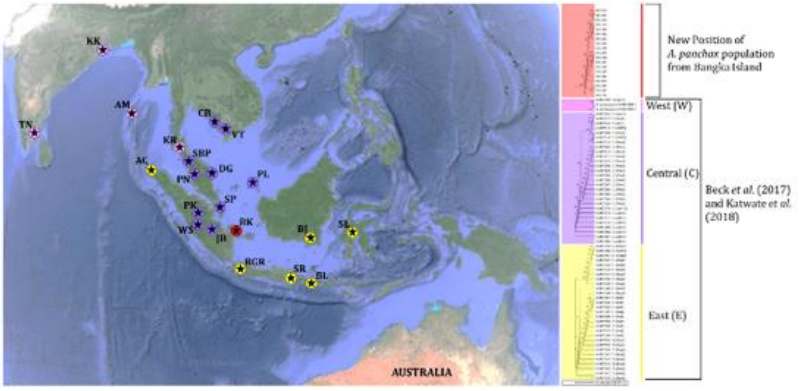

Figure 4. New phylogeographical map for *A. panchax*, particularly Bangka Island populations (red cycle) on global phylogeographic of blue panchax.

Several factors can impact an individual's genetic diversity and population genetic structure, such as climate or environmental change, natural boundaries, environmental variables, movement and migration, and human activities (Nater et al. 2013; Wang et al. 2020). For example, the persistence of climate change is determined by life history characters of organisms such as dispersal ability, generation period, reproductive ability, habitat specialization, organism interactions, genetic diversity, and habitat or migration corridors (Schierenbeck 2017).

The critical part was that the position of *A. panchax* populations from Bangka Island was different from other locations, but they were included as Sundaland region. It indicated that the biogeographic region of Sundaland (Borneo, Sumatra, the Malay Peninsula, Java, Palawan, and associated islands) is essential to understand evolution (Hinckley et al. 2021). As long as chronosequence exists, the ecological component may affect organism diversity. Ecological disturbance is necessary to maintain the dynamism and diversity of ecosystems. The disturbance history may be the primary driver that shapes patterns of genetic diversity in many natural populations through changes (Banks et al., 2013). Beck et al. (2017) explained that the significant mitochondrial clades of *A. Panchax* are consistent with this study. The basal dissimilarity of *A. panchax* mitochondrial ancestries was around 3.5 Mega-annum (Ma).

On the other hand, the subsequent dissimilarity timings of these clades occurred in the early Pleistocene (~2.6 Ma). Ceaseless phylogeographic investigation showed a reasonable west-east dispersal followed by quick radiation across Southeast Asia. Salles et al. (2021) recreate scene evolution, sedimentation, and Sundaland flooding history under tectonic, eustatic, and precipitation constraining conditions. Furthermore, they link Sundaland's flooding history to tectonic and sea-level conditions using three external driving instruments of rainfalls, eustatic sea-level variations, and tectonics. Over the last one million years, these factors caused the Mekong, Johor, Siam, and East Sunda River to merge. Additionally, it causes Bangka Island to be separated from the mainland of Sumatra Island, Malay Peninsula, Java, and Kalimantan, about 400 ka.

Founder effects or events can impact stochasticity in species' genetic population structure at the regional scale (Haileselasie et al. 2018). Even though their populations have decreased in genetic diversity after the founder event, specific individuals that establish well in new places appear to be destined to extinction. The limit of life forms to adjust to new environments can rely upon the organism's capacity of reacting to regular determination, which is dictated by the genetic pattern of the founder populations (Lee 2002; Dlugosch and Parker 2008; Kaňuch et al. 2014). This condition can impact genetic variation in natural populations, mutation, and genetic drift (Star and Spencer 2013). Genetic drift and gene flow shape allele frequencies over time for an extended period (Chen et al. 2019). Ecological and geological factors

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may have contributed to the high degree of divergence in gene COI between *A. panchax* populations from Bangka Island and other sites. Subsequently, *A. panchax* sequences from all clades were also analyzed, with a genetic distance of 0.17% - 110.45%. All intra-species from Bangka Island, East clade, and Central clade populations showed a genetic distance value < 0.05 (Table 2). As a result, a clear genetic gap between 1.42% and 103.87% separated the Bangka Island population from others (Figure 5).

Table 2. The genetic distances between species of *Aplocheilus* on each clade. Values in bold were intra-species distances of *Aplocheilus*

***Aplocheilus panchax* Populations**	**Genetic Distance (%)**			
	[1]	**[2]**	**[3]**	**[4]**
Bangka Island [1]	**0.17 - 1.42**			
East Clade [2]	0.62 - 104.02	**0.17 - 1.41**		
Central Clade [3]	0.44 - 103. 87	0.00 - 1.40	**0.17 - 0.35**	
West Clade [4]	0.027 - 110.45	0. 22 - 7.37	0.22 - 6.98	**0.21* - 4.49****

Note: *) there was indication a differences *A. panchax* and *A. andamanicus* as well as Katwate et al. (2018)

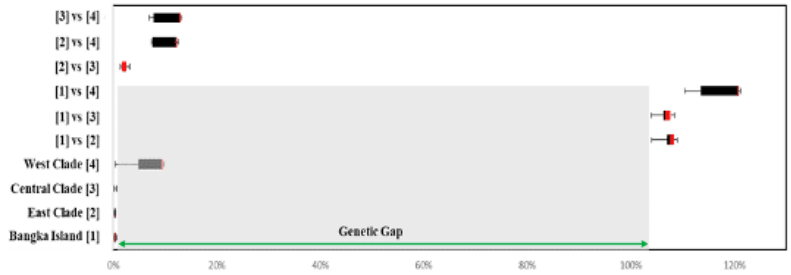

Figure 5. Intra- and inter-species genetic distances for the *A. panchax* populations from Bangka Island and other clades. There was a clear genetic gap, spanning from 1.42% to 103.87%, between the maximum intra-species and minimum inter-species distance that indicates the *A. panchax* populations from Bangka Island were genetically distinct from each other clade. Black lines within the boxes showed the medians, and the red boxes indicated the 75th quartiles

The genetic distances were lower than 2% (0.02), indicating that *A. panchax* populations from Bangka Island did not indicate cryptic species. However, the phylogenetic showed that *A. panchax* populations from Bangka Island differed from others. Intraspecific genetic distances in some species are higher than 2% (Thu et al. 2019) or above 3% (Nascimento et al. 2016), indicating the existence of cryptic diversity within these fishes. The term "cryptic species" has lately replaced the term "siblings" for taxa of this type (Korshunova et al. 2019). Sibling refers to two or more distinct individuals classified as a single species under one or the same scientific name (Bickford et al. 2007; Xiao et al. 2010; Karanovic et al. 2016). These species cannot be confidently separated based on their morphology, yet they were genetically distinct (Boluda et al. 2016; De Oliveira et al. 2017; Faulwetter et al. 2017). The cryptic species were delineated as individuals in the same geographic area but exhibited significant molecular phylogenetic contrasts. However, they are not recognized morphologically and ethologically (Hosoishi and Ogata 2019; Cerca et al. 2020).

The seventy-nine (79) sequences that were analyzed by DNAsp v.5 showed genetic variability of 0.22 for nucleotide diversity (π), 0.895 for Haplotype diversity (Hd), 68.028 for Fu's Fs test, 2.00 ($P < 0.02$) for Fu and Li's D' test, and 2.365 ($P < 0.02$) for Fu and Li's test. There were 28 haplotypes of these specimens among the COI sequences (n = 79) built into a haplotype network (Figure 6). All of the sequences analyzed showed that the haplotype network has a star-shaped topology.

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Table 3. The haplotype of *Aplocheilus panchax* on each clade

Haplotype(s)	Specimen(s)
Hap_1: 5	[BK_A01 BK_A02 BK_E01 BK_E02 BK_G03]
Hap_2: 1	[BK_B01]
Hap_3: 1	[BK_B02]
Hap_4: 1	[BK_C01]
Hap_5: 1	[BK_C02]
Hap_6: 1	[BK_D01]
Hap_7: 1	[BK_D02]
Hap_8: 1	[BK_F01]
Hap_9: 1	[BK_F02]
Hap_10: 1	[BK_G01]
Hap_11: 1	[BK_G04]
Hap_12: 1	[BK_K01]
Hap_13: 1	[BK_K02]
Hap_14: 1	[BK_K03]
Hap_15: 1	[BK_K04]
Hap_16: 9	[KJ957618.1 KJ957549.1 KJ957550.1 KJ957551.1 KJ957552.1 KJ957553.1 KJ957607.1 KJ957591.1 KJ957592.1]
Hap_17: 4	[KJ957540.1 KJ957542.1 KJ957544.1 KJ957545.1]
Hap_18: 2	[KJ957541.1 KJ957543.1]
Hap_19: 10	[KJ957546.1 KJ957547.1 KJ957548.1 KJ957554.1 KJ957555.1 KJ957556.1 KJ957557.1 KJ957558.1 KJ957559.1 KJ957560.1]
Hap_20: 4	[KJ957561.1 KJ957562.1 KJ957563.1 KJ957564.1]
Hap_21: 11	[KJ957565.1 KJ957566.1 KJ957567.1 KJ957568.1 KJ957569.1 KJ957570.1 KJ957571.1 KJ957572.1 KJ957573.1 KJ957616.1 KJ957617.1]
Hap_22: 13	[KJ957582.1 KJ957583.1 KJ957584.1 KJ957585.1 KJ957586.1 KJ957587.1 KJ957589.1 KJ957590.1 KJ957608.1 KJ957609.1 KJ957610.1 KJ957611.1 KJ957612.1]
Hap_23: 2	[KJ957588.1 KJ957613.1]
Hap_24: 1	[KJ957614.1]
Hap_25: 1	[KJ957615.1]
Hap_26: 1	[KJ957593.1]
Hap_27: 1	[*A. andamanicus* MH813789.1]
Hap_28: 1	[*A. andamanicus* MH813790.1]

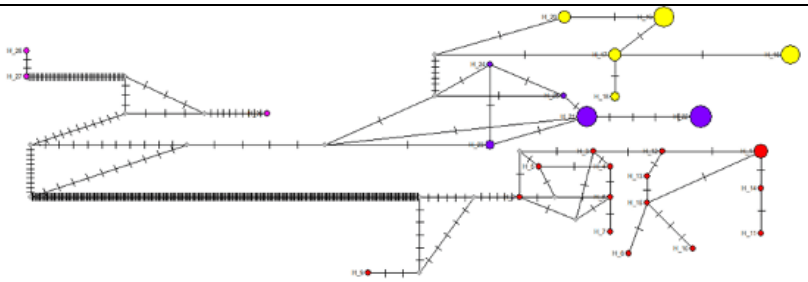

Figure 6. Median-joining network based on COI sequences for the *A. panchax* populations of Bangka Island and other locations from Beck et al. (2017) and Katwate et al. (2018). Numbers correspond to haplotype. Crossing-line indicated mutated positions. Different colors showed the collection sites: red (Bangka Island clade), yellow (East clade), violet (Central clade), and pink (West clade), while grey (median vectors). Colored-circles size was proportional to haplotype frequency (see Table 3).

Hap_1 to Hap_15 showed haplotype of *A. panchax* populations from Bangka Island, while Hap_16 to Hap_28 were from Beck et al. (2017) and Katwate et al. (2018). Current approaches to biodiversity research focus primarily on ecosystems, environmental communities, geographic regions, and species (Coates et al. 2018). The ecological factors such as over-exploitation, pollution, habitat destruction, and climate change can substantially impact intra-population genetic variation (Liu et al. 2013; Martinez et al. 2018). Recent studies have shown a strong linkage between environmental pressures and biodiversity levels, including genes, species, populations, and communities. The relationships between genetic diversity, polymorphism, and ecological stress have been evidenced in natural populations. The biochemical and molecular approaches investigated the correlation between the genetic structure of populations and the environmental characteristics (Cimmaruta et al. 2003; Markert et al. 2010; Schierenbeck 2017; Hu et al. 2020).

The water characteristic of tin mining pits as one of the habitats for *A. panchax* has an acidic pH and heavy metals

contamination (Kurniawan et al. 2019; Kurniawan 2020). A recent study proved that the waters in abandoned tin mining pits are contaminated by heavy metals such as As, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, Sn, Ta, Te, Th, V, and Zn. Heavy metal presence corresponded with pH characteristics (Kurniawan 2020). Therefore, the pH value, acidic pH, specifically acidic mine drainage (AMD) due to the oxidation process of sulfide minerals and potentially acidic formation, is a significant indicator of abandoned post-mining habitats. (PAF) (Tan et al. 2007; Çelebi and Öncel 2016). These conditions cause organisms to adapt to the extreme environment since *A. panchax* was grouped as extremophile fishes (Riesch et al. 2015; Kurniawan and Mustikasari 2021). The environmental factor from Bangka Island as a tin producer may also contribute to the genetic diversity of A. panchax.

Previous studies investigated the correlation between the water quality of abandoned tin mining waters with the presence and morphological characteristics of *A. panchax*. The results showed that *A. panchax* was found in pits with a pH value of 3.81-3.84 and dissolved oxygen (DO) between 5.33 and 5.63. The change of chronosequence's pH impacted the other changes such as DO, BOD, C-organic, total nitrogen, total phosphate, and others (Kurniawan et al. 2019). The presences and phenotypic characters were correlated with the environmental factors, especially pH and heavy metals (Mustikasari et al. 2020a, b). Therefore, these factors strongly contributed to the polymorphism of *A. panchax* populations from Bangka Island.

Genetic diversity is considered an internal contributing element in the susceptibility of organisms to heavy metals-related poison or toxicity levels. The variety in various genes, directly or indirectly included in the metabolism of weighty metals, has been researched by specific studies. For example, metallothioneins (MTs) are proteins that detoxify heavy metals because of a few gene varieties of genomic sequences (Joneidi et al. 2019). Metallothioneins are small cysteine-rich proteins that play significant roles in metal homeostasis and protection for heavy metal toxicity, DNA defect, and oxidative conditions (Si and Lang 2018), cellular processes, cell growth regulation, and well as proliferation and DNA repair (Grennan 2011).

The contribution of MTs with various cell or organelles processes has gotten much consideration while their association with the mitochondria functions has been inadequate. Furthermore, it increases the duration of malfunctioning mitochondrial cells by protecting productive components from the damage caused by reactive oxygen species (ROS) and limiting apoptosis. MTs are also involved in mitochondrial infection, including redox balance, metal homeostasis, enzyme, and transcription factor regulation (Lindique et al. 2010; Kurniawan and Mustikasari 2021). The requirements for obtaining metal specificity and specific novel capacity may drive their enhancement. MTs further enhanced the capability of metal detoxification under ecologically sensitive settings (Nam and Kim 2017). The relationship with mitochondria indicated an extreme environment as in abandoned tin mining waters of Bangka Island to genetic diversity, especially the COI gene of *A. panchax*. The heavy metals contamination and acidic pH in the habitat can cause genetic variations in mitochondrial genes, such as the COI gene. Moreover, heavy metals can reduce genetic variability within natural populations and cause genetic erosion (Ungherese et al. 2010). The evolution chronosequence of Bangka Island and the entire Sundaland may be attributed to the divergence of COI gene changes to diversify *A. panchax* genes.

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REFERENCES

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Banks SC, Cary GJ, Smith AL, Davies ID, Driscoll DA, Gill AM, ..., Peakall R. 2013. How does ecological disturbance influence genetic diversity?. Trends in Ecology & Evolution 28(11): 670-679.

Beck SV, Carvalho GR, Barlow A, Rüber L, Tan HH, Nugroho E, Wowor D, Nor SAM, Herder F, Muchlisin ZA, De Bruyn, M. 2017. Plio-Pleistocene phylogeography of the Southeast Asian blue panchax killifish, *Aplocheilus panchax*. PloS one 12(7): e0179557.

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22(3): 148-155.

Bobo-Pinilla J, Salmerón-Sánchez E, Mendoza-Fernández AJ, Mota JF, Peñas J. 2021. Conservation and phylogeography of plants: from the mediterranean to the rest of the world. Diversity 14(78): 1-23.

Bolotov IN, Konopleva ES, Vikhrev IV, Gofarov MY, Lopes-Lima M, Bogan AE, ..., Kondakov AV. 2020. New freshwater mussel taxa discoveries clarify biogeographic division of Southeast Asia. Scientific Reports 10(1): 1-22.

Boluda CG, Hawksworth DL, Divakar PK, Crespo A. 2016. Microchemical and molecular investigations reveal Pseudephebe species as cryptic with an environmentally modified morphology. The Lichenologist 48(5): 527-543.

Bragança PH, Amorim PF, Costa WJ. 2018. Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. Zoosystematics and Evolution 94(1): 137-145.
Buhay JE. 2009. "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. Journal of Crustacean Biology 29(1): 96-110.
Čekovská K, Šanda R, Eliášová K, Kovačić M, Zogaris S, Pappalardo AM, Soukupová T, Vukić J. 2020. Population genetic diversity of two marine gobies (Gobiiformes: Gobiidae) from the North-Eastern Atlantic and the Mediterranean Sea. Journal of Marine Science and Engineering 8(792): 1-18.
Çelebi EE, Öncel MS. 2016. Determination of acid forming potential of massive sulfide minerals and the tailings situated in lead/zinc mining district of Balya (NW Turkey). Journal of African Earth Sciences 124: 487-496.
Cerca J, Meyer C, Purschke G, Struck TH. 2020. Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (Stygocapitella; Annelida, Sedentaria). Molecular Phylogenetics and Evolution 143(2020): 106663.
Chen N, Juric I, Cosgrove EJ, Bowman R, Fitzpatrick JW, Schoech SJ, ..., Coop G. 2019. Allele frequency dynamics in a pedigreed natural population. Proceedings of the National Academy of Sciences 116(6): 2158-2164.
Chowdhury MM, Rahman AS, Nahar L, Rahman M, Al Reza H, Ahmed MS. 2016. Efficiency of different DNA extraction methods for fish tissues: a comparative analysis. IOSR Journal of Pharmacy and Biological Sciences 11(3): 11-15.
Cimmaruta R, Scialanca F, Luccioli F, Nascetti G. 2003. Genetic diversity and environmental stress in Italian populations of the cyprinodont fish Aphanius fasciatus. Oceanologica Acta 26(1): 101-110.
Coates DJ, Byrne M, Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Frontiers in Ecology and Evolution 6(165): 1-13.
Costa WJEM. 2013. Historical biogeography of aplocheiloid killifishes (Teleostei: Cyprinodontiformes). Vertebrate Zoology 63(2): 139-154.
Costa WJEM. 2016. Comparative morphology and classification of South American cynopoeciline killifishes (Cyprinodontiformes: Aplocheilidae), with notes on family-group names used for aplocheiloids. Vertebrate Zoology 66(2): 125-140.
De Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, den Bergh Gv, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, Von Rintelen T. 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Systematic Biology 63(6): 879-901.
De Oliveira DAS, Decraemer W, Moens T, Dos Santos GAP, Derycke S. 2017. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. BMC Evolutionary Biology 17(1): 1-17.
Dekar M, Sarong MA, Batubara AS, Muchlisin ZA. 2018. Ichthyofauna of Aceh River, Aceh Province, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 216, No. 1, p. 012024). IOP Publishing.
Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17(1): 431-449.
Dorey JB, Groom SV, Freedman EH, Matthews CS, Davies OK, Deans EJ, ..., Schwarz MP. 2020. Radiation of tropical island bees and the role of phylogenetic niche conservatism as an important driver of biodiversity. Proceedings of the Royal Society B 287(1925): 20200045.
Dwiyitno HS, Parmentier K, Van Keer C. 2018. Method comparison of DNA isolation and quantification for fish and seafood authenticity determination. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 13(3): 115-124.
Farmawati DA, Wirajana N, Yowani SC. 2015. Perbandingan kualitas DNA dengan menggunakan metode boom original dan boom modifikasi pada isolat *Mycobacterium tuberculosis*. KIMIA 9(1), 41-46.
Faulwetter S, Simboura N, Katsiaras N, Chatzigeorgiou G, Arvanitidis C. 2017. Polychaetes of Greece: an updated and annotated checklist. Biodiversity Data Journal 5: e20997
Fortes MD, Ooi JLS, Tan YM, Prathep A, Bujang JS, Yaakub SM. 2018. Seagrass in Southeast Asia: a review of status and knowledge gaps, and a road map for conservation. Botanica Marina 61(3): 269-288.
Furness AI, Reznick DN, Springer MS, Meredith RW. 2015. Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. Proceedings of the Royal Society B: Biological Sciences 282(1802): 20142189.
Grennan AK. 2011. Metallothioneins, a diverse protein family. Plant Physiology 155(4): 1750-1751.
Gupta A, Bhardwaj A, Sharma P, Pal Y. 2015. Mitochondrial DNA-a tool for phylogenetic and biodiversity search in equines. J Biodivers Endanger Species S1: S1.006.
Haileselasie TH, Mergeay J, Vanoverbeke J, Orsini L, De Meester L. 2018. Founder effects determine the genetic structure of the water flea Daphnia in Ethiopian reservoirs. Limnology and Oceanography 63(2): 915-926.
Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PD. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. Molecular Ecology Notes 6(4): 959-964.
Hinckley A, Camacho-Sanchez M, Ruedi M, Hawkins MT, Mullon M, Cornellas A, ..., Leonard JA. 2021. Evolutionary history of Sundaland shrews (Eulipotyphla: Soricidae: Crocidura) with a focus on Borneo. Zoological Journal of the Linnean Society 20(2021): 1-14.
Hosoishi S, Ogata K. 2019. Cryptic diversity in the widespread Asian ant Crematogaster rothneyi (Hymenoptera: Formicidae) inferred from morphological and genetic evidence. Zoological Studies 58(11): 1-15.
Hu ZM, Zhong KL, Weinberger F, Duan DL, Draisma SG, Serrao E. 2020. Linking ecology to genetics to better understand adaptation and evolution: a review in marine macrophytes. Frontiers in Marine Science 7: 545102.
Hughes AC. 2017. Understanding the drivers of S outheast A sian biodiversity loss. Ecosphere 8(1): e01624.
Ivanova NV, Zemlak TS, Hanner RH, Hebert PD. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes 7(4): 544-548.
Joneidi Z, Mortazavi Y, Memari F, Roointan A, Chahardouli B, Rostami S. 2019. The impact of genetic variation on metabolism of heavy metals: Genetic predisposition?. Biomedicine & Pharmacotherapy 113: 108642.
Kañuch P, Berggren Å, Cassel-Lundhagen A. 2014. Genetic diversity of a successful colonizer: isolated populations of *Metrioptera roeselii* regain variation at an unusually rapid rate. Ecology and Evolution 4(7): 1117-1126.
Karanovic T, Djurakic M, Eberhard SM. 2016. Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. Systematic Biology 65(2): 304-327.
Katwate U, Kumkar P, Britz R, Raghavan R, Dahanukar N. 2018. The identity *of Aplocheilus andamanicus* (Köhler, 1906) (Teleostei: Cyprinodontiformes), an endemic Killifish from the Andaman Islands, with notes on Odontopsis armata van Hasselt. Zootaxa 4382(1): 159-174.
Korshunova T, Picton B, Furfaro G, Mariottini P, Pontes M, Prkić J, ..., Martynov A. 2019. Multilevel fine-scale diversity challenges the 'cryptic species' concept. Scientific Reports 9(1): 1-23.
Kuffel A, Gray A, Daeid NN. 2021. Impact of metal ions on PCR inhibition and RT-PCR efficiency. International Journal of Legal Medicine 135(1): 63-72.
Kurniawan A, Mustikasari D. 2021. Review tentang kemampuan ikan ekstremofil untuk hidup di perairan asam dan terkontaminasi logam berat pascapenambangan timah. Jurnal Ilmu Lingkungan 19(3): 541-554.
Kurniawan A, Oedjijono O, Tamad T, Sulaeman U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka Regency, Indonesia. Indonesian Journal of Chemistry 19(1): 254-261.

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Kurniawan A, Prasetiyono E, Syaputra D. 2020. Analisis korelasi parameter kualitas perairan kolong pascatambang timah dengan umur berbeda. Samakia: Jurnal Ilmu Perikanan 11(2): 91-100.
Kurniawan A. 2020. The metal oxides of abandoned tin mining pit waters as an indicator for bacterial diversity. AACL Bioflux 13(5): 2982-2992.
Lee CE. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17(8): 386-391.
Lindeque JZ, Levanets O, Louw R, Van Der Westhuizen FH. 2010. The involvement of metallothioneins in mitochondrial function and disease. Current Protein and Peptide Science 11(4): 292-309.
Liu Y, Webber S, Bowgen K, Schmaltz L, Bradley K, Halvarsson P, Abdelgadir M, Griesser M. 2013. Environmental factors influence both abundance and genetic diversity in a widespread bird species. Ecology and Evolution 3(14): 4683-4695.
Lone AR, Thakur SS, Tiwari N, Sokefun OB, Yadav S. 2021. Disentangling earthworm taxonomic stumbling blocks using molecular markers. Journal of Threatened Taxa 13(11): 19566-19579.
Markert JA, Champlin DM, Gutjahr-Gobell R, Grear JS, Kuhn A, McGreevy TJ, Roth A, Bagley MJ, Nacci DE. 2010. Population genetic diversity and fitness in multiple environments. BMC Evolutionary Biology 10(1): 1-13.
Martinez AS, Willoughby JR, Christie MR. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecology and Evolution 8(23): 12022-12031.
Mustikasari D, Nuryanto A, Suryaningsih S. 2020a. The presence of blue panchax (*Aplocheilus panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of different age. Aquaculture, Aquarium, Conservation & Legislation 13(5): 2538-2550.
Mustikasari D, Suryaningsih S, Nuryanto A. 2020b. Morphological variation of blue panchax (*Aplocheilus panchax*) lives in different habitat assessed using truss morphometric. Biosaintifika: Journal of Biology & Biology Education 12(3): 399-407.
Nam YK, Kim EJ. 2017. Diversification and domain evolution of molluskan metallothioneins: a mini review. Fisheries and Aquatic Sciences 20(1): 1-18.
Nascimento MHS, Almeida MS, Veira MNS, Filho DL, Lima RC, Barros MC, Fraga EC. 2016. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. Genetics and Molecular Research 15(3): 1-11.
Nater A, Arora N, Greminger MP, van Schaik CP, Singleton I, Wich SA, Fredrikson G, Perwitasari D, Pamungkas J, Krützen M. 2013. Marked population structure and recent migration in the critically endangered Sumatran orangutan (*Pongo abelii*). Journal of Heredity 104(1): 2-13.
Parenti LR, Hartel KE. 2011. Osteology identifies *Fundulus capensis* Garman, 1895 as a killifish in the family Fundulidae (Atherinomorpha: Cyprinodontiformes). Copeia 2011(2): 242-250.
Pohl M, Milvertz FC, Meyer A, Vences M. 2015. Multigene phylogeny of cyprinodontiform fishes suggests continental radiations and a rogue taxon position of Pantanodon. Vertebrate Zoology 65(1): 37-44.
Pratomo YW, Zahida F, Yuda P. 2021. Perbandingan Metode isolasi DNA sebagai templat PCR untuk identifikasi jenis kelamin Cerek Jawa (*Charadriius javanicus*) secara molekuler menggunakan Primer 2550F/2718R. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati 6(2): 78-86.
Riesch R, Tobler M, Plath M. 2015. Extremophile fishes. Ecology, Evolution, and Physiology of Teleosts in Extreme Environments: Springer.
Rius M, Turon X. 2020. Phylogeography and the description of geographic patterns in invasion genomics. Frontiers in Ecology and Evolution 8(439): 595711.
Rizko N, Kusumaningrum HP, Ferniah RS, Pujiyanto S, Erfianti T, Mawarni SN, Rahayu HT, Khairunnisa D. 2020. Isolasi DNA daun Jeruk Bali Merah (*Citrus maxima* Merr.) dengan modifikasi Metode Doyle and Doyle. Berkala Bioteknologi 3(2): 1-7.
Rosilawati ML, Sudarmono P, Ibrahim F. 2002. Sensitivitas metode PCR (*Polymerase Chain Reaction*) dalam mendeteksi isolat klinis *Mycobacterium tuberculosis*. Jurnal Kedokteran Trisakti 21(1): 7-14.
Ruchi W, Putri DH, Anhar A, Farma SA. 2018. Comparison of three different DNA isolation methods to degradate the Trichoderma fungi cell wall. Bioscience 2(1): 50-59.
Salles T, Mallard C, Husson L, Zahirovic S, Sarr AC, Sepulchre P. 2021. Quaternary landscape dynamics boosted species dispersal across Southeast Asia. Communications Earth & Environment 2(1): 1-12.
Schierenbeck KA. 2017. Population-level genetic variation and climate change in a biodiversity hotspot. Annals of Botany 119(2): 215-228.
Setiaputri AA, Barokah GR, Sahaba MAB, Arbajayanti RD, Fabella N, Pertiwi RM, Nurilmala M, Nugraha R, Abdullah A. 2020. Perbandingan metode isolasi DNA pada produk perikanan segar dan olahan. Jurnal Pengolahan Hasil Perikanan Indonesia 23(3): 447-458.
Si M, Lang J. 2018. The roles of metallothioneins in carcinogenesis. Journal of Hematology & Oncology 11(1): 1-20.
Star B, Spencer HG. 2013. Effects of genetic drift and gene flow on the selective maintenance of genetic variation. Genetics 194(1): 235-244.
Tan GL, Shu WS, Hallberg KB, Li F, Lan CY, Huang LN. 2007. Cultivation-dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China. FEMS Microbiology Ecology 59(1): 118-126.
Thu PT, Huang WC, Chou TK, Van Quan N, Van Chien P, Li F, Shao KT, Liao TY. 2019. DNA barcoding of coastal ray-finned fishes in Vietnam. PloS one 14(9): e0222631.
Ungherese G, Mengoni A, Somigli S, Baroni D, Focardi S, Ugolini A. 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper Talitrus saltator (Montagu) (Crustacea, Amphipoda). Environmental Pollution 158(5): 1638-1643.
Von Rintelen K, Arida E, Häuser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Research Ideas and Outcomes 3: e20860.
Wang XY, Wang MM, Chen C, Wang XQ. 2020. Genetic variation and phylogeographic structure of Spodoptera exigua in western China based on mitochondrial DNA and microsatellite markers. PloS one 15(5): e0233133.
Xiao JH, Wang NX, Li YW, Murphy RW, Wan DG, Niu LM, Hu HY, Fu YG, Huang DW. 2010. Molecular approaches to identify cryptic species and polymorphic species within a complex community of fig wasps. PLoS one 5(11): e15067.
Yang CH, Wu KC, Chuang LY, Chang HW. 2019. Decision theory-based COI-SNP tagging approach for 126 scombriformes species tagging. Frontiers in Genetics 10: 259.

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Phylogeography of the blue panchax, *Aplocheilus panchax*, in Indonesia, with special focus on the Bangka Island population

Abstract. Previous study divided Blue panchax, *Aplocheilus panchax* into three different clades, namely West (W), Central (C), and East (E) clades. Blue panchax populations from Indonesia were belong to Central and East clades. However, that study did not include blue panchax samples from pits with harsh conditions in Bangka Island. Therefore, this study aimed to assess the phylogeography of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. The results showed that genetic distance within Bangka Island population was less than 2%, while genetic distances between Bangka population and other populations were ranged from 103.87% to 122.10%. There was also a clear genetic gap between the Bangka and other populations, with the minimum gap was 101.94%. Furthermore, the seventy-nine sequences analyzed resulted 28 haplotypes with genetic variability of 0.221 for nucleotide diversity (π), 0.923 for Haplotype diversity (Hd), 68.028 for Fu's Fs-test, 2.00 ($P < 0.02$) for Fu and Li's D-test, and 2.365 ($P < 0.02$) for Fu and Li's F-test; and 1.927 for Tajima's D test ($0.10 > P > 0.05$). The Bangka population of *Aplocheilus panchax* established a distinct clade from the Western (W), Eastern (E), and Central (C) clades. Molecular data established that the population on Bangka Island is a novel clade for Indonesia and a global blue panchax phylogeographic.

Keywords: abandoned tin mining pits, genetic distance, haplotype, killifish, new clade position

Running title: phylogeographic position of *Aplocheilus panchax*

INTRODUCTION

Phylogeography analyzes and understands organism diversity and biogeography (Bobo-Pinilla et al., 2021). Furthermore, it is a study of geographical distributions of closely related genetic lineages or geographic ordination of genotypes (Rius and Turon, 2020). Therefore, phylogeographic research is essential to evaluate the geographical distribution of these genetic lineages and their pattern, as well as elaborate ecology factors and organism biodiversity (Lone et al., 2021).

The blue panchax (*Aplocheilus panchax* Hamilton, 1882) is an endemic species to the Oriental region (Costa 2013; Costa 2016; Beck et al. 2017), widely distributed across the Indo-Malayan Islands, including Indonesia, the Indo-China region, and India (Dekar et al. 2018; Bolotov et al. 2020). *Aplocheilus panchax* is a fish from Genus *Aplocheilus*, Family Aplocheilidae, Suborder Aplocheiloidei, Order Cyprinodontiformes, and Class Actinopterygii (Parenti and Hartel 2011; Furness et al. 2015). Fishes belonging to Order Cyprinodontiformes are also known as *Aplocheiloid killifishes* or *livebearer*s (Pohl et al., 2015; Braganca et al., 2018).

Indonesia is one of the world's biodiversity hotspots with many different habitats and a highly complicated geological history (Bruyn et al., *2014;* von Rintelen et al., 2017). Generally, Southeast Asia's complex climatic and geological history caused this region to have high biodiversity (Beck et al., 2017; Fortes et al., 2018). Biogeography, geology, climate, and ecology of Southeast Asia have led to megadiverse organisms' evolution. Additionally, the region is home to several endemic and ecologically well-adapted species (Hughes 2017; von Rintelen et al. 2017). The *Aplocheilus panchax* can be used to understand further how climatic changes and sea-level fluctuations have influenced the species' distribution within this region. Specifically, sea-level variations result from glacial cycles that continued throughout the Pleistocene, interfering with and restricting the network of all populations. These examples show proof of isolation in palaeodrainage basins (Beck et al., 2017).

Previous phylogeographic study using the COI gene placed worlds' *A. panchax* populations into three different clades; West (W), Central (C), and East (E) clades. The *A. panchax* populations from Indonesia were divided into Central and East clades. Central clade consisted of Pekanbaru, Jambi, and West Sumatera populations. East clade was formed by *A. panchax* populations from Java, Bali, Kalimantan, and Sulawesi islands (Beck et al. 2017). Nevertheless, the study by Beck et al. (2017) did not include *A. panchax* samples from Bangka Island. Bangka Island, the biggest tin producer in Indonesia, has unique waters like a lake or pit, known as kolong, formed and abandoned after tin mining activity. This

water has low pH (acid waters), low nutrients, minimum dissolved oxygen (DO), and heavy metals (Ashraf et al. 2011, 2012a, 2012b; Hashim et al. 2018; Koki et al. 2019; Kurniawan 2020). However, *A. panchax*, locally known as ikan Kepala Timah, can live in this extreme habitat (Kuniawan et al. 2019, Kurniawan et al. 2020; Mustikasari et al. 2020a). The genus *Aplocheilus*, which includes *A. panchax*, was classified as an extremophile fish due to its ability to survive in harsh environmental circumstances (Riesch et al., 2015; Kurniawan and Mustikasari 2021). Mustikasari et al. (2020a, b) explored the presence and morphological variety of blue panchax (*A. panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of various ages.

Island biogeography investigates how the richness of the ecosystems and the complexity of the biodiversity may cause speciation. The adaptive radiation, speciation, climate cycles, and topographical complexity can shape island biodiversity (Dorey et al. 2020). Phylogenetic and phylogeographic analyses based on mitochondrial DNA (mt-DNA) are used in island biodiversity exploration. In addition, mt-DNA possesses some characteristics, including cell quantity, genome size, haploid, maternal inheritance, and extremely low probability of paternal leakage, mutation rate, and change mainly caused the mutation. These features make mt-DNA a useful and one of the most often used markers in molecular analysis. The marker has been frequently used to study genetic diversity, population organization, phylogeography, and organism evolution (Gupta et al. 2015).

This study aimed to assess the phylogeography of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. This is the first time the populations from Bangka Island have been genetically analyzed using the cytochrome oxidase I (COI) gene and compared to another on the global distribution of blue panchax. Furthermore, the presence and genetic profile of *A. panchax* can be a model of extremophile fish study. Therefore, it is used as a bioindicator for harsh habitats since the global distribution, ecological and genetic evolution with the Bangka Island population corresponds to others in Indonesian waters.

MATERIALS AND METHODS

Study area

The study was conducted in Pangkalpinang City and Bangka Regency of Bangka Belitung Archipelago Province, Indonesia. Fish samples were collected from abandoned tin mining lakes (pits) of different ages and the Limbung River. Station A and B (< 5 years old), Station C and D (5 -15 years), Station E and F (15 - 25 years), Station G (25 - 50 years), Station H (50 - 100 years), Station I and J (> 100 years), and Limbung River Stream of Bangka Regency as Station K such as shown in Figure 1. These years of research stations indicated the chronosequence of the abandoned tin mining pits were taken place there. There were nothings put in the habitats, due to the chronosequence only explain about a succession that happened naturally. In addition, they were related to our previous studies about their characteristics of water quality and the presence of *Aplocheilus panchax* in the abandoned tin mining pits based on the difference of time (Mustikasari et al. 2020a, b).

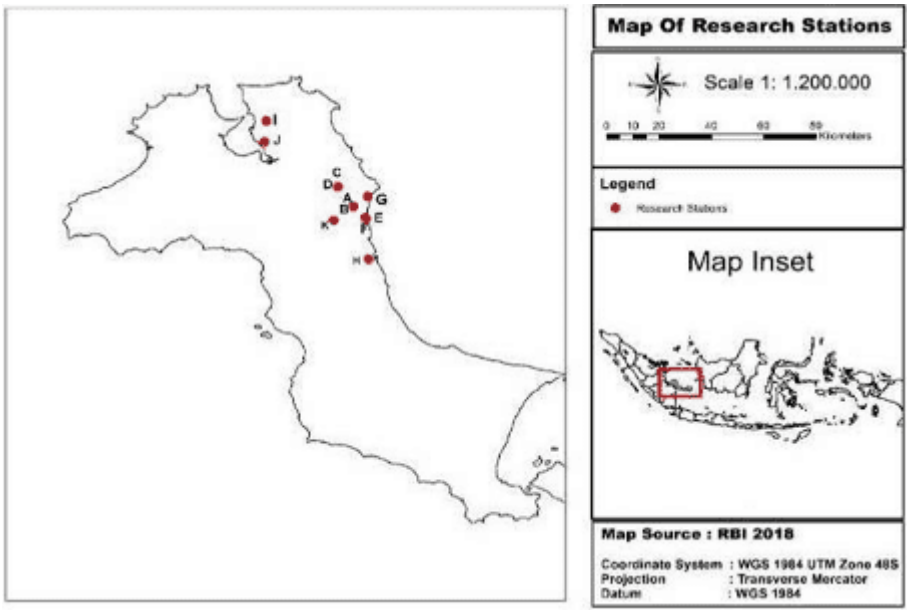

Figure 1. Map of research stations at Bangka Island, Indonesia (Mustikasari et al. 2020a, b)

Procedures

Samples preparation for molecular analysis

The twenty samples were collected at 09.00 am-1.00 pm from closed and open waters of abandoned tin mining lakes (pits) and waters of Limbung River Stream, Bangka Island, using nets with a mesh size of about 0.4 mm. We declared that collected fish as samples were handled in a good manner as explained as Bennett et al. (2016) which cite Canadian Council on Animal Care (2005) and American Fisheries Society (2014) about guidelines for the use of fishshes in research. They were handled with minimises pain, distress, suffering and unnecessary loss of external mucus or scales, minimum of the handling duration, to avoid unnecessary stress, and exposure time. We also gave attention to life-cycle events, such as aggregations of breeding fish and sensitive habitats should be avoided.

Furthermore, this study utilized a 2.0 ml cryotube with ethanol absolute to preserve each of the fish sample for molecular analysis. The pectoral fin about 2 mm from each dead sample of *A. pachax* was taken for the DNA isolation and molecular identification.

Molecular analysis

The genomic DNA extraction was analyzed with gSYNC™ DNA Extraction Kit (Geneaid, GS300). Nucleic acid (genomic DNA) concentration was measured using Nanodrop™ 2000/2000c spectrophotometers. Furthermore, molecular analysis was referred to Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika Science Indonesia, in 2021.

Polymerase chain reaction (PCR) amplification was conducted with (2x) MyTaq HS Red Mix (Bioline, BIO-25048) and KOD FX Neo (Toyobo, KFX-201). The components 1 x 25 µl PCR Master Mix were dd H2O 9.5 µl; MyTaq HS Red Mix, 2x 12.5 µl; 10 µM VF2_t1 0.5 µl; 10 µM Fish F2_t1 0.5 µl; 10 µM Fish R2_t1 0.5 µl; 10 µM Fish FR1d_t1 0.5 µl; and DNA Template 1 µl. Primer sequence of PCR amplification were VF2-t1 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAA GACATTGGCAC-3'; FR1d-t1 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3'; FishR2_t1 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3'; and FishF2_t1 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (Ivanova et al. 2007).

The predenaturation phase initiated the Polymerase Chain Reaction (PCR) cycling for 1 minute (95 °C). Subsequently, the actual PCR amplification was conducted for 35 cycles, denaturation process for 15 seconds (95 °C), annealing process for 15 minutes (50 °C), and extension process for 45 seconds (72 °C). The PCR products (1 µL) were assessed by electrophoresis with 1% TBE agarose with Marker 100bp DNA ladder (loaded 2 µL). Furthermore, the quality and length of the PCR products were analyzed by agarose gel electrophoresis. Bi-directional Sequencing conducted the sequencing step at 1st base Asia.

Data analysis

The contig sequences were obtained from reverse and forward sequences aligned with Program BioEdit. The phylogenetic study was carried out using MEGA XI (Tamura et al. 2021), utilizing the Maximum Parsimony (MP) statistical approach, and a bootstrap consensus tree was constructed using 1,000 replicates. The phylogenetic tree was constructed by comparing sequences of *A. panchax* from Bangka Island with some sequences of *A. panchax* from a previous study (Beck et al. 2017). The study conducted in India (Tamil Nadu and Kolkotta), Cambodia, Vietnam, Thailand (Krabi), Malaysia (Sungai Batu Pahat, Penang, Dungun), Singapore, and Indonesia (Aceh, Pekanbaru, Pulau Laut, West Sumatra, Jambi, Bogor, Surabaya, Banjarmasin, Bali, and Sulawesi) as shown in Figure 2 aim to investigate the position of *A. panchax* population from Bangka Island. Furthermore, it used a sequence of *A. andamanicus* (Katwate et al. 2018). The *A. warneri* (accession number KJ844713.1) was used as outgroup species (Pohl et al. 2015) for this phylogenetic analysis. Sequences metadata from Beck et al. (2017) and Katwate et al. (2018) from NCBI (National Center for Biotechnology Information) were utilized. DnaSP v5 was used to assess the haplotype (Hd) and nucleotide diversity (π), as well as to perform Fu and Li's F and Tajima's D neutrality tests (Čekovská et al. 2020). A phylogenetic network was constructed according to the median-joining method within the software Network 10. The Kimura 2 Parameter (K2P) genetic distance was calculated in the MEGA XI (Tamura et al. 2021). Genetic distances were utilized to estimate the genetic gap between Bangka Island and other clade populations. The lowest genetic distance was estimated by subtracting minimum genetic distance among populations by maximum genetic distance within Bangka Island population.

Figure 2. Sampling locations for *Aplocheilus panchax* over 20 areas, namely Tamil Nadu (TN), Kolkotta (KK), Andaman Island (AM) Cambodia (CB), Vietnam (VT), Krabi (KB), Sungai Batu Pahat (SBP), Aceh (AC), Penang (PN), Dungun (DG), Pulau Laut (PL), Singapore (SP), Pekanbaru (PK), West Sumatera (WS), Jambi (JB), Bogor (BG), Surabaya (SR), Banjarmasin (BJ), Bali (BL) and Sulawesi (SL). (Map was reconstructed from Beck et al. 2017 and Katwate et al. 2018).

RESULTS AND DISCUSSION

DNA quantity and quality

The successfulness of organisms identification methods based on genetic material, such as PCR, relies on the quantity and quality of nucleic acid, purification method, and PCR amplification process. Unfortunately, the low amount and quality of genetic material (DNA) and the appearance of inhibitors inhibit the PCR amplification efficiency (Chowdhury et al., 2016; Dwiyitno et al., 2018; Kuffel et al. 2021). The analysis of the quantity of sample' DNA by Nanodrop spectrophotometer resulted in DNA concentration (ratio $A_{260/280}$) for all samples between 1.65 and 1.99 (Table 1).

Table 1. Quantity of Sample DNA.

No	Sample Code*	$A_{260/280}$	No	Sample Code*	$A_{260/280}$
1	BK_A01	1.72	11	BK_F01	1.87
2	BK_A02	1.90	12	BK_F02	1.65
3	BK_B01	1.91	13	BK_G01	1.75
4	BK_B02	1.87	14	BK_G02	1.59
5	BK_C01	1.87	15	BK_G03	1.81
6	BK_C02	1.91	16	BK_G04	1.88
7	BK_D01	1.93	17	BK_K01	1.65
8	BK_D02	1.99	18	BK_K02	1.83
9	BK_E01	1.90	19	BK_K03	1.71
10	BK_E02	1.89	20	BK_K04	1.70

Note: *) sample' volume (30 µl)

The ratio of absorbance at 260 nm and 280 nm ($A_{260/280}$) is used to assess the purity of material genetic. The measurement of DNA concentration with Nanodrop was conducted at a wavelength of 260 nm. In comparison, the protein was measured at a wavelength of 280 nm with pure DNA having an absorbance ratio of 260/280 between 1.6 and 2.0 (Setiaputri et al. 2020), 1.7-2.0 (Ruchi et al. 2018), or 1.8-20 (Pratomo et al. 2021). The absorbance value below the low absorbance limit indicates the presence of polysaccharides, phenol, and protein contamination. Meanwhile, above the absorbance 2.0 indicates the presence of RNA contamination during the DNA isolation process (Rosilawati et al. 2002; Farmawati et al. 2015; Rizko et al. 2020). The success of a purification extraction and the quantity of DNA is highly dependent on the isolation of the resulting DNA. Therefore, the isolation process using commercial kits is safer from processing errors that cause contamination. However, studies on several specimens with various treatments stated that not all commercial kits can harvest DNA in high concentrations (Hajibabaei et al., 2006; Setiaputri et al., 2020).

The product of PCR also showed that the COI gene length of *A. panchax* populations from Bangka Island was about 700 bp. Meanwhile, the COI gene length for *A. panchax* was around 621 bp in other studies with accession numbers

KJ957593.1, KJ957617.1, and KJ957618.1 from Beck et al. (2017), and also accession number MG813789.1 (Sample *A. andamanicus*) from Katwate et al. (2018). The length showed that the COI gene from Bangka Island was the longest. The mitochondrial gene, namely COI, is commonly used for DNA barcoding, about 650 bp and these gene sequences are also used for ecological and evolutionary studies (Yang et al. 2019). The COI gene length differences of *A. panchax* populations indicate a diversity of *A. panchax* around the Oriental region. It plays a vital role in a global study to collect information about biodiversity and be a key in phylogenetic and phylogeographic analyses (Buhay 2009).

The phylogenetic tree and phylogeographic of *A. panchax* population from Bangka Island

The phylogenetic tree indicated that the ancestral relationship between the *A. panchax* from the distribution sequences was well supported by COI gene sequence analysis based on well-supported clades (>90% bootstrap values). The phylogenetic tree analysis showed *A. panchax* widespread throughout the Oriental region, including Indonesia as a part of Southeast Asia countries. However, the population from Bangka Island has different sequences from others. Bangka Island individuals formed a distinct clade from those previously described by Beck et al. (2017) and Katwate et al. (2018). (Figure 3).

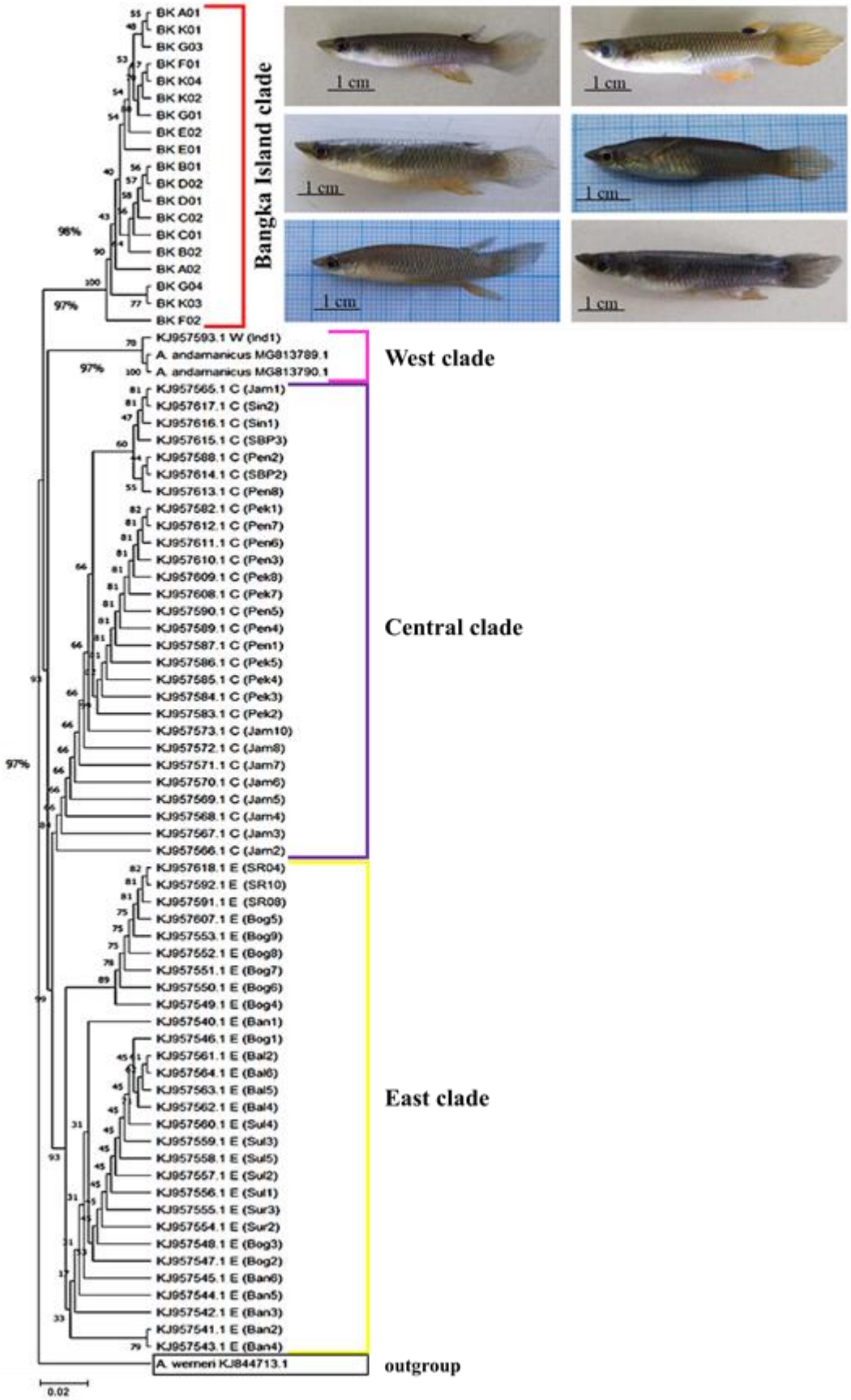

Figure 3. The phylogenetic position of *A. panchax* from Bangka Island is supported by Maximum Parsimony (MP) bootstrap values. Sequences of topotypes of populations from Bangka Island were in red line, while sample's sequences of Beck et al. (2017) and

Katwate et al. (2018) which were cited from the existing databases of COI genes, shown in the pink line (West clade), violet line (East clade), and yellow line (East clade), while *A. warneri* is outgroup.

Beck et al. (2017) revealed their research about Bayesian posterior probabilities that displayed for Western (W) clade, Eastern (E) clade, and Central (C) clade. We added a novel clade to previous phylogenetic tree (Beck et al. 2017; Katwate et al. 2018) by adding samples from Bangka Island (Figure 3). Therefore, the authors also recommended a new map reconstruction of global phylogeographic on blue panchax (*A. panchax*) gene distribution (Figure 4).

A previous study by Katwate et al. (2018) denied the presence of *A. panchax* in the Indo-Malay region. They provided morphological and molecular evidence and demonstrated that *A. andamanicus* and *A. armatus* distinct and valid killifish species in the Indo-Malay region. However, the present study supported Beck et al. (2017) by showing that the killifish from Indonesia, especially populations from Bangka Island, is *A. panchax*. The present result was supported by morphological data that killifish from Bangka Island is *A. panchax* (Mustikasari et al. 2020b).

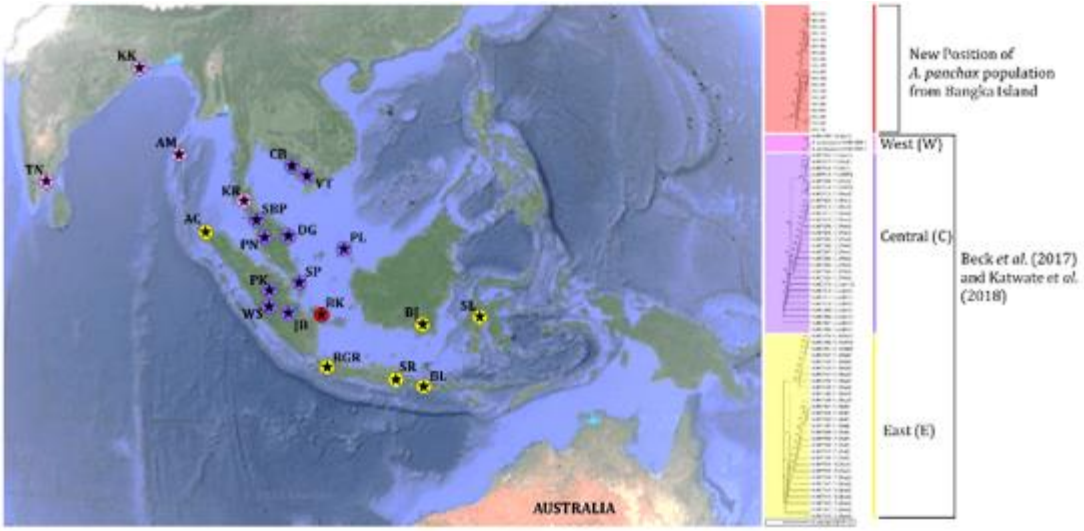

Figure 4. New phylogeographical map for *A. panchax*, particularly Bangka Island populations (red cycle) on global phylogeographic of blue panchax.

Several factors can impact an individual's genetic diversity and population genetic structure, such as climate or environmental change, natural boundaries, environmental variables, movement and migration, and human activities (Nater et al. 2013; Wang et al. 2020). For example, the persistence of climate change is determined by life history characters of organisms such as dispersal ability, generation period, reproductive ability, habitat specialization, organism interactions, genetic diversity, and habitat or migration corridors (Schierenbeck 2017).

The critical part was that the position of *A. panchax* populations from Bangka Island was different from other locations, but they were included as Sundaland region. It indicated that the biogeographic region of Sundaland (Borneo, Sumatra, the Malay Peninsula, Java, Palawan, and associated islands) is essential to understand evolution (Hinckley et al. 2022). As long as chronosequence exists, the ecological component may affect organism diversity. Ecological disturbance is necessary to maintain the dynamism and diversity of ecosystems. The disturbance history may be the primary driver that shapes patterns of genetic diversity in many natural populations through changes (Banks et al., 2013). Beck et al. (2017) explained that the significant mitochondrial clades of *A. Panchax* are consistent with this study. The basal dissimilarity of *A. panchax* mitochondrial ancestries was around 3.5 million years ago (Ma).

On the other hand, the subsequent dissimilarity timings of these clades occurred in the early Pleistocene (~2.6 Ma). Ceaseless phylogeographic investigation showed a reasonable west-east dispersal followed by quick radiation across Southeast Asia. Salles et al. (2021) recreate scene evolution, sedimentation, and Sundaland flooding history under tectonic, eustatic, and precipitation constraining conditions. Furthermore, they link Sundaland's flooding history to tectonic and sea-level conditions using three external driving instruments of rainfalls, eustatic sea-level variations, and tectonics. Over the last one million years, these factors caused the Mekong, Johor, Siam, and East Sunda River to merge. Additionally, it causes Bangka Island to be separated from the mainland of Sumatra Island, Malay Peninsula, Java, and Kalimantan, about 400 thousand years ago (ka).

Founder effects or events can impact stochasticity in species' genetic population structure at the regional scale (Haileselasie et al. 2018). Even though their populations have decreased in genetic diversity after the founder event, specific individuals that establish well in new places appear to be destined to extinction. The limit of life forms to adjust to new environments can rely upon the organism's capacity of reacting to regular determination, which is dictated by the

genetic pattern of the founder populations (Lee 2002; Dlugosch and Parker 2008; Kaňuch et al. 2014). This condition can impact genetic variation in natural populations, mutation, and genetic drift (Star and Spencer 2013). Genetic drift and gene flow shape allele frequencies over time for an extended period (Chen et al. 2019). Ecological and geological factors may have contributed to the high degree of divergence in gene COI between *A. panchax* populations from Bangka Island and other sites. Subsequently, *A. panchax* sequences from all clades were also analyzed, with a genetic distance of 0.00% to 1.93% for within Bangka Island population. Genetic distance between Bangka Island and other clade populations were ranged from 103.87% to 122.10%. Maximum intrapopulation genetic distances within Bangka Island, East clade, and Central clade populations was less than 2%, while within West clade populations had a maximum genetic distance larger than 2% (Table 2). As a result, there was a clear genetic gap between the Bangka Island population and other populations with minimum gap 101.94% (Bangka Island and Central Clade populations) and maximum gap value was 108.52% (Bangka Island and West clade populations). These values made Bangka Island populations were significantly different and separated from other clade populations (Figure 5).

Table 2. The genetic distances within and among clades of *Aplocheilus*

***Aplocheilus panchax* Populations**	**Genetic Distance (%)**			
	[1]	**[2]**	**[3]**	**[4]**
Bangka Island [1]	**0.00 - 1.93**			
East Clade [2]	104.02-110.32	**0.00 - 1.76**		
Central Clade [3]	103.87 - 108. 49	1.40 - 2.48	**0.00 - 1.05**	
West Clade [4]	110.45 - 112.10	7.37 - 12.54	6.98 - 13.22	**0.00* - 9.80****

Note: Values in bold were intra-population genetic distances of *Aplocheilus*, * there was indication a differences *A. panchax* and *A. andamanicus* as well as Katwate et al. (2018)

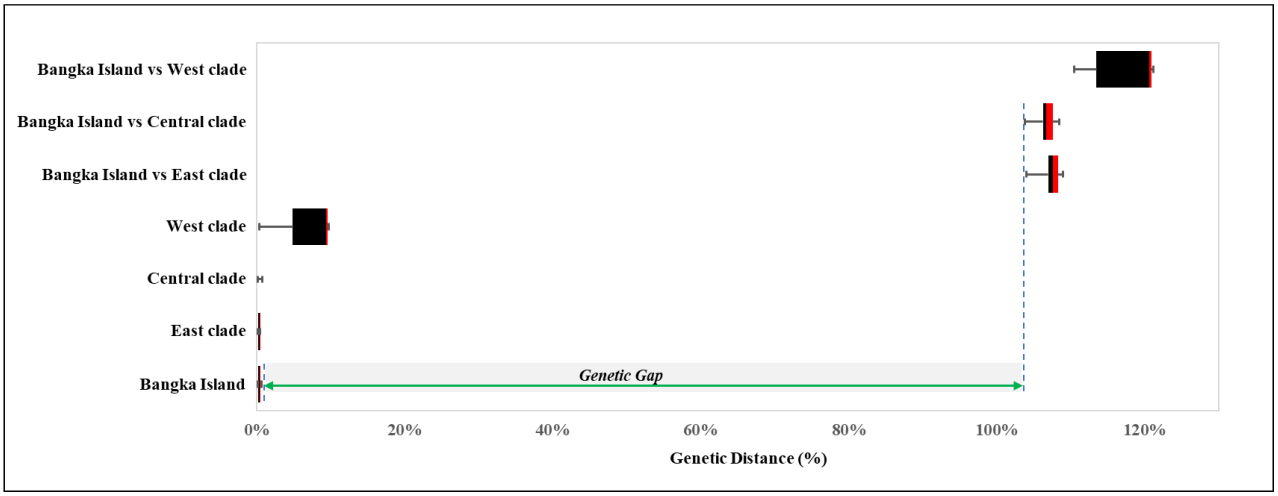

Figure 5. Intra- and inter-species genetic distances for the *A. panchax* populations from Bangka Island and other clades. There was a clear genetic gap, spanning from 1.42% to 103.87%, between the maximum within Bangka Island populations and minimum inter populations distance that indicates the *A. panchax* populations from Bangka Island were genetically distinct from each other clade. Black lines within the boxes showed the medians, and the red boxes indicated the 75th quartiles

The genetic distances were lower than 2% (0.02), indicating that *A. panchax* populations from Bangka Island did not indicate cryptic species. However, the phylogenetic showed that *A. panchax* populations from Bangka Island differed from others. Intraspecific genetic distances in some species are higher than 2% (Thu et al. 2019) or above 3% (Nascimento et al. 2016), indicating the existence of cryptic diversity within these fishes. The term "cryptic species" has lately replaced the term "siblings" for taxa of this type (Korshunova et al. 2019). Sibling refers to two or more distinct individuals classified as a single species under one or the same scientific name (Bickford et al. 2007; Xiao et al. 2010; Karanovic et al. 2016). These species cannot be confidently separated based on their morphology, yet they were genetically distinct (Boluda et al. 2016; De Oliveira et al. 2017; Faulwetter et al. 2017). The cryptic species were delineated as individuals in the same geographic area but exhibited significant molecular phylogenetic contrasts. However, they are not recognized morphologically and ethologically (Hosoishi and Ogata 2019; Cerca et al. 2020).

The seventy-nine (79) sequences that were analyzed by DNAsp v.5 showed genetic variability of 0.22 for nucleotide diversity (π), 0.895 for Haplotype diversity (Hd), 68.028 for Fu's Fs test, 2.00 ($P < 0.02$) for Fu and Li's D' test, and 2.365 ($P < 0.02$) for Fu and Li's test. There were 28 haplotypes of these specimens among the COI sequences ($n = 79$) built into a haplotype network (Figure 6). All of the sequences analyzed showed that the haplotype network has a star-

shaped topology.

Table 3. The haplotype of *Aplocheilus panchax* on each clade

Haplotype(s)	Specimen(s)
Hap_1: 5	[BK_A01 BK_A02 BK_E01 BK_E02 BK_G03]
Hap_2: 1	[BK_B01]
Hap_3: 1	[BK_B02]
Hap_4: 1	[BK_C01]
Hap_5: 1	[BK_C02]
Hap_6: 1	[BK_D01]
Hap_7: 1	[BK_D02]
Hap_8: 1	[BK_F01]
Hap_9: 1	[BK_F02]
Hap_10: 1	[BK_G01]
Hap_11: 1	[BK_G04]
Hap_12: 1	[BK_K01]
Hap_13: 1	[BK_K02]
Hap_14: 1	[BK_K03]
Hap_15: 1	[BK_K04]
Hap_16: 9	[KJ957618.1 KJ957549.1 KJ957550.1 KJ957551.1 KJ957552.1 KJ957553.1 KJ957607.1 KJ957591.1 KJ957592.1]
Hap_17: 4	[KJ957540.1 KJ957542.1 KJ957544.1 KJ957545.1]
Hap_18: 2	[KJ957541.1 KJ957543.1]
Hap_19: 10	[KJ957546.1 KJ957547.1 KJ957548.1 KJ957554.1 KJ957555.1 KJ957556.1 KJ957557.1 KJ957558.1 KJ957559.1 KJ957560.1]
Hap_20: 4	[KJ957561.1 KJ957562.1 KJ957563.1 KJ957564.1]
Hap_21: 11	[KJ957565.1 KJ957566.1 KJ957567.1 KJ957568.1 KJ957569.1 KJ957570.1 KJ957571.1 KJ957572.1 KJ957573.1 KJ957616.1 KJ957617.1]
Hap_22: 13	[KJ957582.1 KJ957583.1 KJ957584.1 KJ957585.1 KJ957586.1 KJ957587.1 KJ957589.1 KJ957590.1 KJ957608.1 KJ957609.1 KJ957610.1 KJ957611.1 KJ957612.1]
Hap_23: 2	[KJ957588.1 KJ957613.1]
Hap_24: 1	[KJ957614.1]
Hap_25: 1	[KJ957615.1]
Hap_26: 1	[KJ957593.1]
Hap_27: 1	[*A. andamanicus* MH813789.1]
Hap_28: 1	[*A. andamanicus* MH813790.1]

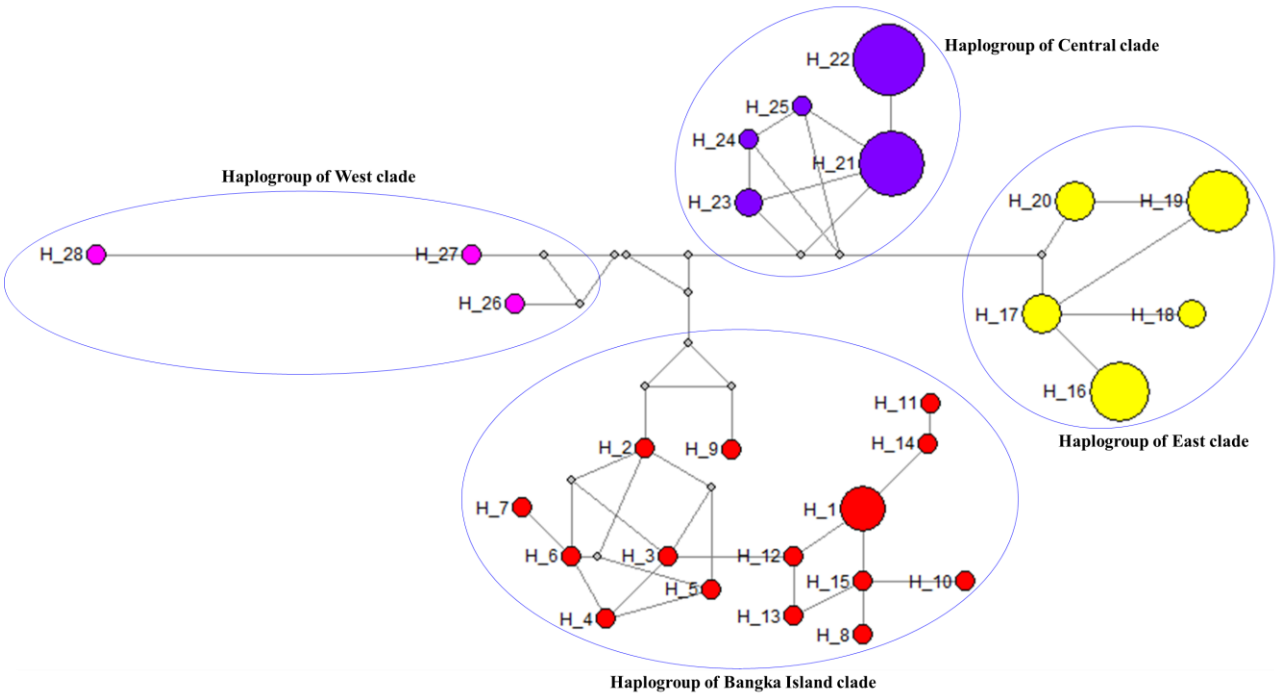

Figure 6. Median-joining network based on COI sequences indicating the *A. panchax* populations of Bangka Island (red haplogroup) formed distinct clade from other clades from Beck et al. (2017) and Katwate et al. (2018). Numbers correspond to haplotype. Crossing-

line indicated mutated positions. Different colors showed the collection sites: red (Bangka Island clade), yellow (East clade), violet (Central clade), and pink (West clade), while grey (median vectors) and blue circle (haplogroup). Colored-circles size was proportional to haplotype frequency (see Table 3).

Hap_1 to Hap_15 showed haplotype of *A. panchax* populations from Bangka Island, while Hap_16 to Hap_28 were from Beck et al. (2017) and Katwate et al. (2018). Current approaches to biodiversity research focus primarily on ecosystems, environmental communities, geographic regions, and species (Coates et al. 2018). The ecological factors such as over-exploitation, pollution, habitat destruction, and climate change can substantially impact intra-population genetic variation (Liu et al. 2013; Martinez et al. 2018). Recent studies have shown a strong linkage between environmental pressures and biodiversity levels, including genes, species, populations, and communities. The relationships between genetic diversity, polymorphism, and ecological stress have been evidenced in natural populations. The biochemical and molecular approaches investigated the correlation between the genetic structure of populations and the environmental characteristics (Cimmaruta et al. 2003; Markert et al. 2010; Schierenbeck 2017; Hu et al. 2020).

The water characteristic of tin mining pits as one of the habitats for *A. panchax* has an acidic pH and heavy metals contamination (Kurniawan et al. 2019; Kurniawan 2020). A recent study proved that the waters in abandoned tin mining pits are contaminated by heavy metals such as As, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, Sn, Ta, Te, Th, V, and Zn. Heavy metal presence corresponded with pH characteristics (Kurniawan 2020). Therefore, the pH value, acidic pH, specifically acidic mine drainage (AMD) due to the oxidation process of sulfide minerals and potentially acidic formation, is a significant indicator of abandoned post-mining habitats (PAF), is a significant indicator of abandoned post-mining habitats (Tan et al. 2007; Çelebi and Öncel 2016). These conditions cause organisms to adapt to the extreme environment since *A. panchax* was grouped as extremophile fishes (Riesch et al. 2015; Kurniawan and Mustikasari 2021). The environmental factor from Bangka Island as a tin producer may also contribute to the genetic diversity of A. panchax.

Previous studies investigated the correlation between the water quality of abandoned tin mining waters with the presence and morphological characteristics of *A. panchax.* The results showed that *A. panchax* was found in pits with a pH value of 3.81-3.84 and dissolved oxygen (DO) between 5.33 and 5.63. The change of chronosequence's pH impacted the other changes such as DO, BOD, C-organic, total nitrogen, total phosphate, and others (Kurniawan et al. 2019). The presences and phenotypic characters were correlated with the environmental factors, especially pH and heavy metals (Mustikasari et al. 2020a, b). Therefore, these factors strongly contributed to the polymorphism of *A. panchax* populations from Bangka Island.

Genetic diversity is considered an internal contributing element in the susceptibility of organisms to heavy metals-related poison or toxicity levels. The variety in various genes, directly or indirectly included in the metabolism of weighty metals, has been researched by specific studies. For example, metallothioneins (MTs) are proteins that detoxify heavy metals because of a few gene varieties of genomic sequences (Joneidi et al. 2019). Metallothioneins are small cysteine-rich proteins that play significant roles in metal homeostasis and protection for heavy metal toxicity, DNA defect, and oxidative conditions (Si and Lang 2018), cellular processes, cell growth regulation, and well as proliferation and DNA repair (Grennan 2011).

The contribution of MTs with various cell or organelles processes has gotten much consideration while their association with the mitochondria functions has been inadequate. Furthermore, it increases the duration of malfunctioning mitochondrial cells by protecting productive components from the damage caused by reactive oxygen species (ROS) and limiting apoptosis. MTs are also involved in mitochondrial infection, including redox balance, metal homeostasis, enzyme, and transcription factor regulation (Lindique et al. 2010; Kurniawan and Mustikasari 2021). The requirements for obtaining metal specificity and specific novel capacity may drive their enhancement. MTs further enhanced the capability of metal detoxification under ecologically sensitive settings (Nam and Kim 2017). The relationship with mitochondria indicated an extreme environment as in abandoned tin mining waters of Bangka Island to genetic diversity, especially the COI gene of *A. panchax.* The heavy metals contamination and acidic pH in the habitat can cause genetic variations in mitochondrial genes, such as the COI gene. Moreover, heavy metals can reduce genetic variability within natural populations and cause genetic erosion (Ungherese et al. 2010). The evolution chronosequence of Bangka Island and the entire Sundaland may be attributed to the divergence of COI gene changes to diversify *A. panchax* genes.

It could be concluded that *Aplocheilus panchax* from the Bangka Island was highly divergence from other populations, including Indonesian populations with high genetic gap. The A. panchax population on Bangka Island formed a novel clade for Indonesia and in a global blue panchax phylogeographic.

ACKNOWLEDGEMENTS

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REFERENCES

Ashraf MA, Maah MJ, Yusoff I. 2011a. Heavy metals accumulation in plants growing in ex tin mining catchment. Int. J. Environ. Sci. Tech. 82: 401-416.

Ashraf MA, Maah MJ, Yusoff I. 2012a. Speciation of heavy metals in the sediments of former tin mining catchment. Iran. J. Sci. Technol. Trans. A Sci. 36(A2): 163-180.

Ashraf MA, Maah MJ, Yusoff I. 2012b. Morphology, geology and water quality assessment of former tin mining catchment. Sci. World J. 2012: 1-15. DOI:10.1100/2012/369206

Banks SC, Cary GJ, Smith AL, Davies ID, Driscoll DA, Gill AM, Lindenmayer DB, Peakall R. 2013. How does ecological disturbance influence genetic diversity?. Trends Ecol. Evol. 28(11): 670-679. DOI: 10.1016/j.tree.2013.08.005

Beck SV, Carvalho GR, Barlow A, Rüber L, Tan HH, Nugroho E, Wowor D, Nor SAM, Herder F, Muchlisin ZA, De Bruyn, M. 2017. Plio-Pleistocene phylogeography of the Southeast Asian blue panchax killifish, *Aplocheilus panchax*. PloS one 12(7): e0179557. DOI: 10.1371/journal.pone.0179557

Bennett, R.H., Ellender, B.R., Mäkinen, T., Miya, T., Pattrick, P., Wasserman, R.J., Woodford, D.J., Weyl, O.L.F. 2016. Ethical considerations for field research on fishes. Koedoe: African Protected Area Conservation and Science 58(1): 1-15. DOI: 10.4102/koedoe.v58i1.1353

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22(3): 148-155. DOI: 10.1016/j.tree.2006.11.004

Bobo-Pinilla J, Salmerón-Sánchez E, Mendoza-Fernández AJ, Mota JF, Peñas J. 2021. Conservation and phylogeography of plants: from the mediterranean to the rest of the world. Diversity 14(78): 1-23. DOI: 10.3390/d14020078

Bolotov IN, Konopleva ES, Vikhrev IV, Gofarov MY, Lopes-Lima M, Bogan AE, LunnZ, Chan Y, Win T, Aksenova OV, Tomilova AA, Tanmuangpak K, Tumpeesuwan S, Kondakov AV. 2020. New freshwater mussel taxa discoveries clarify biogeographic division of Southeast Asia. Sci. Rep. 10(1): 1-22. DOI: 10.1038/s41598-020-63612-5

Boluda CG, Hawksworth DL, Divakar PK, Crespo A. 2016. Microchemical and molecular investigations reveal Pseudephebe species as cryptic with an environmentally modified morphology. The Lichenologist 48(5): 527-543. DOI: 10.1017/S0024282916000426

Bragança PH, Amorim PF, Costa WJ. 2018. Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. Zoosystematics Evol. 94(1): 137-145. DOI: 10.3897/zse.94.22173

Buhay JE. 2009. "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. J. Crus. Biol. 29(1): 96-110. DOI: 10.1651/08-3020.1

Čekovská K, Šanda R, Eliášová K, Kovačić M, Zogaris S, Pappalardo AM, Soukupová T, Vukić J. 2020. Population genetic diversity of two marine gobies (Gobiiformes: Gobiidae) from the North-Eastern Atlantic and the Mediterranean Sea. J. Mar. Sci. Eng. 8(792): 1-18. DOI: 10.3390/jmse8100792

Çelebi EE, Öncel MS. 2016. Determination of acid forming potential of massive sulfide minerals and the tailings situated in lead/zinc mining district of Balya (NW Turkey). J. African Earth Sci. 124: 487-496. DOI: 10.1016/j.jafrearsci.2016.09.014

Cerca J, Meyer C, Purschke G, Struck TH. 2020. Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (Stygocapitella; Annelida, Sedentaria). Mol. Phylogenet. Evol. 143(2020): 106663. DOI: 10.1016/j.ympev.2019.106663

Chen N, Juric I, Cosgrove EJ, Bowman R, Fitzpatrick JW, Schoech SJ, Clark AG, Coop G. 2019. Allele frequency dynamics in a pedigreed natural population. PNAS 116(6): 2158-2164. DOI: 10.1073/pnas.1813852116

Chowdhury MM, Rahman AS, Nahar L, Rahman M, Al Reza H, Ahmed MS. 2016. Efficiency of different DNA extraction methods for fish tissues: a comparative analysis. IOSR-JPBS 11(3): 11-15. DOI: 10.9790/3008-1103041115

Cimmaruta R, Scialanca F, Luccioli F, Nascetti G. 2003. Genetic diversity and environmental stress in Italian populations of the cyprinodont fish Aphanius fasciatus. Oceanol. Acta 26(1): 101-110. DOI: 10.1016/S0399-1784(02)01234-3

Coates DJ, Byrne M, Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Front. Ecol. Evol. 6(165): 1-13. DOI: 10.3389/fevo.2018.00165

Costa WJEM. 2013. Historical biogeography of aplocheiloid killifishes (Teleostei: Cyprinodontiformes). Vertebr. Zool. 63(2): 139-154.

Costa WJEM. 2016. Comparative morphology and classification of South American cynopoeciline killifishes (Cyprinodontiformes: Aplocheilidae), with notes on family-group names used for aplocheiloids. Vertebr. Zool. 66(2): 125-140.

De Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, den Bergh Gv, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, Von Rintelen T. 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63(6): 879-901. DOI: 10.1093/sysbio/syu047

De Oliveira DAS, Decraemer W, Moens T, Dos Santos GAP, Derycke S. 2017. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. BMC Evol. Biol. 17(1): 1-17. DOI: 10.1186/s12862-017-0908-0

Dekar M, Sarong MA, Batubara AS, Muchlisin ZA. 2018. Ichthyofauna of Aceh River, Aceh Province, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 216, No. 1, p. 012024). IOP Publishing. DOI:10.1088/1755-1315/216/1/012024

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol. Ecol.17(1): 431-449. DOI: 10.1111/j.1365-294X.2007.03538.x

Dorey JB, Groom SV, Freedman EH, Matthews CS, Davies OK, Deans EJ, Rebola C, Stevens MI, Lee MSY, Schwarz MP. 2020. Radiation of tropical island bees and the role of phylogenetic niche conservatism as an important driver of biodiversity. Proc. R. Soc. B 287(1925): 20200045. DOI: 10.1098/rspb.2020.0045

Dwiyitno HS, Parmentier K, Van Keer C. 2018. Method comparison of DNA isolation and quantification for fish and seafood authenticity determination. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 13(3): 115-124. DOI: 10.15578/squalen.v13i3.370

Farmawati DA, Wirajana N, Yowani SC. 2015. Perbandingan kualitas DNA dengan menggunakan metode boom original dan boom modifikasi pada isolat *Mycobacterium tuberculosis*. KIMIA 9(1): 41-46. DOI: 10.24843/JCHEM.2015.v09.i01.p07

Faulwetter S, Simboura N, Katsiaras N, Chatzigeorgiou G, Arvanitidis C. 2017. Polychaetes of Greece: an updated and annotated checklist. Biodivers. Data J. 5: e20997. DOI: 10.3897/BDJ.5.e20997

Fortes MD, Ooi JLS, Tan YM, Prathep A, Bujang JS, Yaakub SM. 2018. Seagrass in Southeast Asia: a review of status and knowledge gaps, and a road map for conservation. Botanica Marina 61(3): 269-288. DOI: 10.1515/bot-2018-0008

Furness AI, Reznick DN, Springer MS, Meredith RW. 2015. Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. Proc.R. Soc. B: Biol. Sci. 282(1802): 20142189. DOI: 10.1098/rspb.2014.2189

Grennan AK. 2011. Metallothioneins, a diverse protein family. Plant Physiol. 155(4): 1750-1751. DOI: 10.1104/pp.111.900407

Gupta A, Bhardwaj A, Sharma P, Pal Y. 2015. Mitochondrial DNA-a tool for phylogenetic and biodiversity search in equines. J Biodivers. Endanger. Species S1: S1.006. DOI: 10.4172/2332-2543.S1-006

Haileselasie TH, Mergeay J, Vanoverbeke J, Orsini L, De Meester L. 2018. Founder effects determine the genetic structure of the water flea Daphnia in Ethiopian reservoirs. Limnol. Oceanogr. 63(2): 915-926. DOI: 10.1002/lno.10678

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PD. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. Mol. Ecol. Notes 6(4): 959-964. DOI: 10.1111/j.1471-8286.2006.01470.x

Hashim, M., Nayan, N., Saleh, Y., Mahat, H., Shiang, W. F. 2018. Water quality assessment of former tin mining lakes for recreational purposes in Ipoh City, Perak, Malaysia. Indones. J. Geogr. 50(1): 25-33. DOI: 10.22146/ijg.15665

Hinckley A, Camacho-Sanchez M, Ruedi M, Hawkins MT, Mullon M, Cornellas A, Yuh FTY, Leonard JA. 2022. Evolutionary history of Sundaland shrews (Eulipotyphla: Soricidae: Crocidura) with a focus on Borneo. Zool. J. Linn. Soc. 20(2021): 1-14. DOI: 10.1093/zoolinnean/zlab045

Hosoishi S, Ogata K. 2019. Cryptic diversity in the widespread Asian ant Crematogaster rothneyi (Hymenoptera: Formicidae) inferred from morphological and genetic evidence. Zool. Stud. 58(11): 1-15. DOI: 10.6620/ZS.2019.58-11

Hu ZM, Zhong KL, Weinberger F, Duan DL, Draisma SG, Serrao E. 2020. Linking ecology to genetics to better understand adaptation and evolution: a review in marine macrophytes. Front. Mar. Sci. 7: 545102. DOI: 10.3389/fmars.2020.545102

Hughes AC. 2017. Understanding the drivers of S outheast A sian biodiversity loss. Ecosphere 8(1): e01624. DOI: 10.1002/ecs2.1624

Ivanova NV, Zemlak TS, Hanner RH, Hebert PD. 2007. Universal primer cocktails for fish DNA barcoding. Mol. Ecol. Notes 7(4): 544-548. DOI: 10.1111/j.1471-8286.2007.01748.x

Joneidi Z, Mortazavi Y, Memari F, Roointan A, Chahardouli B, Rostami S. 2019. The impact of genetic variation on metabolism of heavy metals: Genetic predisposition?. Biomed. Pharmacother. 113: 108642. DOI: 10.1016/j.biopha.2019.108642

Kaňuch P, Berggren Å, Cassel-Lundhagen A. 2014. Genetic diversity of a successful colonizer: isolated populations of *Metrioptera roeselii* regain variation at an unusually rapid rate. Ecol. Evol. 4(7): 1117-1126. DOI: 10.1002/ece3.1005

Karanovic T, Djurakic M, Eberhard SM. 2016. Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. Syst. Biol. 65(2): 304-327. DOI: 10.1093/sysbio/syv088

Katwate U, Kumkar P, Britz R, Raghavan R, Dahanukar N. 2018. The identity *of Aplocheilus andamanicus* (Köhler, 1906) (Teleostei: Cyprinodontiformes), an endemic Killifish from the Andaman Islands, with notes on *Odontopsis armata* van Hasselt. Zootaxa 4382(1): 159-174. DOI: 10.11646/zootaxa.4382.1.6

Koki IB, Zain SM, Low KH, Azid A, Juahir H, Abdul ZM. 2019. Development of water quality index of ex-mining ponds in Malaysia. Mal. J. Fund. Appl. Sci. 15(1), 54-60. DOI: 10.11113/mjfas.v15n2019.1079

Korshunova T, Picton B, Furfaro G, Mariottini P, Pontes M, Prkić J, Fletcher K, Malmberg K, Lundin K, Martynov A. 2019. Multilevel fine-scale diversity challenges the 'cryptic species' concept. Scientific Reports 9(1): 1-23. DOI: 10.1038/s41598-019-42297-5

Kuffel A, Gray A, Daeid NN. 2021. Impact of metal ions on PCR inhibition and RT-PCR efficiency. Inter. J. Legal Med. 135(1): 63-72. DOI: 10.1007/s00414-020-02363-4

Kurniawan A, Mustikasari D. 2021. Review tentang kemampuan ikan ekstremofil untuk hidup di perairan asam dan terkontaminasi logam berat pascapenambangan timah. Jurnal Ilmu Lingkungan 19(3): 541-554. DOI: 10.14710/jil.19.3.541-554

Kurniawan A, Oedjijono, Tamad, Sulaeman U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka Regency, Indonesia. Indones. J. Chem. 19(1): 254-261. DOI: 10.22146/ijc.33613

Kurniawan A, Prasetiyono E, Syaputra D. 2020. Analisis korelasi parameter kualitas perairan kolong pascatambang timah dengan umur berbeda. Samakia: Jurnal Ilmu Perikanan 11(2): 91-100. DOI: 10.35316/jsapi.v11i2.824

Kurniawan A. 2020. The metal oxides of abandoned tin mining pit waters as an indicator for bacterial diversity. Aquac. Aquar. Conserv. Legis. 13(5): 2982-2992.

Lee CE. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17(8): 386-391. DOI: 10.1016/S0169-5347(02)02554-5

Lindeque JZ, Levanets O, Louw R, Van Der Westhuizen FH. 2010. The involvement of metallothioneins in mitochondrial function and disease. Curr. Protein Pept. Sci. 11(4): 292-309. DOI: 10.2174/138920310791233378

Liu Y, Webber S, Bowgen K, Schmaltz L, Bradley K, Halvarsson P, Abdelgadir M, Griesser M. 2013. Environmental factors influence both abundance and genetic diversity in a widespread bird species. Ecol. Evol. 3(14): 4683-4695. DOI: 10.1002/ece3.856

Lone AR, Thakur SS, Tiwari N, Sokefun OB, Yadav S. 2021. Disentangling earthworm taxonomic stumbling blocks using molecular markers. J. Threat. Taxa 13(11): 19566-19579. DOI: 10.11609/jott.6888.13.11.19566-19579%20

Markert JA, Champlin DM, Gutjahr-Gobell R, Grear JS, Kuhn A, McGreevy TJ, Roth A, Bagley MJ, Nacci DE. 2010. Population genetic diversity and fitness in multiple environments. BMC Evol. Biol. 10(1): 1-13. DOI: 10.1186/1471-2148-10-205

Martinez AS, Willoughby JR, Christie MR. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecol. Evol. 8(23): 12022-12031. DOI: 10.1002/ece3.4661

Mustikasari D, Nuryanto A, Suryaningsih S. 2020a. The presence of blue panchax (*Aplocheilus panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of different age. Aquac. Aquar. Conserv. Legis. 13(5): 2538-2550.

Mustikasari D, Suryaningsih S, Nuryanto A. 2020b. Morphological variation of blue panchax (*Aplocheilus panchax*) lives in different habitat assessed using truss morphometric. Biosaintifika 12(3): 399-407. DOI: 10.15294/biosaintifika.v12i3.26593

Nam YK, Kim EJ. 2017. Diversification and domain evolution of molluskan metallothioneins: a mini review. Fish Aquatic Sci. 20(1): 1-18. DOI: 10.1186/s41240-017-0054-z

Nascimento MHS, Almeida MS, Veira MNS, Filho DL, Lima RC, Barros MC, Fraga EC. 2016. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. Genet. Mol. Res. 15(3): 1-11. DOI: 10.4238/gmr.15038476.

Nater A, Arora N, Greminger MP, van Schaik CP, Singleton I, Wich SA, Fredrikson G, Perwitasari D, Pamungkas J, Krützen M. 2013. Marked population structure and recent migration in the critically endangered Sumatran orangutan (*Pongo abelii*). J. Hered. 104(1): 2-13. DOI: 10.1093/jhered/ess065

Parenti LR, Hartel KE. 2011. Osteology identifies *Fundulus capensis* Garman, 1895 as a killifish in the family Fundulidae (Atherinomorpha: Cyprinodontiformes). Copeia 2011(2): 242-250. DOI: 10.2307/41261873

Pohl M, Milvertz FC, Meyer A, Vences M. 2015. Multigene phylogeny of cyprinodontiform fishes suggests continental radiations and a rogue taxon position of Pantanodon. Vertebr. Zool. 65(1): 37-44.

Pratomo YW, Zahida F, Yuda P. 2021. Perbandingan Metode isolasi DNA sebagai templat PCR untuk identifikasi jenis kelamin Cerek Jawa (*Charadriius javanicus*) secara molekuler menggunakan Primer 2550F/2718R. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati 6(2): 78-86. DOI: 10.24002/biota.v6i2.3444

Riesch R, Tobler M, Plath M. 2015. Extremophile Fishes. Ecology, Evolution, and Physiology of Teleosts in Extreme Environments: Springer. Netherland.

Rius M, Turon X. 2020. Phylogeography and the description of geographic patterns in invasion genomics. Front. Ecol. Evol. 8(439): 595711. DOI: 10.3389/fevo.2020.595711

Rizko N, Kusumaningrum HP, Ferniah RS, Pujiyanto S, Erfianti T, Mawarni SN, Rahayu HT, Khairunnisa D. 2020. Isolasi DNA daun Jeruk Bali Merah (*Citrus maxima* Merr.) dengan modifikasi Metode Doyle and Doyle. Berkala Bioteknologi 3(2): 1-7.

Rosilawati ML, Sudarmono P, Ibrahim F. 2002. Sensitivitas metode PCR (*Polymerase Chain Reaction*) dalam mendeteksi isolat klinis *Mycobacterium tuberculosis*. Jurnal Kedokteran Trisakti 21(1): 7-14. DOI: 10.18051/UnivMed.2007.v26.1-10

Ruchi W, Putri DH, Anhar A, Farma SA. 2018. Comparison of three different DNA isolation methods to degradate the *Trichoderma* fungi cell wall. Bioscience 2(1): 50-59. DOI: 0201931102859-0-00

Salles T, Mallard C, Husson L, Zahirovic S, Sarr AC, Sepulchre P. 2021. Quaternary landscape dynamics boosted species dispersal across Southeast Asia. Commun. Earth Environ. 2(1): 1-12. DOI: 10.1038/s43247-021-00311-7

Schierenbeck KA. 2017. Population-level genetic variation and climate change in a biodiversity hotspot. Ann. Bot. 119(2): 215-228.

Setiaputri AA, Barokah GR, Sahaba MAB, Arbajayanti RD, Fabella N, Pertiwi RM, Nurilmala M, Nugraha R, Abdullah A. 2020. Perbandingan metode isolasi DNA pada produk perikanan segar dan olahan. Jurnal Pengolahan Hasil Perikanan Indonesia 23(3): 447-458.

Si M, Lang J. 2018. The roles of metallothioneins in carcinogenesis. J. Hemat. Oncol. 11(1): 1-20. DOI: 10.1038/s43247-021-00311-7

Star B, Spencer HG. 2013. Effects of genetic drift and gene flow on the selective maintenance of genetic variation. Genetics 194(1): 235-244. DOI:10.1534/genetics.113.149781/-/DC1

Tan GL, Shu WS, Hallberg KB, Li F, Lan CY, Huang LN. 2007. Cultivation-dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China. FEMS Microbiol. Ecol. 59(1): 118-126. DOI: 10.1111/j.1574-6941.2006.00216.x.

Thu PT, Huang WC, Chou TK, Van Quan N, Van Chien P, Li F, Shao KT, Liao TY. 2019. DNA barcoding of coastal ray-finned fishes in Vietnam. PloS ONE 14(9): e0222631. DOI: 10.1371/journal.pone.0222631

Ungherese G, Mengoni A, Somigli S, Baroni D, Focardi S, Ugolini A. 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). Environ. Pollut. 158(5): 1638-1643. DOI:10.1016/j.envpol.2009.12.007

Von Rintelen K, Arida E, Häuser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. RIO 3: e20860. DOI: 10.3897/rio.3.e20860

Wang XY, Wang MM, Chen C, Wang XQ. 2020. Genetic variation and phylogeographic structure of Spodoptera exigua in western China based on mitochondrial DNA and microsatellite markers. PloS ONE 15(5): e0233133. DOI: 10.1371/journal.pone.0233133

Xiao JH, Wang NX, Li YW, Murphy RW, Wan DG, Niu LM, Hu HY, Fu YG, Huang DW. 2010. Molecular approaches to identify cryptic species and polymorphic species within a complex community of fig wasps. PLoS ONE 5(11): e15067. DOI: 10.1371/journal.pone.0015067

Yang CH, Wu KC, Chuang LY, Chang HW. 2019. Decision theory-based COI-SNP tagging approach for 126 scombriformes species tagging. Front. Genet. 10: 259. DOI: 10.3389/fgene.2019.00259

Reviewer B:

Overall, this study would make an interesting addition to previously published studies. The study system is particularly interesting given their current dist make sense. As such, I had to stop reading before finishing the manuscript.

English needs improving throughout document as lots of spelling errors and sentences that don't make sense.

I don't think the Results and Discussion should be merged as it currently is and strongly recommend the authors to use separate headings for these two

There are statements as to why different sequence lengths show how diverse this population is and how it might have a big ecological effect, but I don't evidence and examples.

I stopped reading at line 187 as I feel the authors need to first go through their manuscript to ensure that the English makes sense and accurately reflect getting lost in translation. There are a lot of big statements that I do not think are justified nor appropriate for this study.

22-26: very broad introduction to phylogeography that is also quite repetitive and simplistic.

29-31: Not sure if the entire classification is needed here as the reader can determine this from its binomial nomenclature.

36: "megadiverse organisms' evolution" doesn't really make sense…perhaps consider changing to something along the lines of "…have all contributed t

42: the previous study did not use the 'worlds' *A. panchax,* just a few populations distributed across SE Asia, but not all.

58-59: I'm not sure what the authors are trying to say here. Are they saying that all Indonesian waters are polluted?

63-66: would be good to have sample sizes for each station

Figure 1: quality needs to be improved as currently quite grainy

71: is that 20 samples for all sites together? Or each site? If it's all sites together, this sample size is quite small

Major concerns:

- Methodological considerations

The authors have minimized methodological features in relation to the molecular phylogenetic and genetic population analyses, being these methods fur in the methods section for the generation of genetic analysis, and some of the statistical additional analyses are required.

1. a) I don´t see in the MS an implementation of the AMOVA analysis. The author must estimate the better grouping hypothesis for samples using different groups hypotheses to maximize the among group variance Φ
2. b) The authors do not include in the study, an indirect estimate of gene flow analyses for the different data sets, which represent important popu scenarios.
3. c) The range of the genetic distances presented here, are very large, and I suspect that there are any problem in the output of the MEGA progr
4. d) What are the bootstrap values to validate the node support in the tree topology?. I see bootstrap values very low in some of them.

- Content issues

1. a) I suggest to improve all the Figure quality because they are the results that must be well exposed in order to facilitate the comprehension of
2. c) It will be desirable a reorganization of the discussion contents and rewording any paragraphs to condense them, because they are very repe
3. d) A better integration of the killifish literature could help to develop the discussion further and increase readership. A large number of annual ar for comparison.

3) Format consideration

1. a) The authors must relocate the Fig. 2 in present version, from the Mat and Methods to the Results_Discussion section, because it constitutes

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Reviewer D:

This manuscript presents some interesting COI data and results on a previously unsampled (at least genetically) population of *Aplocheilus* fishes from B with the current version of the manuscript that need to be addressed before it is suitable for publication. First, the authors need to explain more about th sequences obtained by other aurhors. Is this the result of an indel, or did they simply sequence more of the gene? Second, they need to examine how t the comparisons involving the Bangka samples appear anomalously large (as do some of the mutational distances in the haplotype network), and this co be checked for possible errors. Third, the authors adopt some of the recent taxonomic proposals by Katwate et al. (2018) but not others, and they do no study seem to support the taxonomic proposal that the authors reject (i.e., recognition of *A. armatus* as a separate species from *A. panchax*). Related to *panchax*. In fact, their data suggest that those specimens might represent yet another distinct species. In addition, the English grammar and logic are n manuscript file to improve some of these issues, but the manuscript would benefit from being reviewed for English style and grammar before it is resubm

Recommendation: Resubmit for Review

Reviewer E:

The authors provided an interesting study of the blue panchax (*Aplocheilus panchax*), in Indonesia. Specifically, the authors used a portion of the cytoch unstudied specimens from the pits of Bangka Island. I found the methods to be solid and results easy to interpret but feel that the paper can be shortene the overall quality of the paper. Specific comments are in returned ms.

Recommendation: -

Recommendation: Revisions Required

[biodiv] Editor Decision

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Smujo Editors <smujo.id@gmail.com>
to me

AGUS NURYANTO:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Phylogeography of the blue panchax, Aplocheilu

Our decision is: Revisions Required

Reviewer U:

Dear Authors,

Thank you for preparing and submitting this interesting paper investigating the DOI gene in a novel population of the blue panchax. I found your paper to and the findings should add some value to the current literature in A. panchax.

There are some revisions required in order to consider this manuscript for publication. I have included specific feedback on the word document version c using highlighted text or tracked changes. Additionally, please consider the following key areas when making revisions:

1. Methods. Could you provide some more information on how you handled the fish samples. For example, how much tissue was taken? Where was it ta

2. Dendrograms. You may want to make it a bit clearer as to which are your new samples, and which are from the existing databases of COI genes.

With these revisions the paper should be in a good position for consideration.

Recommendation: Revisions Required

[biodiv] Editor Decision

External Inbox ×

Ayu Astuti <smujo.id@gmail.com> Fri, Apr 8, 2022, 11:44 PM
to DIAH, me, SUHESTRI

DIAH MUSTIKASARI, AGUS NURYANTO, SUHESTRI SURYANINGSIH:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Phylogeography of Aplocheilus panchax in Indonesia, with special focus on the Bangka Island population".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity

agus.nuryanto 1 <agus.nuryanto@unsoed.ac.id> Sat, Apr 9, 2022, 7:28 AM
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Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

Thanks a lot. Congratulations! Thank you!

Reply Forward

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Phylogeography of *Aplocheilus panchax* in Indonesia, with special focus on the Bangka Island population

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Abstract. *Mustikasari D, Nuryanto A, Suryaningsih S. 2022. Phylogeography of* Aplocheilus panchax *in Indonesia, with special focus on the Bangka Island population. Biodiversitas 23: xxxx.* Previous study divided Blue panchax, *Aplocheilus panchax* into three different clades, namely West (W), Central (C), and East (E) clades. Blue panchax populations from Indonesia were belong to Central and East clades. However, that study did not include blue panchax samples from pits with harsh conditions in Bangka Island. Therefore, this study aimed to assess the phylogeography of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. The results showed that genetic distance within Bangka Island population was less than 2%, while genetic distances between Bangka population and other populations were ranged from 103.87% to 122.10%. There was also a clear genetic gap between the Bangka and other populations, with the minimum gap was 101.94%. Furthermore, the seventy-nine sequences analyzed resulted 28 haplotypes with genetic variability of 0.221 for nucleotide diversity (π), 0.923 for Haplotype diversity (Hd), 68.028 for Fu's Fs-test, 2.00 ($P < 0.02$) for Fu and Li's D-test, and 2.365 ($P < 0.02$) for Fu and Li's F-test; and 1.927 for Tajima's D test ($0.10 > P > 0.05$). The Bangka population of *Aplocheilus panchax* established a distinct clade from the Western (W), Eastern (E), and Central (C) clades. Molecular data established that the population on Bangka Island is a novel clade for Indonesia and a global blue panchax phylogeographic.

Keywords: Abandoned tin mining pits, genetic distance, haplotype, killifish, new clade position

INTRODUCTION

Phylogeography analyzes and understands organism diversity and biogeography (Bobo-Pinilla et al. 2021). Furthermore, it is a study of geographical distributions of closely related genetic lineages or geographic ordination of genotypes (Rius and Turon, 2020). Therefore, phylogeographic research is essential to evaluate the geographical distribution of these genetic lineages and their pattern, as well as elaborate ecology factors and organism biodiversity (Lone et al. 2021).

The blue panchax (*Aplocheilus panchax* Hamilton, 1882) is an endemic species to the Oriental region (Costa 2013; Costa 2016; Beck et al. 2017), widely distributed across the Indo-Malayan Islands, including Indonesia, the Indo-China region, and India (Dekar et al. 2018; Bolotov et al. 2020). *Aplocheilus panchax* is a fish from Genus *Aplocheilus*, Family Aplocheilidae, Suborder Aplocheiloidei, Order Cyprinodontiformes, and Class Actinopterygii (Parenti and Hartel 2011; Furness et al. 2015). Fishes belonging to Order Cyprinodontiformes are also known as *Aplocheiloid killifishes* or *livebearer*s (Pohl et al. 2015; Braganca et al. 2018).

Indonesia is one of the world's biodiversity hotspots with many different habitats and a highly complicated geological history (Bruyn et al. 2014*;* von Rintelen et al. 2017). Generally, Southeast Asia's complex climatic and geological history caused this region to have high biodiversity (Beck et al. 2017; Fortes et al. 2018). Biogeography, geology, climate, and ecology of Southeast Asia have led to megadiverse organisms' evolution. Additionally, the region is home to several endemic and ecologically well-adapted species (Hughes 2017; von Rintelen et al. 2017). The *Aplocheilus panchax* can be used to understand further how climatic changes and sea-level fluctuations have influenced the species' distribution within this region. Specifically, sea-level variations result from glacial cycles that continued throughout the Pleistocene, interfering with and restricting the network of all populations. These examples show proof of isolation in palaeodrainage basins (Beck et al. 2017).

Previous phylogeographic study using the COI gene placed worlds' *A. panchax* populations into three different clades; West (W), Central (C), and East (E) clades. The *A. panchax* populations from Indonesia were divided into Central and East clades. Central clade consisted of Pekanbaru, Jambi, and West Sumatera populations. East clade was formed by *A. panchax* populations from Java, Bali, Kalimantan, and Sulawesi islands (Beck et al. 2017). Nevertheless, the study by Beck et al. (2017) did not include *A. panchax* samples from Bangka Island. Bangka Island, the biggest tin producer in Indonesia, has unique waters like a lake or pit, known as kolong, formed and abandoned after tin mining activity. This water has low pH (acid waters), low nutrients, minimum dissolved oxygen (DO), and heavy metals (Ashraf et al. 2011, 2012a, 2012b; Hashim et al. 2018; Koki et al. 2019; Kurniawan 2020). However, *A. panchax*, locally known as ikan Kepala

Timah, can live in this extreme habitat (Kuniawan et al. 2019, Kurniawan et al. 2020; Mustikasari et al. 2020a). The genus *Aplocheilus*, which includes *A. panchax*, was classified as an extremophile fish due to its ability to survive in harsh environmental circumstances (Riesch et al. 2015; Kurniawan and Mustikasari 2021). Mustikasari et al. (2020a, b) explored the presence and morphological variety of blue panchax (*A. panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of various ages.

Island biogeography investigates how the richness of the ecosystems and the complexity of the biodiversity may cause speciation. The adaptive radiation, speciation, climate cycles, and topographical complexity can shape island biodiversity (Dorey et al. 2020). Phylogenetic and phylogeographic analyses based on mitochondrial DNA (mt-DNA) are used in island biodiversity exploration. In addition, mt-DNA possesses some characteristics, including cell quantity, genome size, haploid, maternal inheritance, and extremely low probability of paternal leakage, mutation rate, and change mainly caused the mutation. These features make mt-DNA a useful and one of the most often used markers in molecular analysis. The marker has been frequently used to study genetic diversity, population organization, phylogeography, and organism evolution (Gupta et al. 2015).

This study aimed to assess the phylogeography of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. This is the first time the populations from Bangka Island have been genetically analyzed using the cytochrome oxidase I (COI) gene and compared to another on the global distribution of blue panchax. Furthermore, the presence and genetic profile of *A. panchax* can be a model of extremophile fish study. Therefore, it is used as a bioindicator for harsh habitats since the global distribution, ecological and genetic evolution with the Bangka Island population corresponds to others in Indonesian waters.

MATERIALS AND METHODS

Study area

The study was conducted in Pangkalpinang City and Bangka District of Bangka Belitung Archipelago Province, Indonesia. Fish samples were collected from abandoned tin mining lakes (pits) of different ages and the Limbung River. Station A and B (< 5 years old), Station C and D (5 - 15 years), Station E and F (15 - 25 years), Station G (25 - 50 years), Station H (50 - 100 years), Station I and J (> 100 years), and Limbung River Stream of Bangka District as Station K such as shown in Figure 1. These years of research stations indicated the chronosequence of the abandoned tin mining pits were taken place there. There were nothings put in the habitats, due to the chronosequence only explain about a succession that happened naturally. In addition, they were related to our previous studies about their characteristics of water quality and the presence of *Aplocheilus panchax* in the abandoned tin mining pits based on the difference of time (Mustikasari et al. 2020a, b).

Procedures

Samples preparation for molecular analysis

The twenty samples were collected at 09.00 am-1.00 pm from closed and open waters of abandoned tin mining lakes (pits) and waters of Limbung River Stream, Bangka Island, using nets with a mesh size of about 0.4 mm. We declared that collected fish as samples were handled in a good manner as explained as Bennett et al. (2016) which cite Canadian Council on Animal Care (2005) and American Fisheries Society (2014) about guidelines for the use of fishshes in research. They were handled with minimises pain, distress, suffering and unnecessary loss of external mucus or scales, minimum of the handling duration, to avoid unnecessary stress, and exposure time. We also gave attention to life-cycle events, such as aggregations of breeding fish and sensitive habitats should be avoided.

Furthermore, this study utilized a 2.0 ml cryotube with ethanol absolute to preserve each of the fish sample for molecular analysis. The pectoral fin about 2 mm from each dead sample of A. pachax was taken for the DNA isolation and molecular identification.

Molecular analysis

The genomic DNA extraction was analyzed with gSYNC™ DNA Extraction Kit (Geneaid, GS300). Nucleic acid (genomic DNA) concentration was measured using Nanodrop™ 2000/2000c spectrophotometers. Furthermore, molecular analysis was referred to Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika Science Indonesia, in 2021.

Polymerase chain reaction (PCR) amplification was conducted with (2x) MyTaq HS Red Mix (Bioline, BIO-25048) and KOD FX Neo (Toyobo, KFX-201). The components 1 x 25 µL PCR Master Mix were dd H2O 9.5 µL; MyTaq HS Red Mix, 2x 12.5 µL; 10 µM VF2_t1 0.5 µL; 10 µM Fish F2_t1 0.5 µL; 10 µM Fish R2_t1 0.5 µL; 10 µM Fish FR1d_t1 0.5 µL; and DNA Template 1 µL. Primer sequence of PCR amplification were VF2-t1 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3'; FR1d-t1 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3'; FishR2_t1 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3'; and FishF2_t1 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (Ivanova et al. 2007).

The predenaturation phase initiated the Polymerase Chain Reaction (PCR) cycling for 1 minute (95 °C). Subsequently, the actual PCR amplification was conducted for 35 cycles, denaturation process for 15 seconds (95 °C), annealing process for 15 minutes (50 °C), and extension process for 45 seconds (72 °C). The PCR products (1 µL) were assessed by electrophoresis with 1% TBE agarose with Marker 100bp DNA ladder (loaded 2 µL). Furthermore, the quality and length of the PCR products were analyzed by agarose gel electrophoresis. Bi-directional Sequencing conducted the sequencing step at 1st base Asia.

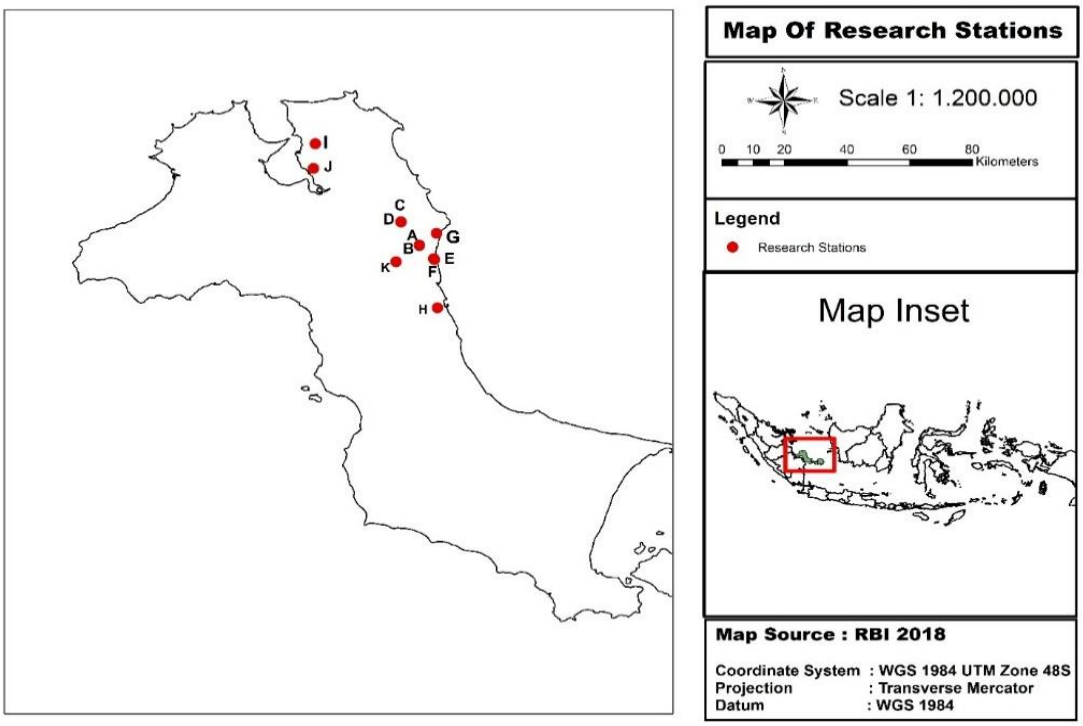

Figure 1. Map of research stations at Bangka Island, Indonesia (Mustikasari et al. 2020a, b)

Data analysis

The contig sequences were obtained from reverse and forward sequences aligned with Program BioEdit. The phylogenetic study was carried out using MEGA XI (Tamura et al. 2021), utilizing the Maximum Parsimony (MP) statistical approach, and a bootstrap consensus tree was constructed using 1,000 replicates. The phylogenetic tree was constructed by comparing sequences of *A. panchax* from Bangka Island with some sequences of *A. panchax* from a previous study (Beck et al. 2017). The study conducted in India (Tamil Nadu and Kolkotta), Cambodia, Vietnam, Thailand (Krabi), Malaysia (Sungai Batu Pahat, Penang, Dungun), Singapore, and Indonesia (Aceh, Pekanbaru, Pulau Laut, West Sumatra, Jambi, Bogor, Surabaya, Banjarmasin, Bali, and Sulawesi) as shown in Figure 2 aim to investigate the position of *A. panchax* population from Bangka Island. Furthermore, it used a sequence of *A. andamanicus* (Katwate et al. 2018). The *A. warneri* (accession number KJ844713.1) was used as outgroup species (Pohl et al. 2015) for this phylogenetic analysis. Sequences metadata from Beck et al. (2017) and Katwate et al. (2018) from NCBI (National Center for Biotechnology Information) were utilized. DnaSP v5 was used to assess the haplotype (Hd) and nucleotide diversity (π), as well as to perform Fu and Li's F and Tajima's D neutrality tests (Čekovská et al. 2020). A phylogenetic network was constructed according to the median-joining method within the software Network 10. The Kimura 2 Parameter (K2P) genetic distance was calculated in the MEGA XI (Tamura et al. 2021). Genetic distances were utilized to estimate the genetic gap between Bangka Island and other clade populations. The lowest genetic distance was estimated by subtracting minimum genetic distance among populations by maximum genetic distance within Bangka Island population.

RESULTS AND DISCUSSION

DNA quantity and quality

The successfulness of organisms identification methods based on genetic material, such as PCR, relies on the quantity and quality of nucleic acid, purification method, and PCR amplification process. Unfortunately, the low amount and quality of genetic material (DNA) and the appearance of inhibitors inhibit the PCR amplification efficiency (Chowdhury et al. 2016; Dwiyitno et al. 2018; Kuffel et al. 2021). The analysis of the quantity of sample' DNA by Nanodrop spectrophotometer resulted in DNA concentration (ratio $A_{260/280}$) for all samples between 1.65 and 1.99 (Table 1).

The ratio of absorbance at 260 nm and 280 nm ($A_{260/280}$) is used to assess the purity of material genetic. The measurement of DNA concentration with Nanodrop was conducted at a wavelength of 260 nm. In comparison, the protein was measured at a wavelength of 280 nm with pure DNA having an absorbance ratio of 260/280 between 1.6 and 2.0 (Setiaputri et al. 2020), 1.7-2.0 (Ruchi et al. 2018), or 1.8-20 (Pratomo et al. 2021). The absorbance value below the low absorbance limit indicates the presence of polysaccharides, phenol, and protein contamination.

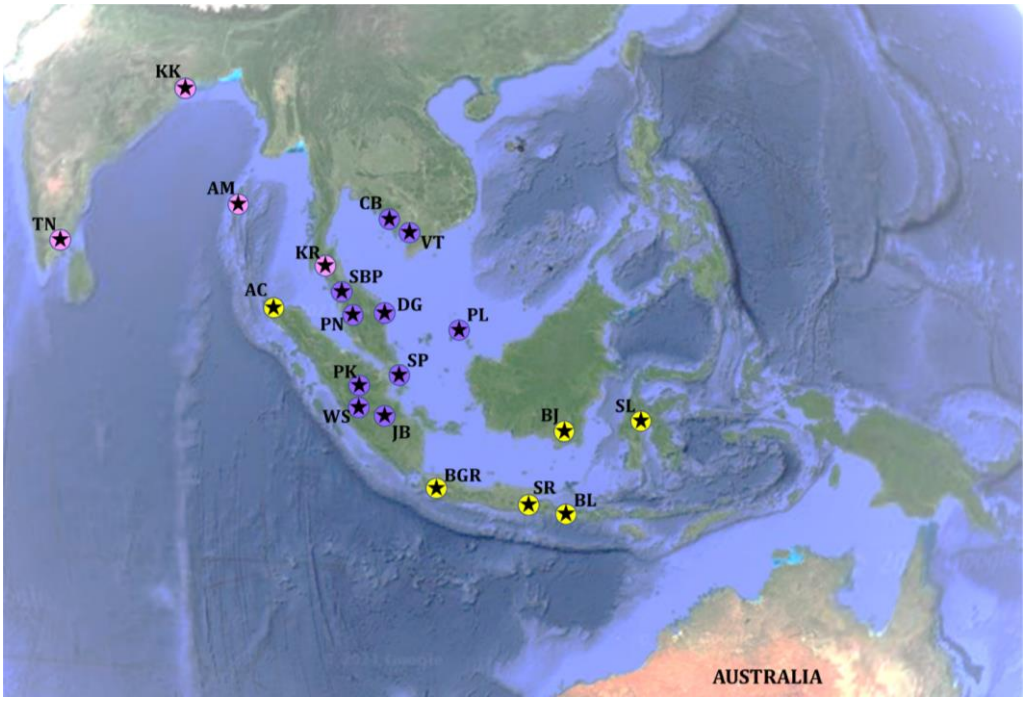

Figure 2. Sampling locations for *Aplocheilus panchax* over 20 areas, namely Tamil Nadu (TN), Kolkotta (KK), Andaman Island (AM) Cambodia (CB), Vietnam (VT), Krabi (KB), Sungai Batu Pahat (SBP), Aceh (AC), Penang (PN), Dungun (DG), Pulau Laut (PL), Singapore (SP), Pekanbaru (PK), West Sumatera (WS), Jambi (JB), Bogor (BG), Surabaya (SR), Banjarmasin (BJ), Bali (BL) and Sulawesi (SL). (Map was reconstructed from Beck et al. 2017 and Katwate et al. 2018)

Table 1. Quantity of Sample DNA

Sample code*	$A_{260/280}$
BK_A01	1.72
BK_A02	1.90
BK_B01	1.91
BK_B02	1.87
BK_C01	1.87
BK_C02	1.91
BK_D01	1.93
BK_D02	1.99
BK_E01	1.90
BK_E02	1.89
BK_F01	1.87
BK_F02	1.65
BK_G01	1.75
BK_G02	1.59
BK_G03	1.81
BK_G04	1.88
BK_K01	1.65
BK_K02	1.83
BK_K03	1.71
BK_K04	1.70

Note: *) sample' volume (30 µL)

Meanwhile, above the absorbance 2.0 indicates the presence of RNA contamination during the DNA isolation process (Rosilawati et al. 2002; Farmawati et al. 2015; Rizko et al. 2020). The success of a purification extraction and the quantity of DNA is highly dependent on the isolation of the resulting DNA. Therefore, the isolation process using commercial kits is safer from processing errors that cause contamination. However, studies on several specimens with various treatments stated that not all commercial kits can harvest DNA in high concentrations (Hajibabaei et al. 2006; Setiaputri et al. 2020).

The product of PCR also showed that the COI gene length of *A. panchax* populations from Bangka Island was about 700 bp. Meanwhile, the COI gene length for *A. panchax* was around 621 bp in other studies with accession numbers KJ957593.1, KJ957617.1, and KJ957618.1 from Beck et al. (2017), and also accession number MG813789.1 (Sample *A. andamanicus*) from Katwate et al. (2018). The length showed that the COI gene from Bangka Island was the longest. The mitochondrial gene, namely COI, is commonly used for DNA barcoding, about 650 bp and these gene sequences are also used for ecological and evolutionary studies (Yang et al. 2019). The COI gene length differences of *A. panchax* populations indicate a diversity of *A. panchax* around the Oriental region. It plays a vital role in a global study to collect information about biodiversity and be a key in phylogenetic and phylogeographic analyses (Buhay 2009).

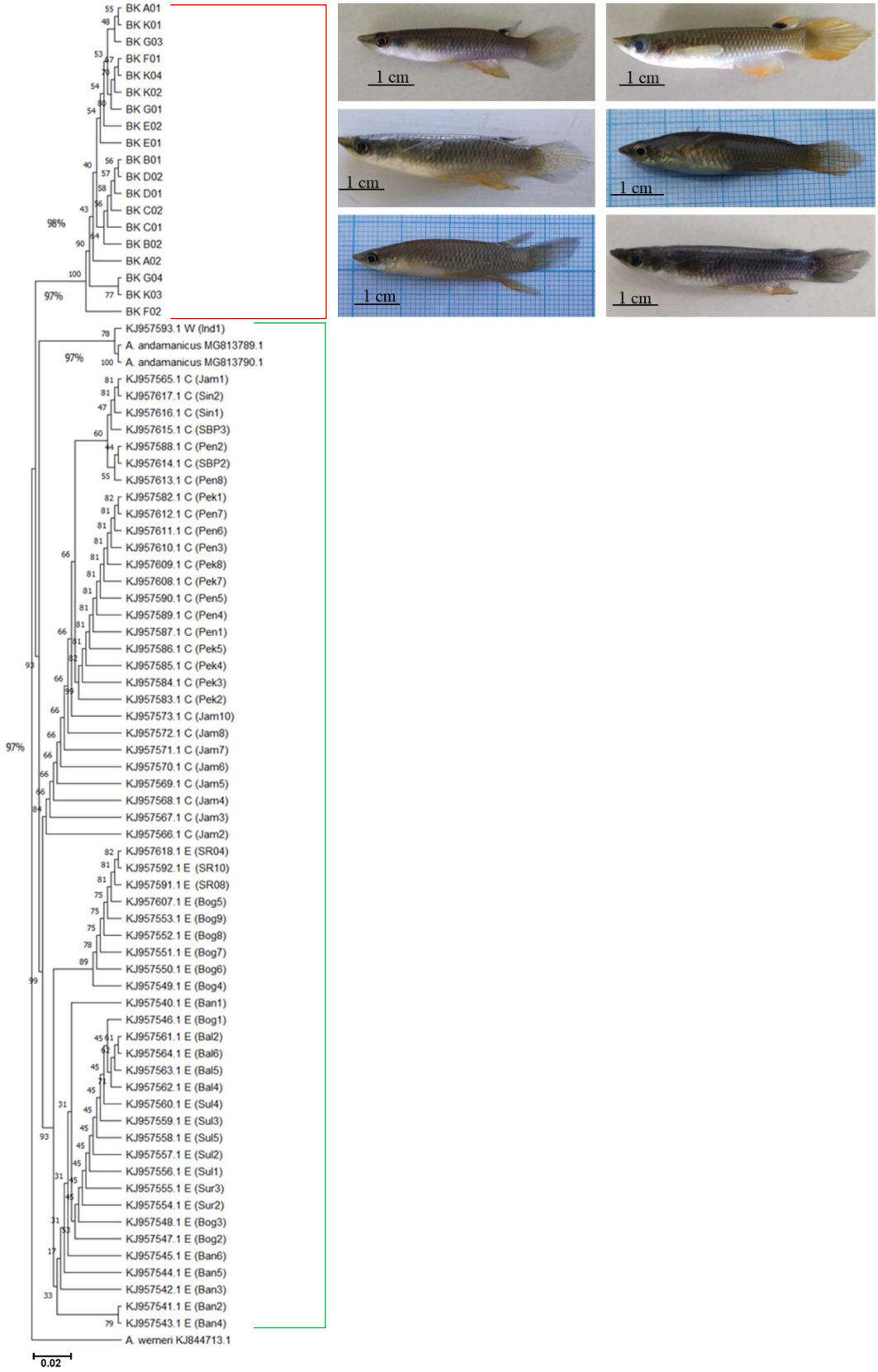

Figure 3. The phylogenetic position of *A. panchax* from Bangka Island is supported by Maximum Parsimony (MP) bootstrap values. Sequences of topotypes of populations from Bangka Island were in red line, while sample's sequences of Beck et al. (2017) and Katwate et al. (2018) which were cited from the existing databases of COI genes, shown in the pink line (West clade), violet line (East clade), and yellow line (East clade), while *A. warneri* is outgroup.

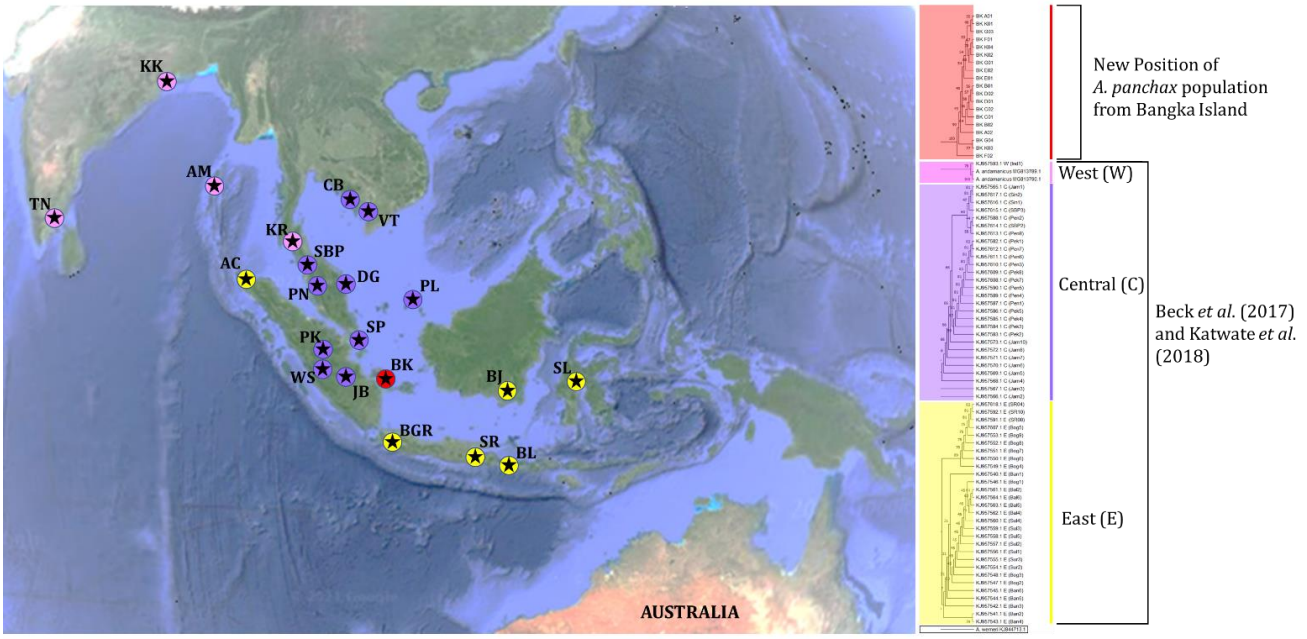

Figure 4. New phylogeographical map for *A. panchax*, particularly Bangka Island populations (red cycle) on global phylogeographic of blue panchax

The phylogenetic tree and phylogeographic of *A. panchax* population from Bangka Island

The phylogenetic tree indicated that the ancestral relationship between the *A. panchax* from the distribution sequences was well supported by COI gene sequence analysis based on well-supported clades (>90% bootstrap values). The phylogenetic tree analysis showed *A. panchax* widespread throughout the Oriental region, including Indonesia as a part of Southeast Asia countries. However, the population from Bangka Island has different sequences from others. Bangka Island individuals formed a distinct clade from those previously described by Beck et al. (2017) and Katwate et al. (2018). (Figure 3).

Beck et al. (2017) revealed their research about Bayesian posterior probabilities that displayed for Western (W) clade, Eastern (E) clade, and Central (C) clade. We added a novel clade to previous phylogenetic tree (Beck et al. 2017; Katwate et al. 2018) by adding samples from Bangka Island (Figure 3). Therefore, the authors also recommended a new map reconstruction of global phylogeographic on blue panchax (*A. panchax*) gene distribution (Figure 4).

A previous study by Katwate et al. (2018) denied the presence of *A. panchax* in the Indo-Malay region. They provided morphological and molecular evidence and demonstrated that *A. andamanicus* and *A. armatus* distinct and valid killifish species in the Indo-Malay region. However, the present study supported Beck et al. (2017) by showing that the killifish from Indonesia, especially populations from Bangka Island, is *A. panchax*. The present result was supported by morphological data that killifish from Bangka Island is *A. panchax* (Mustikasari et al. 2020b).

Several factors can impact an individual's genetic diversity and population genetic structure, such as climate or environmental change, natural boundaries, environmental variables, movement and migration, and human activities (Nater et al. 2013; Wang et al. 2020). For example, the persistence of climate change is determined by life history characters of organisms such as dispersal ability, generation period, reproductive ability, habitat specialization, organism interactions, genetic diversity, and habitat or migration corridors (Schierenbeck 2017).

The critical part was that the position of *A. panchax* populations from Bangka Island was different from other locations, but they were included as Sundaland region. It indicated that the biogeographic region of Sundaland (Borneo, Sumatra, the Malay Peninsula, Java, Palawan, and associated islands) is essential to understand evolution (Hinckley et al. 2022). As long as chronosequence exists, the ecological component may affect organism diversity. Ecological disturbance is necessary to maintain the dynamism and diversity of ecosystems. The disturbance history may be the primary driver that shapes patterns of genetic diversity in many natural populations through changes (Banks et al. 2013). Beck et al. (2017) explained that the significant mitochondrial clades of *A. Panchax* are consistent with this study. The basal dissimilarity of *A. panchax* mitochondrial ancestries was around 3.5 million years ago (Ma).

On the other hand, the subsequent dissimilarity timings of these clades occurred in the early Pleistocene (~2.6 Ma). Ceaseless phylogeographic investigation showed a reasonable west-east dispersal followed by quick radiation across Southeast Asia. Salles et al. (2021) recreate scene evolution, sedimentation, and Sundaland flooding history under tectonic, eustatic, and precipitation constraining

conditions. Furthermore, they link Sundaland's flooding history to tectonic and sea-level conditions using three external driving instruments of rainfalls, eustatic sea-level variations, and tectonics. Over the last one million years, these factors caused the Mekong, Johor, Siam, and East Sunda River to merge. Additionally, it causes Bangka Island to be separated from the mainland of Sumatra Island, Malay Peninsula, Java, and Kalimantan, about 400 thousand years ago (ka).

Founder effects or events can impact stochasticity in species' genetic population structure at the regional scale (Haileselasie et al. 2018). Even though their populations have decreased in genetic diversity after the founder event, specific individuals that establish well in new places appear to be destined to extinction. The limit of life forms to adjust to new environments can rely upon the organism's capacity of reacting to regular determination, which is dictated by the genetic pattern of the founder populations (Lee 2002; Dlugosch and Parker 2008; Kaňuch et al. 2014). This condition can impact genetic variation in natural populations, mutation, and genetic drift (Star and Spencer 2013). Genetic drift and gene flow shape allele frequencies over time for an extended period (Chen et al. 2019). Ecological and geological factors may have contributed to the high degree of divergence in gene COI between *A. panchax* populations from Bangka Island and other sites. Subsequently, *A. panchax* sequences from all clades were also analyzed, with a genetic distance of 0.00% to 1.93% for within Bangka Island population. Genetic distance between Bangka Island and other clade populations were ranged from 103.87% to 122.10%. Maximum intrapopulation genetic distances within Bangka Island, East clade, and Central clade populations was less than 2%, while within West clade populations had a maximum genetic distance larger than 2% (Table 2). As a result, there was a clear genetic gap between the Bangka Island population and other populations with minimum gap 101.94% (Bangka Island and Central Clade populations) and maximum gap value was 108.52% (Bangka Island and West clade populations). These values made Bangka Island populations were significantly different and separated from other clade populations (Figure 5).

The genetic distances were lower than 2% (0.02), indicating that *A. panchax* populations from Bangka Island did not indicate cryptic species. However, the phylogenetic showed that *A. panchax* populations from Bangka Island differed from others. Intraspecific genetic distances in some species are higher than 2% (Thu et al. 2019) or above 3% (Nascimento et al. 2016), indicating the existence of cryptic diversity within these fishes. The term "cryptic species" has lately replaced the term "siblings" for taxa of this type (Korshunova et al. 2019). Sibling refers to two or more distinct individuals classified as a single species under one or the same scientific name (Bickford et al. 2007; Xiao et al. 2010; Karanovic et al. 2016). These species cannot be confidently separated based on their morphology, yet they were genetically distinct (Boluda et al. 2016; De Oliveira et al. 2017; Faulwetter et al. 2017). The cryptic species were delineated as individuals in the same geographic area but exhibited significant molecular phylogenetic contrasts. However, they are not recognized morphologically and ethologically (Hosoishi and Ogata 2019; Cerca et al. 2020).

The seventy-nine (79) sequences that were analyzed by DNAsp v.5 showed genetic variability of 0.22 for nucleotide diversity (π), 0.895 for Haplotype diversity (Hd), 68.028 for Fu's Fs test, 2.00 ($P < 0.02$) for Fu and Li's D' test, and 2.365 ($P < 0.02$) for Fu and Li's test. There were 28 haplotypes of these specimens among the COI sequences ($n = 79$) built into a haplotype network (Figure 6). All of the sequences analyzed showed that the haplotype network has a star-shaped topology.

Hap_1 to Hap_15 showed haplotype of *A. panchax* populations from Bangka Island, while Hap_16 to Hap_28 were from Beck et al. (2017) and Katwate et al. (2018). Current approaches to biodiversity research focus primarily on ecosystems, environmental communities, geographic regions, and species (Coates et al. 2018). The ecological factors such as over-exploitation, pollution, habitat destruction, and climate change can substantially impact intra-population genetic variation (Liu et al. 2013; Martinez et al. 2018). Recent studies have shown a strong linkage between environmental pressures and biodiversity levels, including genes, species, populations, and communities. The relationships between genetic diversity, polymorphism, and ecological stress have been evidenced in natural populations. The biochemical and molecular approaches investigated the correlation between the genetic structure of populations and the environmental characteristics (Cimmaruta et al. 2003; Markert et al. 2010; Schierenbeck 2017; Hu et al. 2020).

Table 2. The genetic distances within and among clades of *Aplocheilus*

***Aplocheilus panchax* Populations**	**Genetic Distance (%)**			
	[1]	**[2]**	**[3]**	**[4]**
Bangka Island [1]	**0.00 - 1.93**			
East Clade [2]	104.02-110.32	**0.00 - 1.76**		
Central Clade [3]	103.87 - 108. 49	1.40 - 2.48	**0.00 - 1.05**	
West Clade [4]	110.45 - 112.10	7.37 - 12.54	6.98 - 13.22	**0.00* - 9.80****

Note: Values in bold were intra-population genetic distances of *Aplocheilus*, * there was indication a differences *A. panchax* and *A. andamanicus* as well as Katwate et al. (2018)

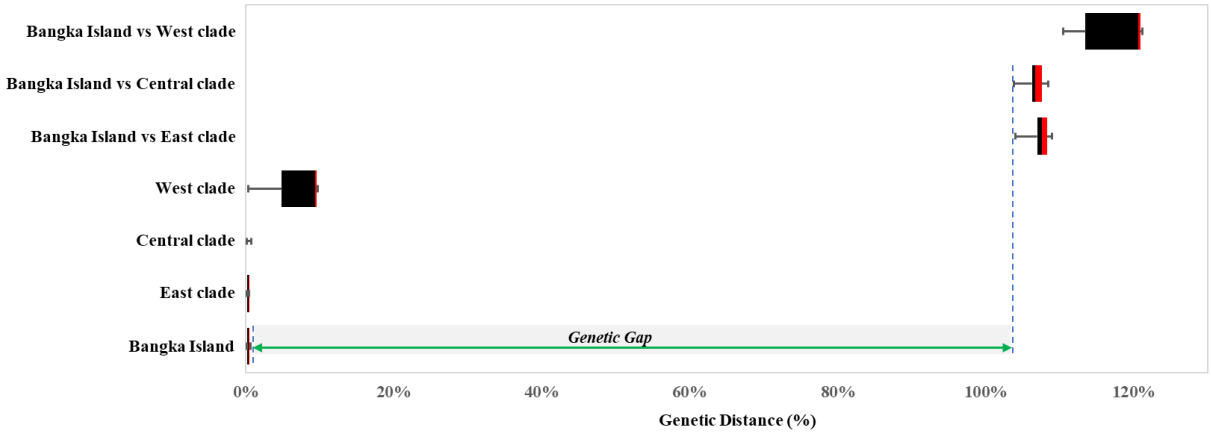

Figure 5. Intra- and inter-species genetic distances for the *A. panchax* populations from Bangka Island and other clades. There was a clear genetic gap, spanning from 1.42% to 103.87%, between the maximum within Bangka Island populations and minimum inter populations distance that indicates the *A. panchax* populations from Bangka Island were genetically distinct from each other clade. Black lines within the boxes showed the medians, and the red boxes indicated the 75th quartiles

Table 3. The haplotype of *Aplocheilus panchax* on each clade

Haplotype(s)	Specimen(s)
Hap_1: 5	[BK_A01 BK_A02 BK_E01 BK_E02 BK_G03]
Hap_2: 1	[BK_B01]
Hap_3: 1	[BK_B02]
Hap_4: 1	[BK_C01]
Hap_5: 1	[BK_C02]
Hap_6: 1	[BK_D01]
Hap_7: 1	[BK_D02]
Hap_8: 1	[BK_F01]
Hap_9: 1	[BK_F02]
Hap_10: 1	[BK_G01]
Hap_11: 1	[BK_G04]
Hap_12: 1	[BK_K01]
Hap_13: 1	[BK_K02]
Hap_14: 1	[BK_K03]
Hap_15: 1	[BK_K04]
Hap_16: 9	[KJ957618.1 KJ957549.1 KJ957550.1 KJ957551.1 KJ957552.1 KJ957553.1 KJ957607.1 KJ957591.1 KJ957592.1]
Hap_17: 4	[KJ957540.1 KJ957542.1 KJ957544.1 KJ957545.1]
Hap_18: 2	[KJ957541.1 KJ957543.1]
Hap_19: 10	[KJ957546.1 KJ957547.1 KJ957548.1 KJ957554.1 KJ957555.1 KJ957556.1 KJ957557.1 KJ957558.1 KJ957559.1 KJ957560.1]
Hap_20: 4	[KJ957561.1 KJ957562.1 KJ957563.1 KJ957564.1]
Hap_21: 11	[KJ957565.1 KJ957566.1 KJ957567.1 KJ957568.1 KJ957569.1 KJ957570.1 KJ957571.1 KJ957572.1 KJ957573.1 KJ957616.1 KJ957617.1]
Hap_22: 13	[KJ957582.1 KJ957583.1 KJ957584.1 KJ957585.1 KJ957586.1 KJ957587.1 KJ957589.1 KJ957590.1 KJ957608.1 KJ957609.1 KJ957610.1 KJ957611.1 KJ957612.1]
Hap_23: 2	[KJ957588.1 KJ957613.1]
Hap_24: 1	[KJ957614.1]
Hap_25: 1	[KJ957615.1]
Hap_26: 1	[KJ957593.1]
Hap_27: 1	[*A. andamanicus* MH813789.1]
Hap_28: 1	[*A. andamanicus* MH813790.1]

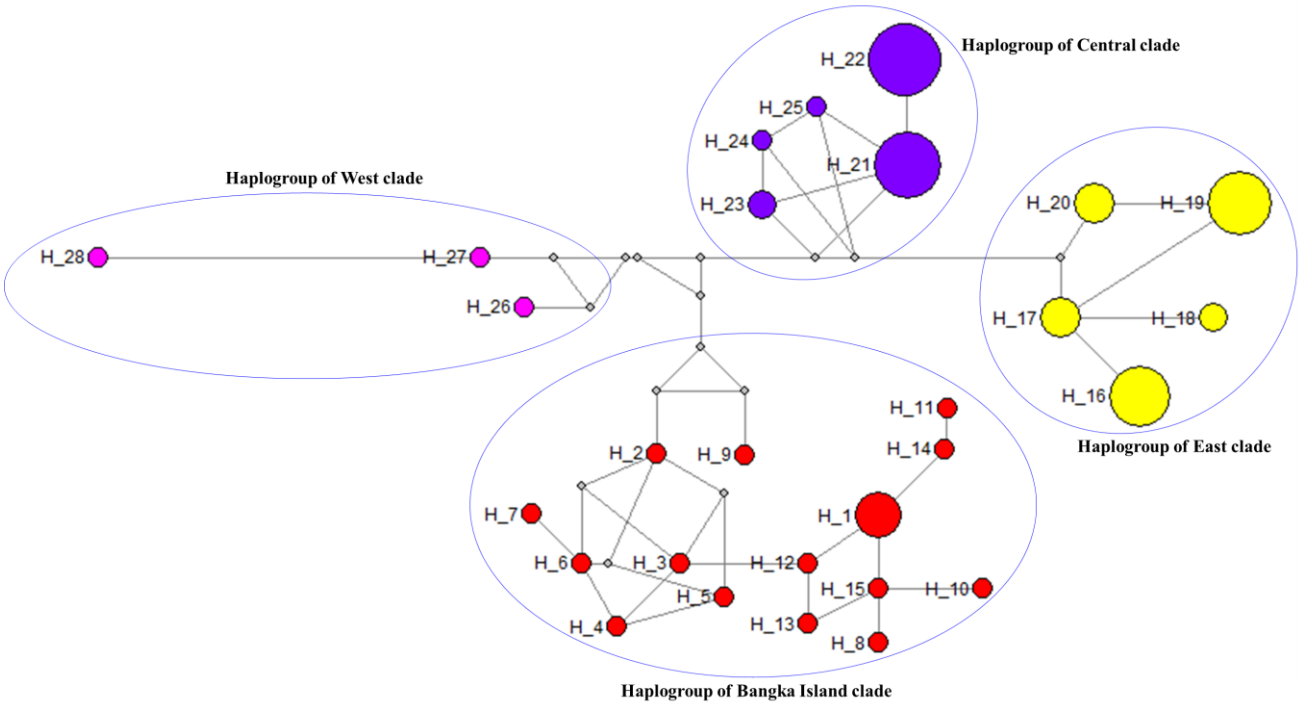

Figure 6. Median-joining network based on COI sequences indicating the *A. panchax* populations of Bangka Island (red haplogroup) formed distinct clade from other clades from Beck et al. (2017) and Katwate et al. (2018). Numbers correspond to haplotype. Crossing-line indicated mutated positions. Different colors showed the collection sites: red (Bangka Island clade), yellow (East clade), violet (Central clade), and pink (West clade), while grey (median vectors) and blue circle (haplogroup). Colored-circles size was proportional to haplotype frequency (see Table 3)

The water characteristic of tin mining pits as one of the habitats for *A. panchax* has an acidic pH and heavy metals contamination (Kurniawan et al. 2019; Kurniawan 2020). A recent study proved that the waters in abandoned tin mining pits are contaminated by heavy metals such as As, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, Sn, Ta, Te, Th, V, and Zn. Heavy metal presence corresponded with pH characteristics (Kurniawan 2020). Therefore, the pH value, acidic pH, specifically acidic mine drainage (AMD) due to the oxidation process of sulfide minerals and potentially acidic formation, is a significant indicator of abandoned post-mining habitats (PAF), is a significant indicator of abandoned post-mining habitats (Tan et al. 2007; Çelebi and Öncel 2016). These conditions cause organisms to adapt to the extreme environment since *A. panchax* was grouped as extremophile fishes (Riesch et al. 2015; Kurniawan and Mustikasari 2021). The environmental factor from Bangka Island as a tin producer may also contribute to the genetic diversity of *A. panchax*.

Previous studies investigated the correlation between the water quality of abandoned tin mining waters with the presence and morphological characteristics of *A. panchax*. The results showed that *A. panchax* was found in pits with a pH value of 3.81-3.84 and dissolved oxygen (DO) between 5.33 and 5.63. The change of chronosequence's pH impacted the other changes such as DO, BOD, C-organic, total nitrogen, total phosphate, and others (Kurniawan et al. 2019). The presences and phenotypic characters were correlated with the environmental factors, especially pH and heavy metals (Mustikasari et al. 2020a, b). Therefore, these factors strongly contributed to the polymorphism of *A. panchax* populations from Bangka Island.

Genetic diversity is considered an internal contributing element in the susceptibility of organisms to heavy metals-related poison or toxicity levels. The variety in various genes, directly or indirectly included in the metabolism of weighty metals, has been researched by specific studies. For example, metallothioneins (MTs) are proteins that detoxify heavy metals because of a few gene varieties of genomic sequences (Joneidi et al. 2019). Metallothioneins are small cysteine-rich proteins that play significant roles in metal homeostasis and protection for heavy metal toxicity, DNA defect, and oxidative conditions (Si and Lang 2018), cellular processes, cell growth regulation, and well as proliferation and DNA repair (Grennan 2011).

The contribution of MTs with various cell or organelles processes has gotten much consideration while their association with the mitochondria functions has been inadequate. Furthermore, it increases the duration of malfunctioning mitochondrial cells by protecting productive components from the damage caused by reactive oxygen species (ROS) and limiting apoptosis. MTs are also involved in mitochondrial infection, including redox balance, metal homeostasis, enzyme, and transcription factor regulation (Lindique et al. 2010; Kurniawan and Mustikasari 2021). The requirements for obtaining metal specificity and specific novel capacity may

drive their enhancement. MTs further enhanced the capability of metal detoxification under ecologically sensitive settings (Nam and Kim 2017). The relationship with mitochondria indicated an extreme environment as in abandoned tin mining waters of Bangka Island to genetic diversity, especially the COI gene of *A. panchax*. The heavy metals contamination and acidic pH in the habitat can cause genetic variations in mitochondrial genes, such as the COI gene. Moreover, heavy metals can reduce genetic variability within natural populations and cause genetic erosion (Ungherese et al. 2010). The evolution chronosequence of Bangka Island and the entire Sundaland may be attributed to the divergence of COI gene changes to diversify *A. panchax* genes.

It could be concluded that *Aplocheilus panchax* from the Bangka Island was highly divergence from other populations, including Indonesian populations with high genetic gap. The A. panchax population on Bangka Island formed a novel clade for Indonesia and in a global blue panchax phylogeographic.

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REFERENCES

Ashraf MA, Maah MJ, Yusoff I. 2011a. Heavy metals accumulation in plants growing in ex tin mining catchment. Int. J. Environ. Sci. Tech. 82: 401-416.

Ashraf MA, Maah MJ, Yusoff I. 2012a. Speciation of heavy metals in the sediments of former tin mining catchment. Iran. J. Sci. Technol. Trans. A Sci. 36 (A2): 163-180.

Ashraf MA, Maah MJ, Yusoff I. 2012b. Morphology, geology and water quality assessment of former tin mining catchment. Sci. World J. 2012: 1-15. DOI:10.1100/2012/369206

Banks SC, Cary GJ, Smith AL, Davies ID, Driscoll DA, Gill AM, Lindenmayer DB, Peakall R. 2013. How does ecological disturbance influence genetic diversity?. Trends Ecol. Evol. 28 (11): 670-679. DOI: 10.1016/j.tree.2013.08.005

Beck SV, Carvalho GR, Barlow A, Rüber L, Tan HH, Nugroho E, Wowor D, Nor SAM, Herder F, Muchlisin ZA, De Bruyn, M. 2017. Plio-Pleistocene phylogeography of the Southeast Asian blue panchax killifish, *Aplocheilus panchax*. PloS one 12 (7): e0179557.
DOI: 10.1371/journal.pone.0179557

Bennett, R.H., Ellender, B.R., Mäkinen, T., Miya, T., Pattrick, P., Wasserman, R.J., Woodford, D.J., Weyl, O.L.F. 2016. Ethical considerations for field research on fishes. Koedoe: African Protected Area Conservation and Science 58 (1): 1-15. DOI: 10.4102/koedoe.v58i1.1353

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22 (3): 148-155. DOI: 10.1016/j.tree.2006.11.004

Bobo-Pinilla J, Salmerón-Sánchez E, Mendoza-Fernández AJ, Mota JF, Peñas J. 2021. Conservation and phylogeography of plants: from the mediterranean to the rest of the world. Diversity 14 (78): 1-23. DOI: 10.3390/d14020078

Bolotov IN, Konopleva ES, Vikhrev IV, Gofarov MY, Lopes-Lima M, Bogan AE, LunnZ, Chan Y, Win T, Aksenova OV, Tomilova AA, Tanmuangpak K, Tumpeesuwan S, Kondakov AV. 2020. New freshwater mussel taxa discoveries clarify biogeographic division of Southeast Asia. Sci. Rep. 10 (1): 1-22. DOI: 10.1038/s41598-020-63612-5

Boluda CG, Hawksworth DL, Divakar PK, Crespo A. 2016. Microchemical and molecular investigations reveal Pseudephebe species as cryptic with an environmentally modified morphology. The Lichenologist 48 (5): 527-543. DOI: 10.1017/S0024282916000426

Bragança PH, Amorim PF, Costa WJ. 2018. Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. Zoosystematics Evol. 94 (1): 137-145. DOI: 10.3897/zse.94.22173

Buhay JE. 2009. "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. J. Crus. Biol. 29 (1): 96-110. DOI: 10.1651/08-3020.1

Čekovská K, Šanda R, Eliášová K, Kovačić M, Zogaris S, Pappalardo AM, Soukupová T, Vukić J. 2020. Population genetic diversity of two marine gobies (Gobiiformes: Gobiidae) from the North-Eastern Atlantic and the Mediterranean Sea. J. Mar. Sci. Eng. 8(792): 1-18. DOI: 10.3390/jmse8100792

Çelebi EE, Öncel MS. 2016. Determination of acid forming potential of massive sulfide minerals and the tailings situated in lead/zinc mining district of Balya (NW Turkey). J. African Earth Sci. 124: 487-496. DOI: 10.1016/j.jafrearsci.2016.09.014

Cerca J, Meyer C, Purschke G, Struck TH. 2020. Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (Stygocapitella; Annelida, Sedentaria). Mol. Phylogenet. Evol. 143(2020): 106663. DOI: 10.1016/j.ympev.2019.106663

Chen N, Juric I, Cosgrove EJ, Bowman R, Fitzpatrick JW, Schoech SJ, Clark AG, Coop G. 2019. Allele frequency dynamics in a pedigreed natural population. PNAS 116(6): 2158-2164. DOI: 10.1073/pnas.1813852116

Chowdhury MM, Rahman AS, Nahar L, Rahman M, Al Reza H, Ahmed MS. 2016. Efficiency of different DNA extraction methods for fish tissues: a comparative analysis. IOSR-JPBS 11(3): 11-15. DOI: 10.9790/3008-1103041115

Cimmaruta R, Scialanca F, Luccioli F, Nascetti G. 2003. Genetic diversity and environmental stress in Italian populations of the cyprinodont fish Aphanius fasciatus. Oceanol. Acta 26(1): 101-110. DOI: 10.1016/S0399-1784(02)01234-3

Coates DJ, Byrne M, Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Front. Ecol. Evol. 6(165): 1-13. DOI: 10.3389/fevo.2018.00165

Costa WJEM. 2013. Historical biogeography of aplocheiloid killifishes (Teleostei: Cyprinodontiformes). Vertebr. Zool. 63(2): 139-154.

Costa WJEM. 2016. Comparative morphology and classification of South American cynopoeciline killifishes (Cyprinodontiformes: Aplocheilidae), with notes on family-group names used for aplocheiloids. Vertebr. Zool. 66(2): 125-140.

De Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, den Bergh Gv, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, Von Rintelen T. 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63(6): 879-901. DOI: 10.1093/sysbio/syu047

De Oliveira DAS, Decraemer W, Moens T, Dos Santos GAP, Derycke S. 2017. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. BMC Evol. Biol. 17(1): 1-17. DOI: 10.1186/s12862-017-0908-0

Dekar M, Sarong MA, Batubara AS, Muchlisin ZA. 2018. Ichthyofauna of Aceh River, Aceh Province, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 216, No. 1, p. 012024). IOP Publishing. DOI:10.1088/1755-1315/216/1/012024

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol. Ecol.17(1): 431-449. DOI: 10.1111/j.1365-294X.2007.03538.x

Dorey JB, Groom SV, Freedman EH, Matthews CS, Davies OK, Deans EJ, Rebola C, Stevens MI, Lee MSY, Schwarz MP. 2020. Radiation of tropical island bees and the role of phylogenetic niche conservatism as an important driver of biodiversity. Proc. R. Soc. B 287(1925): 20200045. DOI: 10.1098/rspb.2020.0045

Dwiyitno HS, Parmentier K, Van Keer C. 2018. Method comparison of DNA isolation and quantification for fish and seafood authenticity determination. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 13(3): 115-124. DOI: 10.15578/squalen.v13i3.370

Farmawati DA, Wirajana N, Yowani SC. 2015. Perbandingan kualitas DNA dengan menggunakan metode boom original dan boom modifikasi pada isolat *Mycobacterium tuberculosis*. KIMIA 9(1): 41-46. DOI: 10.24843/JCHEM.2015.v09.i01.p07

Faulwetter S, Simboura N, Katsiaras N, Chatzigeorgiou G, Arvanitidis C. 2017. Polychaetes of Greece: an updated and annotated checklist. Biodivers. Data J. 5: e20997. DOI: 10.3897/BDJ.5.e20997

Fortes MD, Ooi JLS, Tan YM, Prathep A, Bujang JS, Yaakub SM. 2018. Seagrass in Southeast Asia: a review of status and knowledge gaps, and a road map for conservation. Botanica Marina 61(3): 269-288. DOI: 10.1515/bot-2018-0008

Furness AI, Reznick DN, Springer MS, Meredith RW. 2015. Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. Proc.R. Soc. B: Biol. Sci. 282(1802): 20142189. DOI: 10.1098/rspb.2014.2189

Grennan AK. 2011. Metallothioneins, a diverse protein family. Plant Physiol. 155(4): 1750-1751. DOI: 10.1104/pp.111.900407

Gupta A, Bhardwaj A, Sharma P, Pal Y. 2015. Mitochondrial DNA-a tool for phylogenetic and biodiversity search in equines. J Biodivers. Endanger. Species S1: S1.006. DOI: 10.4172/2332-2543.S1-006

Haileselasie TH, Mergeay J, Vanoverbeke J, Orsini L, De Meester L. 2018. Founder effects determine the genetic structure of the water flea Daphnia in Ethiopian reservoirs. Limnol. Oceanogr. 63(2): 915-926. DOI: 10.1002/lno.10678

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PD. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. Mol. Ecol. Notes 6(4): 959-964. DOI: 10.1111/j.1471-8286.2006.01470.x

Hashim, M., Nayan, N., Saleh, Y., Mahat, H., Shiang, W. F. 2018. Water quality assessment of former tin mining lakes for recreational purposes in Ipoh City, Perak, Malaysia. Indones. J. Geogr. 50(1): 25-33. DOI: 10.22146/ijg.15665

Hinckley A, Camacho-Sanchez M, Ruedi M, Hawkins MT, Mullon M, Cornellas A, Yuh FTY, Leonard JA. 2022. Evolutionary history of Sundaland shrews (Eulipotyphla: Soricidae: Crocidura) with a focus on Borneo. Zool. J. Linn. Soc. 20(2021): 1-14. DOI: 10.1093/zoolinnean/zlab045

Hosoishi S, Ogata K. 2019. Cryptic diversity in the widespread Asian ant Crematogaster rothneyi (Hymenoptera: Formicidae) inferred from morphological and genetic evidence. Zool. Stud. 58(11): 1-15. DOI: 10.6620/ZS.2019.58-11

Hu ZM, Zhong KL, Weinberger F, Duan DL, Draisma SG, Serrao E. 2020. Linking ecology to genetics to better understand adaptation and evolution: a review in marine macrophytes. Front. Mar. Sci. 7: 545102. DOI: 10.3389/fmars.2020.545102

Hughes AC. 2017. Understanding the drivers of S outheast A sian biodiversity loss. Ecosphere 8(1): e01624. DOI: 10.1002/ecs2.1624

Ivanova NV, Zemlak TS, Hanner RH, Hebert PD. 2007. Universal primer cocktails for fish DNA barcoding. Mol. Ecol. Notes 7(4): 544-548. DOI: 10.1111/j.1471-8286.2007.01748.x

Joneidi Z, Mortazavi Y, Memari F, Roointan A, Chahardouli B, Rostami S. 2019. The impact of genetic variation on metabolism of heavy metals: Genetic predisposition?. Biomed. Pharmacother. 113: 108642. DOI: 10.1016/j.biopha.2019.108642

Kaňuch P, Berggren Ä, Cassel-Lundhagen A. 2014. Genetic diversity of a successful colonizer: isolated populations of *Metrioptera roeselii* regain variation at an unusually rapid rate. Ecol. Evol. 4(7): 1117-1126. DOI: 10.1002/ece3.1005

Karanovic T, Djurakic M, Eberhard SM. 2016. Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. Syst. Biol. 65(2): 304-327. DOI: 10.1093/sysbio/syv088

Katwate U, Kumkar P, Britz R, Raghavan R, Dahanukar N. 2018. The identity *of Aplocheilus andamanicus* (Köhler, 1906) (Teleostei: Cyprinodontiformes), an endemic Killifish from the Andaman Islands, with notes on *Odontopsis armata* van Hasselt. Zootaxa 4382(1): 159-174.
DOI: 10.11646/zootaxa.4382.1.6

Koki IB, Zain SM, Low KH, Azid A, Juahir H, Abdul ZM. 2019. Development of water quality index of ex-mining ponds in Malaysia. Mal. J. Fund. Appl. Sci. 15(1), 54-60. DOI: 10.11113/mjfas.v15n2019.1079

Korshunova T, Picton B, Furfaro G, Mariottini P, Pontes M, Prkić J, Fletcher K, Malmberg K, Lundin K, Martynov A. 2019. Multilevel fine-scale diversity challenges the 'cryptic species' concept. Scientific Reports 9(1): 1-23. DOI: 10.1038/s41598-019-42297-5

Kuffel A, Gray A, Daeid NN. 2021. Impact of metal ions on PCR inhibition and RT-PCR efficiency. Inter. J. Legal Med. 135(1): 63-72. DOI: 10.1007/s00414-020-02363-4

Kurniawan A, Mustikasari D. 2021. Review tentang kemampuan ikan ekstremofil untuk hidup di perairan asam dan terkontaminasi logam berat pascapenambangan timah. Jurnal Ilmu Lingkungan 19(3): 541-554. DOI: 10.14710/jil.19.3.541-554

Kurniawan A, Oedjijono, Tamad, Sulaeman U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka District, Indonesia. Indones. J. Chem. 19(1): 254-261. DOI: 10.22146/ijc.33613

Kurniawan A, Prasetiyono E, Syaputra D. 2020. Analisis korelasi parameter kualitas perairan kolong pascatambang timah dengan umur berbeda. Samakia: Jurnal Ilmu Perikanan 11(2): 91-100. DOI: 10.35316/jsapi.v11i2.824

Kurniawan A. 2020. The metal oxides of abandoned tin mining pit waters as an indicator for bacterial diversity. Aquac. Aquar. Conserv. Legis. 13(5): 2982-2992.

Lee CE. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17(8): 386-391. DOI: 10.1016/S0169-5347(02)02554-5

Lindeque JZ, Levanets O, Louw R, Van Der Westhuizen FH. 2010. The involvement of metallothioneins in mitochondrial function and disease. Curr. Protein Pept. Sci 11 (4): 292-309. DOI: 10.2174/138920310791233378

Liu Y, Webber S, Bowgen K, Schmaltz L, Bradley K, Halvarsson P, Abdelgadir M, Griesser M. 2013. Environmental factors influence both abundance and genetic diversity in a widespread bird species. Ecol. Evol. 3(14): 4683-4695. DOI: 10.1002/ece3.856

Lone AR, Thakur SS, Tiwari N, Sokefun OB, Yadav S. 2021. Disentangling earthworm taxonomic stumbling blocks using molecular markers. J. Threat. Taxa 13(11): 19566-19579. DOI: 10.11609/jott.6888.13.11.19566-19579%20

Markert JA, Champlin DM, Gutjahr-Gobell R, Grear JS, Kuhn A, McGreevy TJ, Roth A, Bagley MJ, Nacci DE. 2010. Population genetic diversity and fitness in multiple environments. BMC Evol. Biol. 10(1): 1-13. DOI: 10.1186/1471-2148-10-205

Martinez AS, Willoughby JR, Christie MR. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecol. Evol. 8(23): 12022-12031. DOI: 10.1002/ece3.4661

Mustikasari D, Nuryanto A, Suryaningsih S. 2020a. The presence of blue panchax (*Aplocheilus panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of different age. Aquac. Aquar. Conserv. Legis. 13(5): 2538-2550.

Mustikasari D, Suryaningsih S, Nuryanto A. 2020b. Morphological variation of blue panchax (*Aplocheilus panchax*) lives in different habitat assessed using truss morphometric. Biosaintifika 12(3): 399-407. DOI: 10.15294/biosaintifika.v12i3.26593

Nam YK, Kim EJ. 2017. Diversification and domain evolution of molluskan metallothioneins: a mini review. Fish Aquatic Sci. 20(1): 1-18. DOI: 10.1186/s41240-017-0054-z

Nascimento MHS, Almeida MS, Veira MNS, Filho DL, Lima RC, Barros MC, Fraga EC. 2016. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. Genet. Mol. Res. 15(3): 1-11. DOI: 10.4238/gmr.15038476.

Nater A, Arora N, Greminger MP, van Schaik CP, Singleton I, Wich SA, Fredrikson G, Perwitasari D, Pamungkas J, Krützen M. 2013. Marked population structure and recent migration in the critically endangered Sumatran orangutan (*Pongo abelii*). J. Hered. 104(1): 2-13. DOI: 10.1093/jhered/ess065

Parenti LR, Hartel KE. 2011. Osteology identifies *Fundulus capensis* Garman, 1895 as a killifish in the family Fundulidae (Atherinomorpha: Cyprinodontiformes). Copeia 2011(2): 242-250. DOI: 10.2307/41261873

Pohl M, Milvertz FC, Meyer A, Vences M. 2015. Multigene phylogeny of cyprinodontiform fishes suggests continental radiations and a rogue taxon position of Pantanodon. Vertebr. Zool. 65(1): 37-44.

Pratomo YW, Zahida F, Yuda P. 2021. Perbandingan Metode isolasi DNA sebagai templat PCR untuk identifikasi jenis kelamin Cerek Jawa (*Charadriius javanicus*) secara molekuler menggunakan Primer

2550F/2718R. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati 6(2): 78-86. DOI: 10.24002/biota.v6i2.3444

Riesch R, Tobler M, Plath M. 2015. Extremophile Fishes. Ecology, Evolution, and Physiology of Teleosts in Extreme Environments: Springer. Netherland.

Rius M, Turon X. 2020. Phylogeography and the description of geographic patterns in invasion genomics. Front. Ecol. Evol. 8(439): 595711. DOI: 10.3389/fevo.2020.595711

Rizko N, Kusumaningrum HP, Ferniah RS, Pujiyanto S, Erfianti T, Mawarni SN, Rahayu HT, Khairunnisa D. 2020. Isolasi DNA daun Jeruk Bali Merah (*Citrus maxima* Merr.) dengan modifikasi Metode Doyle and Doyle. Berkala Bioteknologi 3(2): 1-7.

Rosilawati ML, Sudarmono P, Ibrahim F. 2002. Sensitivitas metode PCR (*Polymerase Chain Reaction*) dalam mendeteksi isolat klinis *Mycobacterium tuberculosis*. Jurnal Kedokteran Trisakti 21(1): 7-14. DOI: 10.18051/UnivMed.2007.v26.1-10

Ruchi W, Putri DH, Anhar A, Farma SA. 2018. Comparison of three different DNA isolation methods to degradate the *Trichoderma* fungi cell wall. Bioscience 2(1): 50-59. DOI: 0201931102859-0-00

Salles T, Mallard C, Husson L, Zahirovic S, Sarr AC, Sepulchre P. 2021. Quaternary landscape dynamics boosted species dispersal across Southeast Asia. Commun. Earth Environ. 2(1): 1-12. DOI: 10.1038/s43247-021-00311-7

Schierenbeck KA. 2017. Population-level genetic variation and climate change in a biodiversity hotspot. Ann. Bot. 119(2): 215-228.

Setiaputri AA, Barokah GR, Sahaba MAB, Arbajayanti RD, Fabella N, Pertiwi RM, Nurilmala M, Nugraha R, Abdullah A. 2020. Perbandingan metode isolasi DNA pada produk perikanan segar dan olahan. Jurnal Pengolahan Hasil Perikanan Indonesia 23(3): 447-458.

Si M, Lang J. 2018. The roles of metallothioneins in carcinogenesis. J. Hemat. Oncol. 11(1): 1-20. DOI: 10.1038/s43247-021-00311-7

Star B, Spencer HG. 2013. Effects of genetic drift and gene flow on the selective maintenance of genetic variation. Genetics 194(1): 235-244. DOI:10.1534/genetics.113.149781/-/DC1

Tan GL, Shu WS, Hallberg KB, Li F, Lan CY, Huang LN. 2007. Cultivation-dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China. FEMS Microbiol. Ecol. 59(1): 118-126. DOI: 10.1111/j.1574-6941.2006.00216.x.

Thu PT, Huang WC, Chou TK, Van Quan N, Van Chien P, Li F, Shao KT, Liao TY. 2019. DNA barcoding of coastal ray-finned fishes in Vietnam. PloS ONE 14 (9): e0222631. DOI: 10.1371/journal.pone.0222631

Ungherese G, Mengoni A, Somigli S, Baroni D, Focardi S, Ugolini A. 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). Environ. Pollut. 158(5): 1638-1643. DOI:10.1016/j.envpol.2009.12.007

Von Rintelen K, Arida E, Häuser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. RIO 3: e20860. DOI: 10.3897/rio.3.e20860

Wang XY, Wang MM, Chen C, Wang XQ. 2020. Genetic variation and phylogeographic structure of Spodoptera exigua in western China based on mitochondrial DNA and microsatellite markers. PloS ONE 15(5): e0233133. DOI: 10.1371/journal.pone.0233133

Xiao JH, Wang NX, Li YW, Murphy RW, Wan DG, Niu LM, Hu HY, Fu YG, Huang DW. 2010. Molecular approaches to identify cryptic species and polymorphic species within a complex community of fig wasps. PLoS ONE 5 (11): e15067. DOI: 10.1371/journal.pone.0015067

Yang CH, Wu KC, Chuang LY, Chang HW. 2019. Decision theory-based COI-SNP tagging approach for 126 scombriformes species tagging. Front. Genet. 10: 259. DOI: 10.3389/fgene.2019.00259

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Phylogeography of *Aplocheilus panchax* in Indonesia, with special focus on the Bangka Island population

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Abstract. *Mustikasari D, Nuryanto A, Suryaningsih S. 2022. Phylogeography of* Aplocheilus panchax *in Indonesia, with special focus on the Bangka Island population. Biodiversitas 23: xxxx.* Previous study divided Blue panchax, *Aplocheilus panchax* into three different clades, namely West (W), Central (C), and East (E) clades. Blue panchax populations from Indonesia were belong to Central and East clades. However, that study did not include blue panchax samples from pits with harsh conditions in Bangka Island. Therefore, this study aimed to assess the phylogeography of blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. The results showed that genetic distance within Bangka Island population was less than 2%, while genetic distances between Bangka population and other populations were ranged from 103.87% to 122.10%. There was also a clear genetic gap between the Bangka and other populations, with the minimum gap was 101.94%. Furthermore, the seventy-nine sequences analyzed resulted 28 haplotypes with genetic variability of 0.221 for nucleotide diversity (π), 0.923 for Haplotype diversity (Hd), 68.028 for Fu's Fs-test, 2.00 ($P < 0.02$) for Fu and Li's D-test, and 2.365 ($P < 0.02$) for Fu and Li's F-test; and 1.927 for Tajima's D test ($0.10 > P > 0.05$). The Bangka population of *Aplocheilus panchax* established a distinct clade from the Western (W), Eastern (E), and Central (C) clades. Molecular data established that the population on Bangka Island is a novel clade for Indonesia and a global blue panchax phylogeographic.

Keywords: Abandoned tin mining pits, genetic distance, haplotype, killifish, new clade position

INTRODUCTION

Phylogeography analyzes and understands organism diversity and biogeography (Bobo-Pinilla et al. 2021). Furthermore, it is a study of geographical distributions of closely related genetic lineages or geographic ordination of genotypes (Rius and Turon, 2020). Therefore, phylogeographic research is essential to evaluate the geographical distribution of these genetic lineages and their pattern, as well as elaborate ecology factors and organism biodiversity (Lone et al. 2021).

The blue panchax (*Aplocheilus panchax* Hamilton, 1882) is an endemic species to the Oriental region (Costa 2013; Costa 2016; Beck et al. 2017), widely distributed across the Indo-Malayan Islands, including Indonesia, the Indo-China region, and India (Dekar et al. 2018; Bolotov et al. 2020). *Aplocheilus panchax* is a fish from Genus *Aplocheilus*, Family Aplocheilidae, Suborder Aplocheiloidei, Order Cyprinodontiformes, and Class Actinopterygii (Parenti and Hartel 2011; Furness et al. 2015). Fishes belonging to Order Cyprinodontiformes are also known as *Aplocheiloid killifishes* or *livebearer*s (Pohl et al. 2015; Braganca et al. 2018).

Indonesia is one of the world's biodiversity hotspots with many different habitats and a highly complicated geological history (de Bruyn et al. 2014; von Rintelen et al. 2017). Generally, Southeast Asia's complex climatic and geological history caused this region to have high biodiversity (Beck et al. 2017; Fortes et al. 2018). Biogeography, geology, climate, and ecology of Southeast Asia have led to megadiverse organisms' evolution. Additionally, the region is home to several endemic and ecologically well-adapted species (Hughes 2017; von Rintelen et al. 2017). The *Aplocheilus panchax* can be used to understand further how climatic changes and sea-level fluctuations have influenced the species' distribution within this region. Specifically, sea-level variations result from glacial cycles that continued throughout the Pleistocene, interfering with and restricting the network of all populations. These examples show proof of isolation in palaeodrainage basins (Beck et al. 2017).

Previous phylogeographic study using the COI gene placed worlds' *A. panchax* populations into three different clades; West (W), Central (C), and East (E) clades. The *A. panchax* populations from Indonesia were divided into Central and East clades. Central clade consisted of Pekanbaru, Jambi, and West Sumatera populations. East clade was formed by *A. panchax* populations from Java, Bali, Kalimantan, and Sulawesi islands (Beck et al. 2017). Nevertheless, the study by Beck et al. (2017) did not include *A. panchax* samples from Bangka Island. Bangka Island, the biggest tin producer in Indonesia, has unique waters like a lake or pit, known as kolong, formed and abandoned after tin mining activity. This water has low pH (acid waters), low nutrients, minimum dissolved oxygen (DO), and high heavy metals (Ashraf et al. 2011, 2012a, 2012b; Hashim et al. 2018; Koki et al. 2019; Kurniawan 2020). However, *A. panchax*, locally known as ikan Kepala

Timah, can live in this extreme habitat (Kuniawan et al. 2019, Kurniawan et al. 2020; Mustikasari et al. 2020a). The genus *Aplocheilus*, which includes *A. panchax*, was classified as an extremophile fish due to its ability to survive in harsh environmental circumstances (Riesch et al. 2015; Kurniawan and Mustikasari 2021). Mustikasari et al. (2020a, b) explored the presence and morphological variety of blue panchax (*A. panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of various ages.

Island biogeography investigates how the richness of the ecosystems and the complexity of the biodiversity may cause speciation. The adaptive radiation, speciation, climate cycles, and topographical complexity can shape island biodiversity (Dorey et al. 2020). Phylogenetic and phylogeographic analyses based on mitochondrial DNA (mt-DNA) are commonly used in island biodiversity exploration. In addition, mt-DNA possesses some characteristics, including cell quantity, genome size, haploid, maternal inheritance, and extremely low probability of paternal leakage, mutation rate, and change mainly caused the mutation. These features make mt-DNA a useful and one of the most often used markers in molecular analysis. The marker has been frequently used to study genetic diversity, population organization, phylogeography, and organism evolution (Gupta et al. 2015).

This study aimed to assess the phylogeography of ~~blue~~ Blue panchax in Indonesia with special focus on the Bangka Island population using the cytochrome c oxidase 1 (COI) gene. This is the first time the populations from Bangka Island have been genetically analyzed using the cytochrome oxidase I (COI) gene and compared to ~~an~~other populations on the global distribution of blue panchax. Furthermore, the presence and genetic profile of *A. panchax* can be a model of extremophile fish study. Therefore, it is used as a bioindicator for harsh habitats since the global distribution, ecological and genetic evolution with the Bangka Island population corresponds to others in Indonesian waters.

MATERIALS AND METHODS

Study area

The study was conducted in Pangkalpinang City and Bangka District of Bangka Belitung Archipelago Province, Indonesia. Fish samples were collected from abandoned tin mining lakes (pits) of different ages and the Limbung River. Station A and B (< 5 years old), Station C and D (5 - 15 years), Station E and F (15 - 25 years), Station G (25 - 50 years), Station H (50 - 100 years), Station I and J (> 100 years), and Limbung River Stream of Bangka District as Station K such as shown in Figure 1. These ~~years~~ ages of research stations indicated the chronosequence of the abandoned tin mining pits were taken place there. There were nothings put in the habitats, due to the chronosequence only explain about a succession that happened naturally. In addition, they were related to our previous studies about their characteristics of water quality and the presence of *Aplocheilus panchax* in the abandoned tin mining pits based on the difference of time (Mustikasari et al. 2020a, b).

Procedures

Samples preparation for molecular analysis

The twenty samples were collected at 09.00 am-1.00 pm from closed and open waters of abandoned tin mining lakes (pits) and waters of Limbung River Stream, Bangka Island, using nets with a mesh size of about 0.4 mm. We declared that the collected fish as samples were handled in a good manner as explained as Bennett et al. (2016) which cite Canadian Council on Animal Care (2005) and American Fisheries Society (2014) about guidelines for the use of fishshes in research. They were handled with minimises pain, distress, suffering and unnecessary loss of external mucus or scales, minimum of the handling duration, to avoid unnecessary stress, and exposure time. We also gave attention to life-cycle events, such as aggregations of breeding fish and sensitive habitats ~~should be~~were avoided.

Furthermore, this study utilized a 2.0 ml cryotube with ethanol absolute to preserve each of the fish sample for molecular analysis. The pectoral fin about 2 mm from each dead sample of *A. pachax* was taken for the DNA isolation and molecular identification.

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Molecular analysis

The genomic DNA extraction was analyzed with gSYNC™ DNA Extraction Kit (Geneaid, GS300). Nucleic acid (genomic DNA) concentration was measured using Nanodrop™ 2000/2000c spectrophotometers. Furthermore, molecular analysis was referred to Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika Science Indonesia, in 2021.

Polymerase chain reaction (PCR) amplification was conducted with (2x) MyTaq HS Red Mix (Bioline, BIO-25048) and KOD FX Neo (Toyobo, KFX-201). The components 1 x 25 µL PCR Master Mix were dd H2O 9.5 µL; MyTaq HS Red Mix, 2x 12.5 µL; 10 µM VF2_t1 0.5 µL; 10 µM Fish F2_t1 0.5 µL; 10 µM Fish R2_t1 0.5 µL; 10 µM Fish FR1d_t1 0.5 µL; and DNA Template 1 µL. Primer sequence of PCR amplification were VF2-t1 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3'; FR1d-t1 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3'; FishR2_t1 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3'; and FishF2_t1 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (Ivanova et al. 2007).

The predenaturation phase initiated the Polymerase Chain Reaction (PCR) cycling for 1 minute (95 °C). Subsequently, the actual PCR amplification was conducted for 35 cycles, denaturation process for 15 seconds (95 °C), annealing process for 15 minutes (50 °C), and extension process for 45 seconds (72 °C). The PCR products (1 µL) were assessed by electrophoresis with 1% TBE agarose with Marker 100bp DNA ladder (loaded 2 µL). Furthermore, the quality and length of the PCR products were analyzed by agarose gel electrophoresis. Bi-

directional Sequencing conducted the sequencing step at 1st base Asia.

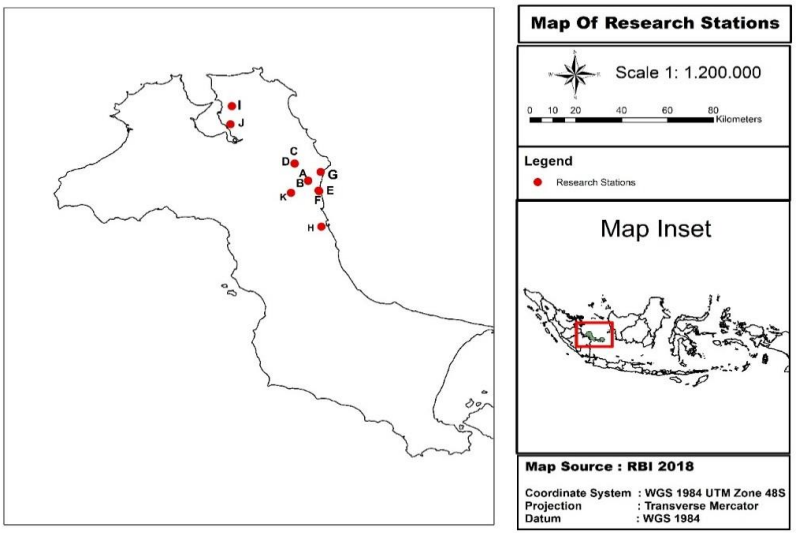

Figure 1. Map of research stations at Bangka Island, Indonesia (Mustikasari et al. 2020a, b)

Data analysis

The contig sequences were obtained from reverse and forward sequences aligned with Program BioEdit (Hall 2011). The phylogenetic study was carried out using MEGA XI (Tamura et al. 2021), utilizing the Maximum Parsimony (MP) statistical approach, and a bootstrap consensus tree was constructed using 1,000 replicates. The phylogenetic tree was constructed by comparing sequences of *A. panchax* from Bangka Island with some sequences of *A. panchax* from a previous study (Beck et al. 2017). The study conducted in India (Tamil Nadu and Kolkotta), Cambodia, Vietnam, Thailand (Krabi), Malaysia (Sungai Batu Pahat, Penang, Dungun), Singapore, and Indonesia (Aceh, Pekanbaru, Pulau Laut, West Sumatra, Jambi, Bogor, Surabaya, Banjarmasin, Bali, and Sulawesi) as shown in Figure 2 aim to investigate the position of *A. panchax* population from Bangka Island. Furthermore, it used a sequence of *A. andamanicus* (Katwate et al. 2018). The *A. warneri* (accession number KJ844713.1) was used as outgroup species (Pohl et al. 2015) for this phylogenetic analysis. Sequences metadata from Beck et al. (2017) and Katwate et al. (2018) from NCBI (National Center for Biotechnology Information) were utilized. DnaSP v5 was used to assess the haplotype (Hd) and nucleotide diversity (π), as well as to perform Fu and Li's F and Tajima's D neutrality tests (Čekovská et al. 2020). A phylogenetic network was constructed according to the median-joining method within the software Network 10. The Kimura 2 Parameter (K2P) genetic distance was calculated in the MEGA XI (Tamura et al. 2021). Genetic distances were utilized to estimate the genetic gap between Bangka Island and other clade populations. The lowest genetic distance was estimated by subtracting minimum genetic distance among populations by maximum genetic distance within Bangka Island population.

RESULTS AND DISCUSSION

DNA quantity and quality

The successfulness of organisms identification methods based on genetic material, such as PCR, relies on the quantity and quality of nucleic acid, purification method, and PCR amplification process. Unfortunately, the low amount and quality of genetic material (DNA) and the appearance of inhibitors inhibit the PCR amplification efficiency (Chowdhury et al. 2016; Dwiyitno et al. 2018; Kuffel et al. 2021). The analysis of the quantity of sample' DNA by Nanodrop spectrophotometer resulted in DNA concentration (ratio $A_{260/280}$) for all samples between 1.65 and 1.99 (Table 1).

The ratio of absorbance at 260 nm and 280 nm ($A_{260/280}$) is used to assess the purity of material genetic. The measurement of DNA concentration with Nanodrop was conducted at a wavelength of 260 nm. In comparison, the protein was measured at a wavelength of 280 nm with pure DNA having an absorbance ratio of 260/280 between 1.6 and 2.0 (Setiaputri et al. 2020), 1.7-2.0 (Ruchi et al. 2018), or 1.8-20 (Pratomo et al. 2021). The absorbance value below the low absorbance limit indicates the presence of polysaccharides, phenol, and protein contamination.

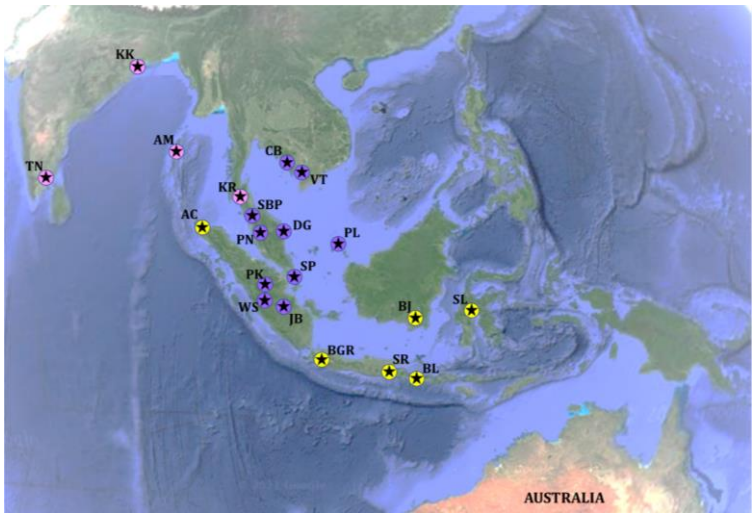

Figure 2. Sampling locations for *Aplocheilus panchax* over 20 areas, namely Tamil Nadu (TN), Kolkotta (KK), Andaman Island (AM) Cambodia (CB), Vietnam (VT), Krabi (KB), Sungai Batu Pahat (SBP), Aceh (AC), Penang (PN), Dungun (DG), Pulau Laut (PL), Singapore (SP), Pekanbaru (PK), West Sumatera (WS), Jambi (JB), Bogor (BG), Surabaya (SR), Banjarmasin (BJ), Bali (BL) and Sulawesi (SL). (Map was reconstructed from Beck et al. 2017 and Katwate et al. 2018)

Table 1. Quantity of Sample DNA

Sample code*	**$A_{260/280}$**
BK_A01	1.72
BK_A02	1.90
BK_B01	1.91
BK_B02	1.87
BK_C01	1.87
BK_C02	1.91
BK_D01	1.93
BK_D02	1.99
BK_E01	1.90
BK_E02	1.89
BK_F01	1.87
BK_F02	1.65
BK_G01	1.75
BK_G02	1.59
BK_G03	1.81
BK_G04	1.88
BK_K01	1.65
BK_K02	1.83
BK_K03	1.71
BK_K04	1.70

Note: *) sample' volume (30 µL)

Meanwhile, above the absorbance 2.0 indicates the presence of RNA contamination during the DNA isolation process (Rosilawati et al. 2002; Farmawati et al. 2015; Rizko et al. 2020). The success of a purification extraction and the quantity of DNA is highly dependent on the isolation of the resulting DNA. Therefore, the isolation process using commercial kits is safer from processing errors that cause contamination. However, studies on several specimens with various treatments stated that not all commercial kits can harvest DNA in high concentrations (Hajibabaei et al. 2006; Setiaputri et al. 2020).

The product of PCR also showed that the COI gene length of *A. panchax* populations from Bangka Island was about 700 bp. Meanwhile, the COI gene length for *A. panchax* was around 621 bp in other studies with accession numbers KJ957593.1, KJ957617.1, and KJ957618.1 from Beck et al. (2017), and also accession number MG813789.1 (Sample *A. andamanicus*) from Katwate et al. (2018). The length showed that the COI gene of *A. panchax* from Bangka Island was the longest. The mitochondrial gene, namely COI, is commonly used for DNA barcoding, about 650 bp and these gene sequences are also used for ecological and evolutionary studies (Yang et al. 2019). The COI gene length differences of *A. panchax* populations indicate a diversity of *A. panchax* around the Oriental region. It plays a vital role in a global study to collect information about biodiversity and be a key in phylogenetic and phylogeographic analyses (Buhay 2009).

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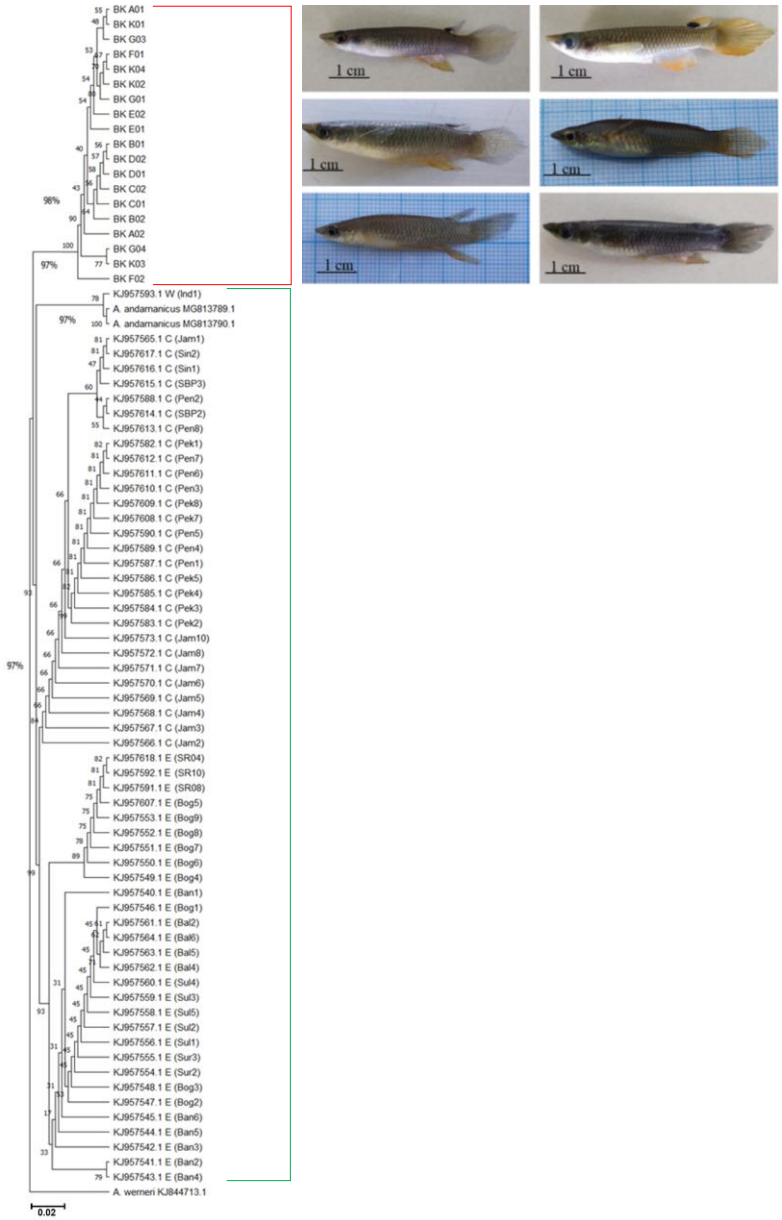

Figure 3. The phylogenetic position of *A. panchax* from Bangka Island is supported by Maximum Parsimony (MP) bootstrap values. Sequences of topotypes of populations from Bangka Island were in red line, while sample's sequences of Beck et al. (2017) and Katwate et al. (2018) which were cited from the existing databases of COI genes, shown in the pink line (West clade), violet line (East clade), and yellow line (East clade), while *A. warneri* is outgroup.

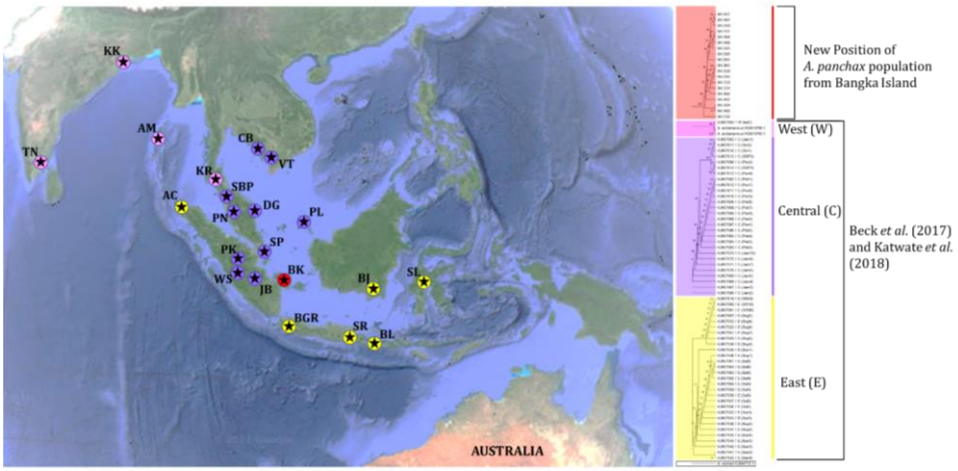

Figure 4. New phylogeographical map for *A. panchax*, particularly Bangka Island populations (red cycle) on global phylogeographic of blue panchax

The phylogenetic tree and phylogeographic of *A. panchax* population from Bangka Island

The phylogenetic tree indicated that the ancestral relationship between the *A. panchax* from all over their geographic~~the~~ distribution based on the COI gene sequences was well supported by ~~COI gene sequence analysis based on well-supported clades~~high bootstrap value (>90% bootstrap values). The phylogenetic tree analysis showed *A. panchax* widespread throughout the Oriental region, including Indonesia as a part of Southeast Asia countries. However, the population from Bangka Island has different COI sequences and separated from others. Bangka Island individuals formed a distinct clade from those previously described by Beck et al. (2017) and Katwate et al. (2018). (Figure 3).

Beck et al. (2017) revealed their research about Bayesian posterior probabilities that displayed for Western (W) clade, Eastern (E) clade, and Central (C) clade ~~of *A. panchax*~~. We added a novel clade to previous phylogenetic tree (Beck et al. 2017; Katwate et al. 2018) by adding samples from Bangka Island (Figure 3). Therefore, the authors also recommended a new map reconstruction of global phylogeographic on blue panchax (*A. panchax*) gene distribution (Figure 4).

A previous study by Katwate et al. (2018) denied the presence of *A. panchax* in the Indo-Malay region. They provided morphological and molecular evidence and demonstrated that *A. andamanicus* and *A. armatus* distinct and valid killifish species in the Indo-Malay region. However, the present study supported Beck et al. (2017) by showing that the killifish from Indonesia, especially populations from Bangka Island, is *A. panchax*. The present result was supported by morphological data that killifish from Bangka Island is *A. panchax* (Mustikasari et al. 2020b).

Several factors can impact an individual's genetic diversity and population genetic structure, such as climate or environmental change, natural boundaries, environmental variables, movement and migration, and human activities (Nater et al. 2013; Wang et al. 2020). For example, the persistence of climate change is determined by life history characters of organisms such as dispersal ability, generation period, reproductive ability, habitat specialization, organism interactions, genetic diversity, and habitat or migration corridors (Schierenbeck 2017).

The critical part was that the position of *A. panchax* populations from Bangka Island was different from other locations, but they were included as Sundaland region. It indicated that the biogeographic region of Sundaland (Borneo, Sumatra, the Malay Peninsula, Java, Palawan, and associated islands) is essential to understand evolution (Hinckley et al. 2022). As long as chronosequence exists, the ecological component may affect organism diversity. Ecological disturbance is necessary to maintain the dynamism and diversity of ecosystems. The disturbance history may be the primary driver that shapes patterns of genetic diversity in many natural populations through changes (Banks et al. 2013). Beck et al. (2017) explained that the significant mitochondrial clades of *A. Panchax* are consistent with this study. The basal dissimilarity of *A. panchax* mitochondrial ancestries was around 3.5 million years ago (Ma).

On the other hand, the subsequent dissimilarity timings of these clades occurred in the early Pleistocene (~2.6 Ma). Ceaseless phylogeographic investigation showed a reasonable west-east dispersal followed by quick radiation across Southeast Asia. Salles et al. (2021) recreate scene

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evolution, sedimentation, and Sundaland flooding history under tectonic, eustatic, and precipitation constraining conditions. Furthermore, they link Sundaland's flooding history to tectonic and sea-level conditions using three external driving instruments of rainfalls, eustatic sea-level variations, and tectonics. Over the last one million years, these factors caused the Mekong, Johor, Siam, and East Sunda River to merge. Additionally, it causes Bangka Island to be separated from the mainland of Sumatra Island, Malay Peninsula, Java, and Kalimantan, about 400 thousand years ago (ka).

Founder effects or events can impact stochasticity in species' genetic population structure at the regional scale (Haileselasie et al. 2018). Even though their populations have decreased in genetic diversity after the founder event, specific individuals that establish well in new places appear to be destined to extinction. The limit of life forms to adjust to new environments can rely upon the organism's capacity of reacting to regular determination, which is dictated by the genetic pattern of the founder populations (Lee 2002; Dlugosch and Parker 2008; Kaňuch et al. 2014). This condition can impact genetic variation in natural populations, mutation, and genetic drift (Star and Spencer 2013). Genetic drift and gene flow shape allele frequencies over time for an extended period (Chen et al. 2019). Ecological and geological factors may have contributed to the high degree of divergence in ~~gene~~ COI gene between *A. panchax* populations from Bangka Island and other sites. Subsequently, *A. panchax* sequences from all clades were also analyzed, with a genetic distance of 0.00% to 1.93% for within Bangka Island population. Genetic distance between Bangka Island and other clade populations were ranged from 103.87% to 122.10%. Maximum intrapopulation genetic distances within Bangka Island, East clade, and Central clade populations was less than 2%, while within West clade populations had a maximum genetic distance larger than 2% (Table 2). As a result, there was a clear genetic gap between the Bangka Island population and other populations with minimum gap 101.94% (Bangka Island and Central Clade populations) and maximum gap value was 108.52% (Bangka Island and West clade populations). These values made Bangka Island populations were significantly different and separated from other clade populations (Figure 5).

The genetic distances were lower than 2% (0.02), indicating that *A. panchax* populations from Bangka Island did not indicate cryptic species. However, the phylogenetic showed that *A. panchax* populations from Bangka Island differed from others. Intraspecific genetic distances in some species are higher than 2% (Thu et al. 2019) or above 3% (Nascimento et al. 2016), indicating the existence of cryptic diversity within these fishes. The term "cryptic species" has lately replaced the term "siblings" for taxa of this type (Korshunova et al. 2019). Sibling refers to two or more distinct individuals classified as a single species under one or the same scientific name (Bickford et al. 2007; Xiao et al. 2010; Karanovic et al. 2016). These species cannot be confidently separated based on their morphology, yet they were genetically distinct (Boluda et al. 2016; De Oliveira et al. 2017; Faulwetter et al. 2017). The cryptic species were delineated as individuals in the same geographic area but exhibited significant molecular phylogenetic contrasts. However, they are not recognized morphologically and ethologically (Hosoishi and Ogata 2019; Cerca et al. 2020).

The seventy-nine (79) sequences that were analyzed by DNAsp v.5 showed genetic variability of 0.22 for nucleotide diversity (π), 0.895 for Haplotype diversity (Hd), 68.028 for Fu's Fs test, 2.00 ($P < 0.02$) for Fu and Li's D' test, and 2.365 ($P < 0.02$) for Fu and Li's test. There were 28 haplotypes of these specimens among the COI sequences (n = 79) built into a haplotype network (Figure 6). All of the sequences analyzed showed that the haplotype network has a star-shaped topology.

Hap_1 to Hap_15 showed haplotype of *A. panchax* populations from Bangka Island, while Hap_16 to Hap_28 were from Beck et al. (2017) and Katwate et al. (2018). Current approaches to biodiversity research focus primarily on ecosystems, environmental communities, geographic regions, and species (Coates et al. 2018). The ecological factors such as over-exploitation, pollution, habitat destruction, and climate change can substantially impact intra-population genetic variation (Liu et al. 2013; Martinez et al. 2018). Recent studies have shown a strong linkage between environmental pressures and biodiversity levels, including genes, species, populations, and communities. The relationships between genetic diversity, polymorphism, and ecological stress have been evidenced in natural populations. The biochemical and molecular approaches investigated the correlation between the genetic structure of populations and the environmental characteristics (Cimmaruta et al. 2003; Markert et al. 2010; Schierenbeck 2017; Hu et al. 2020).

Table 2. The genetic distances within and among clades of *Aplocheilus*

***Aplocheilus panchax* Populations**	**Genetic Distance (%)**			
	[1]	**[2]**	**[3]**	**[4]**
Bangka Island [1]	**0.00 - 1.93**			
East Clade [2]	104.02-110.32	**0.00 - 1.76**		
Central Clade [3]	103.87 - 108. 49	1.40 - 2.48	**0.00 - 1.05**	
West Clade [4]	110.45 - 112.10	7.37 - 12.54	6.98 - 13.22	**0.00* - 9.80****

Note: Values in bold were intra-population genetic distances of *Aplocheilus*, * there was indication a differences *A. panchax* and *A. andamanicus* as well as Katwate et al. (2018)

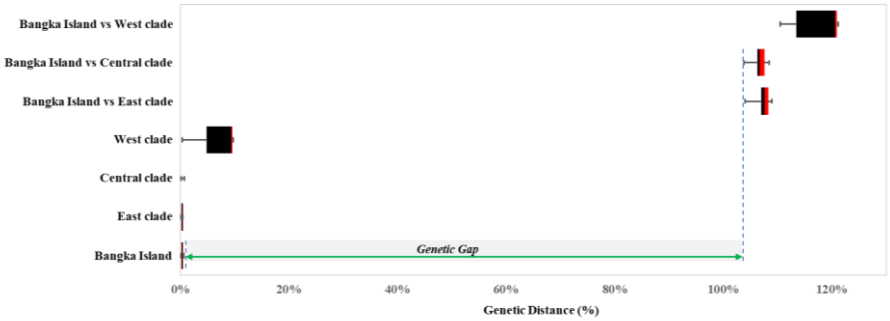

Figure 5. Intra- and inter-species genetic distances for the *A. panchax* populations from Bangka Island and other clades. There was a clear genetic gap, spanning from 1.42% to 103.87%, between the maximum within Bangka Island populations and minimum inter populations distance that indicates the *A. panchax* populations from Bangka Island were genetically distinct from each other clade. Black lines within the boxes showed the medians, and the red boxes indicated the 75th quartiles

Table 3. The haplotype of *Aplocheilus panchax* on each clade

Haplotype(s)	Specimen(s)
Hap_1: 5	[BK_A01 BK_A02 BK_E01 BK_E02 BK_G03]
Hap_2: 1	[BK_B01]
Hap_3: 1	[BK_B02]
Hap_4: 1	[BK_C01]
Hap_5: 1	[BK_C02]
Hap_6: 1	[BK_D01]
Hap_7: 1	[BK_D02]
Hap_8: 1	[BK_F01]
Hap_9: 1	[BK_F02]
Hap_10: 1	[BK_G01]
Hap_11: 1	[BK_G04]
Hap_12: 1	[BK_K01]
Hap_13: 1	[BK_K02]
Hap_14: 1	[BK_K03]
Hap_15: 1	[BK_K04]
Hap_16: 9	[KJ957618.1 KJ957549.1 KJ957550.1 KJ957551.1 KJ957552.1 KJ957553.1 KJ957607.1 KJ957591.1 KJ957592.1]
Hap_17: 4	[KJ957540.1 KJ957542.1 KJ957544.1 KJ957545.1]
Hap_18: 2	[KJ957541.1 KJ957543.1]
Hap_19: 10	[KJ957546.1 KJ957547.1 KJ957548.1 KJ957554.1 KJ957555.1 KJ957556.1 KJ957557.1 KJ957558.1 KJ957559.1 KJ957560.1]
Hap_20: 4	[KJ957561.1 KJ957562.1 KJ957563.1 KJ957564.1]
Hap_21: 11	[KJ957565.1 KJ957566.1 KJ957567.1 KJ957568.1 KJ957569.1 KJ957570.1 KJ957571.1 KJ957572.1 KJ957573.1 KJ957616.1 KJ957617.1]
Hap_22: 13	[KJ957582.1 KJ957583.1 KJ957584.1 KJ957585.1 KJ957586.1 KJ957587.1 KJ957589.1 KJ957590.1 KJ957608.1 KJ957609.1 KJ957610.1 KJ957611.1 KJ957612.1]
Hap_23: 2	[KJ957588.1 KJ957613.1]
Hap_24: 1	[KJ957614.1]
Hap_25: 1	[KJ957615.1]
Hap_26: 1	[KJ957593.1]
Hap_27: 1	[*A. andamanicus* MH813789.1]
Hap_28: 1	[*A. andamanicus* MH813790.1]

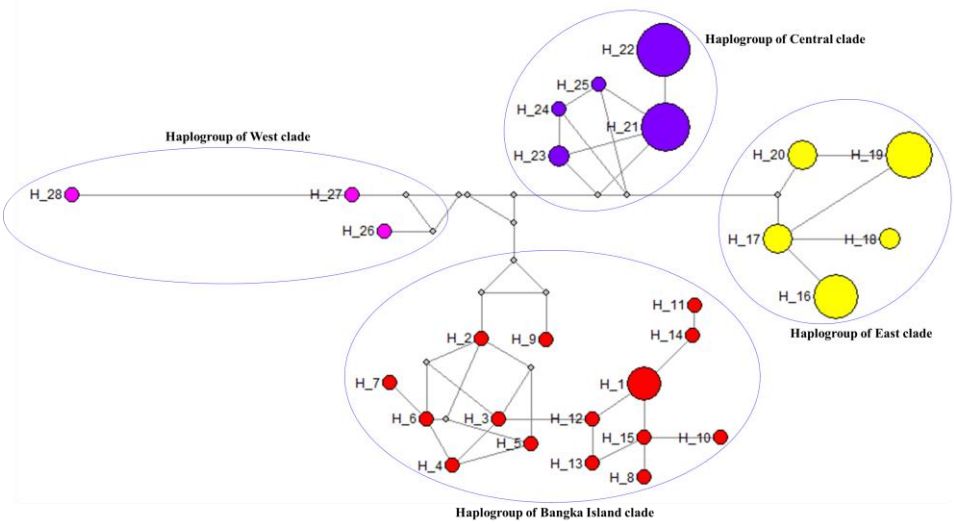

Figure 6. Median-joining network based on COI sequences indicating the *A. panchax* populations of Bangka Island (red haplogroup) formed distinct clade from other clades from Beck et al. (2017) and Katwate et al. (2018). Numbers correspond to haplotype. Crossing-line indicated mutated positions. Different colors showed the collection sites: red (Bangka Island clade), yellow (East clade), violet (Central clade), and pink (West clade), while grey (median vectors) and blue circle (haplogroup). Colored-circles size was proportional to haplotype frequency (see Table 3)

The water characteristic of tin mining pits as one of the habitats for *A. panchax* has an acidic pH and high heavy metals contamination (Kurniawan et al. 2019; Kurniawan 2020). A recent study proved that the waters in abandoned tin mining pits are contaminated by heavy metals such as As, Co, Cr, Cu, Fe, Ga, Hf, Mn, Ni, Pb, Sn, Ta, Te, Th, V, and Zn. Heavy metal presence corresponded with pH characteristics (Kurniawan 2020). Therefore, the pH value, acidic pH, specifically acidic mine drainage (AMD) due to the oxidation process of sulfide minerals and potentially acidic formation ~~, is a significant indicator of abandoned post-mining habitats~~ (PAF), is a significant indicator of abandoned post-mining habitats (Tan et al. 2007; Çelebi and Öncel 2016). These conditions cause organisms to adapt to the extreme environment since *A. panchax* was grouped as extremophile fishes (Riesch et al. 2015; Kurniawan and Mustikasari 2021). The environmental factor from Bangka Island as a tin producer may also contribute to the genetic diversity of *A. panchax*.

Previous studies investigated the correlation between the water quality of abandoned tin mining waters with the presence and morphological characteristics of *A. panchax*. The results showed that *A. panchax* was found in pits with a pH value of 3.81-3.84 and dissolved oxygen (DO) between 5.33 and 5.63. The change of chronosequence's pH impacted the other changes such as DO, BOD, C-organic, total nitrogen, total phosphate, and others (Kurniawan et al. 2019). The presences and phenotypic characters were correlated with the environmental factors, especially pH and heavy metals (Mustikasari et al. 2020a, b). Therefore, these factors strongly contributed to the polymorphism of *A. panchax* populations from Bangka Island.

Genetic diversity is considered an internal contributing element in the susceptibility of organisms to heavy metals-related poison or toxicity levels. The variety in various genes, directly or indirectly included in the metabolism of weighty metals, has been researched by specific studies. For example, metallothioneins (MTs) are proteins that detoxify heavy metals because of a few gene varieties of genomic sequences (Joneidi et al. 2019). Metallothioneins are small cysteine-rich proteins that play significant roles in metal homeostasis and protection for heavy metal toxicity, DNA defect, and oxidative conditions (Si and Lang 2018), cellular processes, cell growth regulation, and well as proliferation and DNA repair (Grennan 2011).

The contribution of MTs ~~with~~ in various cell or organelles processes has gotten much consideration while their association with the mitochondria functions has been inadequate. Furthermore, it increases the duration of malfunctioning mitochondrial cells by protecting productive components from the damage caused by reactive oxygen species (ROS) and limiting apoptosis. MTs are also involved in mitochondrial infection, including redox balance, metal homeostasis, enzyme, and transcription factor regulation (Lindeique et al. 2016; Kurniawan and Mustikasari 2021). The requirements for obtaining metal specificity and specific novel capacity may

drive their enhancement. MTs further enhanced the capability of metal detoxification under ecologically sensitive settings (Nam and Kim 2017). The relationship with mitochondria indicated an extreme environment as in abandoned tin mining waters of Bangka Island to genetic diversity, especially the COI gene of *A. panchax*. The heavy metals contamination and acidic pH in the habitat can cause genetic variations in mitochondrial genes, such as the COI gene. Moreover, heavy metals can reduce genetic variability within natural populations and cause genetic erosion (Ungherese et al. 2010). The evolution chronosequence of Bangka Island and the entire Sundaland may be attributed to the divergence of COI gene changes to diversify *A. panchax* genes.

It could be concluded that *Aplocheilus panchax* from the Bangka Island was highly divergence from other populations, including Indonesian populations with high genetic gap. The *A. panchax* population on Bangka Island formed a novel clade for Indonesia and in a global blue panchax phylogeographic.

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REFERENCES

Ashraf MA, Maah MJ, Yusoff I. 2011a. Heavy metals accumulation in plants growing in ex tin mining catchment. Int. J. Environ. Sci. Tech. 82: 401-416.

Ashraf MA, Maah MJ, Yusoff I. 2012a. Speciation of heavy metals in the sediments of former tin mining catchment. Iran. J. Sci. Technol. Trans. A Sci. 36 (A2): 163-180.

Ashraf MA, Maah MJ, Yusoff I. 2012b. Morphology, geology and water quality assessment of former tin mining catchment. Sci. World J. 2012: 1-15. DOI:10.1100/2012/369206

Banks SC, Cary GJ, Smith AL, Davies ID, Driscoll DA, Gill AM, Lindenmayer DB, Peakall R. 2013. How does ecological disturbance influence genetic diversity?. Trends Ecol. Evol. 28 (11): 670-679. DOI: 10.1016/j.tree.2013.08.005

Beck SV, Carvalho GR, Barlow A, Rüber L, Tan HH, Nugroho E, Wowor D, Nor SAM, Herder F, Muchlisin ZA, De Bruyn, M. 2017. Plio-Pleistocene phylogeography of the Southeast Asian blue panchax killifish, *Aplocheilus panchax*. PloS one 12 (7): e0179557. DOI: 10.1371/journal.pone.0179557

Bennett, R.H., Ellender, B.R., Mäkinen, T., Miya, T., Pattrick, P., Wasserman, R.J., Woodford, D.J., Weyl, O.L.F. 2016. Ethical considerations for field research on fishes. Koedoe: African Protected Area Conservation and Science 58 (1): 1-15. DOI: 10.4102/koedoe.v58i1.1353

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. 22 (3): 148-155. DOI: 10.1016/j.tree.2006.11.004

Bobo-Pinilla J, Salmerón-Sánchez E, Mendoza-Fernández AJ, Mota JF, Peñas J. 2021. Conservation and phylogeography of plants: from the mediterranean to the rest of the world. Diversity 14 (78): 1-23. DOI: 10.3390/d14020078

Bolotov IN, Konopleva ES, Vikhrev IV, Gofarov MY, Lopes-Lima M, Bogan AE, LunnZ, Chan Y, Win T, Aksenova OV, Tomilova AA, Tanmuangpak K, Tumpeesuwan S, Kondakov AV. 2020. New freshwater mussel taxa discoveries clarify biogeographic division of Southeast Asia. Sci. Rep. 10 (1): 1-22. DOI: 10.1038/s41598-020-63612-5

Boluda CG, Hawksworth DL, Divakar PK, Crespo A. 2016. Microchemical and molecular investigations reveal Pseudephebe species as cryptic with an environmentally modified morphology. The Lichenologist 48 (5): 527-543. DOI: 10.1017/S0024282916000426

Braganca PH, Amorim PF, Costa WJ. 2018. Pantanodontidae (Teleostei, Cyprinodontiformes), the sister group to all other cyprinodontoid killifishes as inferred by molecular data. Zoosystematics Evol. 94 (1): 137-145. DOI: 10.3897/zse.94.22173

Buhay JE. 2009. "COI-like" sequences are becoming problematic in molecular systematic and DNA barcoding studies. J. Crus. Biol. 29 (1): 96-110. DOI: 10.1651/08-3020.1

Čekovská K, Šanda R, Eliášová K, Kovačić M, Zogaris S, Pappalardo AM, Soukupová T, Vukić J. 2020. Population genetic diversity of two marine gobies (Gobiiformes: Gobiidae) from the North-Eastern Atlantic and the Mediterranean Sea. J. Mar. Sci. Eng. 8(792): 1-18. DOI: 10.3390/jmse8100792

Çelebi EE, Öncel MS. 2016. Determination of acid forming potential of massive sulfide minerals and the tailings situated in lead/zinc mining district of Balya (NW Turkey). J. African Earth Sci. 124: 487-496. DOI: 10.1016/j.jafrearsci.2016.09.014

Cerca J, Meyer C, Purschke G, Struck TH. 2020. Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (Stygocapitella; Annelida, Sedentaria). Mol. Phylogenet. Evol. 143(2020): 106663. DOI: 10.1016/j.ympev.2019.106663

Chen N, Juric I, Cosgrove EJ, Bowman R, Fitzpatrick JW, Schoech SJ, Clark AG, Coop G. 2019. Allele frequency dynamics in a pedigreed natural population. PNAS 116(6): 2158-2164. DOI: 10.1073/pnas.1813852116

Chowdhury MM, Rahman AS, Nahar L, Rahman M, Al Reza H, Ahmed MS. 2016. Efficiency of different DNA extraction methods for fish tissues: a comparative analysis. IOSR-JPBS 11(3): 11-15. DOI: 10.9790/3008-1103041115

Cimmaruta R, Scialanca F, Luccioli F, Nascetti G. 2003. Genetic diversity and environmental stress in Italian populations of the cyprinodont fish Aphanius fasciatus. Oceanol. Acta 26(1): 101-110. DOI: 10.1016/S0399-1784(02)01234-3

Coates DJ, Byrne M, Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. Front. Ecol. Evol. 6(165): 1-13. DOI: 10.3389/fevo.2018.00165

Costa WJEM. 2013. Historical biogeography of aplocheiloid killifishes (Teleostei: Cyprinodontiformes). Vertebr. Zool. 63(2): 139-154.

Costa WJEM. 2016. Comparative morphology and classification of South American cynopoeciline killifishes (Cyprinodontiformes: Aplocheilidae), with notes on family-group names used for aplocheiloids. Vertebr. Zool. 66(2): 125-140.

De Bruyn M, Stelbrink B, Morley RJ, Hall R, Carvalho GR, Cannon CH, den Bergh Gv, Meijaard E, Metcalfe I, Boitani L, Maiorano L, Shoup R, Von Rintelen T. 2014. Borneo and Indochina are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63(6): 879-901. DOI: 10.1093/sysbio/syu047

De Oliveira DAS, Decraemer W, Moens T, Dos Santos GAP, Derycke S. 2017. Low genetic but high morphological variation over more than 1000 km coastline refutes omnipresence of cryptic diversity in marine nematodes. BMC Evol. Biol. 17(1): 1-17. DOI: 10.1186/s12862-017-0908-0

Dekar M, Sarong MA, Batubara AS, Muchlisin ZA. 2018. Ichthyofauna of Aceh River, Aceh Province, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 216, No. 1, p. 012024). IOP Publishing. DOI:10.1088/1755-1315/216/1/012024

Dlugosch KM, Parker IM. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol. Ecol.17(1): 431-449. DOI: 10.1111/j.1365-294X.2007.03538.x

Dorey JB, Groom SV, Freedman EH, Matthews CS, Davies OK, Deans EJ, Rebola C, Stevens MI, Lee MSY, Schwarz MP. 2020. Radiation of tropical island bees and the role of phylogenetic niche conservatism as an important driver of biodiversity. Proc. R. Soc. B 287(1925): 20200045. DOI: 10.1098/rspb.2020.0045

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Dwiyitno HS, Parmentier K, Van Keer C. 2018. Method comparison of DNA isolation and quantification for fish and seafood authenticity determination. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 13(3): 115-124. DOI: 10.15578/squalen.v13i3.370

Farmawati DA, Wirajana N, Yowani SC. 2015. Perbandingan kualitas DNA dengan menggunakan metode boom original dan boom modifikasi pada isolat *Mycobacterium tuberculosis*. KIMIA 9(1): 41-46. DOI: 10.24843/JCHEM.2015.v09.i01.p07

Faulwetter S, Simboura N, Katsiaras N, Chatzigeorgiou G, Arvanitidis C. 2017. Polychaetes of Greece: an updated and annotated checklist. Biodivers. Data J. 5: e20997. DOI: 10.3897/BDJ.5.e20997

Fortes MD, Ooi JLS, Tan YM, Prathep A, Bujang JS, Yaakub SM. 2018. Seagrass in Southeast Asia: a review of status and knowledge gaps, and a road map for conservation. Botanica Marina 61(3): 269-288. DOI: 10.1515/bot-2018-0008

Furness AI, Reznick DN, Springer MS, Meredith RW. 2015. Convergent evolution of alternative developmental trajectories associated with diapause in African and South American killifish. Proc.R. Soc. B: Biol. Sci. 282(1802): 20142189. DOI: 10.1098/rspb.2014.2189

Grennan AK. 2011. Metallothioneins, a diverse protein family. Plant Physiol. 155(4): 1750-1751. DOI: 10.1104/pp.111.900407

Gupta A, Bhardwaj A, Sharma P, Pal Y. 2015. Mitochondrial DNA-a tool for phylogenetic and biodiversity search in equines. J Biodivers. Endanger. Species S1: S1.006. DOI: 10.4172/2332-2543.S1-006

Haileselasie TH, Mergeay J, Vanoverbeke J, Orsini L, De Meester L. 2018. Founder effects determine the genetic structure of the water flea Daphnia in Ethiopian reservoirs. Limnol. Oceanogr. 63(2): 915-926. DOI: 10.1002/lno.10678

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. Mol. Ecol. Notes 6(4): 959-964. DOI: 10.1111/j.1471-8286.2006.01470.x

Hall T. 2011. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60–61. https://doi.org/10.1017/S0317167100012865

Hashim, M., Nayan, N., Saleh, Y., Mahat, H., Shiang, W. F. 2018. Water quality assessment of former tin mining lakes for recreational purposes in Ipoh City, Perak, Malaysia. Indones. J. Geogr. 50(1): 25-33. DOI: 10.22146/ijg.15665

Hinckley A, Camacho-Sanchez M, Ruedi M, Hawkins MT, Mullon M, Cornellas A, Yuh FTY, Leonard JA. 2022. Evolutionary history of Sundaland shrews (Eulipotyphla: Soricidae: Crocidura) with a focus on Borneo. Zool. J. Linn. Soc. 20(2021): 1-14. DOI: 10.1093/zoolinnean/zlab045

Hosoishi S, Ogata K. 2019. Cryptic diversity in the widespread Asian ant Crematogaster rothneyi (Hymenoptera: Formicidae) inferred from morphological and genetic evidence. Zool. Stud. 58(11): 1-15. DOI: 10.6620/ZS.2019.58-11

Hu ZM, Zhong KL, Weinberger F, Duan DL, Draisma SG, Serrao E. 2020. Linking ecology to genetics to better understand adaptation and evolution: a review in marine macrophytes. Front. Mar. Sci. 7: 545102. DOI: 10.3389/fmars.2020.545102

Hughes AC. 2017. Understanding the drivers of S outheast A sian biodiversity loss. Ecosphere 8(1): e01624. DOI: 10.1002/ecs2.1624

Ivanova NV, Zemlak TS, Hanner RH, Hebert PD. 2007. Universal primer cocktails for fish DNA barcoding. Mol. Ecol. Notes 7(4): 544-548. DOI: 10.1111/j.1471-8286.2007.01748.x

Joneidi Z, Mortazavi Y, Memari F, Roointan A, Chahardouli B, Rostami S. 2019. The impact of genetic variation on metabolism of heavy metals: Genetic predisposition?. Biomed. Pharmacother. 113: 108642. DOI: 10.1016/j.biopha.2019.108642

Kaňuch P, Berggren Å, Cassel-Lundhagen A. 2014. Genetic diversity of a successful colonizer: isolated populations of *Metrioptera roeselii* regain variation at an unusually rapid rate. Ecol. Evol. 4(7): 1117-1126. DOI: 10.1002/ece3.1005

Karanovic T, Djurakic M, Eberhard SM. 2016. Cryptic species or inadequate taxonomy? Implementation of 2D geometric morphometrics based on integumental organs as landmarks for delimitation and description of copepod taxa. Syst. Biol. 65(2): 304-327. DOI: 10.1093/sysbio/syv088

Katwate U, Kumkar P, Britz R, Raghavan R, Dahanukar N. 2018. The identity *of Aplocheilus andamanicus* (Köhler, 1906) (Teleostei: Cyprinodontiformes), an endemic Killifish from the Andaman Islands, with notes on *Odontopsis armata* van Hasselt. Zootaxa 4382(1): 159-174.
DOI: 10.11646/zootaxa.4382.1.6

Koki IB, Zain SM, Low KH, Azid A, Juahir H, Abdul ZM. 2019. Development of water quality index of ex-mining ponds in Malaysia. Mal. J. Fund. Appl. Sci. 15(1), 54-60. DOI: 10.11113/mjfas.v15n2019.1079

Korshunova T, Picton B, Furfaro G, Mariottini P, Pontes M, Prkić J, Fletcher K, Malmberg K, Lundin K, Martynov A. 2019. Multilevel fine-scale diversity challenges the 'cryptic species' concept. Scientific Reports 9(1): 1-23. DOI: 10.1038/s41598-019-42297-5

Kuffel A, Gray A, Daeid NN. 2021. Impact of metal ions on PCR inhibition and RT-PCR efficiency. Inter. J. Legal Med. 135(1): 63-72. DOI: 10.1007/s00414-020-02363-4

Kurniawan A, Mustikasari D. 2021. Review tentang kemampuan ikan ekstremofil untuk hidup di perairan asam dan terkontaminasi logam berat pascapenambangan timah. Jurnal Ilmu Lingkungan 19(3): 541-554. DOI: 10.14710/jil.19.3.541-554

Kurniawan A, Oedjijono, Tamad, Sulaeman U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka District, Indonesia. Indones. J. Chem. 19(1): 254-261. DOI: 10.22146/ijc.33613

Kurniawan A, Prasetiyono E, Syaputra D. 2020. Analisis korelasi parameter kualitas perairan kolong pascatambang timah dengan umur berbeda. Samakia: Jurnal Ilmu Perikanan 11(2): 91-100. DOI: 10.35316/jsapi.v11i2.824

Kurniawan A. 2020. The metal oxides of abandoned tin mining pit waters as an indicator for bacterial diversity. Aquac. Aquar. Conserv. Legis. 13(5): 2982-2992.

Lee CE. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17(8): 386-391. DOI: 10.1016/S0169-5347(02)02554-5

Lindeque JZ, Levanets O, Louw R, Van Der Westhuizen FH. 2010. The involvement of metallothioneins in mitochondrial function and disease. Curr. Protein Pept. Sci 11 (4): 292-309. DOI: 10.2174/138920310791233378

Liu Y, Webber S, Bowgen K, Schmaltz L, Bradley K, Halvarsson P, Abdelgadir M, Griesser M. 2013. Environmental factors influence both abundance and genetic diversity in a widespread bird species. Ecol. Evol. 3(14): 4683-4695. DOI: 10.1002/ece3.856

Lone AR, Thakur SS, Tiwari N, Sokefun OB, Yadav S. 2021. Disentangling earthworm taxonomic stumbling blocks using molecular markers. J. Threat. Taxa 13(11): 19566-19579. DOI: 10.11609/jott.6888.13.11.19566-19579%20

Markert JA, Champlin DM, Gutjahr-Gobell R, Grear JS, Kuhn A, McGreevy TJ, Roth A, Bagley MJ, Nacci DE. 2010. Population genetic diversity and fitness in multiple environments. BMC Evol. Biol. 10(1): 1-13. DOI: 10.1186/1471-2148-10-205

Martinez AS, Willoughby JR, Christie MR. 2018. Genetic diversity in fishes is influenced by habitat type and life-history variation. Ecol. Evol. 8(23): 12022-12031. DOI: 10.1002/ece3.4661

Mustikasari D, Nuryanto A, Suryaningsih S. 2020a. The presence of blue panchax (*Aplocheilus panchax*) in the waters, contaminated by heavy metals, of the abandoned tin mining pits of different age. Aquac. Aquar. Conserv. Legis. 13(5): 2538-2550.

Mustikasari D, Suryaningsih S, Nuryanto A. 2020b. Morphological variation of blue panchax (*Aplocheilus panchax*) lives in different habitat assessed using truss morphometric. Biosaintifika 12(3): 399-407. DOI: 10.15294/biosaintifika.v12i3.26593

Nam YK, Kim EJ. 2017. Diversification and domain evolution of molluskan metallothioneins: a mini review. Fish Aquatic Sci. 20(1): 1-18. DOI: 10.1186/s41240-017-0054-z

Nascimento MHS, Almeida MS, Veira MNS, Filho DL, Lima RC, Barros MC, Fraga EC. 2016. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. Genet. Mol. Res. 15(3): 1-11. DOI: 10.4238/gmr.15038476.

Nater A, Arora N, Greminger MP, van Schaik CP, Singleton I, Wich SA, Fredrikson G, Perwitasari D, Pamungkas J, Krützen M. 2013. Marked population structure and recent migration in the critically endangered Sumatran orangutan (*Pongo abelii*). J. Hered. 104(1): 2-13. DOI: 10.1093/jhered/ess065

Parenti LR, Hartel KE. 2011. Osteology identifies *Fundulus capensis* Garman, 1895 as a killifish in the family Fundulidae (Atherinomorpha: Cyprinodontiformes). Copeia 2011(2): 242-250. DOI: 10.2307/41261873

Pohl M, Milvertz FC, Meyer A, Vences M. 2015. Multigene phylogeny of cyprinodontiform fishes suggests continental radiations and a rogue taxon position of Pantanodon. Vertebr. Zool. 65(1): 37-44.

Pratomo YW, Zahida F, Yuda P. 2021. Perbandingan Metode isolasi DNA sebagai templat PCR untuk identifikasi jenis kelamin Cerek Jawa

(*Charadriius javanicus*) secara molekuler menggunakan Primer 2550F/2718R. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati 6(2): 78-86. DOI: 10.24002/biota.v6i2.3444

Riesch R, Tobler M, Plath M. 2015. Extremophile Fishes. Ecology, Evolution, and Physiology of Teleosts in Extreme Environments: Springer. Netherland.

Rius M, Turon X. 2020. Phylogeography and the description of geographic patterns in invasion genomics. Front. Ecol. Evol. 8(439): 595711. DOI: 10.3389/fevo.2020.595711

Rizko N, Kusumaningrum HP, Ferniah RS, Pujiyanto S, Erfianti T, Mawarni SN, Rahayu HT, Khairunnisa D. 2020. Isolasi DNA daun Jeruk Bali Merah (*Citrus maxima* Merr.) dengan modifikasi Metode Doyle and Doyle. Berkala Bioteknologi 3(2): 1-7.

Rosilawati ML, Sudarmono P, Ibrahim F. 2002. Sensitivitas metode PCR (*Polymerase Chain Reaction*) dalam mendeteksi isolat klinis *Mycobacterium tuberculosis*. Jurnal Kedokteran Trisakti 21(1): 7-14. DOI: 10.18051/UnivMed.2007.v26.1-10

Ruchi W, Putri DH, Anhar A, Farma SA. 2018. Comparison of three different DNA isolation methods to degradate the *Trichoderma* fungi cell wall. Bioscience 2(1): 50-59. DOI: 0201931102859-0-00

Salles T, Mallard C, Husson L, Zahirovic S, Sarr AC, Sepulchre P. 2021. Quaternary landscape dynamics boosted species dispersal across Southeast Asia. Commun. Earth Environ. 2(1): 1-12. DOI: 10.1038/s43247-021-00311-7

Schierenbeck KA. 2017. Population-level genetic variation and climate change in a biodiversity hotspot. Ann. Bot. 119(2): 215-228.

Setiaputri AA, Barokah GR, Sahaba MAB, Arbajayanti RD, Fabella N, Pertiwi RM, Nurilmala M, Nugraha R, Abdullah A. 2020. Perbandingan metode isolasi DNA pada produk perikanan segar dan olahan. Jurnal Pengolahan Hasil Perikanan Indonesia 23(3): 447-458.

Si M, Lang J. 2018. The roles of metallothioneins in carcinogenesis. J. Hemat. Oncol. 11(1): 1-20. DOI: 10.1038/s43247-021-00311-7

Star B, Spencer HG. 2013. Effects of genetic drift and gene flow on the selective maintenance of genetic variation. Genetics 194(1): 235-244. DOI:10.1534/genetics.113.149781/-/DC1

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Moecular evolutionary genetic analysis version 11. Mol. Biol. Evol. 38(7): 3022-3027. doi:10.1093/molbev/msab120

Tan GL, Shu WS, Hallberg KB, Li F, Lan CY, Huang LN. 2007. Cultivation-dependent and cultivation-independent characterization of the microbial community in acid mine drainage associated with acidic Pb/Zn mine tailings at Lechang, Guangdong, China. FEMS Microbiol. Ecol. 59(1): 118-126. DOI: 10.1111/j.1574-6941.2006.00216.x.

Thu PT, Huang WC, Chou TK, Van Quan N, Van Chien P, Li F, Shao KT, Liao TY. 2019. DNA barcoding of coastal ray-finned fishes in Vietnam. PloS ONE 14 (9): e0222631. DOI: 10.1371/journal.pone.0222631

Ungherese G, Mengoni A, Somigli S, Baroni D, Focardi S, Ugolini A. 2010. Relationship between heavy metals pollution and genetic diversity in Mediterranean populations of the sandhopper *Talitrus saltator* (Montagu) (Crustacea, Amphipoda). Environ. Pollut. 158(5): 1638-1643. DOI:10.1016/j.envpol.2009.12.007

Von Rintelen K, Arida E, Häuser C. 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. RIO 3: e20860. DOI: 10.3897/rio.3.e20860

Wang XY, Wang MM, Chen C, Wang XQ. 2020. Genetic variation and phylogeographic structure of Spodoptera exigua in western China based on mitochondrial DNA and microsatellite markers. PloS ONE 15(5): e0233133. DOI: 10.1371/journal.pone.0233133

Xiao JH, Wang NX, Li YW, Murphy RW, Wan DG, Niu LM, Hu HY, Fu YG, Huang DW. 2010. Molecular approaches to identify cryptic species and polymorphic species within a complex community of fig wasps. PLoS ONE 5 (11): e15067. DOI: 10.1371/journal.pone.0015067

Yang CH, Wu KC, Chuang LY, Chang HW. 2019. Decision theory-based COI-SNP tagging approach for 126 scombriformes species tagging. Front. Genet. 10: 259. DOI: 10.3389/fgene.2019.00259

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Dear Editor,

Thank you very much for the information. We would go through the manuscript and make corrections accordingly.

Best regards,
Agus Nuryanto