

RINGKASAN

Ikan kepala timah, *Aplocheilus panchax* (Hamilton, 1822) adalah salah satu kelompok ikan ekstremofil yang mampu beradaptasi dan bertahan hidup di perairan yang memiliki kualitas rendah dan ekstrem. Salah satu habitat *A. panchax* adalah di perairan tertutup danau pascatambang timah di Pulau Bangka yang memiliki pH asam, terkontaminasi logam berat, dan beberapa parameter lainnya yang mengindikasikan kualitas perairan tersebut rendah. Penelitian ini bertujuan untuk (1) menganalisis keberadaan dan kelimpahan populasi *A. panchax* serta karakteristik perairan danau pascatambang timah sebagai perairan tertutup dan aliran sungai sebagai perairan terbuka di Pulau Bangka yang menjadi habitat *A. panchax*; (2) menganalisis karakteristik morfologi *A. panchax* yang ditemukan di perairan tertutup danau pascatambang timah dan perairan terbuka berupa aliran Sungai Limbung berdasarkan truss morfometrik; dan (3) menganalisis karakteristik molekuler dan filogeografi *A. panchax* yang ditemukan di perairan tertutup danau pascatambang timah dan perairan terbuka berupa aliran Sungai Limbung di Pulau Bangka.

Penelitian ini dilaksanakan pada Bulan Mei 2020 hingga November 2021 untuk menganalisis parameter-parameter penelitian sesuai dengan tahapan penelitian. Stasiun penelitian terdiri atas 10 perairan tertutup berupa danau pascatambang timah dengan umur berbeda dan satu perairan terbuka, yaitu aliran Sungai Limbung yang berada di Pulau Bangka, Provinsi Kepulauan Bangka Belitung. Pengambilan sampel air dan tanah dilakukan secara acak pada lima titik di setiap stasiun penelitian. Sampel *A. panchax* dikumpulkan dari semua stasiun penelitian sebagai sampel penelitian sebanyak 70 individu untuk analisis morfologi dan molekuler. Pengukuran dan pengambilan sampel penelitian dilakukan pada pukul 07.30-11.00 WIB.

Instrumen dan metode analisis yang digunakan di dalam penelitian adalah pH meter (PH-009 (I)-A) Sun Care untuk mengukur pH; DO meter Lutron DO-5510 untuk mengukur oksigen terlarut atau *dissolved oxygen* (DO); Lutron WA-2017SD untuk mengukur total padatan terlarut atau *total dissolved solid* (TDS), potensial reduksi oksidasi atau redoks (E_h), suhu air, dan konduktivitas perairan; X-ray fluorescence (Rigaku) untuk mengukur kandungan unsur-unsur kimia (*elements*) atau logam di air; serokan berdiameter 40 cm dengan ukuran mata jaring 0,4 mm untuk menangkap sampel ikan; botol sampel sebagai wadah penyimpanan sampel; kamera untuk dokumentasi; serta peralatan yang digunakan di dalam laboratorium untuk menganalisis tekstur tanah dengan menggunakan metode hydrometer, kebutuhan oksigen biologis atau *biological oxygen demand* (BOD), dan kebutuhan oksigen kimiawi atau *chemical oxygen demand* (COD). Bahan yang digunakan di dalam penelitian, yaitu sampel air dari lokasi penelitian dan *A. panchax*; bahan kimiawi dalam analisis BOD sesuai SNI 6989.72:2009; dan bahan kimia dalam analisis COD sesuai SNI 6989.2:2009.

Analisis morfologi *A. panchax* terdiri atas morfometrik standar dan truss morfometrik. Analisis morfometrik standar berupa panjang total, panjang standar, tinggi tubuh, diameter mata, panjang kepala, tinggi sirip dorsal, panjang sirip pektoral, panjang sirip ventral, dan tinggi sirip anal. Analisis truss morfometrik dilakukan pada 29 karakteristik yang berada di bagian kepala, tubuh, dan ekor.

Isolasi atau ekstraksi DNA dianalisis dengan gSYNC™ DNA Extraction Kit (Geneaid, GS300). Konsentrasi asam nukleat (DNA genomik) diukur dengan menggunakan spektrofotometer Nanodrop™ 2000/2000c. Analisis molekuler merujuk pada Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika

Science Indonesia. Analisis *polymerase chain reaction* (PCR) dilakukan menggunakan agilent surecyclers 8800. Tahapan analisis PCR terdiri atas denaturasi awal (*initial denaturation*) (95 °C, 1 menit, 1 siklus), tahapan denaturasi (*denaturation*) (95 °C, 15 detik), penempelan primer (*annealing*) (50 °C, 15 detik), dan polimerisasi (*extension*) (72 °C, 45 detik) yang seluruh tahapan analisis tersebut dilakukan sebanyak 35 siklus, serta penyimpanan pada suhu 4 °C. Tahapan sekuensing DNA dilakukan di First Base, Malaysia dengan BigDye® Terminator kit dari Applied Biosystems.

Hasil penelitian menunjukkan karakteristik perairan habitat *A. panchax* adalah suhu air (31,28-31,70 °C), pH (3,81-7,79), E_h (0,011-0,198 V), konduktivitas (19,03-198,67 $\mu\text{S}\cdot\text{cm}^{-1}$), TDS (12,40-132,00 ppm), DO (5,33-7,20 ppm), BOD (2,65-4,34 ppm), COD (6,12-7,21 ppm), perairan tidak berarus atau berarus lemah, ditumbuhi sedikit tumbuhan air atau dengan kerapatan yang sedang, tidak terdapat hingga terdapat tumbuhan (pohon) peneduh di pinggiran perairan, serta terdapat sisa kayu, akar, atau rerumputan mati di perairan. *A. panchax* memiliki kemampuan beradaptasi pada perairan yang memiliki kandungan logam berat seperti stannum (Sn), ferrum (Fe), hafnium (Hf), cuprum (Cu), maupun non logam berat seperti magnesium (Mg), aluminium (Al), silikon (Si), chlorine (Cl), sulfur (S), calcium (Ca), phosphorus (P), tantalum (Ta), dan titanium (Ti). Kelimpahan *A. panchax* terbanyak ditemukan di Stasiun G, yaitu perairan tertutup danau pascatambang timah berumur 25-50 tahun, yaitu sebanyak 31 ekor/m². Analisis *principal component analysis* (PCA) menunjukkan bahwa parameter pH dan suhu merupakan variabel yang memiliki hubungan yang paling erat dengan kelimpahan *A. panchax*.

Ikan *A. panchax* memiliki karakteristik morfologi yaitu, terdapat bintik putih keperakan di kepala bagian tengah, mulut bertipe terminal, mulut dapat disembulkan, bentuk tubuh yang menyerupai panah (*sagittiform*), sirip dorsal berukuran kecil dan terletak pada bagian tubuh posterior, terdapat bercak hitam pada pangkal sirip dorsal, sirip anal memanjang dari anus hingga pangkal batang ekor, batang ekor relatif panjang dengan ketinggian relatif pendek, serta bentuk sirip ekor membulat (*rounded*) atau meruncing (*rhomboid*).

Hasil analisis Kruskal-Wallis menunjukkan terdapat 25 karakter truss morfometrik yang berbeda signifikan (adj. sig < 0,05) dari 29 karakter truss morfometrik yang dianalisis. Karakter truss morfometrik yang berbeda signifikan tersebut terdapat di bagian kepala dan tubuh. Karakter truss morfometrik yang tidak berbeda signifikan terdapat pada bagian ekor, yaitu sebanyak 4 karakter truss morfometrik. Hasil uji lanjut Dunn's test menunjukkan adanya korelasi positif antara karakteristik habitat dengan karakter truss morfometrik, yaitu 8 karakter truss morfometrik berbeda signifikan pada perairan tertutup saja dan 17 karakter truss morfometrik berbeda signifikan pada perairan tertutup dan perairan terbuka.

Hasil analisis jarak genetik intraspecies pada klade populasi *A. panchax* dari Pulau Bangka menghasilkan nilai berkisar antara 0,17-1,93%. Analisis jarak genetik interklade antara populasi *A. panchax* dari Pulau Bangka dengan populasi lainnya berkisar 0,17-122,10%. Jarak genetik populasi klade Bangka, klade timur, dan klade tengah sebesar < 2%, sedangkan populasi klade barat sebesar > 2%. Hal ini menyebabkan munculnya celah genetik (*genetic gap*) minimum, yaitu 1,93%-103,87% dari klade Bangka dan klade tengah dan *genetic gap* maksimum antara klade Bangka dan klade barat, yaitu 1,93%-110,45%.

Populasi *A. panchax* dari Pulau Bangka memiliki 15 haplotipe dan haplotipe 1 memiliki jumlah terbanyak, yaitu 5 individu. Uji netralitas menunjukkan keanekaragaman genetik atau *gene diversity* (Hd) populasi *A. panchax* dari Pulau

Bangka sebesar $0,9415 \pm 0,0477$, keanekaragaman nukleotida atau *nucleotide diversity* (π) sebesar $0,007193 \pm 0,004173$, D Tajima's sebesar $-1,29745$ (*p-value* 0,088), dan Fu's FS sebesar $-8,43141$ (*p-value* 0,000). *Analysis of molecular variance* (AMOVA) menunjukkan nilai *fixation index* (FST) populasi *A. panchax* sebesar 0,97019 dengan *p-value* = $0,00000 \pm 0,00000$ ($p < 0,05$) dan nilai *number of migrant* (Nm) sebesar 0,008.

Analisis jarak genetik menegaskan bahwa *A. panchax* merupakan spesies tunggal dan bukan *cryptic species*. Hasil penelitian juga menjelaskan bahwa populasi *Aplocheilus panchax* dari Pulau Bangka memiliki keanekaragaman genetik tinggi, kemampuan adaptasi tinggi, konektivitas genetik dan aliran genetik rendah, terjadi isolasi geografis, dan mengalami strukturisasi genetik spasial (*spatial genetic structure*). Kemampuan adaptasi tinggi didukung oleh ketidakstabilan genetik (*genetic inequilibrium*). Analisis filogenetik mengkonstruksikan bahwa populasi *A. panchax* dari Pulau Bangka membentuk klade baru pada filogeografi *A. panchax* global di Asia dan Asia Tenggara.

Kata Kunci : *Aplocheilus panchax*, danau pascatambang timah, genetika populasi, molekuler, morfologi



SUMMARY

The blue panchax, *Aplocheilichthys panchax* (Hamilton, 1822) is one of the extremophile fish groups that are able to adapt and survive in waters that have low quality and extreme. One of the habitats found by *A. panchax* is the closed waters of ex-tin mining lake in Bangka Island, Indonesia which has an acidic pH, are contaminated with heavy metals, and several other parameters that indicate the quality of the waters is low. This study aimed (1) to analyze the presences and abundance of *A. panchax* population and the waters characteristics of the ex-tine mining lake as closed water and river flow as opened waters in Bangka Island that were the habitat of *A. Panchax*; (2) to analyze the morphology characteristics differences of *A. panchax* in the closed waters of the ex-tin mining lake and opened water of Limbung River flow based on truss morphometric; and (3) to analyze the molecular characteristics and phylographic of *A. panchax* found in the closed waters of the ex-tin mining lake and opened water of Limbung River flow in Bangka Island.

The research was carried out in May 2020 until November 2021 to analyze research parameters according to the research stages. The research station consists of 10 closed waters in the form of ex-tin mining lakes with different ages and one open water, namely the flow of the Limbung River located in Bangka Island, Bangka Belitung Archipelago Province. Sampling of water and soil was carried out randomly at five sampling points at each research station. Samples of *A. panchax* were collected from all research stations as a sample of 70 individuals for morphological and molecular analysis. Measurement and samples collection were taken at 07.30-11.00 WIB.

The instruments and analysis methods used in the study were pH meter (PH-009 (I)-A) Sun Care to measure pH; Lutron DO-5510 DO meter to measure dissolved oxygen (DO); Lutron WA-2017SD to measure total dissolved solids (TDS), potential of reduction oxidation or redox (E_h), water temperature, and water conductivity; X-ray fluorescence (Rigaku) to measure the content of chemical elements or metals in water; beckett nets 40 cm in diameter with a net size of 0.4 mm to collect fish samples; sample bottles as sample storage containers; camera for documentation; and equipment used in the laboratory to analyze soil texture using the hydrometer method, biological oxygen demand (BOD), and chemical oxygen demand (COD). Materials used in the study, namely water samples from the research site and *A. panchax*; chemicals in BOD analysis according to SNI 6989.72: 2009; and chemicals in COD analysis according to SNI 6989.2: 2009.

Morphological analysis of *A. panchax* consists of standard morphometric and truss morphometric. The standard morphometric analysis consists of total length, standard length, body depth, eye diameter, head length, dorsal fin height, pectoral fin length, ventral fin length, and anal fin height. Morphometric truss analysis were carried out on 29 different characteristics of the head, anterior body, posterior body, and caudal peduncle.

DNA isolation or extraction was analyzed with the gSYNC™ DNA Extraction Kit (Geneaid, GS300). The nucleic acid concentration (genomic DNA) was measured using the 2000/2000c Nanodrop™ spectrophotometer. The molecular analysis referred to Protocol Species Barcoding Fish GMS-165, Genetics Laboratory of Genetica Science Indonesia. Polymerase chain reaction (PCR) analysis was performed using agilent surecycler 8800. The PCR stages analysis consist of initial denaturation (95 °C, 1 minute, 1 cycle), denaturation stages (95 °C, 15 seconds), annealing (50 °C, 15

seconds), and extension (72 °C, 45 seconds) all of which were carried out as many as 35 cycles, and storage at 4 °C. DNA sequencing was done at First Base, Malaysia with the BigDye® Terminator kit from Applied Biosystems.

The results showed that the habitat characteristics of *A. panchax* were temperature (31.28-31.70 °C), pH (3.81-7.79), E_h (0.011-0.198 V), conductivity (19.03-198.67 $\mu\text{S}\cdot\text{cm}^{-1}$), TDS (12.40-132.00 ppm), DO (5.33-7.20 ppm), BOD (2.65-4.34 ppm), COD (6.12-7.21 ppm), waters have no current or weak currents, overgrown with few aquatic plants or with moderate density, there were no shade plants (trees) on the edge of the waters, and there were residual wood, roots, or dead grass in the waters. *A. panchax* has the adaptation ability to waters that contain heavy metals such as Sn, Fe, Hf, and Cu as well as non-heavy metals such as Mg, Al, Si, Cl, S, Ca, P, Ta, and Ti. The abundance of *A. panchax* was mostly found in closed waters of ex-tin mining lakes aged 25-50 years, which was 31 individuals/m². The principal component analysis (PCA) showed that pH and temperature were the variables that had the most closely related relationship with the abundance of *A. panchax*.

The morphological characteristics of *A. panchax* were a silvery-white spot on center of the head, the mouth was a terminal type, the mouth can be popped, the body shape was like an arrow (*sagittiform*), the dorsal fins were small and located on the body posteriorly, there was black spot at the base of the dorsal fin, the anal fin extended from the anus to the base of caudal peduncle, the caudal peduncle was relatively long with a relatively short height, and the shape of the caudal fin was rounded or slightly tapered (rhomboid).

The results of the Kruskal-Wallis analysis showed that there were 25 morphometric truss characters that differed significantly (adj. sig <0.05) from the 29 morphometric truss characters were analyzed. Significantly different truss morphometric characters were found in the head and body. The morphometric truss characters that are not significantly different are found in the peduncle, which was 4 morphometric truss characters. The results of the post hoc Dunn's test showed a positive correlation between habitat characteristics and morphometric truss characters, namely 8 morphometric truss characters were significantly different in closed waters only and 17 morphometric truss characters were significantly different in closed waters and open waters.

The results of intraspecies genetic distance analysis in the clade of *A. panchax* population from Bangka Island produced values ranging from 0.17-1.93%. Analysis of interclade genetic distance between *A. panchax* population from Bangka Island with other populations was 0.17-122.10%. The genetic distance between the Bangka clade population, the eastern clade, and the central clade was < 2%, while the western clade population was > 2%. This caused a minimum genetic gap, which was 1.93%-103.87% of the Bangka clade and the central clade, while the maximum genetic gap between the Bangka clade and the western clade was 1.93%-110.45%.

The population of *A. panchax* from Bangka Island has 15 haplotypes and haplotype 1 has the largest number, which was 5 individuals. The neutrality test showed genetic diversity (H_d) of the *A. panchax* population from Bangka Island was 0.9415 ± 0.0477 , nucleotide diversity (π) was 0.007193 ± 0.004173 , Tajima's D was -1.29745 (p-value 0.088), and Fu's FS was -8.43141 (p-value 0.000). Analysis of molecular variance (AMOVA) showed a fixation index (F_{ST}) value of *A. panchax* population was 0.97019 with p-value = 0.00000 ± 0.00000 (p < 0.05) and the value of the number of migrant (N_m) was 0.008.

Molecular analysis confirmed that *A. panchax* was a single species and not a cryptic species. The results also explained that the population of *A. panchax* from Bangka Island has high genetic diversity, high adaptability, undergoes spatial genetic structure and geographical isolation, and also low genetic connectivity and genetic flow. High adaptability was supported by genetic instability (genetic inequilibrium). Phylogenetic analysis constructed that the *A. panchax* population from Bangka Island formed a new clade of global *A. panchax* phylogeography in Asia and Southeast Asia.

Keywords : *Aplocheilus panchax*, ex-tin mining lake, molecular, morphology, population genetics

