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Short Communication: *Aplocheilus panchax* Hamilton, 1822 Found at Acidic Waters of Abandoned Ex-tin Mining Ponds as Insectivorous Fish and Its Role for Water Quality Improvement

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ABSTRAK

Ikan kepala timah adalah salah satu spesies yang ditemukan di perairan asam pascatambang timah yang terabaikan. Ikan kepala timah termasuk ke dalam kelompok ikan ekstremofil yang mampu bertahan hidup pada lingkungan perairan ekstrem yang disebabkan oleh tekanan faktor lingkungan. Penelitian ini bertujuan untuk mengkonfirmasi ikan kepala timah sebagai spesies *Aplocheilus panchax* dan menganalisis kebiasaan makan alaminya. Analisis molekuler melalui *DNA barcoding* dilakukan dengan menganalisis gen COI dan mengkonfirmasi sekuens ikan kepala timah di bank gen pada laman NCBI serta mengkonstruksi pohon filogenetik ikan kepala timah terhadap populasi *A. panchax* lainnya. Analisis kebiasaan makan dilakukan dengan mengidentifikasi isi saluran pencernaan ikan kepala timah yang diamati secara mikroskopis. Hasil penelitian menunjukkan bahwa ikan kepala timah yang ditemukan di perairan asam pascatambang timah yang terabaikan terkonfirmasi sebagai *Aplocheilus panchax* Hamilton, 1822. Sejumlah karakter morfologi mempertegas kemiripan ikan kepala timah dengan *A. panchax* berupa bintik putih keperakan di bagian kepala, tipe mulut terminal, bentuk tubuh sagitiform, sirip dorsal kecil di bagian tubuh posterior, dan sirip ekor membundar atau meruncing. Analisis isi saluran pencernaan menunjukkan kebiasaan makan *A. panchax* di perairan asam pascatambang timah yang terabaikan adalah serangga. Kebiasaan makan ini menjelaskan bahwa *A. panchax* tergolong sebagai pemakan serangga atau larva serangga. Keberadaan *A. panchax* dan aktivitas metabolismenya di perairan asam pascatambang timah yang terabaikan dapat berkontribusi terhadap peningkatan bahan organik di perairan sehingga dapat bermanfaat bagi perbaikan kualitas perairan.

***Kata kunci*:** *Aplocheilus panchax*, ikan kepala timah, morfologi, molekuler, insectivorous atau larvirorous

ABSTRACT

Kepala timah fish or blue panchax is one of the species found at acidic waters of abandoned ex-tin mining pond. Kepala timah fish belongs to the extremophile fishes which can survive in extreme aquatic environments caused by pressure from environmental factors. This study aimed to confirm the kepala timah fish as a species of *Aplocheilus panchax* and to analyze its natural food habits. Molecular analysis through DNA barcoding was carried out by analyzing the COI gene and confirming the sequence of kepala timah fish in the gene bank on the NCBI website and constructing a phylogenetic tree for kepala timah fish to other *A. panchax* populations. Analysis of food habits was carried out by identifying the contents of the digestive tract of kepala timah fish which were observed microscopically. The results showed that kepala timah fish found at acidic waters of abandoned ex-tin mining pond was confirmed as *Aplocheilus panchax* Hamilton, 1822. Some morphological characters confirmed the similarity between kepala timah fish and *A. panchax* such as a silvery white spot on its head, terminal type of the mouth position, body shape was sagittiform, small dorsal fin on posterior body, and the shape of the caudal fin was rounded or rhomboid. Analysis of the contents of digestive tract showed the food habits of *A. panchax* found at acidic waters of ex-tin mining pond was insects. The food habits explained that *A. panchax* was classified as larvivorous or insectivorous fishes. The presence of *A. panchax* and its metabolic activity at abandoned ex-tin mining ponds can contributed to increasing organic matter in the waters so that it can be beneficial for improving water quality.

***Keywords*:** *Aplocheilus panchax*, blue panchax, morphology, molecular, insectivorous or larvirorous

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1. Introduction

The waters of ex-tin mining pond in the Bangka Belitung Archipelago Province known as pit or kolong are one of the interesting environments for ecological study. Some studies have revealed that these waters have acidic characteristics with the potential for heavy metal contamination as a result of the tin (Sn) mineral exploration process (Kurniawan et al. 2019). Mining activities and industrialization can lead to the emergence of *acid mine drainage* (AMD) (Kaur et al. 2018). Industrial waste and mining activities are also the main source of metal contamination in an environment after the mining activity (Guan et al. 2014; Kurniawan 2016) such as Pb, Zn, Mn, Fe, Cr, Cu, Ni, Cd, As, etc (Daniel et al. 2014; Kurniawan 2020). The condition of pressure or stress in the aquatic environment, namely in the form of acidic pH and heavy metal contamination, are indicators that these waters can be classified as extreme environments for organisms in general (Thompson et al. 2017; Hashim et al. 2018; Kurniawan and Mustikasari 2019).

The results of previous studies revealed that one of the fish that can live in the closed waters of the ex-tin mining pond is kepala timah fish (*Aplocheilus panchax*) (Kurniawan and Mustikasari 2019; Mustikasari and Agustiani 2021). The Genus of Aplocheilus is categorized as an extremophile fish because it can survive at extreme water conditions (Riesch et al. 2015). The research of Kurniawan et al. (2020) has explained the physical and chemistry characters of water quality such as pH, temperature, DO, COD, TDS, TSS, Eh, conductivity, total nitrogen, and total phosphate which are associated with the presence of a number of fish in these waters.

A. panchax fish are larvivorous or larval-eating and surface feeder fishes (Chandra et al. 2008; Manna et al. 2011; Putri et al. 2022). *A. panchax* has a habit of eating mosquito larvae in nature so it makes *A. panchax* serving as a natural larvae controller, including malaria mosquito larvae (Lutfi et al. 2019).

The availability of natural foods in extreme environments, including abandoned ex-tin mining water can also contributes to the presence and survivability of *A. panchax*. This study aims to confirm that kepala timah fish is *Aplocheilus panchax* by gene Cytochrome C Oxidase subunit I (COI) and to analyze the natural food of *A. panchax* found at acidic waters of abandoned ex-tin mining pond.

2. Method

This research was conducted exploratoryly with the purposive sampling method, which is to select the acidic waters (pH < 5) of abandoned ex-tin mining pond where *A. panchax* was found in the habitat. Sampling of *A. panchax* was carried out in Rebo Village and Merawang Village, Bangka Regency, Bangka Belitung Archipelago Province.

Fish collection was carried out at 07.30-09.00 am with net fish in 0.5 mm of net size. The *A. panchax* obtained has 2 ± 0.5 cm in length as many as 5-10 fishes with attention of sustainability and abundance of fish in these waters.

Identification molecular of gene COI was analyzed by refers to molecular analysis of protocol PCR species barcoding fish of genetica laboratory, Genetica Science Indonesia. Genomic DNA extraction using ZR Tissue & Insect DNA MiniPrep (Zymo Research, D6016), while amplification PCR using MyTaq Red Mix (Bioline) BIO-25047. Analysis used primer VF2_t1 (TGTAAAACGACG GCCAGTCAACCAACCACAAAGACATTGGCAC), FishF2_ t1 (TGTAAAACGACGGCCAGTCGACTAATCATAAAGATA TCGGCAC), FishR2_t1 (CAGGAAACAGCTATGACACTTC AGGGTGACCGAAGAATCAGAA), & FR1d_t1 (CAGGAA ACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA) .

Polymerase Chain Reaction (PCR) analysis used agilent surecycler 8800 with PCR condition were initial denaturation step (96 °C, 3 min, 1 cycle), denaturation step (94 °C, 10 sec, 35 cycle), annealing step (50 °C, 30 sec, 35 cycle), extension step (72 °C, 45 sec, 35 cycle), and hold step (4 °C, 10 sec, 1 cycle). Sequencing process used the cycle sequencing protocols for the BigDye® Terminator v3.1 at Genetica Science Indonesia.

The phylogenetic tree was constructed by MEGA XI with neighbour-joining tree model, 1000 bootstraps replicates, and Kimua 2 Parameters (K-2P) model. The other sequences of *A. panchax* were taken from gene bank of National Center for Biotechnology Information (NCBI) for verifying and contructing the phylogenetic tree.

Food identification for identifying gastrointestine content was obtained by observation of fish's digestive tract under a microscope binocular 40-100x zoom.

3. Result and Discussion

Kepala timah fish or blue panchax (Figure 1) found at acidic waters of abandoned ex-tin mining was confirmed as *Aplocheilus panchax*. The phylogenetic tree indicated kepala timah fish from Bangka Island has a relationship with *A. panchax* from the other populations, although it was on different clade (Figure 2).

Some of the general morphological characteristics of *A. panchax* were a silvery white spot on its head, terminal type of the mouth position, body shape was sagittiform, small dorsal fin on posterior body, and the shape of the caudal fin was rounded or rhomboid.

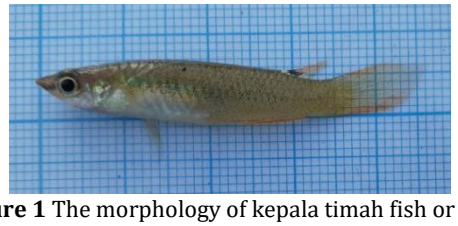

Figure 1 The morphology of kepala timah fish or blue panchax (*A. panchax*) found at acidic waters of abandoned ex-tin mining pond

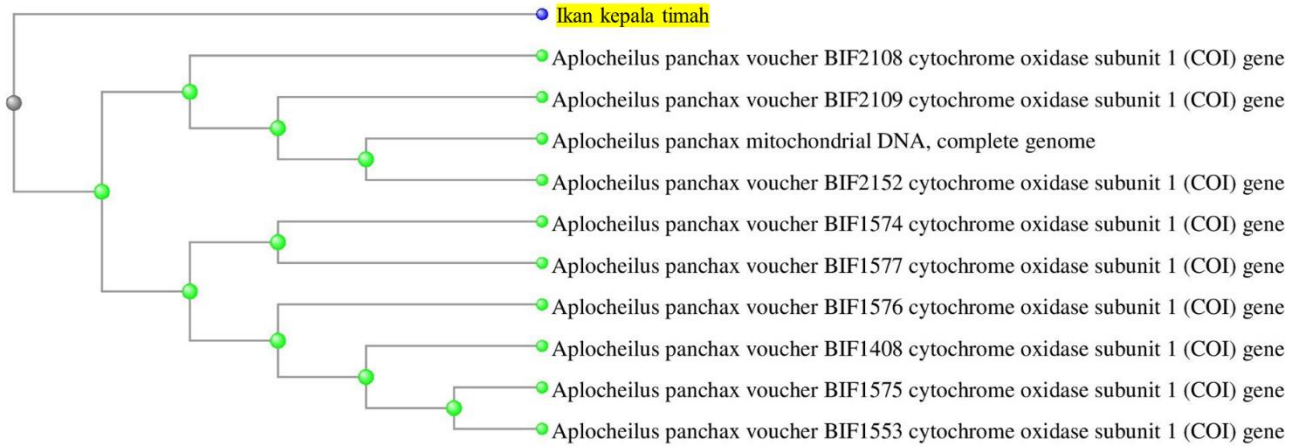

Figure 2 The phylogenetic tree of kepala timah fish (*Aplocheilus panchax*) found at acidic waters of abandoned ex-tin mining pond

The position of the mouth of *A. panchax* which is the terminal type indicates that *A. panchax* has a habit of living and foraging on the surface as surface feeder and is not suitable for bottom feeding (Fugi et al. 2001). The terminal mouth position is generally owned by species that prefer to bite their prey and they are carnivorous fishes (Alves et al. 2021). The sagittiform body shape strengthens the characteristics of *A. panchax* as a predator that quickly ambushes its prey as ambush predator (Senay et al. 2017; Burns and Sidlauskas 2019), especially insects or their larvae that fall on the surface of the water (larvivorous or insectivorous fishes) (Gupta and Banerjee 2013; Ng et al. 2017).

Mustikasari et al (2020) reveal morphological variation of blue panchax lives in different habitat assessed using truss morphometric. There are differences of morphological characters of blue panchax that affected extreme water quality, included acidic water and heavy metal contamination. Mustikasari et al (2022) explain that environment factor impact to morphological characteristics. This condition cause morphological characteristics must be confirmed with molecular analysis to justify species identification.

The food habit of *A. panchax* as surface feeder and larvirous or insectivorous was confirmed by some insects or insect larvae were found in its digestive tract (Figure 3). The ability of the native of *A. panchax* as larvivorous fish, predator of mosquito larvae was assessed (Manna et al. 2011). Study of the comparative has revealed significant higher predation efficiency for *A. panchax* over *Poecilia reticulata* (Gupta and Banerjee 2013). The predation ability of *A. panchax* can be used as biocontrol to reduce the presence of mosquito larvae in an environment (Chakraborty et al. 2008; Putri et al. 2022), the disease vectors of malaria, dengue, chikungunya, filariasis, yellow fever, and etc (Das et al. 2018; Lukas et al. 2021).

Furthermore, the ability of *A. panchax* to survive at extreme environment have to explorated for ecological management. The presence of organisms such as fishes at acidic waters of abandoned ex-tin mining pond can produce organic matters. Tada et al. (2021) explain organic matters from fish such as waste feed (unconsumed feed) and fecal matter can conduct and determine total organic carbon, total nitrogen, and total phosphorous in environment. The presence of organic matters can support a life and biogeochemical cycle in the environment. It impact to increasing of waters quality so the waters can be used for secondary activity such as aquaculture and other activities.

Figure 3 Insect were found in digestive track of *Aplocheilus panchax*

Kurniawan et al (2023) have explained about food chain and energy flow in a pit environment and biogeochemical flow involved some bacterials found in abandoned tin mining pits. Based on this mechanism, we considered that the presence of *A. panchax* at acidic waters of abandoned ex-tin mining ponds can contribute to improving water quality.

We resumed the role of *A. panchax* and its metabolic for improving water quality at ex-mining. We proposed a new scheme or pathway the role of *A. panchax* for water quality management, especially at abandoned ex-tin mining ponds (**Figure 4**).

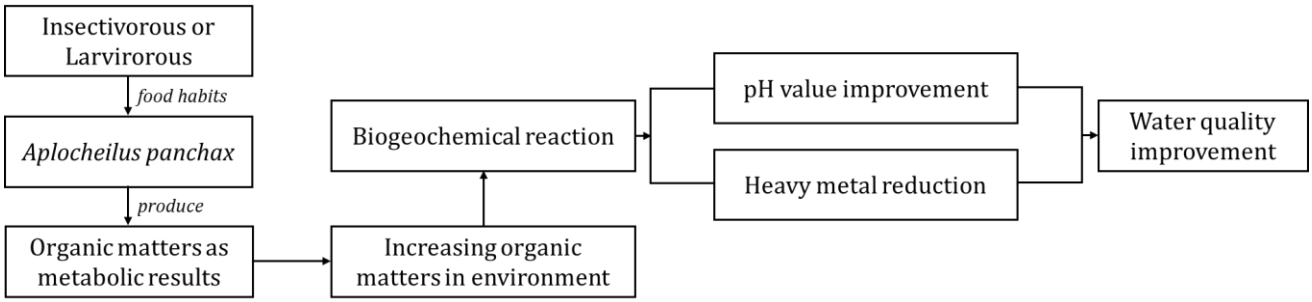

Figure 4 Parthway of the role of *Aplocheilus panchax* at abandoned ex-tin mining ponds

4. Conclusion

This research has disclosed a confirmation about simirality between kepala timah fish and *Aplocheilus panchax* Hamilton, 1822 by molecular and morphological analysis. Furthermore, this research also confirmed *A. panchax* found at acidic waters of abandoned ex-tin mining pond has food habits as insectivorous or larvivorous. Furthermore, our research focused on the effect of acidic waters of ex-tin mining ponds to histological changes of organism's organs.

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Conflict of Interest

The authors declare that there is no conflict of interest in this publication.

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