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HIGHLY COMMERCIAL FISHERIES TAWAR FISH: MOLECULAR ANALYSIS DNA MITOCHONDRIAL COI GENE SEQUENCE AND PROXIMATE ANALYSIS FROM MALACCA STRAIT, RIAU

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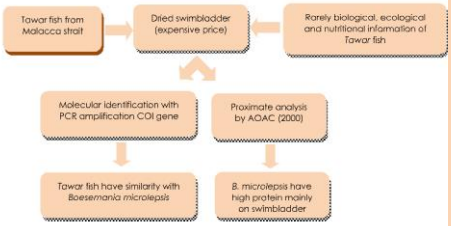
Graphical abstract



Abstract

Tawar fish is one of fish that live at the Coast of Malacca strait, Riau. Even though they live at sea but the local community has mentioned it as "tawar" fish, which means freshwater fish. Tawar fish is not found in the other place. The most interesting thing of this fish is their dried swim bladder that have an expensive price can reach as much as IDR 15 000 000 (± USD 1 200) per kg. The local community usually sell them directly to Singapore. The myth has found that this swim bladder is useful for health and could recover diseases although the actual benefit for health has not been well explored. The aim of this research was to investigate molecular approach with PCR amplification COI gene (Cytochrome Oxidase subunit I) and by means proximate analysis on the content muscle and swim bladder. The result showed that tawar fish (FBR_BCL with accession number LC064301) have 92 % sequence similarity with *Boesemania microlepsis*. While through the proximate analysis, it was found that this species had high protein mainly in swim bladder as much as 36.21 %. *Boesemania microlepsis* (Bleeker, 1858) have an economic value.

Keywords: COI gene, proximate analysis, swim bladder, tawar fish

1.0 INTRODUCTION

Tawar fish is a species suspected native to the waters of Malacca strait, and there is a little biological, ecological or nutritional information on this species. Yet, the existence of tawar fish is vital as a source of livelihood for coastal community of Riau Islands since the fish is apparently highly sought commodity for the Chinese community. Foreigners commonly seeks out tawar fish not as a delicacy. They believe that its swim bladder has properties that may be beneficial and able to enhance health. Tawar fish swim bladder are usually taken out freshly from newly hauled tawar fish and are usually dried under the sun for two until three days. These dried swim bladders are then collected and sold. A tawar fish weighing 5 kg usually yields 3 ounces of swim bladder. Market price for Tawar swim bladder can reach as much as IDR 15 000 000 (± USD 1 200) per kg.

High demands of tawar fish swim bladder encourages the local fishermen in catching them. Yet, the official biological nomenclature for this species remains unknown, and the local community seems to be unaware of why there has been such a lucrative demand for its swim bladder. Tawar fish price is still largely determined by Singaporean consumers. It is known that tawar fish hauls have never been performed in large amount, as hauls cannot be made every day and there are only three to four tawar fish in one haul. This may be a precursory fact of how rare this species is. It is feared

that the increasing demands and catching of this species may lead to its endangerment.

The aforementioned facts demand more study on this species and the phenomenon of the market demand for it. Thus, this research aims to identify the species of tawar fish by means of PCR COI (Cytochrome Oxidase C Subunit-1) amplification and to analyze nutritional contents of its swim bladder by proximate analysis by AOAC. The results of this research is expected to be used as a source of information which will become a starting point for cooperation with local government in following up the existence and catching regulation of tawar fish in its natural habitat.

Recent investigations have suggested the feasibility of creating identification system reliant on the analysis of sequences diversity in small segments of DNA. Previous study proposed that a DNA barcoding system for animal life could be based upon sequence diversity in cytochrome oxidase subunit I (COI) [1]. They established that diversity in the amino acid sequences coded by the 5' section of this mitochondrial gene was sufficient to reliably place species into higher taxonomic categories (from phyla to orders).

Fish comprise nearly half of all vertebrate species, the group includes approximately 15 700 marine and 13 700 freshwater species (FishBase: www.fishbase.org) [2]. Other study also carried out a proof that compiled barcodes for 200 species of commercially important Australian marine fish [3].

The outcome of this research will be a detailed information on tawar fish which is a native species of Malacca strait, with high demands and consumption habits in foreign countries, and of which local communities know very little. It is expected that this study on tawar fish can assist the government in maximizing its economic potential for local communities through further exploration as well as maintaining the conservation effort of this particular species. Furthermore, this research studied information about tawar fish that are found at the coast of Malacca strait and consumed by neighboring countries, yet, has not been explored well by local community. Hence, information from this study would be able to give more advantages for the local community and government to increase the utilization of this fish.

2.0 MATERIALS AND METHODS

2.1 Sampling

Tawar fish were collected at the coast of Malacca strait, Riau. Specimens sample were preserved in 95 % ethanol [4]. Morphological character descriptions, counts, and measurements were performed based on FAO method [5].

2.2 DNA Extraction

Sample was isolated from fragments of white muscle of tawar fish [3]. The DNA was extracted from white muscle using chelex method [6, 7]. Selected white muscle was mixed with 20 % Chelex 100. The mixture was boiled at 95 °C for 45 min and mixed using vortex once after 20 min. The muscle tissue should be mixed perfectly with chelex solution). Afterwards, sample was centrifuged at 5 000 rpm (1 rpm = 1/60 Hz) for 10 min and stored at –4 °C.

The DNA concentrations were quantified and qualified by using NanoDrop 2000 spectrophotometer (Thermo Scientific) [7]. The concentration of 1 µL DNA sample was determined by using the NanoDrop 2000 spectrophotometer (Thermo Scientific). The 260/280 and 260/230 nm ratios was calculated by the NanoDrop spectrophotometer and was used to evaluate the DNA purity and also the concentration of DNA.

2.3 PCR Amplification of COI Gene

The polymerase chain reaction (PCR) was used to amplify the mithocondrial DNA COI gene (Cytochrome oxidase subunit I) [3, 4, 8]. DNA extracts for COI gene were amplified by PCR using selected primers, i.e. FISH BCH (5'-ACTTCYGGGTGRCCRAAR AATCA-3') and FISH BCL (5'-TCAACYAATCA YAAAGATATYGGCAC-3'). The PCR mixture consisted of GoTaq®Green Master Mix Promega (25 µL), primer FISH BCH (0.5 to 5) µL, primer FISH BCL (0.5 to 5) µL, DNA extract (1 to 5) µL, and *Nuclease-Free* Water (20 µL) [9, 10].

The PCR reaction was performed in a KYRATEC using cycling conditions consisting of an initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 40 sec, and extension at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min [3]. The PCR products were analyzed by agarose 1 % gel electrophoresis and the result showed by using UVIDoc HD5 (UVITEC cambridge).

2.4 DNA Sequencing

DNA sequencing was conducted at 1stBASE, Genetika Science. Purification PCR product was used in QIAquick PCR purification KIT (QIAGEN) and PCR sequencing was performed by using Big Dye Terminator v.3.1 and automatically sequences analysis by using ABI 3130XL, Applied Biosystem.

2.5 BLAST Homology

The sequences of amplified COI gene were compared in the GenBank database the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) with the Advanced BLAST search program to identify the sequences of any closely related fishes. The partial COI gene sequence of this fish DNA extraction was deposited in

DDBJ (DNA Data Bank of Japan) to get the accession number.

2.6 Phylogenetic Analysis

The result of DNA sequences were preliminarily aligned with ClustalW Multiple Alignment [11] and the phylogenetic analyses were performed by using MEGA 6 [12]. The phylogenetic tree was determined using the neighbour-joining method with Kimura's two-parameter. The resultant tree topology was evaluated by bootstrap analyses of the neighbour-joining method based on 1 000 resamplings.

2.7 Proxymate Analysis

The proximate compositions were determined as described by AOAC [13]. Protein, fat, carbohydrate, water content, and mineral were calculated for proximate analysis.

3.0 RESULTS AND DISCUSSION

The morphological studies of tawar fish was determined by method developed by FAO [5]. This species belongs to family Sciaenidae based on its morphological trait. The morphological of this fish were moderately elongate and compressed. Head, body and caudal fin are completely scaly, except tip of snout. The teeth are differentiated into large and small size in both upper and lower jaw. Enlarged teeth are located at outer series in upper jaw, while inner series are in lower jaw. Well-developed canines (more than twice as large as other teeth), vomer and palatine without teeth are also found. It has continuous dorsal fin with deep notch between anterior (spinous) and posterior (soft) portions; anterior portion with X slender spines, and posterior portion with I spine and 24 soft rays; base of posterior portion elongate (much longer than anal-fin base), anal fin with II spines and six soft rays, caudal fin rounded, pelvic fins with I spine and five soft rays can be found in this species. It also has scales ctenoid (rough), lateral-line scales extending to hind margin of caudal fin, dorsal side of head (skull) cavernous with a series of bridge-like bony struts and posterior margin of preopercle serrate. Swim bladder is well developed with thick wall and hammer-shaped. Visualization of tawar fish and its swim bladder can be seen in Figure 1.

3.1 DNA Extraction

DNA extraction was conducted by using chelex method. The 260/280 nm and 260/230 nm ratios was calculated using NanoDrop spectrophotometer. This means could also be used to evaluate the DNA purity. The result of the quality and quantity of DNA extraction is on Table 1 while visualization from gel electrophoresis is on Figure 2.

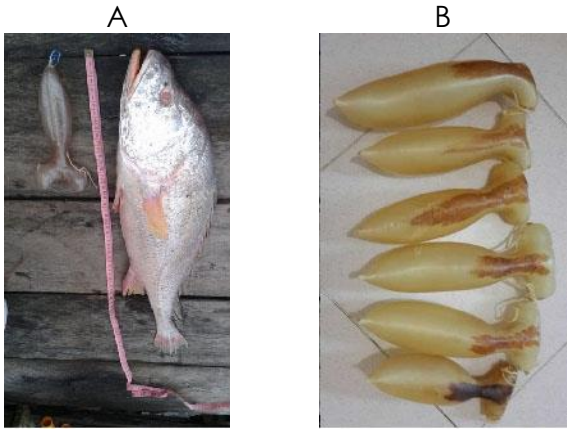


Figure 1 Tawar fish (A) and its swim bladder (B)

Table 1 Quantity and Quality of DNA extraction

No	Code	DNA concentration (ng · µL–1)	260/230	260/280
1	FBR_BCL	24422.4	0.98	1.76

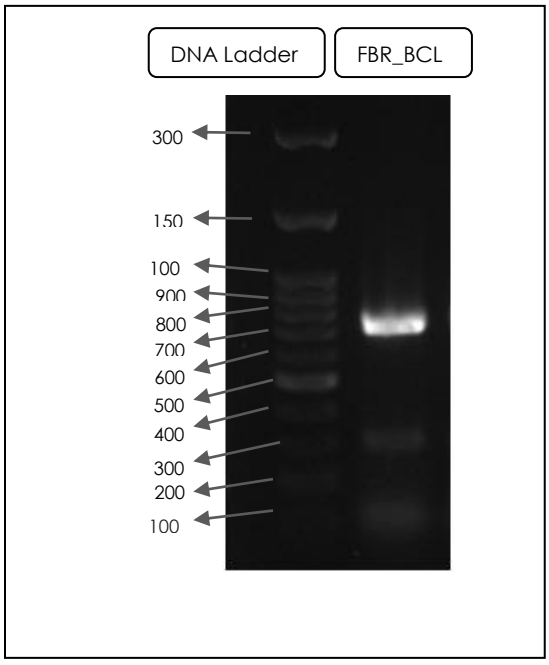


Figure 2 Gel electrophoresis with UVIDoc HD5 (UVITEC cambridge)

3.2 Sequencing

The result of tawar fish DNA (FBR_BCL) was sequenced for a 674 bp DNA sequences by using the DNA mitochondrial COI gene sequences. A large number of studies have emphasized the effectiveness of the COI gene for reliable

discrimination of taxa, in both invertebrates and vertebrates, including a variety of fishes [3, 14–16]. Most COI studies have analyzed a fragment located in the first half of the gene, which is considered to be a "barcode" [3].

Table 2 The result of BLAST homology of FBR_BCL

No	Code	Accession number	Similarity	Percent similarity	Accession number
1	FBR_BCL	LC064301	*Boesemania microlepsis*	92 %	KP722706.1

3.3 BLAST Homology

The result from BLAST homology showed that FBR_BCL was most closely related with *Boesemania microlepsis* (Bleeker, 1859) with 92 % sequence similarities (Table 2). The result shwoed that in the present studies it was possible to identify the fish species using COI gene, thus, reinforcing the efficiency of this marker as a reliable bio-identifier. Tawar fish is most closely related to *B. microlepsis* with 92 % sequence similarities by using amplified DNA mitochondrial COI gene sequence. This fish can only be found at Malacca strait, Riau, Sumatera. Moreover, *B. microlepsis* are also distributed in Thailand to Vietnam and Sumatera [17]. They lived on brackish seas and benthopelagic and are important to fisheries with highly commercial market. Barcoding and morphological analysis should go hand-in-hand for species identification [3].

Oryzias and Xenopoecilus were amplified by using the complete nuclear and mitochondrial DNA with high bootsrap values [18]. The DNA barcode marker was analyzed from 17 representatives of the *Oryzias woworae* species group including the two other species of Oryzias that live in Sulawesi, and *Chanos chanos* as a distant outgroup [4]. Moreover, all previous molecular phylogenetic studies of the Sciaenidae were limited to few taxa, a particular geographical region, and only mitochondrial markers were used to infer the the cytochrome oxidase subunit I (COI) having two important advantages [19]. First, the universal primers for this gene are very robust, enabling recovery of its 5' end from representatives of most, if not all, for animal phyla and for relationships [16, 20].

3.4 Phylogenetic Analysis

The phylogenetic tree was determined using the neighbour-joining method with Kimura's two-parameter. The result of tree topology was evaluated by bootstrap analyses of the neighbour-joining method based on 1 000 resamplings (Figure 3).

COI appears to possess a greater range of phylogenetic signal than any other mitochondrial gene that is about three times greater than that of 12S or 16S rDNA [21]. They are likely applicable for COI identification system on new animal groups and geographical setting suggests the feasibility of creating an identification system for animals at large [1].

Once a global COI barcode database has been established for fish, anyone with direct or indirect access to a DNA sequence will be able to identify. This will be an valuable tool for fisheries managers, fisheries ecologists and fish retailers, and for those wishing to develop fish identification microarrays. The scientific and practical benefits of fish barcoding are mainfold [3].

3.5 Proximate Analysis

The proximate compositions were performed based in AOAC method [13]. The result showed that the content of protein was mostly high, i.e. 18.03 % in muscle, 18.65 % in egg fish, and 36.21 % in swim bladder (Table 3).

Table 3 Result of proximate analysis with AOAC methods

No.	Test parameter	Muscle (%)	Swim bladder (%)	Egg fish (%)
1	Protein	18.03	36.21	18.65
2	Fat	1.20	2.35	2.30
3	Carbohydrate	0.18	13.65	9.75
4	Water content	76.13	26.25	45.30
5	Mineral	2.06	16.28	0.35

The result of proximate analysis showed that the protein of swim bladder was higher than that in muscle of *B. microlepsis*. High content of protein in swim bladder led to higher price. Most nutritionists would recommend that human beings should eat fish every day. Regular consumption of fish can promote the defense mechanism for protection against invasion of human pathogens because fish contains antimicrobial peptide [22]. By consuming fish, risk of heart diseases and other disease such as dementia or Alzheimer's can be reduced.

Various species of fish do not provide the same nutrient profile to their consumer. Moisture, dry matter, protein, lipids, vitamins and minerals are the most important components that act as sources of nutritive value of fish. Among the proximate composition, protein in fish is the excellent source, because of the amino acid composition and degree of digestibility [23]. Principal composition of fish are (16 to 21) % protein; (0.2 to 5) % fat, (1.2 to 1.5) % mineral, (0 to 0.5) % carbohydrate and (66 to 81) % water [24].

Fisheries has received increased attention as a potential source of animal protein and essential nutrients for human diets. The *B.* swim bladder provide high protein (36.21 %), yet low lipid content (2.35 %). Moreover, the protein and lipid content of Scomberoides species were 21.68 % and 0.89 %, respectively [24]. Fish that contain lower calory content per unit of protein are ideal source of animal protein in diets. The proximate composition of fish species greatly varies during the catching season due to physiological reasons and changes in environmental conditions [25].

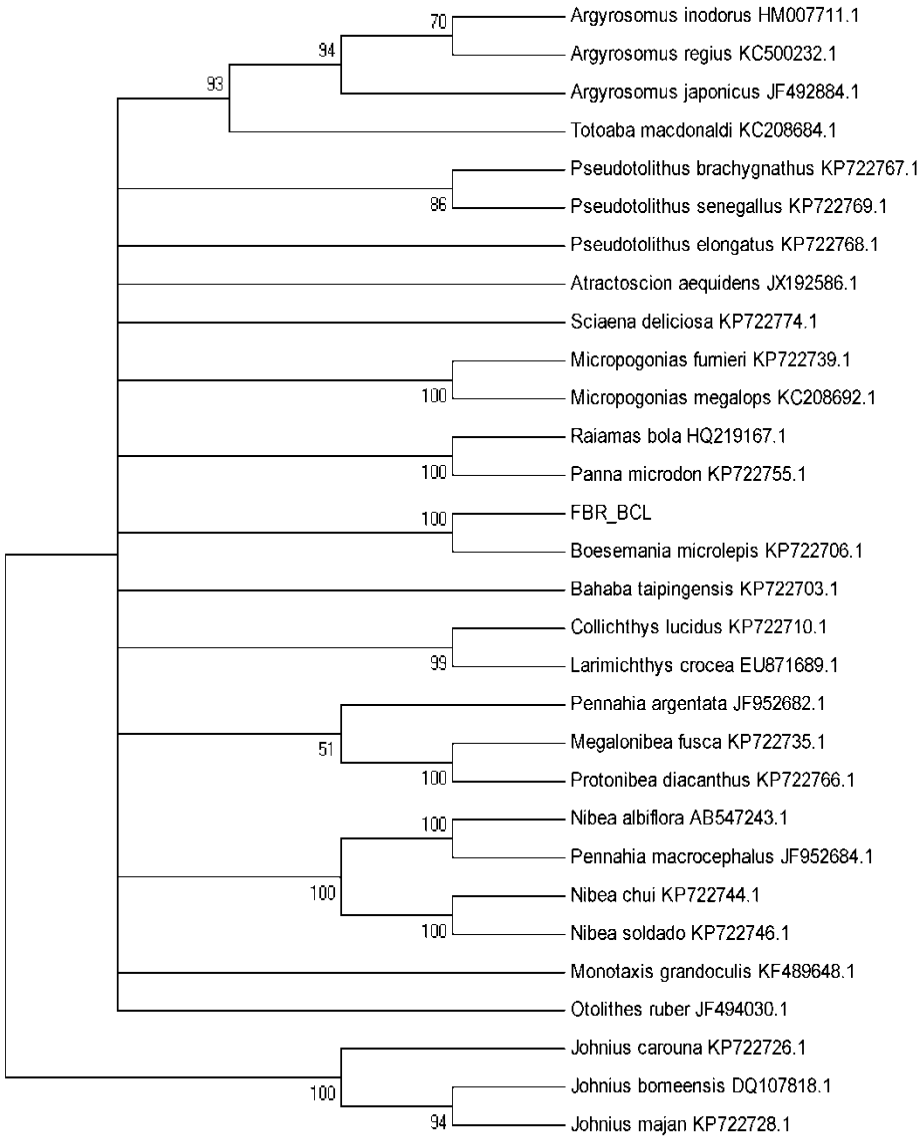


Figure 3 Neighbor-joining phylogenetic tree based on comparative COI gene sequence analysis of *Boesemania microlepsis* showing the phylogenetic affiliation of FBR_BCL

4.0 CONCLUSION

Tawar fish that found at Malacca strait, Riau is most closely related with *Boesemania microlepsis* (Bleeker, 1858), they are belonging to highly commercial in fisheries and have the high contain of protein so that have the high priced for the market purpose.

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