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Species identification by DNA barcoding and sarcoplasmic protein profiles of Indonesian freshwater fish

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Introduction

Freshwater fish are becoming the important source of animal protein along with the worldwide shifting of protein needs from red meat (livestock) to white meat (fish). Freshwater fish have high quality of protein and amino acid composition.

However, not a few species of local freshwater fish have not been characterized, even though they have a high potential as a source of healthy food based on local wisdom information. Thus, the present research aimed to identify freshwater species based on morphology and DNA barcoding, together with the sarcoplasmic protein profiles as visualized by electrophoresis.

Materials and methods

Fresh specimens of local freshwater fish were collected from Tangerang-Banten Province, Borneo Island, and Medan, Sumatera. All the specimens were stored at -20°C as a whole fish until used. The morphological identification of the species was performed by referring to Kottelat [1].

DNA Barcoding
DNA barcoding was performed using a molecular marker cytochrome oxidase subunit 1 (COI). DNA was isolated from the ordinary muscles using a Qiagen DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer's instructions. PCR thermocycling conditions consisted of an initial hot start of 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 1 min, and extension at 72°C for 1 min, with a final extension 72°C for 7 min. PCR products were sequenced by 1st BASE.

Sequences were aligned using MEGA 6.0 with Pairwise Distance. Neighbour joining (NJ) phylogenetic trees were constructed using 1000 bootstraps [2].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)
SDS-PAGE was performed as described by Laemmli [3]. Sarcoplasmic protein fractions from the ordinary muscles were analyzed by 12.5% separating gel. Electrophoresis was performed using an apparatus (SDS-PAGE TV100YK SCIE-PLAS, Bio-Rad) at a constant voltage (150 V) and current density of 15 mA. Proteins were stained with Coomassie Brilliant Blue. Densitometry of the protein bands was carried out using Photocapt, and the image analysis was performed using ImageJ software.

Results and discussion

The specimens examined were identified as striped snakehead (*Channa striata*), Indonesian snakehead (*Channa micropeltes*), marble goby (*Oxyeleotris marmorata*), Asian redtail catfish (*Hemibagrus nemurus*), and soro brook carp (*Tor* sp.) based on the scheme of Kottelat [1]. Namely, triped snakehead gave a color ribbon-shaped like "<" that lead to the front. The caudal rounded, depressed head, body brisket had dark speckles with black and yellow color combination of dark and white on the belly. Indonesian snakehead had small teeth and a line of sharp fangs. Dark patterned lines lengthwise along the body with white color on the belly. Marble goby was characterized by separate ventral fins with 80-90 scales along side of body, with 60-65 scales in front of dorsal fin and no spots (ocellus) on the caudal peduncle. Asian redtail catfish gave a body similar to that of catfish, flattened head slightly flat with the rough skull above the head, not covered by the skin, and medium-sized fat fins behind the dorsal fin. Soro brook carp had a slippery body with no scales on its body, three venomous spines on a pair of pectoral fin and the initial dorsal fin, and heads are not conical.

Striped snakehead had a total length, width, height and weight total respectively 30.30 ± 0.42 cm, 4.70 ± 0.14 cm, 3.35 ± 0.07 cm and 245.00 ± 4.24 g. The values for Indonesian snakehead was 30.45 ± 0.07 cm,

4.30 ± 0.14 cm, 3.85 ± 0.07 cm, and 282.00 ± 11.31 g, respectively. For marble goby, the values were 24.80 ± 0.85 cm, 4.75 ± 0.07 cm, 3.55 ± 0.07 cm and 215.00 ± 5.66 g. Those of Asian redtail catfish were 45.50 ± 0.71 cm, 8.25 ± 0.35 cm, 9.50 ± 0.71 cm, and 935.00 ± 4.24, and for soro brook carp, they were 35.00 ± 0.00 cm, 5.00 ± 0.00 cm, 10.50 ± 0.71 cm, and 519.50 ± 2.12 g, respectively.

Specific primer design was done by aligning nucleotide sequences of five samples. Obtained sequence of striped snakehead has been submitted to GenBank with the accession number (KU204858).

Protein profiles of five species showed species-specificity but also similarity regarding the 15-18 bands in the range of 6.9-156 kDa. The major proteins were 15.6 kDa and 38.3 kDa components for striped snakehead; 11.3-16.4 kDa and 45.7-59.6 kDa components for Indonesian snakehead; 6.9-15.1 kDa components for marble goby; 6.9 kDa and 39.2 kDa components for Asian redtail catfish; 6.9 kDa and 34.3-58.5 kDa for soro brook carp.

Identification of the protein types was carried out based on Gam et al. [6] who performed proteomic analysis on muscle tissue of snakehead. The results gave six group protein functions, namely, structural proteins, hypothetical proteins, enzymes, the transcription factors, the translation factors, and transport proteins.

Conclusions

By morphological identification and DNA barcoding, five species examined were successfully identified. All the fish showed similar protein profiles. These information would facilitate effective utilization of these species. Further studies are needed to identify the species as in the present study targeting more domestic freshwater species.

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