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Control Region-Mitochondrial Partial DNA analysis of Humphead Wrasse [*Cheilinus undulates* (Ruppel, 1835)] from Anambas Islands, Indonesia

Indriatmoko[a]*,Amran Ronny Syam[a], Khairul Syahputra[b]

[a]*Research Institute for Fisheries Enhancement and Conservation, Jl. Cilalawi No 1, Purwakarta-41152, West Java, Indonesia*
[b]*Research Institute for Fish Breeding, Jl. Raya Sukamandi No 2, Subang-41282, West Java, Indonesia*

Abstract

Humphead wrasse [*Cheilinus undulates* (Ruppel, 1835)] known as one of the threatened species and had been being classified as Appendix II-type species. Nevertheless, Indonesia has a unique issue for its species, especially in Anambas Islands. Anambas island's fishermen have been successfully developed ranching-like mechanism of humphead wrasse's culture for last five decades, approximately. Against the major opinions, either juvenile or up to ten kilograms humphead wrasse could be found in Anambas fishermen's cages, abundantly. This investigation reported partial control region-mtDNA sequence analysis provided from caudal fin of Anambas humphead wrasse. Sequence comparisons were conducted by using reference reported sequence provided by NCBI genbank. mtDNA analysis resulted among all examined samples collected from Anambas islands shows no significant genetic variation. This concludes that collected Anambas humphead wrasse samples were indicated as one genetic population. Though, dissimilarities of nucleotides and amino acid sites among Anambas humphead wrasse and reference samples were observed significantly different, genetically. This phenomenon indicated that Anambas humphead wrasse possibly belonging specified genetic characteristic compared to humphead wrasse from reference sequence.

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Keywords: Anambas; *Cheilinus undulatus* (Ruppel, 1835); genetic; control region-mtDNA.

* Corresponding author. Tel.: +62 857 2770 1940
E-mail address: indriatmoko.bp2ksi@outlook.com

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1. Introduction

Humphead wrasse, also known as napoleon fish and by Anambas local fishermen so called *ketepas*, had been being classified as appendix 2- type species by CITES and categorized as wild-type marine commodities (Donaldson and Sadovy, 2001; Sadovy et al., 2003). International regulation considers humphead wrasse as wild-captured fish and tradable in limited quota. A unique phenomenon was captured in Anambas Islands, Indonesia. In these regions, ranching-like culture has been applied by local fishermen. But, very limited information provided in order to elaborate updated data regarding to its extraordinary condition.

One of the approaches to understand Anambas humphead wrasse is by investigating genetic characteristic of the objects. Mitochondrial DNA (mtDNA) prospected by numerous researchers for their advance in describing maternal inheritance, rapid evolutionary rate, and rare recombinant (Craig et al., 2001; Ding et al., 2006; Qi et al., 2013). Thus, mtDNA is considerable potential in analyzing genetic population and evolution (Miya and Nishida, 2000). mtDNA has become one of the most interesting objects to understand genetic characteristic for large type of species (Wolf et al., 2000; Wang et al., 2011; Li et al., 2013). Qi et al. (2013) and Han et al. (2014) previously had been successfully describe the structure of humphead wrasse mtDNA in full sequence genome. Nevertheless, it is observable that those two reported full sequence are distinguishable in certain nucleotide regions, e.g., D-loop control region. Regarding to its condition, therefore, it is important to provide information about Anambas humphead wrasse genetic characteristic.

2. Material and methods

2.1. Sample collections

C. undulatus caudal fins were clipped from three fish collected from Air Sena waters (N 03°15'01.2960" E 106°16'48.9000"), Anambas, Indonesia, by permission of the owner fishermen. According to fishermen information, those collected sample were captured in *Sargassum* sp. beds adjacent to their cages about (2 to 3) inch in size (1 in = 2.54 cm). Then, they had been grown in cages for 5 yr to 7 yr, approximately. 1 cm to 2 cm caudal fins were collected and reserved in 96 % ethanol, aseptically. Prior to analyze, samples were frozen to prevent from DNA damaging.

2.2. DNA extraction, polymerase chain reaction and sequencing

Total genomic DNA was extracted from caudal fin using GeneJet Genomic DNA Purification Kit (Thermo Scientific, Lithuania) following the procedures in the manual. The extracted DNA was then stored at -20 °C until further processing. Polymerase Chain Reaction (PCR) was applied to amplify 560 bp of mitochondrial control region using the primers CRA (5'-TTCCACCTCTAACTCCCAAAGCTAG-3') and CRE (5'-CCTGAAGTAGGAACCAGATG-3') (Lee et al., 1995). PCR reaction was carried out in a final volume of 50 µL containing 100 ng of genomic DNA, 25 µL of Maxima Hot Start Green PCR Master Mix (Thermo Scientific, Lithuania) and 1 µM of each primer (1st BASE, Malaysia). The PCR conditions were set up as an initial denaturation of 95 °C for 4 min, followed by 40 cycles of DNA denaturing at 95 °C for 30 s, primer annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, ending with a final extension of 72 °C for 10 min. PCR products were separated by electrophoresis with 2 % (w/v) agarose gel and visualized using GelRed (Biotium Inc. California, USA) and UV transilluminator. PCR products were sent to First BASE Laboratories Sdn Bhd (Malaysia) for DNA sequencing.

2.3. Data analysis

C. undulatus references sequence were obtained from BLAST searches of NCBI genebank database. Either collected sample sequences or reference sequences were aligned using Multiple Sequence Analysis facilitated by Bioedit (Hall, 1999). Pairwise distance and phylogeny analysis were employed by Mega 3.1(Kumar et al., 1994). Neighbour-Joining (K2P) with 1000 iteration was conducted to provide data illustration. Full mitochondrial sequence, provided by Qi et al.(2013), was annotated using Mitoannotator (Iwasaki et al., 2013).