Phylogenetic Analysis of The Darkfin Hind, *Cephalopholis urodeta* (Serranidae) Using Partial Mitochondrial CO1 Gene Sequences

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Abstract

*Cephalopholis* is one of the largest genera belonging to Subfamily Epinephelinae, which has various species. Phenotypically, an adult *C. urodeta* similar to a juvenile of *C. sonnerati*, since both of them have a striking trait, two white oblique stripes or bands on the caudal fins. This work was conducted to investigate the genetic relationships of this species using CO1 gene segment. Fish were collected from several sampling point in South Sulawesi areas such as Sinjai and Selayar Island. The phenotypic characterizations were identified using the FAO species catalogue of groupers of the world, and the species that seemed to have *C. urodeta* morphology then separated. Tissue samples from dorsal muscle tissue were used as the source of DNA. Using part of the CO1 gene sequence, it can be confirmed that our samples are exactly *C. urodeta* species. The 22 *C. urodeta* sequences from GenBank compared with our sequences. Interestingly, because based on the phylogenetic tree, our sequences clustered with the other *C. urodeta* sequences from several part of the world except the Arabian Sea off the coast of India, which is a separate branch. The present study reveals less genetic distance in *C. urodeta* than some other parts of the ocean as follows; South Pacific Ocean (Polynesia), North Pacific Ocean (Northern Mariana), South China Sea (Philippines), Andaman, west coast of Réunion Island, Arabian Sea and Indo West Pacific (Indonesia). This has implications for understanding the migration pattern of the species and may affect conservation policy decisions.

Keywords: *Cephalopholis urodeta*, CO1, phylogenetics, Serranidae, South Sulawesi
Introduction

The Serranid subfamily Epinephelinae comprises about 159 species of marine fishes in 15 genera, commonly known as groupers, rockcods, hinds, and sebastes (Heemstra and Randall 1993). Various fishes of this family are known as Kerapu or Belon in Indonesian (Burhanudin et al., 1980). Serranidae is a benthic fish that occupies coral reefs and rocky substrate at the sea bottom. Indonesia has vast water areas with coral reefs, so that there are potentially groupers (Syaifudin et al., 2007).

Most members of Serranidae live in the sea, while others inhabit freshwater in tropical and temperate regions in around the world (Craig and Hastings, 2007). Indonesia is one of geographical areas where the Subfamily Epinephelinae including Cephalopholis, is distributed in the Indo-Pacific region.

Cephalopholis is one of the largest genera (besides Mycteroperca and Epinephelus) which has various species. Cephalopholis spp. has often been misidentified as Epinephelus spp., for example, species of Cephalopholis have only IX dorsal-fin spines, and Epinephelus acanthurus of the Eastern Pacific also has the same dorsal-fin spines (Heemstra and Randall, 1993). According to Heemstra and Randall (1993) another useful generic character separating both genera is the presence of 3 to 6 trisegmental pterygiophores in the dorsal fin of Cephalopholis species.

Cephalopholis urodeta, also known as the darkfin hind, flag tailed rockcod (Australia), banded tail coral-cod (Hong Kong), Nijihata (Japan) or flagtail grouper (Micronesia), is a common and widespread species, mainly inhabiting coral reefs in the west-central Pacific and Indian oceans (Carpenter and Niem 1999). Phenotypically, an adult C. urodeta has white oblique bands or stripes on the caudal fins. These stripes also appear on juvenile C. sonnerati. Consequently the phenotypic identification based on the color pattern and morphological characters is often ambiguous between Serranidae genus (Govindaraju and Jayasankar 2004), Cephalopholis characters also show interspecific variation or differences in morphology between juvenile and adult individuals.

Molecular techniques can be a major tool for systematic ichthyology and may also be useful for biological fisheries to quickly solve problems about the taxonomic level of species and populations (Chow et al., 1993; Zemlak et al., 2009). The mitochondrial DNA segment that we use for the identification and phylogenetic analysis of blackfin hind (family Serranidae) is cytochrome c oxidase subunit 1 (CO1). CO1, is one of the genes in the mitochondrial genome (mtDNA) commonly used as a barcode. Segments of the CO1 gene with a size of about 648 bp, have been used for identification in several animal taxa such as insects, birds, and fish (Hebert et al., 2003, 2004; Ward et al., 2005; Hajibabaei et al., 2006; Kerr et al., 2007). The application of barcoding technique is expected to overcome the difficulties in morphological identification and reduce the misidentification of commercially important fish such as grouper in every stage of the development phase from larvae to adult (Sachithanandam et al., 2012).

Materials and Methods

Fish species belonging to family Serranidae were collected from several sampling points in South Sulawesi areas such as Sinjai and Selayar Island (Figure 1), then the species that seemed to be C. urodeta from their morphology. Phenotypic characterization was analysed using the FAO species catalogue of groupers of the world. Tissue samples from the dorsal muscle tissue were used as the source of DNA. All tissue specimens were stored in 95% alcohol.

DNA extraction and PCR reaction

Total DNA was extracted from 0.30 g of ethanol-preserved tissue muscle using a DNA Extraction Kit for animal tissue (Qiagen) following the manufacturer’s protocol. Approximately 650-655 bp were amplified from the 5′ region of the COI gene using combinations of the fish-specific primers FishF1-FishR1 as described in Ward et al. (2005).

The 50 μL PCR mixes included 25 μL 2X ReadyMix KapaTaq DNA polymerase mix (0.05 U μL⁻¹, 3mM Mg²⁺, 0.4 mM each dNTP) (Kapa Biosystems, Boston, USA), 1 μL of each primer (1st BASE, Singapore), 2 μL of DNA template and 21 μL of water (Qiagen). The thermal regime consisted of an initial step of 2 min at 94°C followed by 40 cycles of 0.5 min at 94°C, 0.5 min at 56-57°C, and 1 min at 72°C, followed in turn by 7 min at 72°C and then held at 4.0 in an Applied Biosystems (Foster City, CA, USA) Verity™ thermocycler. Amplicon was performed using 1% agarose gel that run at a voltage of 100 volts for 30 minutes. Afterwards we proceeded with Gel Red (Bio Rad) then visualized and documented under UV Transilluminator.

All amplicon were sequenced through company service sequencing following the manufacturer’s protocol. DNA sequences were proofread, aligned and edited using MEGA 6 (Tamura et al., 2013) and BioEdit (Hall, 1999).
A Kimura 2-parameter (K2P) distance metric was employed for sequence comparisons (Kimura, 1980), including genetic distance calculations and to generate neighbour-joining (NJ) trees based on CO1 region with node frequencies were calculated based on 1000 bootstrap replicates.

**Result and Discussion**

**Sequence composition**

Out of 650-655 nucleotides (nt) basic taxonomy sequence length, it was possible to get 541 nt from all of three C. urodeta aligned sequences. Of the final 504 nt, 465 were conserved, 39 variable and 11 parsimony informative after those three sequences were aligned with 22 C. urodeta reference sequences from GenBank. The average nucleotides composition for each sequence of C. urodeta in this present work is shown in table 1. The G + C content in the three codon positions was different. The G + C content at the first and the second codon positions were similar (54.2% and 43.2%) for those of three C. urodeta sequences, but was different for the IA23 Sinjai sequence (33.4) at the third codon position. The G + C content average for all of the three sequences of C. urodeta in this present work was 43.2%. This result concurs with the Ward et al. (2005) statement, that the content of A + T higher than of G + C in most marine fishes.

**Phylogenetics analysis**

Based on the partial CO1 sequences of the C. urodeta and using Cephalopholis sonneratii as outgroup, a molecular phylogenetic tree was constructed by the Neighbour-Joining method (kimura 2-parameter). The values of bootstrap confidence level of nodes were indicated above the branch (Fig 2). There are two C. urodeta cluster formed. Those of all C. urodeta in the present work obviously grouped in the A cluster. The B cluster consisted of C. urodeta sequences from the Arabian Sea off the coast of India. All sequences of C. urodeta from Andaman (JX674963 - JX674966) formed a separate group from the other congeneric C. urodeta species in the A cluster.

![Figure 1. Map of sampling point in South Sulawesi](image)

**Table 1. Nucleotide composition of G + C content on COI sequences**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>G + C content average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All sites</td>
</tr>
<tr>
<td>IA23 Sinjai</td>
<td>43.3</td>
</tr>
<tr>
<td>IA24 Sinjai</td>
<td>43.1</td>
</tr>
<tr>
<td>IA25 Selayar</td>
<td>43.1</td>
</tr>
<tr>
<td>Average</td>
<td>43.2</td>
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</tbody>
</table>
Phylogenetic analysis of the darkfin hind Cephalopholis urodeta (Serranidae) (Y. Ariyanti et al.)

Although all sequences C. urodeta from Andaman (JX674963 - JX674966) formed a separate group from the other congenetic C. urodeta species in the A group, the value between group mean genetic distance A to B (0.0127) was higher than congenetic from Andaman (0.0104). The mapping of clusters of C. urodeta species from various part of the world is shown in figure 3.

Further, the pairwise genetic distance showed C. urodeta IA23 and IA24 Sinjai was similar to the reference sequences of the same species from various parts of the world such as the Philippines (KF009578, FJ583012, FJ583014, FJ583015), Palawan/Sulu Sea (KC970463), and Northern Mariana Island (KF929702). The sequence of FJ583012, FJ583014, FJ583015 and KF929702 has a smaller value of pairwise genetic distance with IA23 and IA24 as amounts of 0.00199 and 0.0000 respectively. While the sequences from Réunion (JQ349869-JQ349873) in Madagascar area forms a separate group that is closely related to the IA23 and IA24 group except the JQ349871 that is closer to IA25 Selayar group.

The sequence of IA25 Selayar was grouped with same species from other countries as follows; French Polynesia (JQ431577: 0.00199) and Manila (Philippines) (FJ583013: 0.00199) which has a smaller value than three others in the same species from the Philippines (KF009579: 0.00599), New Caledonia (KM077911: 0.00398), and French Polynesia (JQ431578: 0.00398). The genetic value between group mean distance C. urodeta Sinjai and Selayar Island is 0.0090. Whereas the pairwise genetic distance is 0.00199, 0.01000 and 0.00800 between IA23 to IA24, IA23 to IA25 and IA24 to IA25, respectively.

Figure 2. Neighbour-joining tree based on the mtDNA COI nucleotide sequences of Cephalopholis species analyzed in the present work and of GenBank species. Numbers at nodes are bootstrap values on 1000 replicates.
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The A cluster comprises C. urodeta sequences from various country such as the Philippines, Northern Mariana Island, Réunion and Andaman, whereas the B cluster consists of sequences from the Arabian Sea off the coast of India. Mapping points based on the sample source proposed two different oceans (Pacific and Indian) as an origin of the samples that caused a separated cluster.

Even though the Andaman sequences belonging to the A cluster and form a separate group from the others, it did not indicate as a difference species. It might be necessary more assessment to clarify it was the difference species. Based on this result, the separated traits of C. urodeta between Pacific and Indian Ocean as described before, become interesting to learn. Previously, in 1991 Randall and Heemstra said that Cephalopholis nigripinnis (from the Indian Ocean) was a synonym for Cephalopholis urodeta, so that Heemstra and Randall (1993) proposed C. urodeta had two different type of morphology based on geographical distribution.

The caudal fin of the Indian Ocean fish is dark reddish brown to almost black and covered with small pale spots while the caudal fin of the Pacific fish has 2 white to bluish white bands that converge posteriorly. Recently the lack of a banded tail showed it was not C. urodeta and it was recognized as a valid species Cephalopholis nigripinnis, furthermore separation of these species was enhanced by mitochondrial (16S and 12S) and the nuclear (Tmo-4C4 and Histone H3) genes (Craig and Hastings, 2007; Gaither and Rocha, 2013). Thus, identification of the samples from several places in the Indian Ocean is needed.

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The East Indies Triangle (EIT) or Indo-Malayan Triangle is the designation for an area rich in marine biodiversity which is located in the Indo-Malayan region. The EIT region covers Malaysia, Philippines, Indonesia, and Papua New Guinea. This area is thought to be a center of diversity for tropical marine species that overlap (Gaither and Rocha, 2013). Due to it’s dependence on the presence of coral reefs, it has recently been referred to as the Coral Triangle (Allen, 2000; Veron et al., 2009; Carpenter et al., 2011). Sinjai and Selayar Island is a source in this present work which includes the Coral Triangle areas south of Sulawesi.

In 2008, C. urodeta is listed as Least Concern by IUCN assessors, because it is a widespread and not known to be in serious decline at present. This species is sensitive to habitat degradation and may become threatened in the future (IUCN).

Conclusion

C. urodeta under investigation show less genetic distance than those from other parts of the
oceans as follows; South Pacific Ocean (Polynesia), North Pacific Ocean (Northern Mariana), South China Sea and Sulu Sea (Philippines), Bay of Bengal (Andaman), Réunion, Arabian Sea and Indo West Pacific (Indonesia). That has implications for understanding the migration patterns and for policy considerations for habitat treatment of these species.

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References


