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

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Abstrak

Analisis Filogenetik Cephalopholis urodeta (Serranidae) Menggunakan Runutan Gen CO1 Mitokondria Parsial

Cephalopholis merupakan salah satu genera terbesar dalam subfamili Epinephelinae yang memiliki banyak species. Secara fenotip, C. urodeta dewasa mirip dengan juvenil C. sonnerati karena memiliki ciri mencolok yaitu garis yang menyudut pada sirip ekor. Untuk memahami hubungan genetik pada spesies ikan ini, maka dilakukan analisis molekuler menggunakan ruas gen CO1. Sejumlah spesies ikan (famili Serranidae) dikumpulkan dari wilayah Sulawesi Selatan seperti Sinjai dan Kepulauan Selayar. Karakter fenotip diidentifikasi menggunakan buku katalog spesies kerapu dunia FAO, kemudian sampel yang diduga C. urodeta secara morfologi dipisahkan. Jaringan yang digunakan sebagai sumber DNA adalah jaringan otot bagian dorsal. Berdasarkan sebagian runutan gen CO1, diyakini bahwa sampel tersebut adalah C. urodeta. Runutan basa nukleotida dari sampel dibandingkan dengan 22 runutan basa nukleotida C. urodeta dari GenBank. Berdasarkan rekonstruksi pohon filogeni, C. urodeta dari Sinjai dan Kepulauan Selayar mengelompok dengan C. urodeta dari berbagai tempat seperti Polynesia, Mariana Utara, Filipina, pulau-pulau di sekitar Madagascar (Ouest, St. Gilles, Canyon, Cimetiere, Jaune) dan Adaman, sedangkan sampel dari Laut Arab di lepas pantai India berada pada cabang yang terpisah. Penelitian ini menyatakan bahwa C. urodeta yang melibatkan beberapa tempat dari berbagai perairan seperti Samudera Pasifik bagian Selatan (Polynesia), Samudera Pasifik bagian Utara (Northern Mariana), Laut China Selatan (Filipina), Teluk Bengal (Andaman), Laut Laccadive (reunion of Ouest, St. Gilles and Cimetiere), Laut Arab dan Indo Pasifik Barat (Indonesia) memiliki perbedaan jarak genetik yang kecil. Hal ini berimplikasi pada pemahaman pola migrasi spesies tersebut dan sebagai bahan pertimbangan pengambilan kebijakan konservasi.

Kata kunci: *Cephalopholis urodeta, CO1, filogenetik, Serranidae, Sulawesi Selatan*

Abstract

Cephalopholis is one of the largest genera belonging to Subfamilly 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 GeneBank 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*

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Introduction

The Serranid subfamily Epinephelinae comprises about 159 species of marine fishes in 15 genera, commonly known as groupers, rockcods, hinds, and seabasses (Heemstra and Randall 1993). Various fishes of this family are known as Kerapu or Belong 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 *Chepalopholis* have only IX dorsal-fin spines, and *Epinephelus acanthistius* 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 ptetygiophores 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 mithochondrial 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 UμL^{-1}, 3mM Mg^{2+}, 0.4 mM each dNTP) (Kapa Biosystems, Boston, USA), 1 µL of each primer (1^{st} 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^{o}C followed by 40 cycles of 0.5 min at 94^{o}C, 0.5 min at 56-57^{o}C, and 1 min at 72^{o}C, followed in turn by 7 min at 72^{o}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* seqences, 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 congenetic *C. urodeta* species in the A cluster.

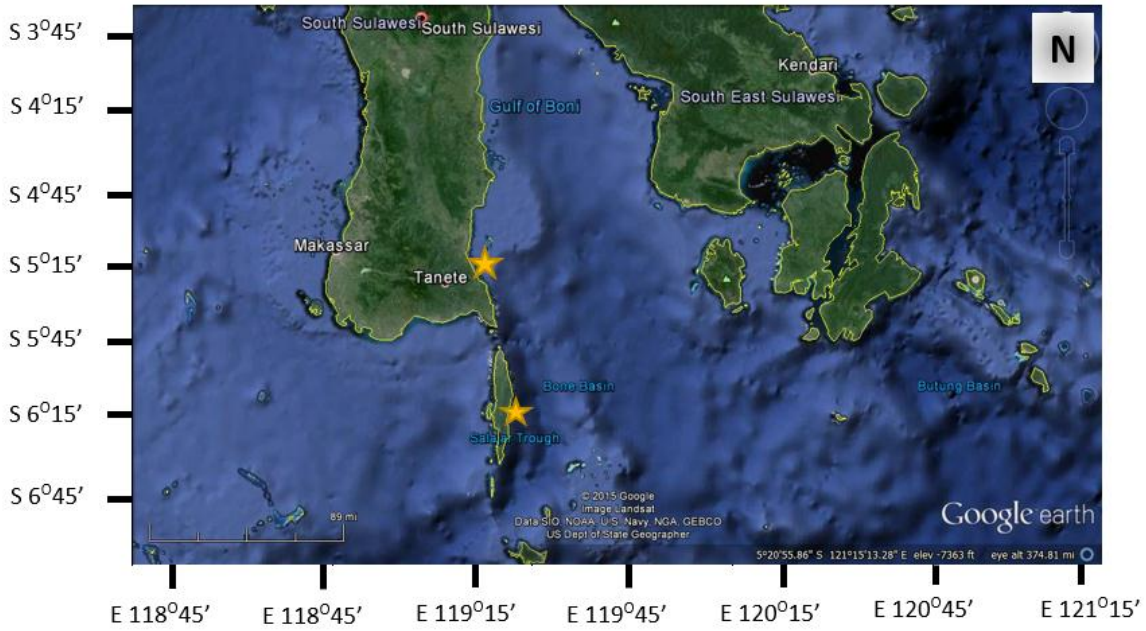

Figure 1. Map of sampling point in South Sulawesi

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

Sequence	G + C content average (%)			
	All sites	1st codon	2nd codon	3rd codon
IA23 Sinjai	43.3	54.2	42.3	33.4
IA24 Sinjai	43.1	54.2	42.3	32.8
IA25 Selayar	43.1	54.2	42.3	32.8
Average	43.2	54.2	42.3	33.7

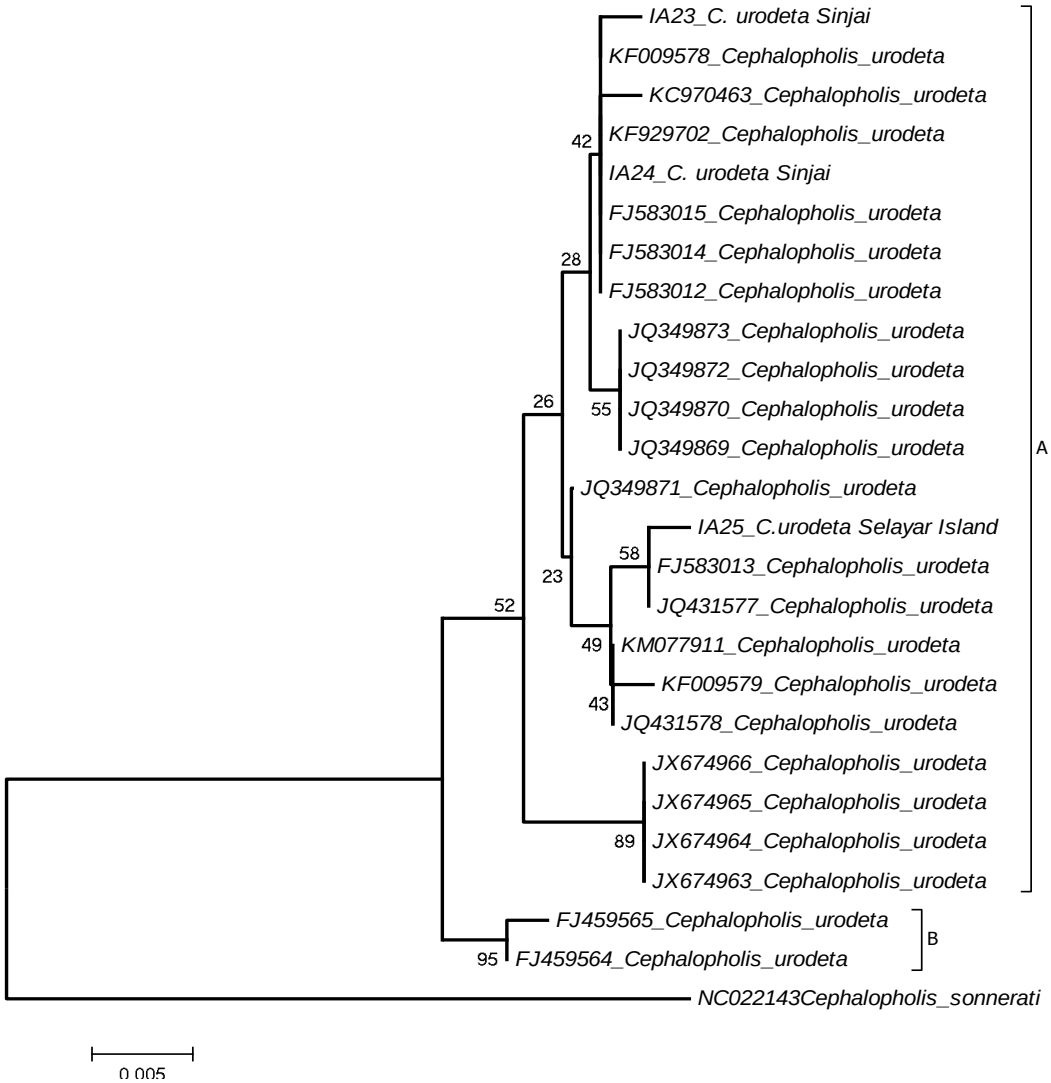

Figure 2. Neighbour-joining tree based on the mtDNA COI nucleotide sequences of *Cephalopholis* species analyzed in the present work and of GenBank species. Numbers at nodes are bootstrap values on 1000 replicates.

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 3. Mapping of clusters *C. urodeta* species from various part of the world. = A Cluster, = B Cluster

Cephalopholis is one of the largest genera (besides *Mycteroperca* and *Epinephelus*) belonging to Subfamilly Epinephelinae, which has various species. Phenotypically, an adult *C. urodeta* seem like a juvenile of *C. sonnerati*, because both of them have two white oblique stripes on the caudal fins. Using a partial CO1 gene sequence, we are convinced that our samples are exactly *C. urodeta* species. We compared our sequences with 22 *C. urodeta* sequences from GeneBank. It was interesting because based on the phylogenetic tree, our sequences clustered with the other *C. urodeta* sequences from several parts of the world except the Arabian Sea off of coast of India which is in separate branch.

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.

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, Philipines, 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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