

Short Communication

The Analysis of Morphological and Genetic Characteristics of Yellowstripe Scad from Muara Baru Modern Fish Market in North Jakarta

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Abstract

Yellowstripe scad is one of the commercially important fish from the *Carangidae* family, which is marketed at Muara Baru Modern Fish Market, North Jakarta. Information regarding the presence of *Selaroides leptolepis* fish in the waters allows for effective conservation, and management of marine resources. A morphological identification of fish species is still considered inaccurate, so the molecular analysis is necessary. This study aims to identify commercially important fish species deriving from Muara Baru Modern Fish Market, North Jakarta, employing morphological, and molecular analysis. A total of 30 specimens were collected from the field. The morphological analysis utilized a visual identification method, and morphometric measurement, while molecular analysis with DNA barcoding employed the mitochondrial cytochrome oxidase subunit I (COI) gene. The results of the morphological analysis indicated that the fish species were yellowstripe scad (*S. leptolepis*). It also followed the results of molecular analysis of DNA barcoding that the fish was a *S. leptolepis* species. Therefore, the combination of morphological and genetic analysis has succeeded in identifying the fish species of *S. leptolepis*.

1. Introduction

Marine fishery resources in Indonesian waters cover 37% of the world's fish species. The potential marine fishery resources expanded in the territorial waters of Indonesia, and the waters of the Indonesian Exclusive Economic Zone (ZEE) are estimated at 12.54 million tons per year. It is reported that the total capture fisheries production has increased to 7.2 million tons (KKP, 2018).

Muara Baru Modern Fish Market is a marine and fishery business center in DKI Jakarta providing the fishery products which are high quality, safe for consumption, and at affordable prices. The fish in this market come from Lampung, Banten, West Java, Central Java, and East Java (along the north coast of Java Island). In 2018, the average number of fish traded in Muara Baru Modern Fish Market was 400 tons per day (KKP, 2018).

One of the fish species found in Muara Baru Modern Fish Market is yellowstripe scad (*S. leptolepis*). The fish is known as one of the pelagic fish from the family *Carangidae* and includes a commercially important fish (Kempton et al., 2015). The high market demand cannot be balanced by fish production in nature. According to (Sharfina et al., 2014), one of these fishing areas is Sunda Strait, where overfishing conditions are reported. The catch from these waters is marketed at Muara Baru modern fish market.

One of the important aspects of studying a biological species is the identification of the species itself. The accurate identification of fish species can assist in longterm fisheries management and will enhance the research on the ecological, ecosystem, and conservation aspects. Traditional fish identification method, such as morphological identification requires taxonomic expertise and are also considered inaccurate (Wang et al., 2018). However, currently, the molecular identification method of fish species has been developed and proven with more accurate results, namely the DNA barcoding method (Leray and Knowlton, 2015)

DNA barcoding is a sequencing based method for the species identification using a universal primer set to target a short, standardized genome region. One of the most frequently used genetic markers is the mitochondrial cytochrome oxidase subunit I (COI) gene. The COI gene is employed as an effective genetic marker for the identification and classification of various species using mitochondrial genes (Hebert et al., 2003).

According to Kamal et al. (2019), the DNA barcoding method with COI genetic markers can be utilized as an instrument in species utilization and protection. This research was conducted to apply morphological and DNA barcoding analysis to economically important fish (*S. leptolepis*) from Muara Baru Modern Fish Market, North Jakarta.

2. Materials and Methods

2.1 Materials

Materials used in this study included morphological analysis (caliper and analytical balance), and molecular analysis (surgical scissors, sample bottles, 96% ethanol, gSYNCTM DNA Extraction Kit, DIAB Mastercycler DNA Engine Thermal Cycler, 1% agarose gel, GelRedTM (Biotium®), and UV transilluminator).

2.2 Methods

2.2.1 Morphological Analysis

A total of 30 (n=30) yellowstripe scads were measured for their length and weight. Meanwhile, their body characteristics and morphometrics were observed (Table 1). The length of the fish body was measured by utilizing calipers, while the body weight of the fish was measured by using analytical scales. The morphometric characters observed including Total Length (TL), Standard Long (SL), Head Long (HL), Head Depth (HD), Body Depth (BD), Caudal Peduncle Depth (CPD), Eye Diameter (ED), Eyes Interval (IE), Body Width (BW), Pre Pelvic Length (PPL), Pre Ventral Length (PVL), Pre Anal Length (PAL), Dorsal Basic Length (DBL), Ventral Basic Length (VBL), Pectoral Basic Length (PBL), Upper Caudal Length (UCL), Middle Caudal Length (MCL), Lower Caudal Length (LCL), Eyes Interval to Gill Cover (EIGC), and Anal Basic Length (ABL) (Figure 1).

2.2.2 Molecular Analysis

Regarding molecular analysis, the fish that had been morphologically analyzed were taken from the caudal fin (muscle tissue) using surgical scissors. Then the tissue was inserted into a sample bottle containing 96% ethanol solution. For one specimen of molecular analysis, 25 mg of caudal fin's muscle tissue was isolated using the gSYNCTM DNA Extraction Kit. The mitochondrial cytochrome oxidase subunit I (COI) gene was directly amplified by PCR using a primer designed by Ward et al. (2005): FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3'); FishR1 (5'-TAGACTTCTGGGTGGCCAAGAATCA3').

The amplification reaction was carried out in a total volume of 25 µl containing 12.5 µl of My Taq, 1.25 µl of forward (F1) and reverse (F2), 9 µl ddH₂O, 1 µl of template DNA, respectively. The amplification was processed employing DIAB Mastercycler DNA Thermal Cycler Engine and followed the temperature profile: the initial stage was predenaturation at a temperature of 94°C for 30 seconds; followed by 38 cycles of each stage of denaturation (94°C, 30 seconds), annealing (50°C, 1 minute) and extension (72°C, 1 minute); and a final extension at 72 °C for 7 minutes. PCR samples were filtered to obtain PCR results at 1% agarose gel. Furthermore, PCR results were visualized by electrophoresis in 1% agarose gel (120°C, 20 minutes). The agarose gel was stained using GelRed™ (Biotium®) and observed under a UV transilluminator. The amplification results were then sent to the 1st BASE® in Singapore for direct sequencing analysis.

2.3 Data Analysis

2.3.1 Morphological analysis

Fish samples were documented using an iPhone 6s+ camera, and analyzed using the ImageJ application. Morphological identification by matching it with the *Market Fishes of Indonesia* identification book [White et al. \(2013\)](#), and [FAO \(1974\)](#). Morphological visualization was digitized using CorelDraw X7 software. The result calculation of the morphometric analysis was calculated using Ms. Excel 2010.

2.3.2 Molecular Analysis

The DNA sequences obtained were processed using the MEGA 6.0 application. The results acquired were then identified by equating the data available on GenBank at NCBI using the BLAST (Basic Local Alignment Search Tool) (<https://www.ncbi.nlm.nih.gov>), and compared with the reference sequence.

3. Results and Discussion

3.1 Morphology of Yellowstripe Scad

Table 1. Data of morphometric of *Selaroides leptolepis* (n=30)

	Morphometric	Average ± SD (cm)
TL	Total Length	11.76 ± 0.73
SL	Standard Long	9.93 ± 0.76
HL	Head Long	2.26 ± 0.33
HD	Head Depth	2.28 ± 0.21
BD	Body Depth	3.26 ± 0.16
CPD	Caudal Penduncle Depth	0.41 ± 0.08
ED	Eye Diameter	0.82 ± 0.22
EI	Eye Interval	1.02 ± 0.11
BW	Body Width	1.05 ± 0.12
PPL	Pre Pelvic Length	3.58 ± 0.55
PVL	Pre Ventral Length	3.24 ± 0.36
PAL	Pre Anal Length	5.70 ± 0.48
DBL	Dorsal Basic Length	2.76 ± 0.54
VBL	Ventral Basic Length	1.46 ± 0.49
PBL	Pectoral Basic Length	2.57 ± 0.43
UCL	Upper Caudal Length	2.18 ± 0.23
MCL	Middle Caudal Length	1.12 ± 0.26
LCL	Lower Caudal Length	2.12 ± 0.25
EIGC	Eyes Interval to Gill Cover	1.12 ± 0.29
ABL	Anal Basic Length	1.89 ± 0.44
BW	Body Weight	21.23 ± 3.04*

*) measured in gram

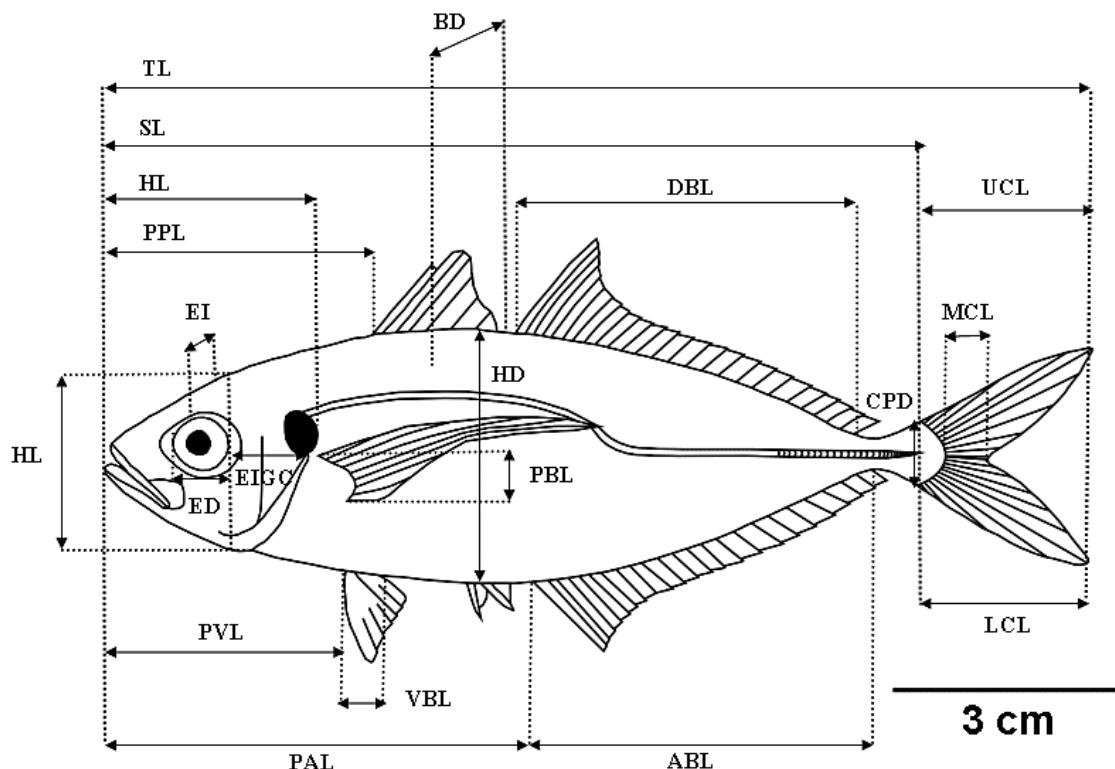


Figure 1. Morphometric of *Selaroides leptolepis*

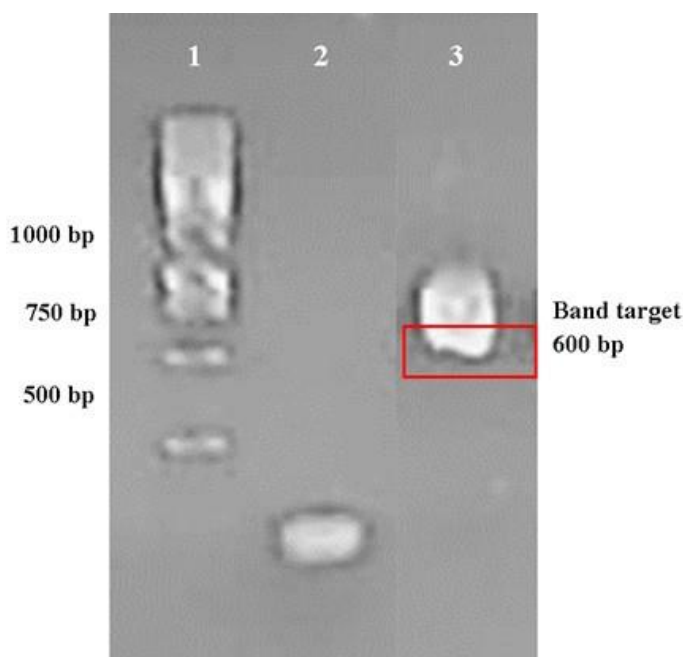


Figure 1. Electrophoresis results (1) DNA ladder; (2) negative control; (3) target sample

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AGTAATTTGGTGCTTGAGCCGGAATAGTGGGA C-
AGCTTTAAGCTTACTCATCCGAGCAGAACTAAGCC
AACCTGGCGCCCTTCTAGGGGACGAT CAG ATTTA-
TAACGTA ATCGTTACGGCCCAC GCCTT-TGTA ATA
AT CTTCTTTATAGTA AT GCCAATCATGATT-
GGTGGCTTCGGAAACTGACTCATCCCCCTAATGAT
CGGAGCCCCCGACATGGCATTCCCCCGAATGAACA
ATATGAGCTTCTGACTCCTTCCTCCTTCTTTCTTCT
TCTTCTGGCCTCTTCAGGGGTCTGAAGCTGGAGCAG
GAACTGGGTGAACAGTCTACCCCCACTAGCCGGC
AACCTGGCCCATGCTGGAGCATCCGTAGACTTAAC
C AT CTTCTCCCTTCACTTAGCAGGTGTT-TC ATCC
ATTCTAGGGGCC ATTA ACTTTATTACC-ACA
ATTATCAAC ATGAAAC CCCCCGCAGTTTCA AT
GTATCAA ATCCCACTATTCGTATGGGCCGTA-CTA
ATTACGGCTGTTCTTCTCCTACTTTCACTTCCCCTC
CTTGCAGCCGGG ATTACA ATGCTTCTCAC-AG
ATCGAAATCTAAACACTGCCTTCTTCGACCCGGCA
GGGGGTGGAGACCCA ATCCTTTAC CA A CA
CCTCTTCTG ATTCTTTG G CCA CCAGAAAAGTC
TAA
    
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Figure 2. The sequence of the COI gene sequence for the type *Selaroides leptolepis* from the Muara Baru Modern Fish Market, North Jakarta

Table 2. BLAST of base nucleotide from GenBank

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<i>S. leptolepis</i> voucher DBMF-M77 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1188	1188	95%	0.0	99.85%	HQ561005.1
<i>S. leptolepis</i> isolate FSCS085-06 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1182	1182	95%	0.0	99.84%	EF607546.1
<i>S. leptolepis</i> voucher DBMF-M294 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1177	1177	94%	0.0	99.84%	JX261019.1
<i>S. leptolepis</i> isolate FSCS179-06 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1177	1177	95%	0.0	99.69%	EF607549.1
<i>S. leptolepis</i> isolate SL4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1175	1175	94%	0.0	99.84%	KJ502083.1
<i>S. leptolepis</i> voucher CEW0049 cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1173	1173	98%	0.0	98.64%	KU179063.1
<i>S. leptolepis</i> voucher DBMF-M475 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1173	1173	94%	0.0	99.69%	JX261265.1
<i>S. leptolepis</i> voucher BBWC35 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1171	1171	93%	0.0	100.00%	KY372157.1

Table 3. Comparison of long range of fish in several locations

Location	Total Length (mm)		Source
	Min	Max	
Fish Market of Tanjungpiang, Kepri	145	310	(Febrianti <i>et al.</i> , 2013)
Sunda Strait	75	174	(Sharfina <i>et al.</i> , 2014)
Pemalang Waters	123	130	(Andriani <i>et al.</i> , 2015)
Kei Islands	100	247	(Supeni & Almohdar, 2017)
Fish Market of Muara Baru, Jakarta	106	129	This study

The morphological identification results noted that the species from the samples taken from the Muara Baru Modern Fish Market in North Jakarta is *Selaroides leptolepis*. The results were congruent with the morphological characteristics in the fish identification book by [FAO \(1974\)](#), stating that the morphological characteristics of the *S. leptolepis* were the lateral side of the eye to the caudal fin is golden yellow which is a distinctive characteristic of this species. Besides, the dorsal part is green and blue, the ventral remains are silvery, a slightly black spot on the operculum is spotted, and the caudal fin is yellow. In addition, according to ([White et al., 2013](#)), the fish have no teeth in the upper jaw, the rim (under the operculum) is smooth without papilla, the yellow line extends laterally, and it lives in a group at a depth of 0-50 meters in the soft substrates. It is distributed throughout the Indo-West Pacific waters.

The morphological characteristics of *S. leptolepis* in the form of length signified the differences in size from previous studies. The fish length size on [Table 3](#) revealed that *S. leptolepis* caught in the Sunda Strait had the smallest size by 75 mm, and the largest size by 310 mm found in the fish auction house of Tanjungpinang village unit cooperative. In this study, the range of the fish length size exposed that the size of the lengths between the minimum, and maximum fish were not overly different. The range of fish size could be said to be close to the homogeneous size. In other words, the fish size caught was close to the uniform length. Internal and external factors could attribute the difference in length size of *S. leptolepis*. Internal factors include heredity, sex, age, and disease, while external factors include food availability and water quality ([Andriani et al., 2015](#)). Additionally, the use of fishing gear that was not environmentally friendly as well as high exploitation pressures, such as fishing dominated by immature gonad fish or spawning, caused the differences in the fish length. Furthermore, the growth overfishing affected the recruitment process in the population ([Agustina et al., 2015](#)).

3.2 Molecular Analysis

The sequencing results obtained indicated a DNA length by 675bp ([Figure 3](#)), and the nucleotide composition of the *S. leptolepis* samples reached 187 or 27.70% Thymine, 197 (29.19%) Cytosine, 161 or 23.85% Adenine, and 130 or 19.26% Guanine. The value of the cytosine nucleotide base was the highest, amounting to 29.19%. The number of base pairing is in accordance with the COI primary target, which was >600bp. In previous research by [Jaafar et al. \(2019\)](#), the number of base pairing in *S. leptolepis* from the sequencing results was 650bp.

Although the number of base pairing in this study was different from previous studies, the fish species identified remained the same. According to [Madduppa \(2014\)](#), the different/varying lengths of base pairing were inflicted by the different number of samples.

Based on DNA barcoding analysis employing the COI gene in *Selaroides* sp., after being compared with the NCBI GenBank database, it is obtained the highest similarity percentage with the *S. leptolepis* species with the same Max score and total score of 1,188, Query cover 95%, Evaluate 0.0, and Per. Ident 99.85%. According to [Triandiza and Madduppa \(2018\)](#), the Max score, and total score with the same value, Query cover nearly reached 100%, Evaluate was close to 0, and Per. Ident approached 100% in each database, denoting the most similar GenBank sequences ([Table 2](#)).

The results of the molecular analysis suggested that the identified fish species was *S. leptolepis*. The results were in accordance with the morphological analysis conducted. Therefore, this species was genetically confirmed taxonomically, and supported morphological identification.

4. Conclusion

The results of morphological and genetic analysis employing DNA barcoding reveal the same species, namely *S. leptolepis*. Molecular test results utilizing COI (mitochondrial cytochrome oxidase subunit I) indicate similarity in species (Per. Ident 99.85%).

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