

Molecular approach for identification of Asian seabass (*Lates calcarifer* Bloch 1790) based on COI gene sequence from Java, Indonesia

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Abstract. Asian seabass or barramundi in Australia, valid as *Lates calcarifer* (Bloch 1790) is one of the most important food fishes found in tropical and semi-tropical regions of the Indo-West Pacific. This species has been utilized in aquaculture for the past several decades in many countries including Indonesia. In order to support the species success in aquaculture, it is necessary to conduct some research, one of which is species identification for the initial selection of parent candidates by the molecular approach based on the COI gene sequence using the DNA Barcoding method. The two analyzed specimens of Asian seabass from Java, Indonesia were identical to the *Lates calcarifer* from Australia, with a matching percentage of about 99.83-100% and with 0.000–0.007 genetic distance.

Key Words: barcoding, mitochondrial DNA, phylogenetic, catadromous.

Introduction. The online Catalog of Fishes (Eschmeyer 2020) published eleven valid species of the *Lates* genus. Two species are the catadromous species *Lates calcarifer* (Bloch 1790) and *Lates japonicus* (Katayama & Taki 1984) from Japan (Otero 2004), seven species are freshwater species restricted to the African Rift lakes such as *Lates niloticus* (Seegers et al 2003; Neuman et al 2016), *Lates macrophthalmus* (Worthington 1929), *Lates longispinis* (Worthington 1932), *Lates mariae* (Steindachner 1909; Kullander & Roberts 2012), *Lates microlepis* (Boulenger 1898), *Lates stappersii* (Boulenger 1914), and two recently classified species *Lates lakdiva* (Pethiyagoda & Gill 2012) in freshwater and brackish water of Sri Lanka and *Lates uwisara* (Pethiyagoda & Gill 2012) in freshwater and brackish water of Myanmar.

Asian seabass or barramundi in Australia valid as *Lates calcarifer* (Bloch 1790), with two synonyms, *L. darwiniensis* (Macleay 1878) from the Australasian region and *Pseudolates cavifrons* (Alleyne & Macleay 1877) from the Torres Strait or in the Gulf of New Guinea (Ward et al 2008) is one of the most important food fishes in Asian countries bordering the Indian Ocean and tropical Australasia (Pender & Griffin 1996). This species has been raised in aquaculture systems for the past several decades (Pethiyagoda & Gill 2012) in many countries including Indonesia, because of the species high economic value. *L. calcarifer* is widely distributed, with the range covering the following regions: the westwards Queensland coast of Australia (Ward et al 2008; Pethiyagoda & Gill 2012; Larson et al 2013; Pusey et al 2017), Indo-West Pacific such as South Sulawesi Indonesia (Iwatsuki et al 2000), New Guinea (Allen et al 2008), Malaysia (Mohsin and Ambak 1996), Myanmar and Sri Lanka (Pethiyagoda & Gill 2012), South China Sea (Randall & Lim 2000), Taiwan (Campbell et al 2019), Hongkong (Sadovy & Cornish 2000), South Pakistan (Psomadakis et al 2015), Bangladesh (Ataur 1989), India (Mirsha et al 1999;

Bijukumar & Sushama 2000), Arabian Sea (Manilo & Bogorodsky 2003), to the Persian Gulf east (Eagderi et al 2019).

In order to support the success of *Lates calcarifer* aquaculture, it is necessary to conduct some research, one of which is species identification for the initial selection of parent candidates. Identification is carried out by the molecular approach based on the COI gene sequence using the DNA Barcoding method (Hebert et al 2003). Region of the mitochondrial cytochrome c oxidase I gene (COI or coxI) is successful to characterize and identify Australian fishes including *L. calcarifer* (Ward et al 2005; Ward et al 2008), which is capable of producing base lengths of up to 650 base pairs (bp).

Material and Method

The fish sampling and description of the study sites. Specimens were obtained from the Java Sea, Indonesia on 20 February 2020. Two specimens are consisting of one male and one female. The dorsal fin of the specimens was preserved in 96% alcohol solution (Hasan & Tamam 2019) for molecular identification.

DNA extraction, isolation and amplification. The sample extraction of DNA was using the Genomic QIAgen DNeasy Blood & Tissue Kit. The mitochondrial cytochrome oxidase I (COI) gene was amplified by polymerase chain reaction (PCR) following the BIONESIA protocols with the Fish_F1 and Fish_R1 primers (Ward et al 2005). The amplification parameters were an initial denaturation of 94°C for 3 min, 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds, with a final extension of 72°C for 2 min. The PCR results were visualized in 1% agarose gel by staining Nucleic Acid Gel Stain (GelRed®) via electrophoresis.

Data analysis. Species were identified to the NCBI (National Center for Biotechnology Information) to analyze a sequence homology of the species via BLASTn (Basic Local Alignment Search Tool-nucleotide) method. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987) with the bootstrap test with 10000 replicates (Felsenstein 1985). The evolutionary distances were computed using the p-distance method (Nei & Kumar 2000). Evolutionary analyses were conducted using MEGA6 software (Tamura et al 2013).

Results. The average base pairs of the Asian seabass from Java sea, Indonesia using Fish_F1 and Fish_R1 primers (Ward et al 2005) were 680 bp (Table 1). According to Ward et al (2008), Asian seabass (*Lates calcarifer*) from Queensland Australia, Myanmar and Tanzania had 651 bp aligned across all specimens and sequences have been deposited in the Barcode of Life Database (www.barcodinglife.org) (2008) and GenBank (www.ncbi.nlm.nih.gov/Genbank). The two specimens of Asian seabass from Java had an identical COI barcode sequence, and this was very similar to about 99.83-100% to the *Lates calcarifer* from Australia (KU496218.1) (Table 2). According to Hebert et al (2003), species with 99-100% similarity level are identical and fragments that have more than 658 base pairs COI genes can be used as a standard for differentiating between animals.

Table 1
Sequences result of Asian seabass *Lates calcarifer* in Java

Sample	Sequences result
Asian seabass <i>Lates calcarifer</i> (Female) 680bp	CCTTTNCAGTATTTGGTGCTTGAGCCGGAATAGTGGGCACCGCTCTTAGCTTGCTCATCCGAGC AGAACTTAGTCAGCCCGGCTCACTCCTGGGGGATGACCAAATCTACAACGTAATCGTCACCGCC CACGCTTTCGTGATAATTTTCTTTATAGTAATGCCAATCATGATCGGAGGCTTCGGAAACTGAC TAGTCCCTTAATAATTGGAGCACCAGACATGGCATTTCCTCCCGAATGAACAATATAAGCTTCTG ACTTCTCCCCCTTCTTCTCCTTCTCCTGGCTTCTTCTGGGGTAGAGGCTGGAGCTGGGACC GGTTGGACTGTATACCCTCCCTTGCTAGCAACCTCGCTCACGCTGGAGCATCCGTCGATCTGA CCATCTTTTCTCTCCACTTAGCGGGTGTCTCCTCAATTCTTGGAGCCATTAACCTTTATCACGAC CATTATTAACATAAAAACCGCTACCGTCTCCATATATCAAATCCCCCTATTCGTCTGAGCAGTC TTGATTACAGCCGTTCTTCTTCTACTCTCCCTCCAGTCCTAGCCGCCGGTATCACCATGCTTC

Asian seabass
Lates calcarifer
(male)
682bp

TTACAGATCGTAACCTAAACACAGCATTCTTCGATCCTGCCGGAGGGGAGACCCGATTCTCTA
TCAACATCTGTTCTGATTCTTTGGCCACCAGAAAGTCTAA
CCCTTTNCAGTATTTGGTGCTTGAGCCGGAATAGTGGGCACCGCTCTTAGCTTGCTCATCCGAG
CAGAACTTAGTCAGCCCGGCTCACTCCTGGGGGATGACCAAATCTATAACGTAATCGTCACCGC
CCACGCTTTCGTGATAATTTCTTTATAGTAATGCCAATCATGATCGGAGGCTTCGGAAACTGA
CTAGTCCCCTTAATAATTGGAGCACCAGACATGGCATTTCCTCGAATGAACAATATAAGCTTCT
GACTTCTCCCCCTTCTTCTCCTTCTCCTGGCTTCTTCTGGGGTAGAGGCTGGAGCTGGGAC
CGGTTGGACTGTATACCCTCCCCTTGCTAGCAACCTCGCTCACGCTGGAGCATCCGTCGATCTG
ACCATCTTTTCTCTCCACTTAGCGGGTGTCTCCTCAATTCTTGGAGCCATTAACCTTTATCACGA
CCATTATTAACATAAAAACCCGCTACCGTCTCCATATATCAAATCCCCCTATTCGCTGAGCAGT
CTTGATTACAGCCGTCCTTCTTCTACTCTCCTTCCCAGTCCCTAGCCGCCGGTATCACCATGCTT
CTTACAGATCGTAACCTAAACACAGCATTCTTCGATCCTGCCGGAGGGGAGACCCGATTCTCT
ATCAACATCTGTTCTGATTCTTTGGCCACCAGAAANNNTCTAA

Table 2
Species identification and similarity of Asian seabass *Lates calcarifer* in Java

Sample	Similarity Gene bank	Species study and identified	Common name	Sub-Phylum	Class	Order	Family
Asian seabass (Female)	100%	<i>Lates calcarifer</i>	Seabass	Vertebrata	Pisces	Percomorphi	Centropomidae
Asian seabass (male)	99.83%	<i>Lates calcarifer</i>	Seabass	Vertebrata	Pisces	Percomorphi	Centropomidae

The *Lates* genus evolutionary analyses were conducted using MEGA6 software (Tamura et al 2013), and evolutionary history by Neighbor-Joining method based on Saitou & Nei (1987) with the bootstrap test with 10000 replicates (Felsenstein 1985) and evolutionary distances with the p-distance method (Nei & Kumar 2000). The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 575 positions in the final dataset. *Cheilinus undulatus* serves as an outgroup for the tree. Asian seabass from the Java Sea, Indonesia is in a group with *Lates calcarifer*, which means Asian seabass from the Java Sea is a species of *Lates calcarifer* (Figure 1).

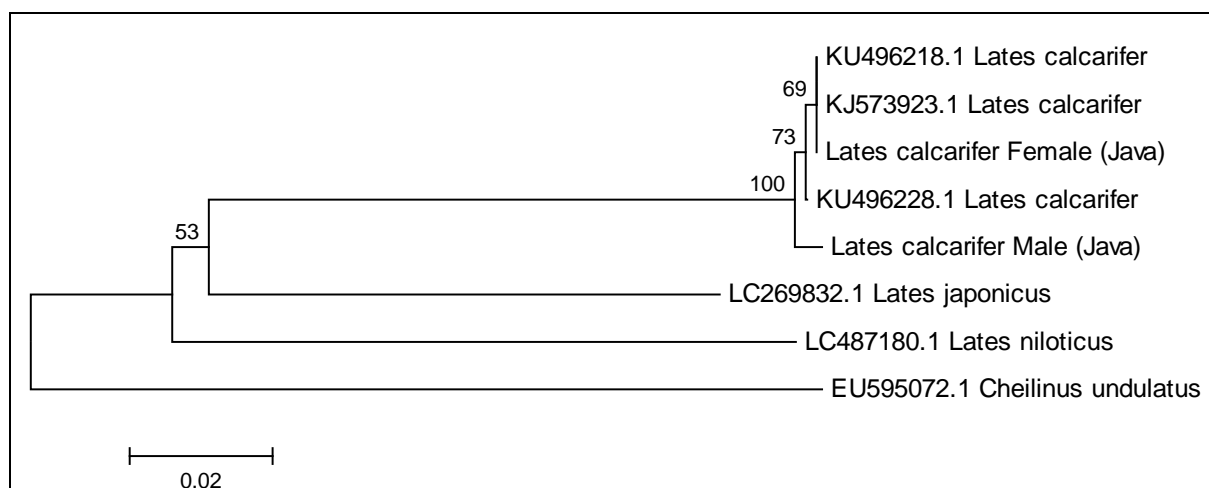


Figure 1. Phylogenetic tree of the Asian seabass *Lates calcarifer* based on COI Gene.

The number of base differences per site between the male and the female of *Lates calcarifer* from the Java Sea, Indonesia and other species of the *Lates* genus were conducted in MEGA6 software (Tamura et al 2013). The male and the female of *Lates calcarifer* from Java, Indonesia had 0.007 genetic distance (Table 3) with four (4) mutations (Table 4) from a total of 575 positions in the final dataset. The female of *Lates*

calcarifer from Java had 100% similar to the *Lates calcarifer* from Australia (KJ573923.1) with 0.000 genetic distance, which means there are no mutations and different bases in the two species. The male of *Lates calcarifer* from Java had 99.83% similar to the *Lates calcarifer* from Australia (KJ573923.1) with 0.007 genetic distance and four (4) mutations among them.

Table 3

Estimates of evolutionary divergence between sequences

	1	2	3	4	5	6	7
1 <i>Lates calcarifer</i> Female (Java)							
2 <i>Lates calcarifer</i> Male (Java)	0.007						
3 KU496218.1 <i>Lates calcarifer</i>	0.000	0.007					
4 KJ573923.1 <i>Lates calcarifer</i>	0.000	0.007	0.000				
5 KU496228.1 <i>Lates calcarifer</i>	0.002	0.005	0.002	0.002			
6 LC487180.1 <i>Lates niloticus</i>	0.179	0.176	0.179	0.179	0.177		
7 LC269832.1 <i>Lates japonicus</i>	0.157	0.157	0.157	0.157	0.155	0.158	

Table 4

Polymorphic sites of Asian seabass *Lates calcarifer* in Java

Sample	Haplotype	Nucleotide Position			
		75	198	429	508
Asian seabass <i>Lates calcarifer</i> (Female)	1	C	C	T	C
Asian seabass <i>Lates calcarifer</i> (Male)	2	T	T	C	T

There are three parts of DNA monomers, namely pentose sugar, nitrogen bases (Adenine, Thymine, Guanine, Cytosine) and phosphate groups. The average nucleotide composition or nitrogen bases of the *Lates calcarifer* in the mitochondrial cytochrome C oxidase subunit I (COI) gene from Java, Indonesia were conducted in MEGA6 software (Tamura et al 2013). The average nitrogen bases of *Lates calcarifer* in Java, Indonesia are Guanine+Cytosine 49.20%, less than Adenine+Thymine which amounts to 50.80% (Table 5).

Table 5

Nucleotide composition of Asian seabass *Lates calcarifer* in Java

Sample	T(U)	C	A	G	Total
Asian sea bass <i>Lates calcarifer</i> Female (sample)	28.7	31.5	21.9	17.9	575.0
Asian sea bass <i>Lates calcarifer</i> Male (sample)	29.0	31.1	21.9	17.9	575.0
Average	28.9	31.3	21.9	17.9	575.0

Conclusions. The two specimens of Asian seabass from the Java Sea, Indonesia had an identical COI barcode sequence, and is very similar to about 99.83-100% to *Lates calcarifer* from Australia with 0.000–0.007 genetic distance. The male and female of the *Lates calcarifer* from the Java Sea, Indonesia had 0.007 genetic distance with four (4) mutations from a total of 575 positions in the final dataset.

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