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Distribution and characteristics of Asian seabass (*Lates calcarifer* Bloch, 1790) in South Sulawesi

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Abstract. The Asian seabass, *Lates calcarifer* (Perciformes, Latidae), known as barramundi in Australia, as *salamata* and *bale kanja* in South Sulawesi, was first described by Bloch in 1790 under the name *Holocentrus calcarifer*. The euryhaline *L. calcarifer* is a high-value marine and freshwater fish, on a par with salmon and tuna. This species has a widespread distribution, but there has been a lack of research on the distribution and identification of the genus *Lates* in Indonesia, including in South Sulawesi. This research aimed to track the distribution of Asia seabass in South Sulawesi (Makassar Strait, Gulf of Bone and Flores Sea) with identification based on DNA barcoding and characterization based on morphological parameters. The results show that Asian seabass can be found from Takalar to Pinrang Regency in the Makassar Strait, along the coast of Bone Bay from Bone to Luwu Regency, and from Bantaeng Regency to Selayar Island. Nucleotide sequences of COI mtDNA representing four Asian seabass populations in the Makassar Strait and Gulf of Bone had 99.12-99.41% identity with *Lates calcarifer* sequences from Singapore, Malaysia and Australia (query cover 99-100%). *L. calcarifer* captured at Cenrana in Bone District had a distinctive silvery-yellowish-white colouring, unlike the silvery-greenish-white observed at the other sites. Body depth (BD) of *L. calcarifer* was 30.10-32.20% SL and on average caudal peduncle depth was 45.65 % BD. There were several morphological differences between *L. calcarifer* in this study and *L. calcarifer* from Thailand: dorsal spine and ray counts, anal ray count, and number of vertebrae.

1. Introduction

The Asian seabass, *Lates calcarifer* Bloch (1790), also known as giant sea perch or barramundi, and in Indonesia as *ikan kakap putih*, is a commercially important fish species found in the tropics and subtropics. The Asian seabass is an important species for both aquaculture and capture fisheries, not only in Southeast Asia and Australia but also in North America and Europe [1-5]. Aside from being a popular food fish, like other seabasses, the Asian seabass is also a target for recreational fisheries/sport fishing.

Asian seabass belong to the Order Perciformes, the largest of the vertebrate orders [6]. In the past, *Lates calcarifer* had several no-longer valid synonyms, through having been assigned to several genera, including *Perca*, *Pseudolates*, *Holocentrus*, *Plectropoma*, and *Plectropomus* [7]. Cuvier & Valenciennes (1828) proposed the genus *Lates* to include several other species, such as the Nile perch



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(*Lates niloticus*) [8]. In South Sulawesi, local names for the Asian seabass depend on size: *pica-pica* (fingerlings), *salamata* (juvenile fish), and *bale cannabis* (adult fish). In Japan, *L. calcarifer* is known as *akame*, which means red eyes, or *Lates japonicus*. The Asian seabass has a wide geographical distribution, from western India, around Sri Lanka to the Bay of Bengal, throughout the Southeast Asian region, to Taiwan, Papua New Guinea and Northern Australia [7-10]. Asian seabass occupy saline and freshwater habitats, including rivers, lakes, estuaries, coastal waters, and lagoons, down to depths of 10-50 meters. The Asian seabass in Southeast Asia and Taiwan form a stock which is separate from Asian seabass in Australia [5]. Asian seabass in India and Myanmar is thought to be a different species from Asian seabass in Southeast Asia and North Australia [8-12].

Asian seabass stocks in Indonesia are poorly managed and under-utilized, even though in 2017, the Indonesian Ministry of Maritime Affairs and Fisheries developed offshore aquaculture of *L. calcarifer* in Sabang, Aceh Province, the Southern Ocean waters off Java Island, and around Karimun Jawa Island in Central Java Province. In Indonesian capture fisheries, Asian seabass are considered as non-target fish (by-catch). Meanwhile, in brackish-water shrimp and milkfish ponds (*tambak*) in Indonesia, juvenile Asian seabass, which have not reached first sexual maturity, are poisoned together with other predatory fishes because they are considered pests for shrimp and milkfish culture.

Although *L. calcarifer* has a wide geographical distribution, maps showing the geographical distribution of Asian seabass stocks in Indonesia are not yet available. Asian seabass stock distribution maps could have many benefits, one of which is would be to provide vital information needed to plan management and conservation strategies, as well as to provide information on genetic diversity and suitable sources of broodstock for Asian seabass breeding activities in Indonesia. Based on these considerations, this research was conducted in South Sulawesi coastal waters, within the Wallacea region, with the following aims: (1) to map Asian seabass population spots; (2) to identify Asian seabass with barcoding methods; and (3) to characterize the morphology of Asian seabass in the study area.

2. Materials and methods

2.1. Asian seabass distribution

The methods used to track Asian seabass were based on the characteristics of the Asian seabass as a catadromous migratory predator that uses brackish-water ponds as a feeding ground at some stages in their life cycle. Therefore, the tracking and collection of Asian seabass for the distribution database used Google Map to obtain information on the coordinates of ponds (*tambak*) and estuaries. Information from Google Map was then used to sample Asian seabass from ponds, estuaries, and coastal areas by using fishing gears, including gill nets, stationary fish traps (*sero*), handlines, and set nets. Asian seabass tracking was also conducted through direct interviews with fishermen and recreational anglers who catch Asian seabass. Because the number of fish caught was not large, all fish caught were taken for analysis. The distribution of Asian seabass in the waters of the coast of South Sulawesi was presented in the form of a map.

2.2. Asian seabass identification

Asian seabass from each population were identified through a DNA barcoding method [11-14], using the primer pair FishF2 and FishR2 which targets a segment of the COX1 gene about 650 bp long [15]. Chromatograms produced from Sanger sequencing were edited using GeneStudio™ Professional (GeneStudio, Inc., USA). The Asian seabass forward and reverse nucleotide sequences for each specimen were aligned with ClustalW, combined and trimmed in MEGA X 10.1 [16]. The sequences obtained were then aligned with nucleotide sequences of *Lates calcarifer* deposited in the National Centre for Biotechnology Information (NCBI) GenBank database using the Nucleotide BLAST program. Specimen species identity was determined based on the values of the parameters % identity and % query cover.

2.3. Morphological Characterisation of Asian seabass

Asian seabass morphological characterization employed morphometric and meristic methods [14-18]. The morphometric characters measured and analyzed were body depth, pre-anal length, distance from the base of the ventral fin to the base of the anal fin, length of the base of the dorsal fin, as well as the length and width of the caudal peduncle. Meristic characters counted and analyzed included dorsal fin spines and rays, anal fin spines and rays, pectoral fin spines and rays, pelvic fin spines and rays, caudal fin rays, and a number of gill rakers. Another morphological character that was analyzed was the position of the pectoral fin relative to the ventral fin.

2.4. Data Analysis

Data on the morphological characteristics of *L. calcarifer* was analyzed descriptively and displayed graphically. The body depth range for *L. calcarifer* individuals within each population was presented as a boxplot produced in SPSS ver.16.

3. Results and discussion

3.1. Asian seabass distribution

The results of Asian seabass tracking in the waters of South Sulawesi show that in the Makassar Strait, the Asian seabass population was spread latitudinally between 3°38'-5°34'S longitude and longitudinally between 119°16'-119°27'E; in the Gulf of Bone the distribution was 3°14'-5°01'S and 120°18'-120°30'E; and in the Flores Sea, the distribution was 5°35'-6°05'S and 119°55'-120°27'E (Figure 1). Asian seabass specimens collected in this study came from shrimp and milkfish ponds, lakes connected to the coast, estuaries, rivers, coastal waters, and seagrass meadows. Asian seabass not only has a wide geographical distribution [10], it also uses a wide variety of habitats, including seas, estuaries and coastal waters, as well as rivers, lakes and billabongs [19-20].

The Asian seabass is classified as a protandrous hermaphrodite, that is, a fish that undergoes sex change (sex reversal) where the fish first matures as a male and later transforms into the female phase [21,22,23]. The total length (TL) and weight ranges of Asian seabass caught in ponds were 171-381 mm and 0.06-0.87 kg, respectively. Asian seabass caught on the coast with a set net fishing gear (locally called *belle*) measured between 255-510 mm TL and 0.20-1.98 kg in weight. All Asian seabass caught in ponds and coastal waters were male, with gonad maturity stage (GMS) of I or II. These results are similar to those of Asian seabass studies in Western Papua where 99.92% (out of 1202 Asian seabass samples) with TL below 730 mm (about 5 years old) were male with GMS I-II, and only 0.08 % or one fish (420 mm TL) was female (about 2 years old) [24].

In this study, Asian seabass caught in seagrass beds at depths of 2-4 meters were caught with gill nets or with hook and line fishing gear. The fish were caught during the breeding season (full moon and new moon). The fish caught weighed 19.40-26.40 kg and were mostly females with GMS III and IV. Studies on Asian seabass migrating at four locations in the South China Sea (Thailand, Malaysia, Singapore, and Indonesia) showed that at the age of 3-4 years the female: male ratio of Asian seabass migrating at the locations was female-biased (F:J = 1.36) [7]. In contrast, research in the Fly River, Papua New Guinea, found that Asian seabass first maturity as male fish occurred around the age of 3-4 years [25]. These findings indicate that Asian seabass in the South China Sea and the waters of South Sulawesi experience sex reversal earlier than Asian seabass in the Fly River in Papua New Guinea.

Asian seabasses are catadromous fish, growing in freshwater habitat and migrating to saline waters for gonad maturation, breeding and spawning. Asian seabass eggs are pelagic and hatch at around 24 hours old. Once the larvae have hatched, they inhabit mangroves and coastal areas up to 400 meters from the shore. At around one year of age, the juveniles will then migrate into brackish waters and then into rivers, to grow and develop there for 3 to 4 years [20,26].



Figure 1. Distribution of Asian seabass, *Lates calcarifer* Bloch, 1790 in South Sulawesi waters

Information on the distribution and presence of Asian seabass populations, as described above, is very important for managing and conserving Asian seabass populations in their natural environment. In addition, the distribution map will also be very useful for sourcing broodstock for Asian seabass aquaculture, which is currently starting to develop in Indonesia, including in South Sulawesi.

3.2. Identification

The alignment of 683 bp COX1 gene nucleotide sequences of Asian seabass caught in the coastal waters of Takalar, Maros, Bone and Wajo Regencies shows that the Asian seabass in these waters are *Lates calcarifer*. COX1 nucleotide sequences of Asian seabass gene from coastal waters of Takalar and Maros in the Makassar Strait and coastal waters of Bone and Wajo Regencies in the Gulf of Bone had a high homology (> 99%) with COX1 L nucleotides calcarifer from Singapore, Malaysia and Australia, with query cover of 99-100% (Table 1). These results indicate that Asian seabass in the waters of the Makassar Strait and the Gulf of Bone are *L. calcarifer*. These results are consonant with the results of research [8], which found that Asian seabass in the waters of Singapore, Malaysia, Thailand, Indonesia and North Australia belong to the same Asian seabass species. In contrast, the results of another study [5] which found that Asian seabass groups from Southeast Asia and Taiwan were in a separate clade from Asian seabass living in Australian coastal waters. The identification of Asian seabass from the Flores Sea is not shown because at the time of writing, the identification process was still ongoing.

Table 1. BLAST alignment results for COX1 gene nucleotide sequences from four Asian seabass specimens, two from the Makassar Strait and two from Bone Bay, with COX1 gene *Lates calcarifer* sequences deposited in the NCBI GeneBank.

Query Cover (%)		Identity (%)	E-value	Accession Number & Country
Makassar Straits				
Ta1-W_ Asian seabass from coastal waters of Takalar Regency	99	99.41	0.0	DQ010541.1 – <i>L. calcarifer</i> from Singapore
	99	99.27	0.0	KY213962.1 – <i>L. calcarifer</i> from Malaysia
	99	99.27	0.0	KR349919.1 – <i>L. calcarifer</i> from Australia
Ma1-W_ Asian seabass from coastal waters of Maros Regency	100	99.26	0.0	DQ010541.1 – <i>L. calcarifer</i> from Singapore
	100	99.12	0.0	KY213962.1 – <i>L. calcarifer</i> from Malaysia
	100	99.12	0.0	KR349919.1 – <i>L. calcarifer</i> from Australia
Gulf of Bone				
Bo2-W_ Asian seabass from coastal waters of Bone Regency	100	99.26	0.0	DQ010541.1 – <i>L. calcarifer</i> from Singapore
	100	99.12	0.0	KY213962.1 – <i>L. calcarifer</i> from Malaysia
	100	99.12	0.0	KR349919.1 – <i>L. calcarifer</i> from Australia
Wa2-W_ Asian seabass from coastal waters of Wajo Regency	100	99.26	0.0	DQ010541.1 – <i>L. calcarifer</i> from Singapore
	100	99.12	0.0	KY213962.1 – <i>L. calcarifer</i> from Malaysia
	100	99.12	0.0	KR349919.1 – <i>L. calcarifer</i> from Australia

3.3. Morphological Characteristics

Lates calcarifer caught in the coastal waters of Makassar Strait and the Gulf of Bone had an elongated body form. The Head profile was concave with protruding mouth and upper jaw extending behind the eye. The body colour was greenish silver, except for *L. calcarifer* from the coastal waters of Cenrana, Bone Regency, which had a yellowish silver body colour. The fins were brownish to black. Dorsal fin spine count varied: D.VI-VIII.I-II with 10-12 soft rays (D.VI-VIII.I-II.10-12). Six to eight dorsal fin spines were separated from the soft portion of the fin and one last spine was fused with the soft rays (Table 2). The dorsal fin character of *L. calcarifer* from South Sulawesi was more varied than in *L. calcarifer* from Phuket, Thailand (D.VII.I.11) and *L. japonicus* from Japan (D.VII-VIII.I.11) [7]. In general, in *L. calcarifer* the 3rd dorsal spine is the longest and the first dorsal spine is the shortest (Figure 2; Table 2).

The anal fin comprised three spines joined to 7-10 rays (A.III.7-10). The third anal fin spine was longer than the second and first (A.III>II>I). The anal fin spine and ray count of Asian seabass in this study were similar to those reported for *L. calcarifer* from Phuket, Thailand and *L. japonicus* from Japan (both A.III.8), however, there was a difference in the relative length of the anal fin spines between

L. calcarifer and *L. japonicus*, where the second spine of *L. japonicus* is longer than the third (A.II>III>I) [7]. The anal fin spine characteristics of *L. calcarifer* in this study were similar to those of *L. lakdiva*, a recently described *Lates* species from Western Sri Lanka, but the second anal spine characteristics differ from *L. uwisara*, a recently described *Lates* species from eastern Myanmar, in which the second anal spine is longer than the third (A.II>III>I). The caudal ray count was 16-20 and the number of vertebrae was 23 (Figure 2).



Figure 2. X-ray showing meristic characters of *Lates calcarifer* Bloch, 1790 from South Sulawesi coastal waters

Table 2. Meristic characters of Asian seabass (*Lates calcarifer* Bloch, 1790) from South Sulawesi coastal waters (Roman numerals represent spines; Arabic numerals represent rays)

Population	Dorsal fin	Anal fin	Pectoral fin	Pelvic fin	Caudal fin	Number of gill-rakers	Scales above lateral line	n
Bone	VII.I.11-12	III.8-9	15-16	I.5-6	17-19	3-4	4-6	9
Siwa-Wajo1	VII.I.12	III.9-10	15-17	I.5	17	4	4	13
Akkotengeng-Wajo2	VI-VII.I-II.10-12	III.7-9	13-18	I.4-6	16-19	4	5-6	40
Takalar	VI-VIII.I.10-12; VIII.12	III.9	15-17	I.5-8	16-18	4	4-5	8
Pinrang	VII.I.12	III.9-10	15-21	I.5-6	16-20	4	4-6	31

The average body depth of *L. calcarifer* in this study ranged from 30.10-32.20% of the standard length (SL) and the average height of the caudal peduncle was around 45.65% of body depth. *L. calcarifer* from the coastal waters of Takalar Regency had the lowest mean body depth (30.10% SL) and the body depth was greatest (32.20% SL) in *L. calcarifer* from the coastal waters of Akkotengengeng Village, Wajo Regency (Wajo2). Six *L. calcarifer* specimens were outliers based on body depth, namely one specimen from Pinrang with a body depth of 25.60% SL, one specimen from the coastal waters of Siwa, Wajo Regency (Wajo1) with a body depth of 41.40% SL and four specimens from Akkotengengeng (Wajo2) with a body depth ranging from 33.20-33.80% SL (Figure 3). The body depth of *L. lakdiva* is reported as 26.6-27.6% SL and the body depth of *L. uwisara* is reported as 28.4-34.5% SL [12].

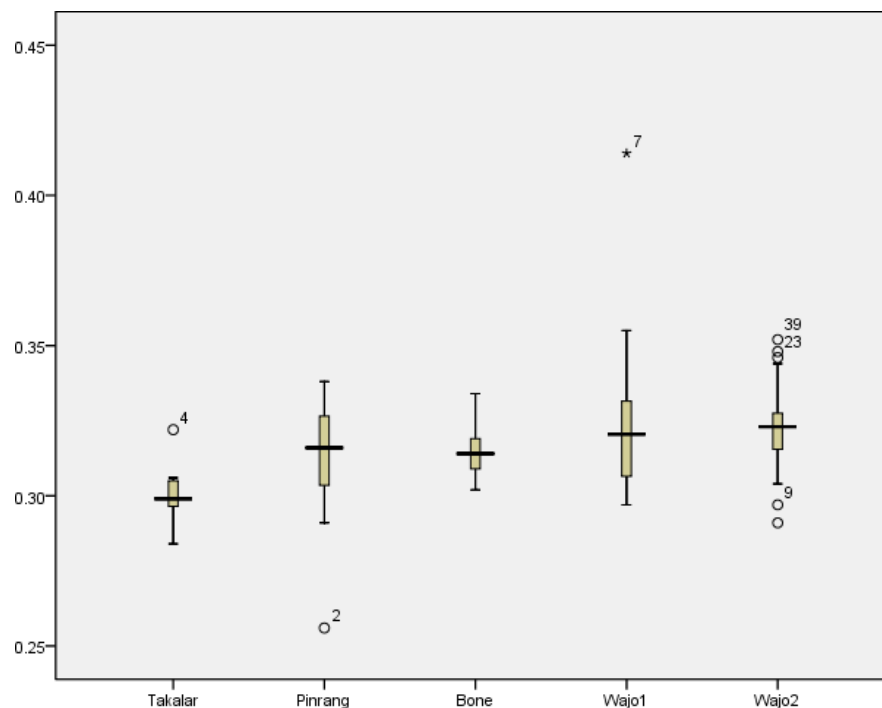


Figure 3. Boxplot of the body depth character of *L. calcarifer* Bloch, 1790 from South Sulawesi coastal waters

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