Marine Research
of the National Academy of Marine Research
ISSN: 2709-6629 (Vol.5 Iss.1)
GPN: 2011200033

DOI: http://dx.doi.org/10.29677/MR.202506_5(1).0005
2025, 5(1), 141-160

DNA Barcoding and Morphology of the Spine Bahaba, *Bahaba polykladiskos* (Actinopterygii, Sciaenidae), from Thailand and Borneo, with Notes on Its Taxonomic Status

Shu-Hui Liu [1] Soranuth Sirisuay [2] Wen-Bin Yeh [1] Shih-Chia Wu [3] Jongkar Grinang [4]
Hin-Kiu Mok [5] Yen-Wei Chang [6*]

[1] *Department of Entomology, National Chung-Hsing University, Taichung, Taiwan*
[2] *Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand*
[3] *Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan*
[4] *Institute of Biodiversity and Environmental Conservation, Universiti Malaysia, Sarawak, Malaysia*
[5] *Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan; National Museum of Marine Biology and Aquarium, Pingtung, Taiwan; Institute of Biodiversity and Environmental Conservation, Universiti Malaysia, Sarawak, Malaysia*
[6] *Seafood Technology Division, Fisheries Research Institute, Ministry of Agriculture, Keelung, Taiwan*

ABSTRACT

In this study, we examined the external morphology of croaker specimens with distinct golden body coloration collected from Thailand's Bang Pakong River, along with one specimen of the spine bahaba, *Bahaba polykladiskos* (Bleeker), from the Sibuti River Estuary in northern Sarawak, Borneo. These specimens differed in coloration from previous descriptions of *Ba. polykladiskos*, including the specimen from Borneo, but shared other morphological characteristics, particularly a longer interocular distance, that closely aligned them with *Ba. polykladiskos*. Phylogenetic analyses and species delimitation based on DNA analysis of the COI gene sequence confirmed that the golden-colored croaker from the Bang Pakong River and *Ba. polykladiskos* specimens from Borneo are conspecific, forming a sister group to the Boeseman croaker, *Boesemania microlepis* (Bleeker). An exceptional laterodorsal enlargement of the swim bladder head in both *Ba. polykladiskos* and *Bo. microlepis* further supports their close phylogenetic affinities. This study also discusses the taxonomic status of *Ba. polykladiskos*, and the genera *Bahaba and Boesemania.*

Keywords: *Boesemania microlepis*, swim bladder, body coloration, sister group, molecular phylogeny.

* Corresponding author, e-mail: ywchang@mail.tfrin.gov.tw

Received December 21, 2024; accepted March 18, 2025.

1 INTRODUCTION

Sciaenids are of considerable importance in coastal and riverine regions because of their high species richness, and many of these species are commercially valuable. Known for their active sound production during the reproductive season, these fish possess a unique sound-producing apparatus. This apparatus consists of a swim bladder and intrinsic or extrinsic sonic muscles, which are attached to the swim bladder and peritoneal wall, respectively. This structure was comprehensively described by Ladich and Fine (2006). The swim bladders of sciaenids exhibit a wide range of morphological variations in shape, number, length, and branching patterns of their swim bladder appendages, also known as diverticula, as documented by Chao (1978, 1986) and Sasaki (2001).

Seven sciaenid species have been identified in the Northern Gulf of Thailand (locally known as the Inner Gulf of Thailand) (Yoshida et al., 2013). Of these, four are confined to coastal and estuarine habitats. However, three species—the prickly croaker, *Aspericorvina jubata* (Bleeker); the spine bahaba, *Bahaba polykladiskos* (Bleeker); and the Boeseman croaker, *Boesemania microlepis* (Bleeker)—are notable for their distribution, extending beyond these habitats and potentially into river systems, including the Bang Pakong River. Geographically, these species occur across various regions, including the Gulf of Thailand, Sumatra, Borneo, Sulawesi, and Malaya for the first species; Vietnam, Cambodia, and Borneo for the second species; and mainland Southeast Asia (Cambodia, Laos, Vietnam, and Thailand) and Sumatra (Indonesia) for the third species (https://en.wikipedia.org/wiki/Boesemania; https://fishbase.mnhn.fr/Summary/SpeciesSummary.php?id=15224&lang=chinese). The holotype of *Ba. polykladiskos* was collected from the Barito River in Banjarmasin, Borneo, Indonesia. The Sarawak waters of Borneo (adjacent to Indonesia) contain at least 11 species (Lim & Gambang, 2009), including *Ba. polykladiskos*.

Local fishermen in the Bang Pakong River have reported observing croakers with an unusual orange to golden coloration, referring to them as "golden croaker." These sightings typically occur during the flood season, specifically between September and November in brackish water regions (estuaries) and from January to October in the lower sections of the river.

This coloration deviates from the typically described gray-to-brown dorsal and whitish-to-yellowish silver ventral hues (Fowler, 1933; Yoshida et al., 2013). Consequently, there is a need for taxonomic identification of these "golden croakers." *Aspericorvina jubata* was excluded from this study because of its distinctive large dark blotch on the opercle, a feature that distinguishes it from other freshwater croakers (Yoshida et al., 2013). This left *Ba. polykladiskos and Bo. microlepis* as the primary candidates for comparison.

Previous descriptions of the morphometric and meristic characteristics of *Bo. microlepis* and *Ba. polykladiskos*, primarily based on the holotype, were provided by Sasaki (2001), Fowler (1933), Wongratana (1985), Trewavas (1977), and Yoshida et al. (2013). However, morphological data for the latter species are limited. In addition, COI gene sequences, which are widely used for species identification, are not available in the GenBank database for either *Ba. polykladiskos* or Chaptis bahaba (*Bahaba chaptis* (Hamilton)), a congeneric relative.

The aim of this study was to determine the taxonomic status of the "golden croaker," defined here as an operational taxonomic unit (OTU) occurring in the Bang Pakong River, Thailand, characterized by a golden body coloration. We compared the meristic and morphometric characters, swim bladder morphology, and COI gene sequences of the "golden croaker" and *Bo. microlepis* from the Bang Pakong River, along with a *Ba. polykladiskos* specimen from Borneo, to infer the species status of the "golden croaker" and elucidate its phylogenetic position.

Marine Research of the National Academy of Marine Research
DOI: 10.29677/MR.202506_5(1).0005

2 RESEARCH METHODS

2.1 Sample Collection and Morphological Analyses

Bo. microlepis and the "golden croaker" were collected from the Bang Pakong River, Bang Pakong District, Chachoengsao Province, Thailand, between 2013 and 2020. A *Ba. polykladiskos* specimen was obtained from a fisherman who caught it in the Sibuti River Estuary, approximately 45 km west of Miri, Sarawak, Malaysia, in April 2023 (Figure 1).

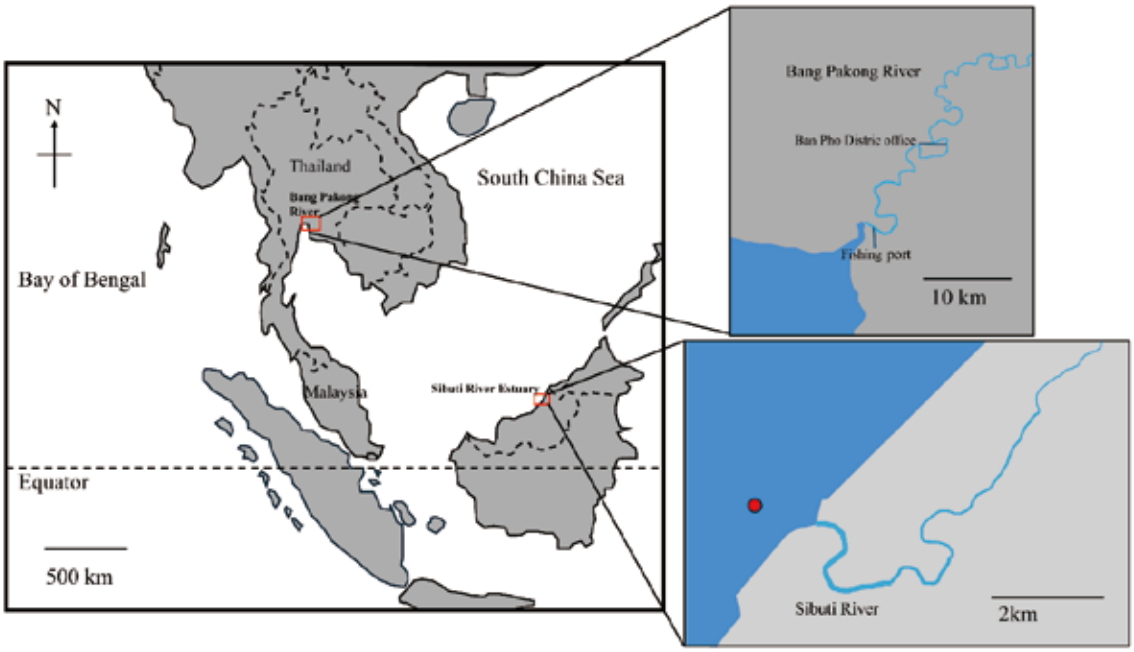

Figure 1. Locality of the study sites in Bang Pakong River, Thailand, and the Sibuti River, Sarawak, Malaysia. Sampling sites included the Ban Pho District Office and the fishing port in the Bang Pakong River (above), and a site in the Sibuti River (below). Red circles indicate sampling locations. Adapted from Map data © 2024 Google (https://www.google.com.tw/maps/).

The "golden croaker" specimens are currently housed in the Kasetsart University Museum of Fisheries (Natural History) [KUMF(N.H.)], cataloged under numbers KUMF 7739 and 7740, and in the National Museum of Marine Biology and Aquarium (NMMBA), cataloged under number NMMB-P040184. The *Ba. polykladiskos* specimens were deposited at the Universiti Malaysia Sarawak Institute of Biodiversity and Environmental Conservation under collection catalog number UNIMAS.P.00447. Two *Ba. polykladiskos* specimens (NMMBA 31398) were collected from the Muar River, Malaysia. Additionally, *Bahaba chaptis* (Hamilton) and the Chinese bahaba, *Bahaba taipingensis* (Herre), were also included in the morphological comparison based on relevant data sourced from the literature.

Muscle tissues were collected from fresh specimens for molecular analysis. Voucher specimens were fixed in 10% formalin and preserved in 70% ethanol.

DOI: 10.29677/MR.202506_5(1).0005

Meristic counts and morphometric measurements of the "golden croaker," *Ba. polykladiskos*, and *Bo. microlepis* were recorded following Trewavas (1977), Wongratana (1985), Sasaki (2001), Yoshida et al. (2013), and Lokamn et al. (2024). The methods used for counts and measurements followed Sasaki and Kailola (1988). Measurements included 16 morphometric characteristics taken from the left side of each individual. All measurements were expressed as percentages of standard length (SL) or head length (HL). X-ray radiography was used to examine the position of the front part of the swim bladder relative to the vertebral column in *Bo. microlepis*, "golden croaker," and *Ba. polykladiskos*.

2.2 DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, and DNA Sequencing for COI Gene

Muscle samples from *Bo. microlepis*, *Ba. polykladiskos*, and the "golden croaker" were preserved in 95% ethanol for molecular phylogenetic analysis. The total genomic DNA from *Bo. microlepis* and the "golden croaker" was extracted using the Epicentre Genomic DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) or the Promega Wizard Genomic DNA Purification Kit following the manufacturers' instructions. *Bahaba polykladiskos*' genomic DNA was extracted using the Maxwell R.S.C. Blood DNA Kit (Promega, USA) as per the protocol provided.

The COI gene was selected as a molecular marker to clarify the taxonomic status and infer the phylogenetic relationships of the species. A closely related group comprising *Bo. microlepis*, *Ba. taipingensis*, and *Ba. polykladiskos* were included in the phylogenetic tree. Three additional species, *Panna microdon*, *Atrobucca nibe*, and *Chrysochir aureus*, were designated as outgroups, based on the phylogenetic framework inferred by Lo et al. (2015, 2017).

PCR was used to amplify the 652 bp barcode region of the COI gene using the universal primer set FishF1/FishR1 for *Ba. polykladiskos* (performed at the National Sun Yat-sen University) and three sets (FishF1/FishR1, FishF2/R1, and FishF1/R2) for *Bo. microlepis* and the "golden croaker" (performed at National Chung-Hsing University) (Ward et al., 2005). The PCR mixtures and conditions, as well as the thermocycling parameters, varied depending on the machines used at different laboratories.

Following PCR, the nucleotide sequences of the amplified DNA fragments were determined using an ABI 3730xl Analyzer (Applied Biosystems, Foster City, CA, USA) at the Sequencing Core Facilities of the National Yang-Ming University Genome Center, Taipei, Taiwan.

The DNA sequences were edited using BioEdit 7.2.5 (Hall, 1999), aligned using default settings, and compared with GenBank-archived sequences via using BLAST. All sequencing data will be deposited in GenBank and made available upon publication.

COI sequences for *Bo. microlepis*, *Ba. taipingensis*, *P. microdon*, *C. aureus*, and *A. nibe* were downloaded from GenBank to identify and verify the taxonomic status of the "golden croaker." Both neighbor-joining and maximum-likelihood analyses were conducted using MEGA v 7.0.20 software (Kumar et al., 2016).

DOI: 10.29677/MR.202506_5(1).0005

Molecular analysis for species delimitation is necessary to determine whether the "golden croaker" is an undescribed species or a morphological variant of another sciaenid. The Automatic Barcode Gap Discovery (ABGD) method, an exploratory tool based on pairwise genetic distances (Puillandre et al., 2012), was used to detect significant intra- and interspecific variation, known as the barcode gap. ABGD analysis was conducted using a web interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the following default settings: a relative gap width (X) of 1.5, an intraspecific divergence (P) value ranging from 0.001 to 0.1, and 50 steps under both available distance metrics and the Kimura two-parameter (K80) model (TS/TV = 2.0). Additionally, putatively closely related taxa of the "golden croaker," identified through BLAST searches, were included in the ABGD analysis to investigate its taxonomic status further.

3 RESULTS AND DISCUSSION

3.1 Molecular Phylogenetic Analysis

3.1.1 BLAST Homology

For the additional sequences used in this study, please refer to Appendix. COI gene sequence analysis revealed that *Ba. polykladiskos* specimens from Sarawak and the "golden croaker" from the Bang Pakong River belong to the same species, exhibiting 99% sequence similarity to three GenBank sequences (LC064031, ON695922, and ON695924) labeled *Bo. microlepis*. Pringgenis and Susilowati (2016) identified a voucher specimen (accession no. LC064301) as *Bo. microlepis* based on only 92% sequence similarity to other *Bo. microlepis* sequences available in the GenBank database. Subsequently, Zhang et al. (2022) identified voucher specimens derived from fish maws (accession nos. ON695922 and ON695924) as *Bo. microlepis*, with 99.69% and 99.53% sequence similarity to LC064301, respectively. However, photographs of the swim bladders from these three specimens (Figure 1B, Pringgenis & Susilowati, 2016; Figures 3A & 3C, Zhang et al., 2022) revealed hammer-shaped swim bladders with no more than three pairs of diverticula and a distinct neck zone. These morphological characteristics suggest that the three voucher specimens were *Ba. polykladiskos*.

3.1.2 Phylogenetic relationship inferring

Analysis of the aligned 652 bp COI gene sequences from the "golden croaker," *Ba. polykladiskos, Bo. microlepis, Ba. taipingensis,* and the outgroups (i.e., *A. nibe, C. aureus, and P. microdon*) revealed six distinct lineages using both neighbor-joining and maximum-likelihood methods (Figure 2). Three of these lineages were designated as outgroups, while the remaining three comprised *Bo. microlepis* from Thailand and Vietnam in Group 1; the "golden croaker," *Ba. polykladiskos*, and *Ba. polykladiskos* (LC064301 from Sumatra, ON695922, and ON695924; see above 3.1.1) in Group 2; and *Ba. taipingensis* in Group 3. The "golden croaker" specimens formed a monophyletic group with *Ba. polykladiskos*, receiving strong support (bootstrap = 100). This analysis supported the classification of the "golden croaker" and *Ba. polykladiskos* as conspecifics. In addition, a sister-group relationship between *Bo. microlepis* and *Ba. polykladiskos* was supported by a high bootstrap value of 96. Therefore, the genus *Bahaba* belongs to the paraphyletic group.

DOI: 10.29677/MR.202506_5(1).0005

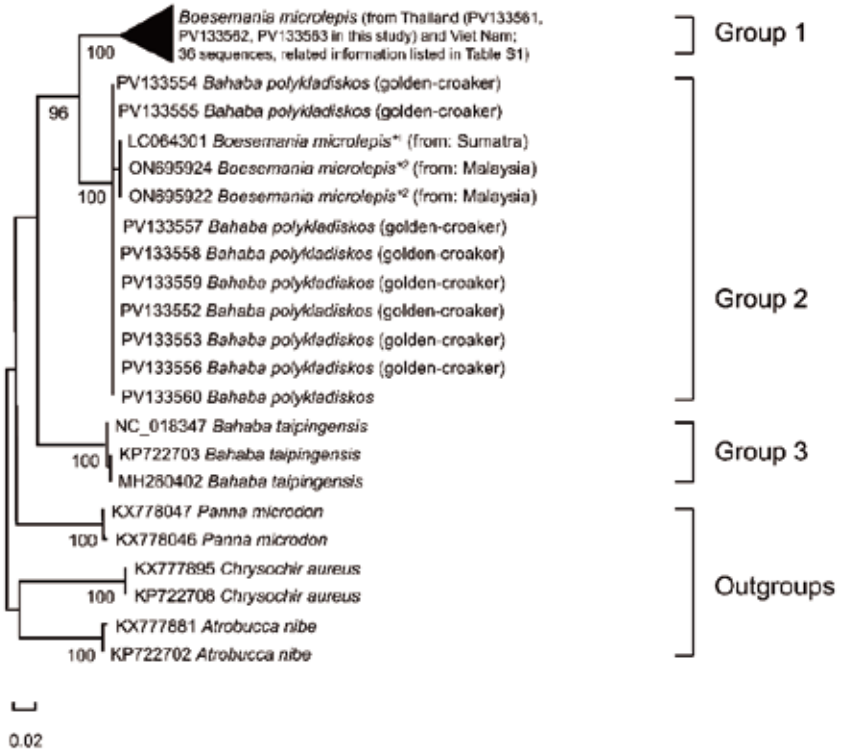

Figure 2. Phylogenetic relationships among *Boesemania, Bahaba*, and the "golden croaker." This figure illustrates the phylogenetic relationship based on neighbor-joining and maximum-likelihood analyses. Three species, i.e., *Panna microdon*, *Atrobucca nibe*, and *Chrysochir aureus*, were selected as outgroups for the phylogenetic tree. Branch lengths correspond to the number of substitutions per nucleotide position (scale bar = 0.02 substitutions). Bootstrap values are represented as percentages at the nodes, with values <90% not displayed. *See Section 3.1.1 BLAST Homology for details about species identification. 1, Pringgenis and Susilowati, 2016; 2, Zhang et al., 2022.

DOI: 10.29677/MR.202506_5(1).0005

3.1.3 Species delimitation

ABGD analysis partitioned the individuals into six groups based on a barcode gap distance of 0.041. The results aligned with the inferences from the neighbor-joining and maximum-likelihood analyses, indicating a barcode gap range of 0.01 to 0.07 (Figure 3). This analysis grouped the "golden croaker" and *Ba. polykladiskos* as conspecifics, thus confirming their close relationship.

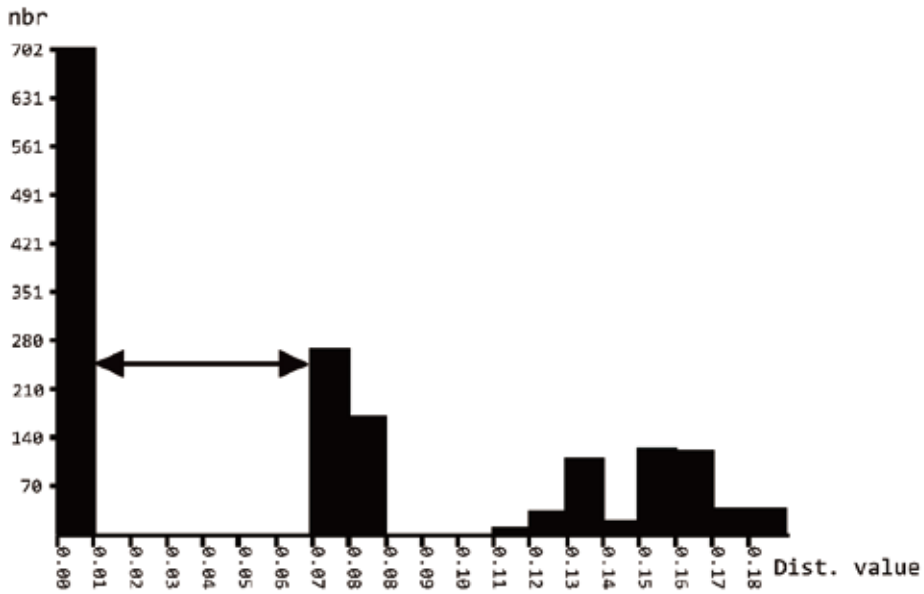

Figure 3. Frequency histogram of K80 pairwise distances in ABGD species delimitation of *Boesemania* and *Bahaba*. The histogram displays the K80 pairwise distances used in the ABGD species delimitation. A double-headed arrow highlights the gap separating intraspecific (left side) from interspecific (right side) distances within the COI region. Notations include nbr (number of branches) and Dist. value (distance value).

3.2 Morphological Description of the "Golden Croakers" from Thailand

Eight "golden croaker" specimens from the Bang Pakong River, deposited at KUMF and NMMBA, were examined. These specimens exhibited a range of morphological characteristics, as listed in Table 1.

DOI: 10.29677/MR.202506_5(1).0005

Table 1. Morphometric and meristic properties. This table provides detailed morphometric and meristic values of the golden croaker *Bahaba polykladiskos*, *Ba. taipingensis*, *Ba. chaptis*, and *Boesemania microlepis*.

	Bahaba polykladiskos						*Bahaba chaptis*[1]	*Bahaba taipingen*	*Boesemania microlepis*	
	Golden croaker (n=8)	**This study (n = 1)[a]**	**This study (n = 2)[b]**	**UMKL 12883[5]**	**RMNH 5983[1]**				**This study (n = 1)**	
Total length (cm)	41.7–48.6	52.4		-	22.6	43.21	-	-	36.4	-
Standard length (cm)	36.6–41.5	48.3	28.1–28.2	34	19.5	38.21	10.5–22.2	13.85–14.90	30	13.1–32.8[2]
Percentages of standard length										
Head length	25.8–29.7	27.1	29.8–31.0	33.5	32	-	-	-	28	29.3–33.0[2]
Body depth	24.4–27.1	27.9	24.8–24.9	28.4	30.8	-	-	-	25.7	24.7–29.3[2]
Body width	12.3–13.9	-	12.4–12.5	18.3	-	-	-	-	10.7	13.0–14.6[2]
Caudal peduncle	6.3–6.9	6.6	6.7–6.8	7	7.1	-	-	-	7.3	5.3–7.4[2]
Caudal peduncle	9.8–11.5	26.9[#]	-	-	11.5	26–28[3#]				
Snout length	5.3–7.1	7.7		-	11.5	-	-	-	6	7.0–9.0[2]
Eye diameter	3.4–3.9	3.1	4.3	4.3	-	-	-	-	4.7	4.8–7.2[2]
Interorbital width	5.7–8.2	7		-	-	-	-	-	4.7	4.7–8.3[2]
Upper jaw length	7.8–12.0	9.5		-	-	-	-	-	8.4	11.4–12.5[2]
Pectoral fin length	21.5–23.1	-	23.1–23.4	23.8	23.4	-	-	-	14	-
Pelvic fin length	16.9–22.9	15.5	25.9–28.8	23.4	-	-	-	-	16.3	-
2nd anal fin spine	15.7–18.4	14.9			18.4	171	12.5–16.0	16.5–16.6	13	12.7–14.8

DOI: 10.29677/MR.202506_5(1).0005

	Bahaba polykladiskos						*Bahaba chaptis*[1]	*Bahaba taipingen*	*Boesemania microlepis*	
	Golden croaker (n=8)	**This study (*n* = 1)[a]**	**This study (*n* = 2)[b]**	**UMKL 12883[5]**	**RMNH 5983[1]**				**This study (*n* = 1)**	
Percentages of head length										
Eye diameter	11.6–14	11.5	13.8–14.3	-	19.1	14.81	22.0–25.5	21.4–24.4	16.7	20–26[1]
Preorbital depth		-	-	-	13.6	13.61	(9.1) 9.6–	12.6–13.4		
Interorbital width	21–28.3	26	23.8–24.1	-	22.6	241	13.7–16.7	16.3–17.1	16.7	17–19.5[1]
Upper jaw length		35.1	-	-	39	36.11	40.0–44.0	42.6–45.7		
Lower jaw length		-	-	-	46.6	45.51	51.0–53.3	51.7–55.0		
Length of snout		28.2	-	-	27.1	-	-	-		
2nd anal fin spine length	54.2–65.4	49.6	63.2–65.5	-	57.5	55–63[3]	-	-	46.4	41.6–49.0 (54.3)[1]; 44–53[3]
Counts										
Dorsal-fin spine and rays	X+I, 26–29	IX+I, 29	X+I, 30	X+I, 28	X+I, 27–29[1]; X+I, 27–30[3, 4]		X+I, 24–26	VII+I, 22–25	X+I, 33	X;I, 1, 28–32[1†]; IX~X(X)+I, 27-34[3]
Anal fin rays	II, 6–7	II, 7	II, 7	II, 7	-	II, 7[3, 4]	-	-	II, 7	II, 7–8(+1)[2]; II, 7[3]
Pectoral fin rays	17–19	17	18	18	-	-	-	-	16	2,16–18[2]
Lateral line scales	48–51	58	50	46+9	49	491	51–52	55–61	50	47–53[2]

DOI: 10.29677/MR.202506_5(1).0005

	Bahaba polykladiskos						*Bahaba chaptis*[1]	*Bahaba taipingen*	*Boesemania microlepis*	
	Golden croaker (n=8)	**This study (*n* = 1)[a]**	**This study (*n* = 2)[b]**	**UMKL 12883[5]**	**RMNH 5983[1]**				**This study (*n* = 1)**	
Scales above	7–9	7	7	7	9	-	-	-	12	7–11[2]
Scales below	11–13	11	7	7	6	-	-	-	19	8–16[2]
Pores on snout	5+3	-	-	-	5+5	5+5[3]	-	-		
Mental pores	6	-	-	-	6	63	-	-		
Gill-rakers	4+1+(5–7)	-	-	-	5+1+7	7–8[3, 4]	-	-		
Vertebrae	11+(12–13)	-	-	-	25(11+14)	-	-	-	-	11+14[1]

Counts and measurements were reported in 1. Trewavas, 1977; 2. Wongratana, 1985 (n = 18); and 3. Sasaki, 2001; 4. Yoshida et al., 2013;5. Lokman et al. 2024.

† It may be a mistake for 'X+I, 28–32'.

#This measurement was performed from the posterior anal end to the final vertebra.

a Sarawak, Malaysia. UNIMAS.P.00447

b Mura, Malaysia. NMMBP 31398

3.2.1 Description

Body moderately elongate and compressed. Snout relatively rounded, with five pores on the tip (upper rostral pores) and three on the lower edge (marginal pores); mental pores present in three pairs.

Mouth terminal; cleft angle when mouth closed ca. 30°. Upper jaw slightly projecting beyond lower jaw; maxillary reaching the level of the anterior of the eye. Upper jaw with an outer row of slightly enlarged conical teeth and an inner band of small conical teeth. Lower jaw with an outer band of small conical teeth and an inner row of slightly enlarged conical teeth.

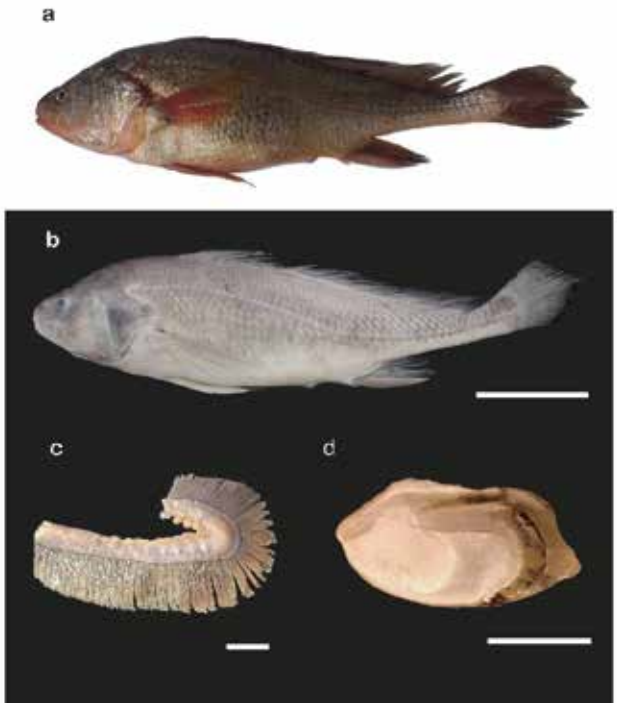

Figure 4. "Golden croaker" from Thailand. This figure presents various aspects of the "golden croaker": (a) fresh specimen; (b) specimen preserved in alcohol (scale bar = 10 cm); (c) gill arch (scale bar = 1 cm); and (d) sagitta (scale bar = 1 cm).

Eye small. Interorbital region broad (21–28.3% HL), convex, and about twice the length of the eye. Anterior nostril rounded and smaller; posterior nostril rounded or ovate. Gill rakers 4+1+(5–7), stiff, >1/2 length of the gill filaments at the junction between the upper and lower limbs of the gill arch. Gill rakers on the epibranchial very short, often club-shaped; the ones on the ceratobranchial mostly finger-shaped, followed by a few denticulated stumps located below the seventh ceratobranchial. Both epibranchial and ceratobranchial bear a row of sclerotic protruding bosses between the bases of the gill rakers and the filaments (Figure 4c).

Dorsal fin X+I, 26–29; anal fin II, 6–7; pelvic fin I, 5; pectoral fin 17–19. Scales cycloid on the snout, cheek, and anterior part of the chest, and ctenoid elsewhere. Lateral-line scales larger, leaving no normal scales between pored lateral-line scales. Lateral-line scales reaching to the tip of the pointed caudal fin, which is densely covered with small scales; lateral-line scales 48–51; scales above lateral line 7–9, below 11–13.

Pectoral fins moderately long, extending backward below the ninth dorsal fin spine; pelvic fin equal to or slightly shorter than pectoral fins. The second anal fin spine is massive, long, and approximately equal to the first soft ray. Caudal fin rhomboid, with a posterior margin pointed.

Sagitta approximately oblong, with raised antero-dorsal and postero-dorsal angles; posterior tip pointed and thick. The "head" of the tadpole-shaped impression relatively small and mushroom-shaped; the "tail" long, deep, and sharply bent, with the distal end close to the ventral edge (Figure 4d).

Swim bladder elongate (Figure 5), with a clearly restricted neck zone at approximately 1/3 the distance from the head end. The portion anterior to this zone slightly tilted upwards with two sub-spherical sacs at the head end, between which the first 3–4 vertebrae are positioned. The posterior tip of the swim bladder acute. A single appendage on the left side (32-cm individual); one to two pairs (the number might be size-dependent, i.e., a 36.6-cm individual with one pair, whereas the 38.9-cm SL individual had two pairs of unbranched tubular appendages) extending caudally from the anterolateral side of the head lobes, ending near the neck zone.

DOI: 10.29677/MR.202506_5(1).0005

Figure 5. Swim bladder of the "golden croaker" from Thailand and *Bahaba polykladiskos* from Borneo. The figure showcases multiple views of the swim bladder of the "golden croaker": (a) dorsal, (b) ventral, (c) lateral, (d) dorsal-lateral, and (f) radiograph in lateral view. It also includes the ventral view of the *Bahaba polykladiskos* from Borneo (e). All views are scaled at 1 cm.

DOI: 10.29677/MR.202506_5(1).0005

Color when fresh. Golden-gray dorsally, paler below; dorsal, anal, and caudal fins with the same color as the body; pectoral fins, pelvic fins, mouth cavity, and gill cover orange (Figure 4a). Color in alcohol. Body background grayish-purple or brownish-cream (Figure 4b).

Distribution in the Bang Pakong River

The "golden croakers" were found in the brackish section of the river, extending up to 20 km from the river mouth (Figure 1). They were observed migrating to coastal areas during flooding in November and December.

3.2.2 Morphological description of a *Bahaba polykladiskos* specimen from the Sibuti River estuary, Borneo

The swim bladder of a single specimen from the Sibuti River Estuary (Figure 1) displayed distinctive morphological features, while other external characteristics were consistent with the descriptions provided by Trewavas (1977) and others (Table 1). Notable features include swim bladder bulbs, a narrow neck region, and an extended interocular distance, with a light yellowish coloration observed in the lower jaw and isthmus region. This species is commonly referred to as Ikan Terusan by fishermen in Sarawak. However, inconsistencies in their descriptions suggest that the local name may refer to multiple large-bodied croaker species or to variations within the spine bahaba, potentially influenced by developmental stage and habitat conditions.

3.2.3 Morphological comparison between the "golden croaker" and *Bahaba polykladiskos* (Table 1)

D X–I, 26–29; A II, 6–7; LGR 5–7 (vs. D X–I, 27–30; A II, 7; LGR 7–8); lateral-line scales 48–51:7–9 (above): 11–13 (below) (vs. 49:9:6); scales cycloid on snout, cheek and anterior part of chest, ctenoid elsewhere (vs. ctenoid except on snout and under eye); number of vertebra 23–24; 11+(12–13) (vs. 25; 11+14); similar in upper-jaw tooth pattern; larger teeth in outer row and smaller teeth in inner row on upper jaw (vs. teeth well differentiated into large and small in both jaws). Interocular distance (21–28.3% HL vs. 22.6–26.0% HL). Color: Golden-gray dorsally, paler below; dorsal, anal, and caudal fins with the same color as the body; pectoral fins, pelvic fins, mouth cavity, and gill cover orange (vs. body gray dorsally, silver ventrally; dorsal, pectoral, pelvic, and anal fins translucent white; caudal fin yellowish-white). This detailed comparison of the "golden croaker" and *Ba. polykladiskos* revealed differences in vertebrae count, tooth patterns, and coloration. Despite these differences, the shared derived character of a broader interocular distance in both the "golden croakers" and *Ba. polykladiskos* supports the notion that they are conspecifics.

DOI: 10.29677/MR.202506_5(1).0005

3.2.4 Morphological comparison among *Bahaba* species and the monophyletic status of the genus *Bahaba* (Table 1)

Unlike other *Bahaba* species, *Ba. polykladiskos* exhibits a wider interocular distance. The swim bladder of *Ba. polykladiskos*, initially described as carrot-shaped, displays distinct profiles when viewed dorsally and laterally (Figure 5). The positioning of the swim bladder appendages varies within the genus *Bahaba*, indicating interspecific inconsistencies in appendage placement relative to the swim bladder and abdominal wall muscles. The monophyletic nature of *Bahaba* remains unsupported by current molecular data, and its synapomorphic morphological characteristics (s) remain to be revealed.

3.2.5 Morphological comparison between *Bahaba polykladiskos* and *Boesemania microlepis*

Distinct morphological differences were observed between *Ba. polykladiskos* and *Bo. microlepis*. These included variations in swim bladder structure, lateral-line scales, eye size, interocular distance, anal spine length, and coloration (Table 1; Figure 6). The well-developed bulbs of the swim bladder and dorsal tilting are treated as synapomorphic morphological characteristics shared by *Ba. polykladiskos* and *Bo. microlepis*.

Marine Research of the National Academy of Marine Research
DOI: 10.29677/MR.202506_5(1).0005

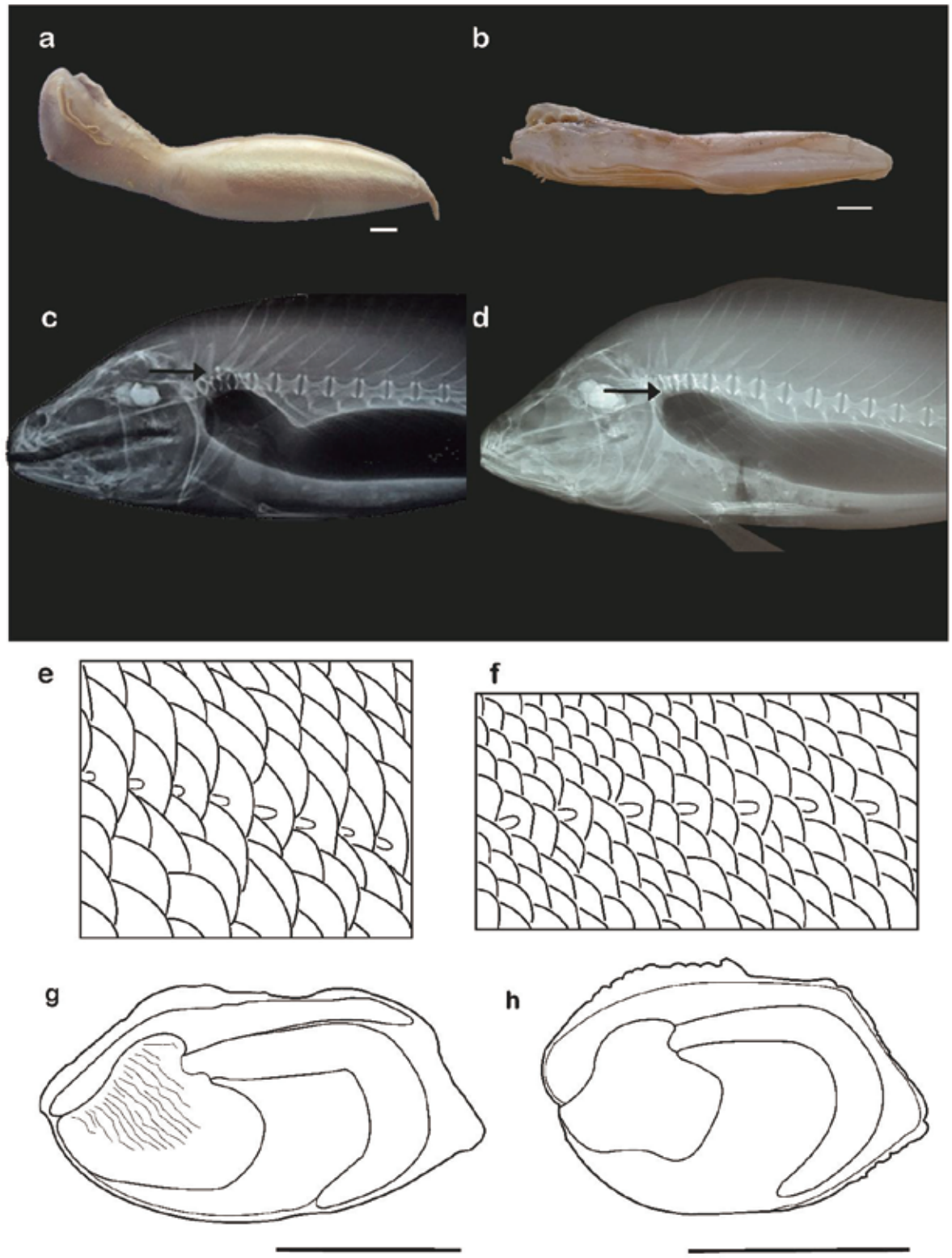

Figure 6. Morphological comparison between *Bahaba polykladiskos* and *Boesemania microlepis*. This figure compares the morphology of *Bahaba polykladiskos* and *Boesemania microlepis*. Included are images of their swim bladders: (a) *Ba. polykladiskos*, (b) *Bo. microlepis*; radiographs of swim bladders: (c) *Ba. polykladiskos*, (d) *Bo. microlepis* (with arrows pointing to the top of the swim bladder); lateral-line scales: (e) *Ba. polykladiskos*, (f) *Bo. microlepis*; and sagittae: (g) *Ba. polykladiskos*, (h) *Bo. microlepis*.

DOI: 10.29677/MR.202506_5(1).0005

3.3 Discussion

Analysis of COI gene sequences indicated that the *Ba. polykladiskos* specimen from Sarawak and the "golden croakers" from the Bang Pakong River are conspecific. Although the COI gene sequences of *Ba. polykladiskos* provided in this study are not novel, we identified the correct source species for the three related sequences published by Pringgenis and Susilowati (2016) and Zhang et al. (2022) and confirmed the COI gene sequence of *Ba. polykladiskos*.

The analytical results of the barcoding data in this study also support a hypothesis regarding the sister-group relationship between *Bo. microlepis* and *Ba. polykladiskos*, which challenges the monophyletic status of the genera *Boesemania* and *Bahaba*. Further analyses incorporating mitochondrial and nuclear genes from related genera are required to test this hypothesis, and a taxonomic revision of these genera is necessary.

Despite differences in coloration and some morphological characteristics between the "golden croakers" and previously described *Ba. polykladiskos* specimens—including the Sarawak specimen in this study—a longer interocular distance, a unique character state shared between the "golden croaker" and *Ba. polykladiskos* but absent in *Bo. microlepis* and other *Bahaba* species, strongly supports their classification as conspecific.

4 CONCLUSIONS

Morphological characteristics, particularly a longer interocular distance, closely align the "golden croaker" from the Bang Pakong River with *Ba. polykladiskos*. COI gene sequence analysis revealed that the spine bahaba specimen from Borneo and the "golden croakers" from the Bang Pakong River are conspecific and form a sister group to *Bo. microlepis*, a species within the monotypic genus *Boesemania*. The exceptionally enlarged laterodorsal region of the head swim bladder in both *Ba. polykladiskos* and *Bo. microlepis* further supports their close phylogenetic affinities. However, this phylogenetic hypothesis needs further validation through additional morphological and molecular evidence.

ACKNOWLEDGEMENTS

The authors wish to thank K.Y. Soong for funding support during the field collection trip to Thailand. Teng Sing Tung and Law Ing Kuo assisted with the molecular analysis of *Ba. polykladiskos* specimens from Borneo.

AUTHOR CONTRIBUTIONS

S.H.L. generated and analyzed the molecular data, performed morphological and anatomical examinations, and wrote the manuscript. S.S. initiated the study, collected specimens, and performed morphological examinations. W.B.Y. and S.C.W. generated and analyzed the molecular data. J.G. collected specimens and performed morphological and anatomical examinations. H.K.M. performed the morphological and anatomical examinations, analyzed the data, and wrote the manuscript. Y.W.C analyzed the data and revised the manuscript. All authors have read and approved the final version of this manuscript.

REFERENCES

Chao, L. N. (1978). *A basis for classifying Western Atlantic Sciaenidae (Teleostei: Perciformes)* (Report No. NOAA Technical Report NMFS Circular No. 415). U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB289330.xhtml

Chao, N. L. (1986). A synopsis on zoogeography of Sciaenidae. In T. Uyeno, R. Arai, T. Taniuchi, & K. Matsuura (Eds.), *Indo-Pacific fish biology: Proceedings of the second international conference on Indo-Pacific fishs* (pp. 570–589). Ichthyological Society of Japan.

Fowler, H. W. (1933). *Contributions to the biology of the Philippine Archipelago and adjacent regions. The fishes of the families Banjosidae, Lethrinidae, Sparidae, Girellidae, Kyphosidae, Oplegnathidae, Gerridae, Mullidae, Emmelichthyidae, Sciaenidae, Sillaginidae, Arripidae and Enoplosidae, collected by the United States Bureau of Fisheries steamer "Albatross," chiefly in Philippine Seas and adjacent waters.* (Report No. U.S. National Museum Bulletin, 100(12)). Smithsonian Institution. https://library.si.edu/digital-library/book/bulletinunitedst100121933unit

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution, 33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Ladich, F., & Fine, M. L. (2006). Sound generating mechanisms in fishes: A unique diversity in vertebrates. In F. Ladich, S. P. Collin, P. Moller, & B. G. Kapoor (Eds.), *Communication in fishes* (Volume 1, pp. 1–41). Science Publishers.

Lim, A. P. K., & Gambang, A. C. (2009). *Field guide to marine & estuarine fishes of Sarawak*. Fisheries Research Institute Bintawa.

Lo, P. C., Liu, S. H., Chao, N. L., Nunoo, F. K. E., Mok, H. K., & Chen, W. J. (2015). A multi-gene dataset reveals a tropical New World origin and Early Miocene diversification of croakers (Perciformes:Sciaenidae). *Molecular Phylogenetics and Evolution, 88*, 132–143. https://doi.org/10.1016/j.ympev.2015.03.025

Lo, P. C., Liu, S. H., Nor, S. A. M., & Chen, W. J. (2017). Molecular exploration of hidden diversity in the Indo-West Pacific sciaenid clade. *PLoS ONE, 12*(4), Article e0176623. https://doi.org/10.1371/journal.pone.0176623

Lokman, I. N. M., Faudzir, N. M., Hidayah, H., Nursyuhada, O., Farhana, R. F., Hartini, S. N., Abdullah, H. M. R., Fawwazah, A. F. N. A., & Bakar, A. L. M. A. (2024). Ethnoichthyology of the first record of spine Bahaba (*Bahaba polykladiskos*) from Muar, Johor, Malaysia. *Borneo Journal of Resource Science and Technology, 14*(1), 18–29. https://doi.org/10.33736/bjrst.5849.2024

Pringgenis, D., & Susilowati, R. (2016). Highly commercial fisheries tawar fish: Molecular analysis DNA mitochondrial COI gene sequence and proximate analysis from Malacca Strait, Riau. *Jurnal Teknologi, 78*(4–2). https://doi.org/10.11113/jt.v78.8149

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology, 21*(8), 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Sasaki, K. (2001). Sciaenidae: Croakers (drums). In K. E. Carpenter & V. H. Niem (Eds.), *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific* (Volume 5, Bony fishes part 3 [*Menidae to Pomacentridae*], pp. 3117–3174). Food and Agriculture Organization. https://openknowledge.fao.org/handle/20.500.14283/y0770e

Sasaki, K., & Kailola, P. J. (1988). Three new Indo-Australian species of the sciaenid genus *Atrobucca*, with a reevaluation of generic limit. *Japanese Journal of Ichthyology, 35*(3), 261–277. https://doi.org/10.11369/jji1950.35.261

Trewavas, E. (1977). The sciaenid fishes (croakers or drums) of the Indo-West Pacific. *The Transactions of the Zoological Society of London, 33*(4), 253–541. https://doi.org/10.1111/j.1096-3642.1977.tb00052.x

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B, Biological Sciences, 360*(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wongratana, T. (1985). *Boesemania microlepis* (Bleeker), a common but misidentified riverine drumfish (Pisces: Sciaemindae) from Thailand and Mekong River. *Proceedings of the 23rd Kasetsart University Conference Fisheries Section, Bangkok, Thailand, 23*, (pp. 3–20).

Yoshida, T., Motomura, H., Musikasinthorn, P., & Matsuura, K. (Eds.). (2013). *Fishes of northern Gulf of Thailand* (pp. 154). National Museum of Nature and Science, Tsukuba, Research Institute for Humanity and Nature, Kyoto, and Kagoshima University Museum. https://www.museum.kagoshima-u.ac.jp/staff/motomura/ThaiFG_low.pdf

Zhang, J., Deng, Q., Liu, X., Wu, M., Ma, Z., Shaw, P. C., Zhang, Y., & Cao, H. (2022). Molecular identification of commercial fish maws by DNA sequencing of 16S rRNA and cytochrome c oxidase I genes. *Journal of Food Protection, 85*(10), 1439–1445. https://doi.org/10.4315/JFP-22-121

Appendix

Cytochrome oxidase subunit I (COI) sequences used in this study. Sequences marked with * indicate those uploaded in this study, while the others were downloaded from GenBank or BOLD.

Accession No.	Species name in GenBank	Species name in this study	Country
LC064301	*Boesemania microlepis*	*Bahaba polykladiskos*	Indonesia
ON695924	*Boesemania microlepis*	*Bahaba polykladiskos*	No record
ON695922	*Boesemania microlepis*	*Bahaba polykladiskos*	No record
MH721187	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
KP722706	*Boesemania microlepis*	*Boesemania microlepis*	Thailand
MT627387	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627386	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627385	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627384	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627383	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627382	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627381	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627380	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627379	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627378	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627377	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627376	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627375	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627374	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627373	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627372	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627371	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627370	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627369	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627368	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627367	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627366	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627365	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627363	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627362	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam

DOI: 10.29677/MR.202506_5(1).0005

Accession No.	Species name in GenBank	Species name in this study	Country
MT627361	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627360	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627359	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627358	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
MT627364	*Boesemania microlepis*	*Boesemania microlepis*	Viet Nam
ON695923	*Boesemania microlepis*	*Boesemania microlepis*	No record
PV133561*	*Boesemania microlepis*	*Boesemania microlepis*	Thailand
PV133562*	*Boesemania microlepis*	*Boesemania microlepis*	Thailand
PV133563*	*Boesemania microlepis*	*Boesemania microlepis*	Thailand
PV133552*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133553*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133554*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133555*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133556*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133557*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133558*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133559*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Thailand
PV133560*	*Bahaba polykladiskos*	*Bahaba polykladiskos*	Malaysia
NC_018347	*Bahaba taipingensis*	*Bahaba taipingensis*	No record
KP722703	*Bahaba taipingensis*	*Bahaba taipingensis*	No record
MH260402	*Bahaba taipingensis*	*Bahaba taipingensis*	No record
KX778046	*Panna microdon*	*Panna microdon*	Malaysia
KX778047	*Panna microdon*	*Panna microdon*	Thailand
KX777895	*Chrysochir aureus*	*Chrysochir aureus*	Taiwan
KP722708	*Chrysochir aureus*	*Chrysochir aureus*	No record
KX777881	*Atrobucca nibe*	*Atrobucca nibe*	Taiwan
KP722702	*Atrobucca nibe*	*Atrobucca nibe*	No record