SYSTEMATICS AND BIOGEOGRAPHY OF THE GENUS *CHITALA* (OSTEOGLOSSOMORPHA: NOTOPTERIDAE) IN MALAYSIA USING AN INTEGRATIVE APPROACH

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by

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LIST OF SYMBOLS

n	The number of specimens
μ	Mean of data / average
μL	Micro-litre
&	Ampersand
~	Approximately

LIST OF ABBREVIATIONS

ABA	Amiruddin Bin Ahmad
AFB	Anal Fin Base
aff.	*Affinis*; related to or resembling
AFL	Anal Fin Length
AFR	Anal Fin Rays
ASAP	Assemble Species by Automatic Partitioning
BD	Body Depth
BDD	Body Depth at Dorsal Fin Insertion
bp	Base pairs
bPTP	Bayesian Poisson Tree Process
C. blanci	*Chitala blanci*
C. borneensis	*Chitala borneensis*
C. chitala	*Chitala chitala*
C. hypselonotus	*Chitala hypselonotus*
C. ornata	*Chitala ornata*
CIA	Chloroform-Isoamyl Alcohol
COI	Cytochrome c Oxidase subunit I
Coll.	Collectors
CTAB	2X Trimethylammonium Bromide
cytb	Cytochrome b
ddH2O	Double distilled water
DE	Distance from the snout to the posterior part of the eye
DFB	Dorsal Fin Base

DFL	Dorsal Fin Length
DFR	Dorsal Fin Rays
DNA	Deoxyribonucleic Acid
DS	Scales from dorsal fin insertion to the lateral line
ED	Eye Diameter
EtOH	Ethyl Alcohol / Ethanol
ex.	Formerly known as
GMYC	Generalized Mixed Yule Coalescent
GR	Gill Rakers
HD	Head Depth
HL	Head Length
IR	Interorbital Ridge
JAMFJ	Jamsari Amirul Firdaus Jamaluddin
JL	Jaw Length
LHR	Luqman Hakim Ruzman
C. Lopis	*Chitala lopis*
LS	Scales along lateral line
MAN	Mohamad Aqmal-Naser
ML Tree	Maximum Likelihood Tree
MLIN	Mohd Lokman Ilham-Norhakim
MRAH	Abdullah Halim Muhammad-Rasul
mtDNA	Mitochondrial Deoxyribonucleic Acid
myBis	Malaysian Biodiversity Information System
MZK	Md Zain Khaironizam
N. borneensis	*Notopterus borneensis*

N. C. borneensis	*Notopterus Chitala borneensis*
N. C. chitala	*Notopterus Chitala chitala*
N. chitala	*Notopterus chitala*
NO.	Number or Code
N. notopterus	*Notopterus Notopterus*
OTU	Operational Taxonomic Unit
PAL	Pre-anal Length
PC1	Principle Component 1
PC2	Principle Component 2
PC3	Principle Component 3
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PDL	Pre-dorsal Length
PFL	Pectoral Fin Length
PFR	Pectoral Fin Rays
PM	Peninsular Malaysia
PoHD	Post-orbital Head Depth
PPL	Pre-pectoral Length
ppm	Parts per Million
PrHD	Pre-orbital Head Depth
PVL	Pre-ventral Length
rDNA	Ribosomal Deoxyribonucleic Acid
rpm	Rotations per minutes
s.d.	Standard Deviation
scu	Abdominal Scutes

sensu	In the sense of; as interpreted by
SL	Standard Length
SLV	Sébastien Lavoué
sp.	Unidentified or undescribed species
TBE Buffer	Tris/Borate/EDTA Buffer
TL	Total Length
TS	Tedjo Sukmono
USMFC	Universiti Sains Malaysia Fish Collection
VFL	Ventral Fin Length
VS	Scales from pelvic fin insertion to the lateral line

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SISTEMATIK DAN BIOGEOGRAFI BAGI GENUS *CHITALA* (OSTEOGLOSSOMORPHA: NOTOPTERIDAE) DI MALAYSIA MENGGUNAKAN PENDEKATAN INTEGRATIF

ABSTRAK

Taksonomi bagi genus ikan belida air tawar *Chitala* (Osteoglossomorpha: Notopteridae) masih belum dapat diselesaikan sepenuhnya kerana membezakan dan menamakan spesies *Chitala* berdasarkan morfologi semata-mata telah membawa kepada hipotesis yang berbeza. Dalam kajian ini, bilangan dan identiti spesies *Chitala* yang terdapat di Semenanjung Malaysia telah dikenal pasti dengan menggunakan pendekatan taksonomi integratif yang menggabungkan morfologi dan dua penanda mitokondria: gen subunit I sitokrom c oksidase (COI) dan sitokrom b. Sebanyak 60 spesimen *Chitala* telah diperoleh dari seluruh Semenanjung Malaysia. Pada mulanya, semua spesimen dibahagikan kepada tiga kumpulan berbeza berdasarkan corak dan tanda pada badan ikan. Analisis filogenetik molekul menunjukkan bahawa setiap kumpulan ini adalah monofiletik, dan kaedah pembahagian spesies automatik turut mengesahkan setiap kumpulan sebagai spesies yang berbeza. Keputusan kajian ini menyokong kewujudan tiga spesies *Chitala* di Semenanjung Malaysia, walaupun analisis morfometrik multivariat gagal untuk membezakan ketiga-tiga spesies ini sepenuhnya. Perbandingan dengan deskripsi asal spesies dan data genetik daripada 70 spesimen tambahan *Chitala* dari negara sekeliling membolehkan spesies ini dikenali sebagai *Chitala lopis*, *Chitala borneensis*, dan *Chitala ornata*. Kajian ini juga menunjukkan kewujudan dua spesies yang belum dibincangkan di Indonesia.

SYSTEMATICS AND BIOGEOGRAPHY OF THE GENUS *CHITALA* (OSTEOGLOSSOMORPHA: NOTOPTERIDAE) IN MALAYSIA USING AN INTEGRATIVE APPROACH

ABSTRACT

The taxonomy of the freshwater featherback fish genus *Chitala* (Osteoglossomorpha: Notopteridae) remains unsettled because delimiting and naming *Chitala* species based solely on morphology led to different hypotheses. In this study, the number and identity of *Chitala* species present in Peninsular Malaysia were determined by employing an integrative taxonomic approach that combines morphological characters and two mitochondrial markers: the cytochrome c oxidase subunit I (COI) and cytochrome b genes. A total of 60 specimens of *Chitala* were collected throughout Peninsular Malaysia. Initially, all specimens were sorted into three distinct categories based on their body marking patterns. A molecular phylogenetic analysis revealed that each of these groups was monophyletic, and an automated species partition method further recognized them as distinct species. These results support the presence of three *Chitala* species in Peninsular Malaysia, even though a multivariate morphometric analysis failed to fully differentiate each of these three species. Comparison with original species descriptions and genetic data from an additional 70 *Chitala* specimens from neighbouring regions allowed to name these species as *Chitala lopi*s, *Chitala borneensis*, and *Chitala ornata*. This study also showed the presence of two undescribed species in Indonesia.

CHAPTER 1

INTRODUCTION

Among the largest freshwater fishes around the world, featherback knifefish from genus *Chitala* Fowler 1934 (commonly called as Ikan Belida) can be found in Malaysian waters. *Chitala* are one of the four genera in the family Notopteridae that falls under order Osteoglossiformes, a group of primitive-looking ray fin fishes (Actinopterygii). Notopteridae comprises eleven species occurring in Tropical Asia (genera *Chitala* and *Notopterus*) and Tropical Africa (genera *Papyrocranus* and *Xenomystus*) (Fricke et al., 2025; Hilton & Lavoué, 2018). *Chitala lopis* (Bleeker 1851), the largest species, can grow up to 1.5 meter in length and can weight more than 20 kilograms which makes it one of the largest freshwater fishes in Southeast Asia freshwaters (Rainboth, 1996; Roberts, 1992). *Chitala* is identifiable by strong laterally compressed head and body, anal fin confluent with caudal fin, strong concavity of anterior dorsal part of the body, rudimentary pelvic fin and short dorsal fin (Kottelat & Widjanarti, 2005; Rainboth, 1996).

Fowler (1934) diagnosed the genus *Notopterus* Lacépède 1800 by having opercular scales not larger that body, 10 to 22 transverse scales at the preopercle as well as 37 to 45 abdominal scutes. Fowler (1934) erected *Chitala* as a subgenus of the genus *Notopterus*. From Roberts (1992), *Chitala* is elevated at the genus level as the sister group of *Notopterus* sensu Roberts (1992). Asian notopterids (genera *Chitala* and *Notopterus*) form a monophyletic group (Hilton & Lavoué, 2018). Adults and subadults of *Chitala* can be easily distinguished from adults of *Notopterus* by having strong concave profile at the anterior dorsal part of the body (absent in *Notopterus* species). Currently there are six valid species of *Chitala*

(Fricke et al., 2025, Froese & Pauly, 2025) and the classification for the genus *Chitala* is as follows:

Kingdom	: Animalia
Phylum	: Chordata
Class	: Actinopterygii
Order	: Osteoglossiformes
Family	: Notopteridae
Genus	: *Chitala*
Species	: *Chitala chitala* (Hamilton 1822)
	: *Chitala lopis* (Bleeker 1851)
	: *Chitala hypselonotus* (Bleeker 1852)
	: *Chitala borneensis* (Bleeker 1851)
	: *Chitala ornata* (Gray 1831)
	: *Chitala blanci* (d'Aubenton 1965)

The species diversity within genus *Chitala* has been debated for a long time, causing instability in the number of valid *Chitala* species recognized by taxonomists. Although the diversity of large fishes is better known than that of smaller fishes, as indicated by the trend in their respective rate of species description (Beukes et al., 2020), the taxonomy of *Chitala* is challenging due to the low morphological variation among species of *Chitala* (*Chitala* represents a case of morphological stasis). Thus, it is difficult to delimitate species of *Chitala*, and different species partition schemes have been proposed (Günther, 1868; Kottelat & Widjanarti, 2005; Roberts, 1992). A short review of the evolution of the classification of the species of *Chitala* is presented **Table 1.1**.

Table 1.1 History of the validity of nominal species of Notopteridae currently classified into the genus *Chitala* Fowler 1934.

Nominal Species	Günther (1868)	Fowler (1934)	Roberts (1992)	Kottelat (2013) Fricke et al. (2025)
Mystus chitala Hamilton 1822	Valid as *N. chitala*	Valid as *N. C. chitala*	Valid as *C. chitala*	Valid as *C. chitala*
Notopterus ornatus Gray 1831	Synonym of *N. chitala*	Synonym of *N. C. chitala*	Valid as *C. ornata*	Valid as *C. ornata*
Notopterus lopis Bleeker 1851a	Synonym of *N. chitala*	Synonym of *N. C. chitala*	Valid as *C. lopis*	Valid as *C. lopis*
Notopterus borneensis Bleeker 1851b	Valid as *N. borneensis*	Valid as *N. C. borneensis*	Synonym of *C. lopis*	Valid as *C. borneensis*
Notopterus maculosus Bleeker 1851b	Synonym of *N. borneensis*	Synonym of *N. C. borneensis*	Synonym of *C. lopis*	Synonym of *C. borneensis*
Notopterus hypselonotus Bleeker 1852	Synonym of *N. chitala*	Synonym of *N. C. chitala*	Synonym of *C. lopis*	Valid as *C. hypselonotus*
Notopterus blanci d'Aubenton 1965	/	/	Valid as *C. blanci*	Valid as *C. blanci*

Roberts (1992) suggested that there are only four valid species of *Chitala* (**Table 1.1**). Because morphometric and meristic counts were found not taxonomically informative to delimitate species, Roberts (1992) mostly used body color pattern to diagnose species of *Chitala*. The four valid species of *Chitala* recognized by Roberts (1992), are *Chitala chitala* (Hamilton 1822) that can be found endemic to the Indian subcontinent, *Chitala ornata* (Gray 1831) in mainland Southeast Asia waters, *Chitala blanci* (d'Aubenton 1965) endemic to Mekong River and *Chitala lopis* (Bleeker 1851) widely distributed in Southeast Asia. Roberts (1992) noted that *Chitala lopis* exhibits four body colour patterns depending on size (i.e., ontogenetic colour phases). For Roberts (1992), *Chitala borneensis* (Bleeker 1851), *Notopterus maculosus* (Bleeker 1851) and *Chitala hypselonotus* (Bleeker 1852) are different ontogenetic stages of *Chitala lopis.* Consequently, these three species names are regarded as junior synonyms of *Chitala lopis*.

Kottelat and Lim (1996) challenged the *Chitala lopis* classification scheme of Roberts (1992). Kottelat and Lim (1996) argued that all ontogenetic phases of *Chitala lopis* as identified by Roberts (1992) can be observed in specimens ranging from 15.0 to 20.0 cm standard length (SL). They concluded that body colour variation is not consistent with size. Therefore, variation may indicate the presence of more than one species within *Chitala lopis* of Roberts (1992). Kottelat and Widjanarti (2005) then proposed that *Chitala lopis* (as recognized by Roberts, 1992) are made up of two or four species. After Kottelat and Widjanarti (2005), the number and identity of species of *Chitala* without large ocellated spots in Peninsular Malaysia were unsettled with several names used, such as *Chitala chitala* (Shaharom, 2012), *Chitala lopis* (Rashid et al., 2015), *Chitala* aff. *lopis* (Ng et al., 2019) *Chitala ornata* (Zakaria-Ismail et al., 2019) and *Chitala lopis* (Aqmal-Naser et al., 2023).

1.1 Problem Statement

Although *Chitala* comprises some of the largest freshwater fishes in Malaysia, a taxonomic revision of this genus is very lacking to reveal how many species occur. This situation (i.e., the uncertainties regarding the number of *Chitala* species) impedes conservation efforts needed to implement effective measures. Thus, in this research, we aim to accurately determine the taxonomic richness of *Chitala* residing in Peninsular Malaysia as well as their identities by using a modern integrative taxonomic approach which combines two different sources of information, morphological evidence and molecular evidence. Because none of the species of *Chitala* were initially described from Peninsular Malaysia, it was necessary to analyse newly generated data for this region in a larger taxonomic and geographic

context, by examining data from specimens collected in neighbouring regions, especially from Indonesia where four nominal species were described. The taxonomic and phylogenetic results will serve to discuss the biogeography and conservation of these fishes in Peninsular Malaysia and elsewhere in Southeast Asia.

1.2 Research Questions

a) What is the taxonomic richness of the species of the giant featherback fishes (genus *Chitala*) in Peninsular Malaysia?
b) What is the precise distribution of species of *Chitala* in Peninsular Malaysia?

1.3 Research Objectives

a) To solve the taxonomy of *Chitala* in Peninsular Malaysia using an integrative taxonomic method which combines morphological and molecular approaches.
b) To provide reliable identification characteristics for each species of *Chitala* occurring in Peninsular Malaysia along with their precise distribution.

CHAPTER 2

LITERATURE REVIEW

2.1 Classification and Taxonomy of Notopteridae

Notopteridae is a family under the order Osteoglossiformes comprising four valid genera and eleven valid species (Fricke et al., 2025). Three species are distributed in Africa and eight species in Asia. It is divided into two clades; the subfamily Xenomystinae (genera *Xenomystus* and *Papyrocranus*) that resides in African region and the subfamily Notopterinae (genera *Chitala* and *Notopterus*) found in Asia (Lavoué & Sullivan, 2004). The three valid species in African waters are known as *Xenomystus nigri* (Günther 1868), *Papyrocranus congoensis* (Nichols & LaMonte 1932) and *Papyrocranus afer* (Günther 1868). The classification of Asian Notopterinae is unsettled because the number of species within the genus *Chitala* is still unknown (Barby et al., 2019) even if it has been occasionally reviewed in the past (Guo-Qing et al., 1997; Lim & Furtado, 1986; Roberts, 1992). However, based on previous studies, *Chitala* has been demonstrated to be monophyletic based on several characteristics (Lal et al., 2006). *Chitala* is considered as the sister group of the genus *Notopterus* (Inoue et al., 2009; Lavoué & Sullivan, 2004). There are six current valid species of *Chitala* which is *Chitala blanci* (d'Aubenton 1965), *Chitala borneensis* (Bleeker 1851), *Chitala chitala* (Hamilton 1822), *Chitala hypselonotus* (Bleeker 1852), *Chitala lopis* (Bleeker 1851) and *Chitala ornata* (Gray 1831) while the genus *Notopterus* consists of two species, *Notopterus notopterus* (Pallas 1769) and *Notopterus synurus* (Bloch & Schneider 1801) (Fricke et al., 2025; Lavoué et al. 2020).

The classification of Notopteridae (to the genus level) is as follows (Fricke et al., 2025):

Kingdom	: Animalia
Phylum	: Chordata
Class	: Actinopterygii
Order	: Osteoglossiformes
Suborder	: Notopteroidei
Family	: Notopteridae Bleeker 1859
Genera	: *Chitala* Fowler 1934
	: *Notopteru*s Lacepède 1800
	: *Papyrocranus* Greenwood 1963
	: *Xenomystus* Günther 1868

Often commonly referred as “featherbacks” or “Old World knifefishes” because of their elongated anal fin and very short dorsal fin, the family Notopteridae is known from the Cretaceous Period (Forey, 1997; Taverne & Maisey, 1999). The systematics of this group has been occasionally revised in the past and although the monophyly of this family is well supported, there are still competing hypotheses regarding the intra-familial relationships (Lavoué & Sullivan, 2004). One of the hypotheses is considered by Greenwood (1963), where *Xenomystus* is a different group from the remaining Notopteridae. Furthermore, Taverne (1998) suggested that all notopterids form a clade apart from *Chitala*, and *Chitala* is considered the sister group to all other notopterids. African members of Notopteridae (genera *Xenomystu*s and *Papyrocranus*), share with the African weakly electric fish of the superfamily Mormyroidea, ampullary type electroreceptors that are absent in Asian notopterids (Alves-Gomes, 2001; Braford, 1986). Ampullary type electroreceptors were likely lost in the ancestors of Asian notopterids (Lavoué & Sullivan, 2004).

2.2 General Morphology of Notopteridae

The external morphology of Notopteridae is unlike that of the other families in the order Osteoglossiformes, being relatively simplified. Notopterids have a laterally compressed body, a head consisting of small cycloid scales, and an elongated anal fin confluent with the caudal fin (Ahmadi et al., 2019; Hilton & Lavoué, 2018; Lavoué & Sullivan, 2004). The very short dorsal fin (absent in *Xenomystus*) is tall and rounded with narrow base while its pelvic fin is vestigial (Roberts, 1992). The members of *Chitala* have a "hunch" or dorsal concavity at its nape and it is more defined in adult specimens (no marked concavity in juvenile *Chitala*) (Hilton & Lavoué, 2018). Furthermore, all notopterid species have spiny abdominal scutes that extend from behind isthmus up to the anterior part of the pelvic fin origin (Hilton & Lavoué, 2018; Musikasinthorn & Ngamtampong, 2022).

2.3 Taxonomy of the Genus *Chitala*

Bleeker (1851a, 1851b, 1852) are some of the earliest taxonomic works on the genus *Notopterus*, which is currently, in part, recognized as the genus *Chitala*. Bleeker (1851a, 1851b, 1852) recognized six different species of *Notopterus*, namely *Mystus chitala* (currently reclassified into the genus *Chitala* as *Chitala chitala*), *Notopterus ornatus* (*Chitala ornata*), *Notopterus lopis* (*Chitala lopis*), *Notopterus borneensis* (*Chitala borneensis*), *Notopterus maculatu*s (currently considered as a junior synonym of *Chitala borneensis*), and *Notopterus hypselonotus* (*Chitala hypselonotus*). All these species were initially described based on morphological characteristics. The illustrations of four species of *Notopterus* described by Bleeker (1851a, 1851b, 1852) from Indonesia can be referred to in **Figure 2.1.** The

translation from Latin to English of the four species descriptions of *Chitala* by Bleeker in **Appendix 1**. *Notopterus lopis* differ from *Notopterus hypselonotus* primarily by the absence of a black spot at the base of the pectoral fin (present in *Notopterus hypselonotus*). Both species have a similar jaw characteristic, where the maxillary jaw extends beyond the posterior part of the eye (a characteristic absent in *Notopterus borneensis* and *Notopterus maculatus*). *Notopterus* species of Bleeker with a jaw length that does not extend beyond the posterior part of the eye are identified as *Notopterus borneensis*. *Notopterus maculatus* shares a similar jaw length characteristic with *N. borneensi*s, but black dots on the posterior half of the body are more numerous and larger than those of *Notopterus borneensis* (**Figure 2.1**). A difference which was not considered of species-level by Günther (1868) who considered *Notopterus maculatus* as a junior synonym of *Notopterus borneensis*.

The taxonomy of *Chitala* has changed over time. Günther (1868) and Fowler (1934) recognized only two species of *Chitala* under the genus *Notopterus*: *Notopterus chitala* and *Notopterus borneensis*. *Notopterus maculosus* was recognized as a junior synonym of *Notopterus borneensis*, while the other three species of *Notopterus* were synonymized with *Notopterus chitala* (Günther, 1868; Fowler, 1934). In his 1934 work, Fowler considered *Notopterus borneensis* as a subspecies of *Notopterus chitala*, as *Notopterus chitala borneensis*. It was not until Roberts (1992) that the subgenus *Chitala* was recognized as a distinct genus, separated from *Notopterus*. In his revision, Roberts also clarified the taxonomy of *Chitala*, synonymizing *Chitala borneensis* (previously *Notopterus borneensis*) and *Chitala hypselonotus* (previously *Notopterus hypselonotus*) with *Chitala lopis* (the senior synonym).

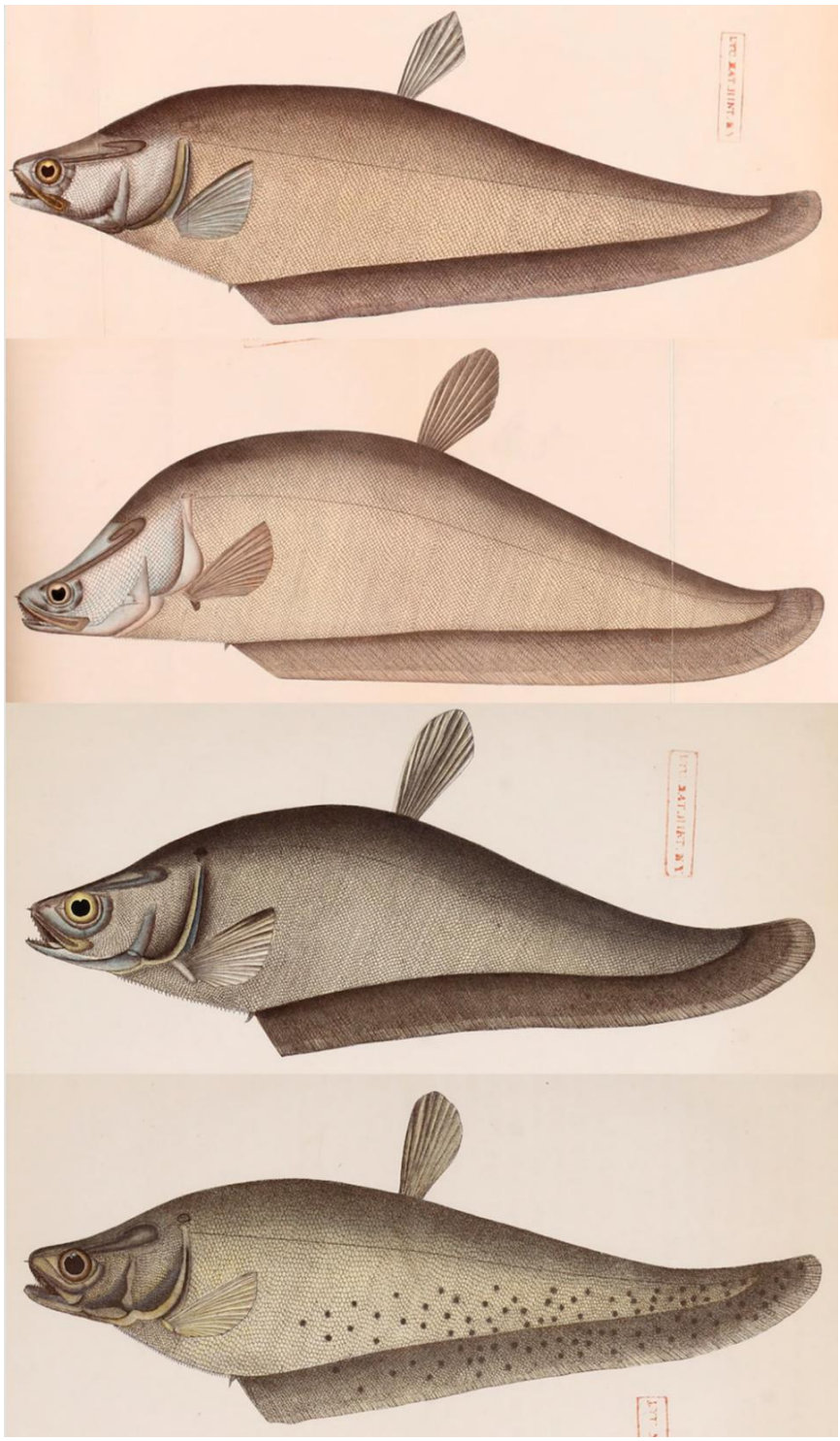

Figure 2.1 Reproduction of the historical illustrations of specimens of the four nominal species of *Notopterus*, currently classified into *Chitala*, described by Pieter Bleeker from Indonesia. These illustrations were published in Bleeker (1854). From top to bottom: *Notopterus lopis* (currently *Chitala lopis*), *Notopterus hypselonotus* (*Chitala hypselonotus*, a junior synonym of *Chitala lopis*), *Notopterus borneensis* (*Chitala borneensis*), and *Notopterus maculosus* (a junior synonym of *Chitala borneensis*). Note the absence of a black mark at the insertion of pectoral fin in *Notopterus lopis* (vs. present in *Notopterus hypselonotus*) and the maxillary extending below the eye in *Notopterus borneensis* and *Notopterus maculosus* versus extending behind the eye in *Notopterus lopis* and *Notopterus hypselonotus*.

Roberts (1992) represents the most comprehensive taxonomic revision of the genus *Chitala*, based on morphological examination. This author recognized four species in the genus *Chitala*, namely *Chitala chitala* (occurring only in the Indian subcontinent), *Chitala ornata* (occurring only in mainland Southeast Asia), *Chitala blanci* (endemic to the Mekong River) and *Chitala lopis* (all populations of *Chitala* found in Malaysia and Indonesia along with some populations of the lower reaches of the Mekong, Chao Phraya and Maeklong Rivers). The delimitation of *Chitala lopis* proposed by Roberts (1992) challenged the previous classifications proposed by authors such as Günther (1868) who recognized three distinct species based on colour differences (i.e. *Chitala lopis* sensu stricto, *Chitala hypselonotus* and *Chitala borneensis*). Roberts (1992) did not find any significant morphological or meristic difference which could be used to delimitate more than one species of *Chitala* in Malaysia and Indonesia. He interpreted the black marking variability as of intraspecific level, defining different ontogenetic phases within the same species. According to Roberts (1992), specimens of *Chitala lopis* smaller than 250 mm in length belong to the "maculosus color phase" in having the entire body covered with numerous small black dots; specimens between 250-300 mm in length belong to the "lopis color phase" and are characterized by a total absence of black mark on the body. Between 300-600 mm in length, specimens have a black conspicuous spot at pectoral-fin base and numerous very small dark dots on the lowermost posterior part of the body ("borneensis color phase") and finally above 600 mm, the largest specimens have no mark on the body excepted for a black mark at pectoral-fin base ("hypselonotus color phase").

The conclusions of Roberts (1992) were first challenged by Kottelat and Lim (1996) who noticed that the four marking patterns of *Chitala lopis* are known in

specimens within the range of 150-250 mm in length. Later, Kottelat and Widjanarti (2005) further suggested that four species of *Chitala* should be recognized in the Sundaic region, each having a distinct geographical distribution: *Chitala borneensis* in Borneo, Sumatra and Peninsular Malaysia, exhibiting several small black dots in the posterior part of the body, *Chitala hypselonotu*s in Sumatra and Borneo having a large black dot at pectoral-fin base, *Chitala lopis* endemic to Java (type locality) having no black mark and, a fourth species, still undescribed, in Peninsular Malaysia and Mekong basin similar to *Chitala hypselonotus*. According to Kottelat and Widjanarti (2005), this undescribed species is morphologically similar to *Chitala hypselonotus*, but can be distinguished based on differences in juvenile colour pattern, which they noted as the only reliable character separating the two. The classification suggested by Kottelat and Widjanarti (2005) is similar to that of earlier authors (Bleeker, 1870; Günther, 1868). However, Kottelat and Widjanarti (2005) called for a comprehensive taxonomic revision of *Chitala* occurring in Malaysia and Indonesia, because of the low morphological variation within *Chitala* making difficult to reliably delimitate species.

2.4 Distribution of *Chitala* Species

The distribution of Notopteridae is quite large covering tropical Africa and tropical Asia (Fricke et al., 2025). The genera *Papyrocranus* and *Xenomystu*s only occur in Africa. *Papyrocranus afer* (type locality: Sierra Leone) and *Papyrocranus congoensis* (type locality: Congo River) are distributed in Western and Central Africa, respectively (Froese & Pauly, 2025). *Xenomystus nigri* (type locality: Niger River) are distributed in Western and Central Africa (Roberts, 1992).

The genera *Chitala* and *Notopterus* are exclusively distributed in Asia (Fricke et al., 2025; Roberts, 1992). *Chitala lopis*, *Chitala hypselonotus* and *Chitala borneensis* are known to occur in Malay Peninsula as well as parts of Indonesian islands (Anjarsari et al., 2021). *Chitala lopis* (type locality: Java) is found in Sumatra, Java and Borneo, *Chitala hypselonotus* (type locality: Musi River) occurs in Borneo and Sumatra rivers, *Chitala borneensi*s (type locality: Sambas) is found in east Malay peninsula, western Borneo and Sumatra. *Chitala ornata* (type locality: Mekong) and *Chitala blanci* (type locality: Mekong) are distributed in mainland Southeast Asia whereas *Chitala chitala* is the only species of *Chitala* endemic to the Indian subcontinent where it is found in the Indus, Ganges-Brahmaputra, and Mahanadi River basins (Fricke et al., 2025; Froese & Pauly, 2025). *Notopterus* has the widest distribution where *Notopterus synurus* is found occurring in South Asia (i.e., mosly India, Pakistan, and Nepal) while *Notopterus notopterus* is found in all coastal river basins of peninsular Thailand and Malaysia, Mekong, Chao Phraya and Maeklong basins as well as Java and Sumatra, but not Borneo (Fricke et al., 2025; Roberts, 1992).

2.5 Biology and Importance of *Chitala* Species

Reaching up to 1.5 m in length (Kottelat et al., 1993), large water drainage is the preferable habitat for species of *Chitala*, such as natural and artificial lakes, canals, ponds, reservoirs and mainstreams that have slow moving water or stagnant backwaters (Mitra et al., 2018). Mitra et al. (2018) mentioned that these fishes have a bladder modification acting as an accessory respiratory organ allowing these fishes to live in low-oxygenated waters (less than 0.5 parts per million [ppm] of dissolved

oxygen). Species of *Chitala* are known to be ferocious predators where fishes, shrimps and small vertebrates constitute their diet. *Chitala* are nocturnal organisms, actively hunting during night (Rainboth, 1996; Vidthayanon, 2005; Wibowo et al., 2014).

The large size of these fishes makes them more vulnerable to several anthropogenic threats including habitat degradation and overfishing as they are often targeted for their size and nutritive quality. These fishes are important commercially in Malaysia and Indonesia where large and 'meaty' species, such as *Chitala lopis*, are commonly consumed as a delicacy (Ahmadi et al., 2019; Christensen, 1993; Lymer et al., 2008; Rainboth, 1996). On the other hand, *Chitala ornata* and *Chitala blanci* are popular as ornamental species due to their pretty appearance (Lal et al., 2006; Sarkar et al., 2009).

2.6 Diversity of *Chitala* in Malaysia

In Malaysia, four species of *Chitala* are currently reported (Malaysia Biodiversity Information System "myBis" at https://www.mybis.gov.my/one/; consulted in January 2025): *Chitala chitala*, *Chitala ornata*, *Chitala lopis* and *Chitala borneensis*. However, previous records of *Chitala chitala* referred in fact to the species currently identified as *Chitala lopis*, and *Chitala ornata* is considered as an introduced species (Zakaria-Ismail et al., 2019). The genus *Chitala* seems to be widely distributed in Malaysia: In Peninsular Malaysia, Ng et al. (2019) reported the presence of *Chitala* aff. *lopis* in Perak River, Khan et al. (1996) and Rashid et al. (2015) identified *Chitala lopis* from Pahang River, Ng and Tan (1999) reported *Chitala lopis* from Endau River whereas Lim and Furtado (1986) listed *Notopterus*

aff. *chitala* from the same drainage. Shaharom (2012) listed *Chitala chitala* from Lake Kenyir, Terengganu River, although the photo of the specimen published in this work shows a different species of *Chitala*. In Sarawak, Parenti and Lim (2005) and Kottelat and Lim (1996) listed *Chitala borneensis* from Rajang and Niak Rivers, respectively. All these records indicate that the genus *Chitala* is widely distributed in Malaysia but none of the authors of these records provided morphological information on specimens. Furthermore, the use of different binomial names either referring to different species or to the same species, adds more confusion to the taxonomic situation.

There are only few species-level and population genetic studies on the genus *Chitala* which, altogether, provide only limited insight into the taxonomy and distribution of these fishes. Inoue et al. (2009) examined the phylogeny of the family Notopteridae using complete mitogenomes and three species of *Chitala* (*Chitala ornata*, *Chitala blanci* and one specimen of *Chitala lopis* of unknown origin). These authors found that the genus *Chitala* is monophyletic and sister group of the genus *Notopterus* as found in Lavoué and Sullivan (2004). Both genera diverged to each other about 50 million years ago and the genus *Chitala* started to diversify after the Eocene (about 30 million years ago). Two studies have provided molecular markers for the identification of *Chitala ornata* (Panprommin et al. 2019; from Kwan Phayao, northern Thailand) and *Chitala chitala* (Lakra et al. 2016; from India). Barby et al. (2018, 2019) found that the population of *Chitala lopis* from the Mekong River is distinctive from those of other species (i.e., *Chitala ornata*, *Chitala blanci*, and *Chitala chitala*) in having less chromosomes (2n=38 versus 2n=42). Wibowo et al. (2010) is the first genetic study on Indonesian giant featherback fishes. These authors examined the diversity of *Chitala* from three localities in east Sumatra plus one

locality in Borneo (i.e., Barito River; Kalimantan Tengah) using the mitochondrial D-loop region and more than 50 specimens. Four main mitochondrial lineages were identified, three of them occurring in sympatry in the Kampar River (Riau province). These results are particularly interesting because Wibowo et al. (2010) demonstrated that *Chitala lopis*, as recognized by Roberts (1992), comprises more than one species. However, Wibowo et al. (2010) did not provide any morphological characteristic which could allow to assign any of these lineages to described species (e.g., *Chitala lopis*, *Chitala borneensis* and *Chitala hypselonotus*). Wibowo et al. (2010) noted that populations of *Chitala* in Sumatra are spatially highly structured suggesting that local (instead of regional) scale management plans are necessary for long-term sustainability. Such situation may also apply to Malaysia.

In Malaysia, so far, genetic data are very scarce, and they provide limited clue on the diversity of *Chitala*. Sultana et al. (2018) designed new mini-COI barcodes for "*Chitala lopis*" collected in Malaysia but the precise locality of the used specimens is unknown. In absence of taxonomic revision, it is uncertain how these barcodes can be applied to populations/species of *Chitala* in Malaysia. There is one COI sequence archived in GenBank without precise origin (GenBank accession number: KT001050).

2.7 Molecular Taxonomy

Twenty years ago, taxonomic research has evolved rapidly with the introduction of genetic marker for delimitating and identifying species. Often referred as DNA barcoding, a short DNA fragment has been used as a molecular marker to delimitate species accurately (Hebert et al., 2003a, 2003b). If each species

has a unique DNA “barcode”, which diverge “significantly” to any other species, the basis of molecular taxonomy is that the DNA “barcodes” of individuals allow their taxonomic classification (Aquilino et al., 2011). The barcode act as an identity card that provide fast, accurate and reliable information on the taxonomical identity of the species and this helped researchers in understanding more about the evolutionary relationships of the species (Hebert & Gregory, 2005).

Before the introduction of molecular taxonomy, the identification of a species was determined solely based on the morphological examination since it was the only source of characters available. However, Hubert et al. (2016) and Kadarusman et al. (2012) who studied the ichthyofauna in Indonesia waters, showed that there are limitations on relying solely on morphological examination. Thus, by utilizing modern taxonomic techniques such as DNA barcoding identification, it becomes possible to address certain challenges that arise when relying solely on morphology. For example, intraspecific morphological variation commonly occurs and may overlap with interspecific variation, this may cause confusion to identify closely similar species (Hebert et al., 2004; Packer et al., 2009; Viswambharan et al., 2015). In addition, morphology-based taxonomy is limited to the adult life stage of an organism while DNA barcodes can provide taxonomic information regardless of the life stages of the animal (Hubert et al., 2010; Ko et al., 2013). DNA barcodes also provide taxonomic information from any biological tissue available, and it is useful in forensic sciences (Holmes et al., 2009; Wong & Hanner, 2008). Finally, morphologically cryptic species can be discovered when applying a molecular taxonomic approach (Hubert et al., 2012; Janzen et al., 2005; Mat Jaafar et al., 2012; Smith et al., 2007, 2008).

Several taxonomists adopted a modern taxonomic method by including DNA barcoding approach on top of morphological examination. That led to the discovery of new species by revealing interspecific divergence (Hebert & Gregory, 2005; Ward, 2009; Ward et al., 2005, 2009). Aquilino et al. (2011) and Decru et al. (2016) demonstrated DNA barcoding efficiency in identifying species reaching a 100% success rate with 80% to 100% accuracy. Viswambharan et al. (2015) and Trivedi et al. (2016) resolved long-standing taxonomic ambiguities which were the consequence of the presence of cryptic species. In addition, traditional morphology methods often fail to identify fish larvae and juveniles (even adult specimens are at risk of being misidentified when using only morphological method). According to Ko et al. (2013), morphological identification only achieved a very low percentage of successfulness in identifying larval fishes with only 13.5% of fishes identified. In contrast, DNA barcoding method can accurately and quickly provide species names (Viswambharan et al., 2015).

Mitochondrial and nuclear markers are currently easily available for molecular taxonomy. Among those genes, the mitochondrial protein-coding gene are widely used due to their advantages when compared to nuclear genes (protein-coding or ribosomal RNA genes). Mitochondrial genes are known to be maternally inherited, meaning the intraspecific species diversity will be fixed since there is no recombination of DNA (Avise et al., 1984). Mitochondrial DNA (mtDNA) is proved to be good in delimitation species level organisms due to its higher rate of nucleotide substitution compared to nuclear genes. In addition, the nature of mtDNA that has less insertions and deletions of nucleotides is also one of the advantages of mtDNA in molecular taxonomy. In addition to having lower likelihood for mutations, if by any chance a mutation occurs, it usually involves the addition or deletion of a codon

(consist of three nucleotides) altogether which subsequently will make the alignment of amino acid sequence unambiguous (Thacker, 2003).

The part of mitochondrial DNA genome that will be used for DNA barcoding must be chosen carefully. Since each part of the mitochondrial genome has its own rate of molecular evolution, the barcode needs to be able to precisely resolve taxonomic ambiguities among closely related species (Johns & Avise, 1998; Moore, 1995): the intraspecific (i.e., within species) genetic divergence must be lower than the interspecific (i.e., between species) genetic divergence (Hebert et al., 2003a). The barcode (i.e., molecular marker) should be able to be sequenced directly in a single reaction, and it should be restricted by two conserved regions for universal primers to bind to it during PCR (Kress & Erickson, 2008). The most practical and suitable gene regions that can be used as DNA barcodes are the cytochrome c oxidase subunit I gene (COI gene) and the cytochrome b gene (cytb gene). These two genes satisfy the criteria to be utilized as DNA barcode.

2.7.1 Cytochrome c oxidase subunit I gene (COI gene)

The cytochrome c oxidase subunit I (COI) gene is widely used in molecular taxonomy (to delimitate and identify species) and in phylogenetics (to infer the evolutionary relationships among species). As a component of the mitochondrial DNA, COI is highly conserved across many taxa, which allows for the identification of evolutionary relationships among species (Hebert et al., 2003b). Its rapid mutation rate makes COI particularly effective for discerning species boundaries, especially in groups with cryptic diversity (Kress et al., 2015). For instance, in fish, COI has been successfully applied to resolve ambiguities in species identification and assess

biodiversity within ecosystems, providing critical insights into conservation efforts (Ward et al., 2005).

So, to achieve fish species-level identification, the 3' part of the mtDNA cytochrome c oxidase I (COI) gene is utilized by taking approximately 650 base pairs (bp) to be the 'barcode fragment' for species identification. The COI gene is one of the standard and practical barcode fragments that can be used in fish species identification (Hebert et al., 2003b). Due to its short length (~650bp), it is much easier to compare with other species than other mtDNA markers (Valentini et al., 2008). Hence, this easier way of sequencing combined with the ability to identify species in a variety of taxa are one of the reasons why the COI gene is nowadays so popular in molecular taxonomy (Johns & Avise, 1998; Moore, 1995). Lastly, detailed information on phylogenetic diversity and evolutionary history of each species also can be mapped out using DNA barcoding which can be used for conservation of species (Viswambharan et al., 2015).

The advantages of using COI in molecular analysis include its ability to provide a DNA barcode for species identification, which has been instrumental in biodiversity assessments. This approach simplifies the process of distinguishing between closely related species, as demonstrated in various studies of freshwater fish, where COI sequences successfully differentiate between morphologically similar species (Meyer & Paulay, 2005). Additionally, COI data can now be obtained from preserved museum specimens, making it a valuable tool for historical biodiversity studies (Hajibabaei et al., 2006).

2.7.2 Cytochrome b gene (cytb gene)

The mtDNA cytochrome b (cytb) gene was utilized in molecular analyses for fish species delimitation due to its advantageous characteristics and efficiency, in particular for vertebrates, including fish (Kocher et al., 1989). The cytb gene is highly conserved among vertebrates, making it suitable for phylogenetic studies while displaying sufficient variation to distinguish closely related species (Kocher et al., 1989). This gene encodes a protein involved in the electron transport chain, playing a critical role in cellular respiration, which ensures its conservation across species.

One of the key advantages of using the cytb gene in molecular taxonomy is its ability to provide deeper phylogenetic insights compared to other markers, especially in cases where species have diverged long ago (Hebert et al., 2003a). Its slower mutation rate allows for the resolution of evolutionary relationships at the species level and can facilitate the identification of cryptic diversity, often overlooked when using faster-evolving markers (Hebert et al., 2003a). Studies have shown that the cytb gene provides reliable phylogenetic data, yielding consistent results across various fish taxa (Kocher et al., 1989). This includes the genus *Chitala*, whose species can be difficult to differentiate based on morphology alone due to their similar physical characteristics (Dutta et al., 2020).

The cytb gene has been shown to yield better resolution in species complexes compared to the commonly used cytochrome c oxidase subunit I (COI) gene, which is often employed for barcoding and can sometimes lead to ambiguous results due to saturation effects in rapidly evolving lineages (Kocher et al., 1989). While COI is commonly used for species identification due to its high variability across species, the cytb gene provides more detailed insights into evolutionary relationships,

including intraspecific variation and population structure, making it particularly useful for studies of genetic diversity within and among fish populations (Hebert et al., 2003a).

CHAPTER 3

MORPHOLOGICAL TAXONOMY OF *CHITALA* IN PENINSULAR MALAYSIA

The genus *Chitala*, belonging to the family Notopteridae, includes a group of freshwater featherback fishes found throughout Southeast Asia (Fricke et al., 2025). These species are distinguished by their elongated, compressed bodies and long anal fins, which contribute to their characteristic knife-like shape (Ahmadi et al., 2019; Hilton & Lavoué, 2018; Lavoué & Sullivan, 2004). Morphological traits, including body proportions, fin ray counts, and scale patterns, have historically been used to differentiate species within this genus (Roberts, 1992; Kottelat & Widjanarti, 2005). However, morphological variation within the genus *Chitala* presents challenges for species delimitation. There are no clear or distinct morphological differences among species of *Chitala* and this makes precise identification difficult (Roberts, 1992). Hence, there is a lack of comprehensive morphological studies on this genus, and the apparent absence of differentiation may only be the result of this situation (Hubert et al., 2016; Kadarusman et al., 2012).

This study aims to address the taxonomy of giant featherback fishes in Malaysia through a comprehensive morphological analysis. One objective is to identify and describe reliable morphological characteristics that can distinguish each species within the genus *Chitala*, focusing on features such as morphometric measurements, meristic counts, and body marking patterns. Additionally, the research seeks to establish a robust morphological framework that allows for precise species identification, which is particularly valuable in areas lacking access to molecular tools. By integrating this information, this study will provide practical

morphological keys for the accurate classification and conservation management of featherback fishes in Malaysian freshwater ecosystems.

3.1 Materials and Methods

All specimens *Chitala* collected for this study were mainly obtained from fish market around Peninsular Malaysia. Other than that, some specimens were obtained by casting and traditional fishing traps operated by local fishermen. All specimens from fish markets and fishermen were already dead before collection. Then, each specimen was sorted and tagged with its own catalogue number. To preserve the specimens for morphological examination, all specimens were fixed in 10% formalin for several weeks and soaked in tap water for three days to remove excess formalin. Then, the specimens were preserved in 70% ethyl alcohol for future morphological examination.

3.1.1 Specimen Collection and Data

Sixty-four specimens of *Chitala* from Peninsular Malaysia, collected between 2020 and 2023, were examined in this study. Most specimens were collected between 2021 and 2022, although there was no fixed sampling schedule. Weekly visits were made to various fish markets to check for the availability of specimens. Additionally, several specimens from 2020 collected by co-authors were included and used for the first time in this study. All specimens were deposited in the ichthyological collection of the Makmal Rujukan Zoologi (Zoological Reference Laboratory), Universiti Sains Malaysia (museum code: USMFC). Two unregistered