

Redefining Chitala and Notopterus genera in Indonesian Freshwate Ecosystem

Final Report

Prepared by: Arif Wibowo, Haryono, Kurniawan Vitas Atmadi Prakoso, Hadi Dahruddin, Indah Lestari, Pesigrihastamadya Normakristagaluh, Yulia Aris Kartika

FAO Ifish

Project : GCP/INS/303GFF- Mainstreaming Biodiversity Conservation and Sustainable Use into Inland Fisheries Practices in Freshwater Ecosystem of High Conservation Value

Acknowledgment

This project was carried out to support the strategic program of mainstreaming biodiversity conservation and sustainable use into inland fisheries practice in freshwater ecosystem of high conservation value in Indonesia funded by FAO I-Fish Project. We would like to thank Sudarsono as National Project Manager I-FISH program FAO Indonesia and FAO Indonesia's staff.

We would also like to acknowledge the assistance and information provided by all stakeholders, in particular, from the following institution and organization: Head of the Research Center of Fisheries, Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia (MMAF) and staff, Director of Marine Biodiversity Conservation of the Ministry of Maritime Affairs and Fisheries (MMAF) of the Republic of Indonesia and staff, Head of the Pontianak Coastal and Marine Resources Management Center (BPSPL Pontianak) and staff, Head of BPSPL Padang and staff, Chief of the Selaras Hijau Indonesia Foundation, Head of Computing Research Center of National Research and Innovation Agency (BRIN) Republic of Indonesia, Head of Artificial Intelligence and Cybersecurity Research Center (BRIN), Head of Biosystematics and Evolution Research Center (BRIN), Head of Research Center for Conservation of Marine and Inland Waters Resources (BRIN), Fisheries Extensions at Research Institute for Freshwater Aquaculture and Fisheries Extensions, Bogor; Center for Fisheries Training and Extension, Tegal and Center for Fisheries Training and Extension, Banyuwangi, Research Institute for Marine Aquaculture and Fisheries Extension, Gondol, Research Institute for Inland Fisheries and Fisheries Extension in Palembang, Fisheries and Marine Office Tebo Regency, Local Government of Food Security Service, Agriculture, Fisheries of South Barito Regency, Fisheries and Marine Office of Seruyan Regency, Manager of Cooperation and Coorporate Social Responsibility of PT Kilang Pertamina Internasional Refinery Unit III Plaju, and Mr. Marimin (Fishing Community Cisadane-Tangerang, Banten).

We would also like to acknowledge the valuable facilitation provided by Data and Information Center of BRIN for the provision of the forIDN website and the Director of Scientific Collection Management of BRIN for utilizing the featherback fish collection at the Bogor Zoological Museum and our colleagues for the valuable feedback Dr. Nicholas Hubart (Institute of Des Sci-ences De L'evolution De Montpellier, France), Dr. Harald Ahnelt (University of Wienna, Vienna), Dr. Meaghan Duncan (Department of Primary Industries, New South Wales, Australia), and Dr. Satoshi Nagai (Japan Fisheries Research and Education Agency).

ABBREVIATIONS AND ACRONYMS

ABD	Antonion Dody Donth
	Anterior Body Depth
ABL	Anal Base Length
AFL	Anal Fin Length
ASAP	Assemble Species by Automated Partitioning
ASNL	Anterior Snout Length
BD	Body Depth
BIC	Bayesian Information Criterion
BIN	Barcode Index Numbers
BOLD	Barcode Of Life Data Systems
BPSPL	Pontianak Coastal and Marine Resources Management / Balai Pengelolaan Sumber Daya Pesisir dan Laut Pontianak
BRIN	National Research and Innovation Agency/ Badan Riset dan Inovasi Nasional
BW	Body Width
C. borneensis	Chitala borneensis
C. hypselonotus	Chitala hypselonotus
C. lopis	Chitala lopis
Chitala spp.	Chitala spesies
cm ²	Square Centimeter
COI	Cytochrome Oxidase Sub Unit 1
CPD	Caudal Peduncle Depth
CPL	Caudal Peduncle Length
CSR	Coorporate Social Responsibility
DFA	Discriminant Function Analysis
DFBL	Dorsal Base Length
DFL	Dorsal Fin Length
DNA	Deoxyribonucleic Acid
ED	Eye Diameter
FAO	Food And Agriculture Organization of The United Nations
forIDN	Fish Occurrence Records of Indonesia / Https://Foridn.Brin.Go.Id/
GMYC	General Mixed Yule-Coalescent
HD	Head Depth
HL	Head Length
IUCN	The International Union For The Conservation Of Nature
IW	Eye Distance
JABA	Population Of Chitala and Notopterus in West Java
JATE	Population Of Chitala and Notopterus in Central Java
JATI	Population Of <i>Chitala</i> and <i>Notopterus</i> in East Java
K2P	Kimura 2-Parameter
LJL	Lower Jaw Length
LMPG	Population Of <i>Chitala</i> and <i>Notopterus</i> in Lampung
mGMYC	Multiple General Mixed Yule-Coalescent

ML	Maximum Likelihood
MMAF	Ministry of Marine Affairs and Fisheries, Republic of Indonesia
MOTU	Molecular Operational Taxonomic Unit
mPTP	Multiple Poisson Tree Process
mtDNA	Mitochondrial DNA/ Mitochondrial Deoxyribonucleic Acid
MZB	Museum Zoologicum Bogoriense /Museum of Zoology
N. Notopterus	Notopterus Notopterus
ND	Nucleotide Difference
PAL	Pre-Anal Length
PBD	Posterior Body Depth
PCA	Principle Component Analysis
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDL	Pre-Dorsal Length
PEFL	Pectoral Fin Length
PEPL	Pectoral-Pelvic Length
PFL	Pelvic Fin Length
PLMB	Population of Chitala and Notopterus in Palembang
PPAL	Pelvic-Anal Length
PPEL	Pre-Pectoral Length
PPL	Pre-Pelvic Length
PTP	Poisson Tree Process
PVL	Pre-Ventral Length
RESL	Refined Single Linkage
sGMYC	Single General Mixed Yule-Coalescent
SL	Standard Length
SNL	Snout Length
sPTP	Single Poisson Tree Process
UCLN	The Uncorrelated Lognormal Clock
UJL	Upper Jaw Length
VFL	Ventral Fin Length

LIST OF CONTENTS

Exec	utive Summary	8
I.]	Introduction	12
II. 1	Material and Methods	14
Sa	mpling and Collection Management	14
M	orphology Analysis	18
M	olecular Analysis	20
	Polymerase Chain Reaction (PCR), Sequencing and International Repositories	20
	Genetic Species Delimitation and Phylogenetic Inferences	21
Ca	ttaloging Sample Collection of Chitala and Notopterus Genera In Museum Zoological	
Bo	ogorianse (Cataloging In MZB)	23
De	evelopment of Fish Record Database Platform	24
Pr	ocess Related to Existing Samples originating from Palembang	24
III. I	Results	26
Ch	nitala	26
	Morphometric Analysis	26
	Molecular Analysis	29
Na	otopterus	32
	Morphology Analysis	32
	Molecular Analysis	34
En	vironmental DNA survey	36
Sc	ale Surface Morphology and Microstructure Examination Using Scanning Electron	
M	icroscopy (SEM)	39
Up	odating Specimen Collection for MZB	41
De	evelopment of Fish Record Database Fish Occurrence Records of Indonesia (forIDN)	
Pla	atform	42
IV. I	Discussion	45
Re	e- Description of Chitala Genera in Indonesia	45

Review for IUCN Status of Chitala	48
Updating Molecular and Morphological Aspect of Notopterus	49
Chitala And Notopterus Genera Prospective Distribution According To Environm	ental DNA
And Their Genetic Links Using SNP Analysis	50
Elaboration Outcomes And Possible Implications Arising From Results	53
Contribution of External Expert in Research	57
V. Conclusion and Recommendation	57
Conclusion	58
Recommendation	58
VI. References	59
VII.Appendix I Form Questionnaire	66
VIII. Appendix II List of Catalog Number MZB Labelling on Sample	75
IX. Appendix III Distribution and Abundance of Chitala Genera using Questionn	aire81
X. Appendix IV Phylogenic Tree of SNP Anaysis	

Figures and Tables

List of Figures

Figure 1 Pre-Survey Coordination Meeting (Online via Zoom Platform) in Java Region15
Figure 2 Pre-Survey Coordination Meeting (Online via Zoom Platform) Kalimantan Region15
Figure 3 Location of Water Habitat of South Kalimantan and Central Kalimantan16
Figure 4 Fishery Market in South Kalimantan region (left) and Central Java Region (right)16
Figure 5 Interview with local community and local stakeholder in Java Region
Figure 6 Sampling site of Family Notopteridae (Chitala sp. and Notopterus) in Indonesia17
Figure 7 Collecting muscle tissue of specimen for Molecular Analysis and Labeling Process18
Figure 8 Morphological Measurement of Specimen20
Figure 9 Preparation and Extraction Sampel for PCR
Figure 10 Preserved and Labeld Specimen
Figure 11 Discussion with Other Researcher regarding Morphological Phenomena24
Figure 12 Examination Process of Existing Samples in Palembang, South Sumatera25
Figure 13 C.hypselonotus from Langam, Sungai Kampar Kanan, Riau
Figure 14 C.hypselonotus from Kuala Tolam, Sungai Kampar Kanan downstream, Riau
Figure 15 Principle Component Analysis (PCA) of <i>Chitala</i> Characters
Figure 16 The Presence of <i>Chitala</i> based on Field Survey and Fishermen Information30
Figure 17 Mitochondrial gene tree for the 184 DNA barcodes of Chitala spp
Figure 18 Distribution Range for <i>C. lopis</i> Widely Distributed in Java, Sumatra and Borneo31
Figure 19 The Presence of <i>Notopterus</i> based on Field Survey and Fishermen Information32
Figure 20 Result of PCA for <i>Notopterus</i> Species
Figure 21 Mitochondrial gene tree for the 161 DNA barcodes of <i>N. notopterus</i>
Figure 22 Haplotype network reconstructed based on the 117 sequences
Figure 23 Barplot Relative Abundance of Identified Genus in 9 Sampling Area

Figure 24 Pie Chart Relative Abundance of Identified Species in 9 Sampling Area
Figure 25 Species Occupancy in 9 Sampling Area
Figure 26 Scanning Microstructure of Scale41
Figure 27 Voucher Chitala and Notopterus Specimen Collection
Figure 28 View Menu of "Collector"
Figure 29 Three main buttons on the "collector" menu
Figure 30 View Menu of "Fish"
Figure 31 Three main buttons on the "Fish" menu44
Figure 32 Search results using the keyword "Chordata"
Figure 33 Example for Summary Result Page for "Chordata" key words44
Figure 34 Photograph of selected specimens of <i>Chitala lopis</i> 46
Figure 35 Complete MtDNA sequences in <i>Chitala</i> 47
Figure 36 Phylogenetic dendrogram showing the sequences of <i>C. hypselonotus</i> at its locality47
Figure 37 Notopterus Notopterus
Figure 38 Chitala hypselonotus
Figure 39 Chitala lopis
Figure 40 Chitala borneensis

List of Table

Table 1 Location and Time Period of Field Survey	14
Table 2 Site Exploration in Palembang	26
Table 3 Morphometric Analysis Result of Chitala	26
Table 4 Barcode Gap of Chitala Species	31
Table 5 The Different Characters Result of SEM Analysis	40

Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem

Executive Summary

Fishes of the genus *Chitala* and *Notopterus* belong to the family Notopteridae, a commonly known family as an iconic lineage of tropical freshwater fishes inhabiting the lowlands of Africa and Asia. This group is among the most ancient group of extant freshwater fish lineages with an origin tracing back to the Early Cretaceous. Three species of *Chitala* have been reported in Java, Sumatra and Borneo, including *Chitala lopis*, *Chitala hypselonotus*, and *Chitala borneensis*. According to the IUCN, *Chitala* species are of least concern in Indonesia, excepting *C. lopis*, which is now considered extinct. Recently, all Notopterid species are currently fully protected by the Indonesian Ministry of Marine Affairs and Fisheries (MMAF) to prevent their extinction. On the other hand, the taxonomy of *Chitala* species is also confusing in Indonesia, the three species being either considered synonyms or considered valid species. The Food and Agriculture Organization of the United Nations collaborated with The Ministry of Marine Affairs and Fisheries, Yayasan Selaras Hijau Indonesia, and The National Agency for Research and Innovation (BRIN) to solve these issues and conducting research and activities through key main activities including:

- Updated taxonomic status of *C. lopis* in Indonesia, which can be used as a reference to review IUCN extinction status and existing national regulation
- 2) Internationally published scientific paper(s) reviewing taxonomic status of *Chitala* and *No-topterus* genera in Indonesia through genetic and morphological approaches
- Inventoried scientific collection of complete Notopteridae specimens from Indonesian inland water in Museum Zoologicum Bogoriense- BRIN
- 4) Fully functioned, open-source digital database platform to input, store, process, and display various information about *Chitala* and *Notopterus* genera

We undertook field surveys in three main islands of Indonesia to collect new specimen of the fishes in 34 locations and distributed questionnaires to detect the present population status of the featherback through 152 fishermen in 55 locations including Java, Sumatra and Borneo. In total, there were 209 notoptorids fish collected during the surveys covering 74 *Chitala* genera and 135 *Notopterus* genera. The specimens were deposited at the Museum Zoologicum Bogoriense -BRIN. Furthermore, DNA barcode, morphometric and meristic analysis was conducted to clarify the taxonomic and genetic status of the featherback.

The recent re-discovery of *C. lopis* in its type locality after several decades finally enabled a comprehensive comparative study of the three species. By examining 150 mitochondrial sequences

from all known species of *Chitala*, including sequences from the type localities of the three species, the three species are recognized by tight clusters of sequences, which were recovered by sequencebased species delimitation methods. The analyses of 22 morphomeristic measurements identified several diagnostic characters between *C. lopis* and *C. borneensis*. In terms of *Notopterus* genera, seventy-three Indonesian sequences of *Notopterus Notopterus* were divided into two major haplotype groups. The result of phylogenetic tree topology confirmed monophyly of the genus *Notopterus* and it reveals that all individuals originating from Indonesia represent monophyletic entities, showed genetic differences and the sequences were unique from their relatives from Southeast Asia ancestors.

The present study provides evidence supporting the recognition of existing three species of *Chitala* in Indonesia namely *C. lopis*, *C. borneensis*, and *C. hypsolonetus*, thanks to the rediscovery of *C. lopis* in its type locality, and as such puts an end to two decades to taxonomic confusion in the group. The presence of *C. lopis* in the Cisadane River in Java finally confirms that the extinction status of *C. lopis* in the IUCN needs to be revised. Species range distribution is profoundly revised for each of the three species here, *C. lopis* being the most widespread *Chitala* species in Indonesia as this species was previously only recorded in Java. Therefore, the distribution of *C. lopis* would encompass Java, Sumatra and Borneo. The study largely revises known species range distribution as *C. borneensis* appears to be widespread in West Borneo and Jambi, while *C. hypselonotus* appears to be endemic to its type locality in Musi River, South Sumatra. Present evidence argues for an urgent revision of the IUCN conservation status of the three species. *Notopterus Notopterus* is found to be a single species form with different genetic structures within population of Sumatra, Java, and Borneo.

As they are, present results indicate that the IUCN conservation status of *C. borneensis* and *C. hypselonotus* should be urgently revised, while the wide distribution of *C. lopis* calls for locally adapted conservation plans. The present study further provides the first comprehensive DNA barcode reference library for *Chitala* spp., enabling automated identification of *Chitala* species in the future, a tool that opens new perspectives in terms of conservation and management.

Ringkasan Singkat

Genus *Chitala* dan *Notopterus* termasuk dalam famili Notopteridae, famili yang umumnya dikenal sebagai keturunan ikonik ikan air tawar tropis yang menghuni dataran rendah Afrika dan Asia. Kelompok ini adalah salah satu kelompok paling purba dari garis keturunan ikan air tawar yang masih ada dengan asal usul yang ditelusuri kembali ke zaman *Cretaceous*. Tiga spesies *Chitala* telah dilaporkan di Jawa, Sumatera dan Kalimantan, termasuk *Chitala lopis, Chitala hypselonotus*, dan *Chitala borneensis*. Menurut IUCN, spesies *Chitala* termasuk dalam status *least concern* di Indonesia, kecuali *C. lopis* yang kini dianggap punah. Saat ini, status seluruh spesies Notopterid dilindungi sepenuhnya oleh Kementerian Kelautan dan Perikanan (KKP) Indonesia untuk mencegah kepunahan spesies tersebut. Di sisi lain, taksonomi spesies *Chitala* juga yang belum jelas di Indonesia juga menjadi permasalahan dimana ketiga spesies tersebut dianggap sinonim atau spesies yang valid. Oleh karena itu, *Food and Agriculture Organization of the United Nations* bersama dengan Kementerian Kelautan dan Perikanan, Yayasan Selaras Hijau Indonesia, dan Badan Riset dan Inovasi Nasional (BRIN) bekerjasama untuk memecahkan masalah ini dan melakukan penelitian dan kegiatan melalui kegiatan utama yaitu:

- 1) Memperbaharui status taksonomi *C. lopis* di Indonesia, yang dapat digunakan sebagai acuan untuk meninjau status kepunahan IUCN dan peraturan nasional yang ada
- Mempublikasikan karya tulis ilmiah yang diterbitkan secara internasional untuk meninjau status taksonomi genus *Chitala* dan *Notopterus* di Indonesia melalui pendekatan genetik dan morfologi
- Inventarisasi koleksi ilmiah spesimen Notopteridae lengkap dari perairan pedalaman Indonesia di Museum Zoologicum Bogoriense-BRIN
- Merancang platform bebas berbasis data digital yang berfungsi penuh untuk memasukkan, menyimpan, memproses, dan menampilkan berbagai informasi tentang genus *Chitala* dan *No-topterus*

Survei lapangan dilakukan pada tiga pulau utama di Indonesia untuk mengumpulkan spesimen ikan dengan jenis tersebut di 34 lokasi dan menggunakan kuesioner untuk mendeteksi status populasi *Chitala* dan *Notopterus* saat ini melalui 152 nelayan di 55 lokasi termasuk Jawa, Sumatera dan Kalimantan. Total sampel *Chitala* yang terkumpul selama survei sebanyak 209 ekor yang meliputi 74 *Chitala* dan 135 *Notopterus*. Spesimen disimpan di Museum Zoologicum Bogoriense -BRIN. Selanjutnya, DNA barcode, analisis morfometrik dan meristik dilakukan untuk mengklarifikasi status taksonomi dan genetik bulu. Penemuan kembali *C. lopis* pada studi ini dalam beberapa dekade akhir ini, menciptakan studi komparatif yang komprehensif dari ketiga spesies tersebut. Pemeriksaan 150 sekuens mitokondria dari semua spesies *Chitala* yang diketahui, termasuk sekuens dari tipe lokalitas dari tiga spesies, ketiga spesies tersebut dikenali lewat pengelompokan sekuens yang rumit, yang ditemukan dengan metode delimitasi spesies berbasis sekuens. Analisis dari 22 pengukuran morfomeristik mengidentifikasi beberapa karakter diagnostik antara *C. lopis* dan *C. borneensis*. Pada genus *Notopterus*, 73 sekuens *Notopterus Notopterus* Indonesia dibagi menjadi dua kelompok haplotipe utama. Hasil topologi pohon filogenetik mengkonfirmasi monofili dari genus *Notopterus* dan mengungkapkan bahwa semua individu yang berasal dari Indonesia mewakili entitas monofiletik, menunjukkan perbedaan genetik dan sekuens yang unik dari kerabat mereka dari nenek moyang Asia Tenggara.

Penelitian ini memberikan bukti yang mendukung adanya tiga spesies *Chitala* di Indonesia yaitu *C. lopis*, *C. borneensis*, dan *C. hypselonotus*, dan adanya penemuan kembali *C. lopis* di lokalitas tipenya, dengan demikian mengakhiri dua dekade untuk kebingungan taksonomi dalam kelompok spesies *Chitala*. Kehadiran *C. lopis* di Sungai Cisadane di Jawa akhirnya menegaskan bahwa status keberadaan *C. lopis* di IUCN perlu direvisi. Penyebaran spesies direvisi secara mendalam untuk masing-masing dari tiga spesies di sini, *C. lopis* menjadi spesies *Chitala* yang paling tersebar luas di Indonesia yang sebelumnya hanya tercatat di Jawa. Dengan demikian, dapat dikatakan persebaran *C. lopis* meliputi Jawa, Sumatera dan Kalimantan. Studi ini sebagian besar merevisi distribusi spesies yang diketahui karena *C. borneensis* berada tersebar luas di Kalimantan Barat dan Jambi, sementara *C. hypselonotus* menjadi endemik di lokasi aslinya yaitu Sungai Musi, Sumatera Selatan dan Sungai Kampar, Riau.. Bukti saat ini dapat mendukung revisi mendesak status konservasi IUCN dari ketiga spesies tersebut. Sedangkan untuk *Notopterus Notopterus* ditemukan dalam bentuk spesies tunggal dengan struktur genetik yang berbeda pada populasi Sumatera, Jawa, dan Kalimantan.

Seperti yang telah diungkapkan sebelumnya, hasil saat ini menunjukkan bahwa status konservasi IUCN untuk *C. borneensis* dan *C. hypselonotus* harus segera direvisi, sedangkan distribusi *C. lopis* yang luas membutuhkan rencana konservasi yang diadaptasi secara lokal. Studi ini juga memberikan pustaka referensi kode genetik DNA komprehensif pertama untuk *Chitala* spp., yang memungkinkan identifikasi secara otomatis spesies *Chitala* di masa depan, suatu alat yang membuka perspektif baru dalam hal konservasi dan pengelolaan.

I. Introduction

Known as featherback, knife fish, or locally as featherback, the genus *Chitala* belongs to family Notopteridae, which is commonly known as an iconic lineage of tropical freshwater fishes inhabiting the lowlands of Africa and Asia. The family comprises four genera namely *Chitala* and *Notopterus*, distributed across Asia from India to Southeast Asia, and *Xenomystus* and *Papyrocranus*, occurring in West and Central Africa (Roberts 1992; Inoue et al. 2009; Froese and Pauly 2020). This family is among the most ancient among extant freshwater fish lineages with an origin tracing back to the Early Cretaceous, and associated with the splitting of the African (*Papyrocranus* and *Xenomystus*) vs Asian (*Notopterus*, *Chitala*) clades (Inoue et al. 2009). Its ancient origin, confinement to freshwater ecosystems and restricted distribution to the tropics of Africa and Asia suggest its current distribution is refugial. The four genera comprise only a handful of species ranging from one in *Xenomystus* (Eschmeyer et al. 2018; Froese and Pauly 2020), two in *Notopterus* (Lavoué et al. 2020) and *Papyrocranus* (Eschmeyer et al. 2018; Froese and Pauly 2020) to six in *Chitala* (Eschmeyer et al. 2018; Froese and Pauly 2020). Despite the low diversity in this family, taxonomic confusion has remained for decades, particularly within the Asian lineage (Roberts 1992; Kottelat et al. 1993; Kottelat 2005; Lavoué et al. 2020).

Of the four genera of knife fish, two occur in Indonesian freshwater ecosystems, namely *Chi-tala* and *Notopterus*. Both genera are widely distributed in Sundaland, over the islands of Sumatra, Java and Borneo (Kottelat et al. 1993; Hubert et al. 2015), and are represented by four species including *Chitala lopis, Chitala hypselonotus, Chitala borneensis*, and *Notopterus Notopterus*. According to the International Union for the Conservation of Nature (IUCN), *Chitala* species are of least concern in Indonesia, except *C. lopis*, which is now considered extinct, and none of them are listed in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). However, rapid stock decline has been observed in Sumatra and Java during the last two decades (Hubert et al. 2015; Dahruddin et al. 2017) and all Notopterid species are currently protected by the Ministry of Marine Affairs and Fisheries (MMAF) to prevent their extinction. In the case of *C. lopis*, it is only known from the Cisadane river, its type locality, where the species haven't been observed for decades now (Ng 2022).

Uncertainties about the conservation status of *Chitala* species are to be related to the confusion around species identity and distribution in Sundaland, which prevents relevant stakeholders from reviewing and adjusting their protection status. Robert (Roberts 1989), stated that Asian Notopterids are not widely known and the entire group must be revised before species can be properly identified in their natural habitat. Later, Roberts (Roberts 1992) stated that Indonesia likely hosts a single species, namely *C. lopis*, and other species represent distinct phases of *C. lopis* life cycle, varying only in subtle color differences and size. This synonymizing of *C. borneensis* and *C. hypselonotus* with *C. lopis* by Roberts (Roberts 1992) was later challenged by Kottelat and Widjanarti (2005), who pointed out inconsistencies in the association between coloration patterns and size, and concluded that until further evidence, the three species should be considered as valid. This debate casted doubt *C. borneensis* and *C. hypselonotus* are valid species, a confusion which has lasted until now. Type localities of the three species occur in Sundaland. The type locality of *C. borneensis* is in Sambas in Western Borneo, while the type locality of *C. hypselonotus* is Musi River in Palembang and type locality of *C. lopis* is Cisadane river in Java. However, the historical lack of observation of *C. lopis* in the Cisadane river and the recent apparent disappearance of *Chitala* from the Musi River due to overharvesting (Hubert et al. 2015; Dahruddin et al. 2017) have prevented the comparison of specimens from type localities and allowed the perpetuation of taxonomic confusion among the three species.

In order to enable the development of effective conservation plans for the *Chitala* species in Indonesia, species boundaries and distribution range of *Chitala* species should be urgently characterized. DNA barcoding, the use of the mitochondrial Cytochrome c oxidase I (COI) gene as an internal tag for animal species identification, recently helped resolve similar controversies in other problematic fish groups in Sundaland. By sequencing COI gene for multiple individuals and populations across species range distribution, including type localities, and applying DNA-based species delimitation, the identity of species and their distribution can be clarified (Keith et al. 2017; Hubert et al. 2019; Sholihah et al. 2020; Dahruddin et al. 2021) and diagnostic morphological characters proposed (Keith et al. 2017, 2020; Mennesson et al. 2021). Based on the recent observation of *Chitala* specimens in the Cisadane river together with a comprehensive DNA barcoding and morphometric study of *Chitala* species across the range distribution of the genus in Sundaland, a thorough re-examination of species boundaries and range distribution is presented and implications for conservation are discussed.

The bronze featherback, *Notopterus Notopterus*, is currently the only valid species name of the genus *Notopterus*, a species widely distributed from the Indus (Pakistan and India) in the westernmost to the Mekong region and to Java (Indonesia) in the southernmost (Lavoue 2020). Wibowo *et al.* (2009), found individual samples of this species from east Borneo, although previously the bronze featherback had never been recorded from the island of Borneo (Roberts 1992; Kottelat and Widjanarti 2005; Parenti and Lim 2005). Specimens of *N. Notopterus* can be clearly identified from all other freshwater fish by their tapered tail and the corner of their mouth under the eyes (not behind as in the genus *Chitala*). It has been reported that this species reaches a standard length of up to 60 cm (Roberts 1992). Talwar & Jhingran (1991) observed that *N. Notopterus* had a maxilla that extended only to the middle of the eye, the dorsal fin was inserted more towards the tip of the snout and the craniodorsal profile of the fish was almost straight. In addition, this species is distinguished by a jaw that continues to grow throughout its lifetime, which is limited to the posterior border of the eye. *N. Notopterus* is categorized as a complex species, in a valid sense the species still needs to be further identified, so it is placed in the category of Least Concern by the IUCN. These species are at high risk of extinction due to overfishing and pollution through factory and agricultural wastes (Ng 2010).

II. Material and Methods

Sampling and Collection Management

Sampling was conducted between February 2022 and September 2022 throughout the range distribution of the genus *Chitala* in Sundaland (Roberts 1992; Kottelat et al. 1993; Kottelat 2005; Hubert et al. 2015). Sampling locations were selected based on literature review, questionnaires, and interviews with experts and local people in every location. Detection of featherback also conducted by interviews and questionnaire with 152 fishermen distributed in 55 locations on three islands (Java, Borneo, and Sumatera). Table 1 shows the location and period of field survey.

No.	Province / Location	Period	
1.	South Sumatra- Lampung	4- 12 April 2022	
2.	West Java, Banten, Tegal, DKI Jakarta	10 – 17 March 2022	
3.	Central Java- East Java	10 – 17 March 2022	
4.	Riau-Jambi	3-11 June 2022	
5.	West Kalimantan	12-20 August 2022	
6.	East Kalimantan – Central Kalimantan	12-19 August 2022	
7.	Tangerang, Banten	10 - 25 September	

Table 1 Location and Time Period of Field Survey

In summary, the survey carried out in two stages, there are:

Preliminary Survey

Preliminary survey was carried out in 7-10 days before departure to re-ascertain site location, equipment and materials, as well as communication and correspondence in the form of discussions, interviews with central and regional stakeholders also fisheries extension workers in each region. The sampling location was chosen based on the expert judgment, literature review (journals, scientific and research publications, fisheries data from each location, Statistic Bereau data), and information from fisheries extension and producers in the site.

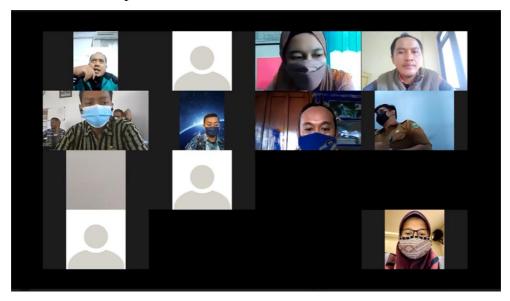


Figure 1 Pre-Survey Coordination Meeting (Online via Zoom Platform) in Java Region with Extension Workers and Regional Fisheries Service



Figure 2 Pre-Survey Coordination Meeting (Online via Zoom Platform) Kalimantan Region with Extension Workers and BPSPL-KKP

Communication with fisheries extension workers and other stakeholders began prior to the survey's departure to discuss the survey's schedule and mechanism, the availability and ordering of specimen samples, and the distribution of questionnaires. Respondens target of questionnaire are fishermen, fish finders (hobby and non-permanent), the community, local small scale business of fisheries (particularly belida and putak fish) and carried out with the assistance of extension workers in each region.

Field Survey

There were two main activities in field survey; sample preparation and sample preservation. The results of the field survey are as follows:

- 1. Samples of belida fish and/or putak fish
- 2. Coordinate points of waters and/a tau sampling
- 3. Sample documentation with measuring instruments
- 4. Water samples (Java waters)
- 5. Water quality data
- 6. Data on the results of questionnaires and / or the results of interviews with extension workers / communities / local small scale buseiness / stakeholders

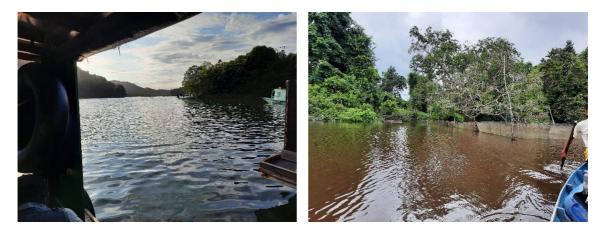


Figure 3 Location of Water Habitat of Right Riam Reservoir, South Kalimantan (left) and Katingan, Central Kalimantan (right)



Figure 4 Fishery Market in South Kalimantan region (left) Fish Auction Place in Central Java Region (right)

During the field survey, in addition to taking water coordinates and samples, other team members conducted interviews with extension workers, or local communities such as local fisherman. Qualitative data were collected using two methods: online questionnaires (<u>https://bit.ly/KuesionerOnlinebelida</u>) and in-person interviews. The total number of online questionnaire respondents from all survey locations was 152. Some of the respondents also became informants as a result of direct interviews. The interview was semi-structured, with the questions formatted in the online questionnaire section (Appendix 1) which included in survey questionnaire. The pictures below depicts the interview process with members of the local community and stakeholders in the surrounding area.



Figure 5 Interview with local community and local stakeholder in Java Region

Specimens were collected in-situ using an assortment of fishing gear including fishing rods, nets, traps gill nets and cast nets across 34 locations in Sundaland (Fig 6). The collected specimens were photographed and individually labeled, and geographic information was recorded including geocoordinates (Fig 7). Voucher specimens and tissues collection were deposited at the Research Center for the Conservation of Marine and Inland Water Resources, National Research and Innovation Agency

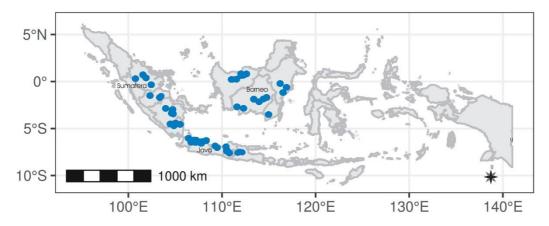


Figure 6 Sampling site of Family Notopteridae (Chitala sp. and Notopterus) in Indonesia

In order to fully document the presence of species in their distribution locations, we collected specimens based on in-situ caught or fisherman collection (available in aquariums or ponds originally from previous caught). The type of giant featherback fishing gear used varies depending on the specific location of the catch area, such as fishing rods, nets, traps widely used in Sumatra and Java and gill nets and cast nets are used in various places in Borneo.



Figure 7 Collecting muscle tissue of specimen for Molecular Analysis (left) and Labeling Process

The collected specimens were subjected to a label attributed (specific location and number), and documented based on the standard taxonomy photograph which is the head positioned on the left side and the rest of body lied on its back. For the purposes of DNA analysis, about 1 cm² of tissue was taken from the known marked specimen's muscle, dissolved in a 1.5 ml tube containing 96% absolute alcohol, for each specimen had at least one replication. Each vial tube containing the preserved specimen tissue was placed in a cryo safe box, a total of 96 tubes per box and then stored in the collection room of the Research Center for the Conservation of Marine and Inland Water Resources (BRIN). Following the completion of the process, all samples (preserved-ready specimens) were formalin preserved by injecting 40% formalin into the fish body and immersing it in 10% formalin.

Morphology Analysis

A total of 22 morphometric measurements were recorded using a dial caliper as follow: standard length (SL), from the anterior pre-maxilla to the caudal fin's central base; head length (HL), from the tip of the snout to the posterior border of the occiput; head depth (HD), measured along a line traversing perpendicularly the top of the head just above the eye and passing through the middle of the eye, to the bottom of the lower jaw; upper jaw length (UJL), from the tip of the snout to the posterior border

of the maxilla; anterior snout length (ASNL), from the tip of the snout to the rearmost nostril; snout length (SNL), from the tip of the snout to the anterior front edge of eye; eye diameter (ED), from the upper to lower border of the orbital cavity; pre-pectoral length (PPEL), from the posterior of the snout to the bottom of first pectoral fin ray; pre-pelvic length (PPL), from the tip of the snout to the bottom of anterior pelvic fin ray; pre-anal length (PAL), from the tip of the snout to the base of anterior anal fin ray; pre-dorsal length (PDL), from the tip of the snout to the bottom of first dorsal fin ray; pectoral-pelvic length (PEPL), from the base of the first pectoral ray and to the base of the first pelvic fin ray; pelvic-anal length (PPAL), from the base of the initial pelvic fin ray to the base of the final anal fin ray; posterior body depth (PBD), measured vertically from the base of the first pectoral and first pelvic fin rays; anterior body depth (ABD), maximal value measured vertically from the abdominal region to the dorsal surface in front of the pelvic fin base; pectoral fin length (PEFL), from bottom to the top of first pectoral fin ray; pelvic fin length (PFL), from bottom to the top of first pelvic fin ray; anal fin length (AFL), from first to last anal fin ray; dorsal fin length (DFL), from first to last dorsal fin ray; caudal peduncle depth (CPD), maximal value measured vertically from the caudal peduncle ventral base to its dorsal border; caudal peduncle length (CPL), from the base of posterior dorsal fin ray to the central base of the caudal fin.

All measurements other than the standard length are presented in proportion to a reference length, either standard or head length. Measurements on the lateral side of the body as well as the length of the head are presented as a percentage of standard length. All other measurements of the head are presented as a percentage of head length. Detailed morphological analysis is supported by microstructural examination of the scale surface using Scanning Electron Microscopy (SEM) on numerous individual samples representing each species in each region in addition to direct analysis of morphological parameters. The genetic laboratory BRIN-Cibinong performed this SEM analysis.

Data were analyzed by Principal Component Analysis (PCA) for *Chitala* and Discriminant Function Analysis (DFA) for *Notopterus* using MINITAB Statistical Software version 17 package. All measurements were log-transformed Measurement of morphology specimen was conducted in Fish Laboratory – Widyasatwaloka Building, BRIN. Figure 8 below is the process of measurement and analysis of sampel.



Figure 8 Morphological Measurement of Specimen

Molecular Analysis

Polymerase Chain Reaction (PCR), Sequencing and International Repositories

For the purpose of genetic analysis, a muscle biopsy of about 1 cm² of tissue was taken and preserved in a 1.5 ml tube containing 96% ethanol. Voucher specimen were preserved in a 5% formalin solution. Genomic DNA was extracted from the muscle tissue samples using a Qiagen DNeasy 96 tissue extraction kit following manufacturer's specifications. A 652-bp segment from the 5' region of the cytochrome oxidase I gene (COI) was amplified using the primer pair FishF1/FishR1 (Ward et al. 2005) or the primer cocktail C_FishF1t1/C_FishR1t1 (Ivanova et al. 2007). PCR amplifications were done on a Veriti 96-well Fast thermocycler (ABI-AppliedBiosystems) with a final volume of 10.0µl containing 5.0µl Buffer 2X, 3.3µl ultrapure water, 1.0µl each primer (10µM), 0.2µl enzyme Phire Hot Start II DNA polymerase (5U) and 0.5µl of DNA template (~50 ng). PCR amplification with the primer pair FishF1/FishR1 were done with a final volume of 25.0µl containing 12.5µl of Taq ready mix, 9.5µl ultrapure water, 1.0µl each primer (10µM) and 1µl of DNA template. Figure 9 is the process of molecular analysis in laboratory BRIN-Cibinong.



Figure 9 Preparation and Extraction Sampel for PCR

The following thermocycler regime was used with the primer cocktail: initial denaturation at 98°C for 5 min followed by 30 cycles denaturation at 98°C for 5s, annealing at 56°C for 20s and extension at 72°C for 30s, followed by a final extension step at 72°C for 5 min. The following thermocycler regime was used with primer pair FishF1/FishR1: initial denaturation at 95°C for 10 min followed by 35 cycles denaturation at 94°C for 60s, annealing at 48°C for 60s and extension at 72°C for 00s, followed by a final extension step at 72°C for 7 min. PCR products were purified with ExoSap-IT (USB Corporation, Cleveland, OH, USA) and sequenced in both directions. Sequences and collateral information were deposited on BOLD (Ratnasingham and Hebert 2007) and GenBank (Table S1).

Genetic Species Delimitation and Phylogenetic Inferences

Several methods for species delineation based on DNA sequences have been proposed (Pons et al. 2006; Ratnasingham and Hebert 2013a; Kapli et al. 2017; Puillandre et al. 2021). Each of these has different properties, particularly when dealing with singletons (i.e. lineages represented by a single sequence) or heterogeneous speciation rates among lineages (Luo et al. 2018). A combination of different approaches is increasingly used to overcome potential pitfalls arising from uneven sampling (Kekkonen and Hebert 2014; Shen et al. 2019; Sholihah et al. 2020; Arida et al. 2021). We used six different sequence-based methods of species delimitation to identify Molecular Operational Taxonomic Unit (MOTU): (1) Refined Single Linkage (RESL) as implemented in BOLD and used to generate Barcode Index Numbers (BIN) (Ratnasingham and Hebert 2013b), (2) Assemble Species by Automated Partitioning (ASAP) (Puillandre et al. 2021), (3) Poisson Tree Process (PTP) in its single (sPTP) and multiple rates version (mPTP) as implemented in the stand-alone software mptp_0.2.3 (Zhang et al. 2013; Kapli et al. 2017), (4) General Mixed Yule-Coalescent (GMYC) in its single

(sGMYC) and multiple threshold version (mGMYC) as implemented in the R package Splits 1.0-19 (Fujisawa and Barraclough 2013). Both the mPTP algorithm and the GYMC use phylogenetic trees as input file. We reconstructed a maximum likelihood (ML) tree for the former using IQ-TREE (Nguyen et al. 2015) with the most-likely substitution model according to ModelFinder following the Bayesian Information Criterion (BIC) (Kalyaanamoorthy et al. 2017) available at <u>http://iqtree.cibiv.univie.ac.at</u> (Trifinopoulos et al. 2016). For the GYMC algorithm we calculated an ultrametric, fully resolved tree using the Bayesian approach implemented in BEAST 2.6.2 (Bouckaert et al. 2014). Sequences were collapsed into haplotypes prior to reconstructing the ultrametric tree using the AL-TER online portal (http://www.sing-group.org/ALTER/), and Bayesian reconstructions were based on a strict-clock prior of 1.2% per million year (Bermingham et al. 1997). Two Markov chains of 20 million each were ran independently using Yule pure birth and GTR+I+F substitution models. Trees were sampled every 5,000 states, after an initial burning period of 5 million. Both runs were examined using Tracer 1.7.1 (Rambaut et al. 2018) (ESS>200), and combined using LogCombiner 2.6.2, and the maximum credibility tree was constructed using TreeAnnotator 2.6.2 (Bouckaert et al. 2014).

A final COI gene tree was reconstructed using the Species Tree UCLN algorithm of the Star-BEAST2 package (Ogilvie et al. 2017). This approach implements a mixed-model including a coalescent component within species and a diversification component between species that allows accounting for variations of substitution rates within and between species (Ho and Larson 2006). Species Tree UCLN jointly reconstructs gene trees and species trees, and as such requires the designation of species, which were determined using the consensus of our species delimitation analyses. The Species Tree UCLN analysis was performed with the same parameters as mentioned above.Kimura 2-parameter (K2P) (Kimura 1980) pairwise genetic distances were calculated using the R package Ape 5.4 (Paradis and Schliep 2019). Maximum intraspecific and nearest neighbor genetic distances were calculated from the pairwise K2P distance matrix using the R package Spider 1.5 (Brown et al. 2012).

Molecular and e-DNA analysis were performed using facilities with complementary activities. The following is a description of the analysis:

- 1. BRPBATPPP Genetic Laboratory- KKP, Bogor:
 - Extraction muscle tissue sampel
 - PCR
- 2. Wildlife Management and Reproduction Laboratory BRIN, Cibinong
 - Extraction muscle tissue sampel
 - PCR
- 3. PT Genetika Science Indonesia, Tangerang, Banten:

- CO1 Sequencing muscle tissue sampel Belida and Putak
- 4. PT Oceanogen Baruga, Bogor
 - Sequencing e-DNA sample
- 5. DArt Australia
 - Sequencing muscle tissue sampel for SNP Analysis

<u>Cataloging Sample Collection of Chitala and Notopterus Genera In Museum Zoological Bogorianse (Cataloging In MZB)</u>

Preservation of fish specimens started with the early treatment during the fieldwork by using 10% formalin for fixation. Hereafter, the fish specimens were processed in the laboratory by washing and soaking in the running tap water for approximately one hour. Then, the specimens were sorted based on location and morphological similarity, placed in the jar full with 70% ethanol, and labelled by exact locality (name of the water body and its location), date collected, and name of the collector. Furthermore, the observation of meristic and morphometric was conducted with the exact parameters according to the measurement data sheet.



Figure 10 Preserved and Labeld Specimen

Following discussions with the Museum of Vienna via Dr. Haralt, one of the fish morphology experts. It is sufficient to exchange samples digitally, namely by sending fresh and preserved belida fish morphometric data from the same sample, as well as photos of the belida fish. Furthermore, discussions about the exchange of morphometric and meristic data, as well as documentation (photos) of samples taken and morphometric data of meristic specimens at the Vienna Museum, were held

online (zoom). The following is documentation of a discussion with a team of experts from the Vienna Museum.



Figure 11 Discussion with Other Researcher regarding Morphological Phenomena

Development of Fish Record Database Platform

The Featherback frameworks platform is built using open source software which consists of:

- 1. PHP programming language with Laravel 8 framework.
- 2. MySQL databases
- 3. JavaScript
- 4. Operating system: Linux

This platform consists of several menus which are divided into three main sections, namely:

- Reference Menu: this menu is useful for filling in, updating, deleting reference data used in "fish" data. This reference menu consists of: Collector, Phylum, Class, Order, Family, Genus, Species, Province, Regency, District, Village, CITES Status, Conservation Status, Distribution Status, IUCN Status, and Occurrence Status.
- Fish Menu: this menu is useful for filling in fish-related property data, such as General, Taxonomy, DNA, Meristic, Color Pattern, and Morphometric data.
- 3. Search menu: this menu is useful for searching for Featherback data that is already in the database and displaying it in the form of Tables and Pie Bars.

Process Related to Existing Samples originating from Palembang

Based on field collection in Palembang between 2006 and 2010, the total of belida fish carcass was 77 fish which consist with various sizes and immerged in 70 % alcohol solution in every stored box. According to the collection book, all of them have been labeled with Dymo, which refers to the

location of the collection and has a variety of sizes for both youth and adult groups. The team arrived in Palembang on April 5, 2022 to collect the carcass sample, but all belida fish specimens did not meet the requirements as a scientific collection, but we have 9 belida fish tissue samples and documentation (photos), four of which are C. hypsolonetus.



Figure 12 Examination Process of Existing Samples of Fish Carcass in Palembang, South Sumatera

These nine belida fish sample tissues are accompanied by photos, including one of C. hypselonatus, which is certain to have a significant impact on resolving the unclear belida fish taxonomy in this activity, given that a sample of this belida type was not guarantee during the survey.



Figure 13 C.hypselonotus from Langam, Sungai Kampar Kanan, Riau (Source: Documentation A. Wibowo, 2006)



Figure 14 C.hypselonotus from Kuala Tolam, Sungai Kampar Kanan downstream, Riau (Source: Documentation A. Wibowo, 2006)

We explored new locations to increase sample diversity and representativeness. The sampling was expanded to 5 locations with different coordinate points to represent each region in South Sumatra where Belida and putak fish species exist. Table below is showing coordinate for new locations.

No.	Location	Coordinate Points	Species	
		2°52'21.9"S	Notopterus Notopterus and Chi-	
1	Muaro Belida River,	104°00'12.8"E	tala lopis	
		3°00'59.9"S	Notopterus Notopterus and Chi-	
2	Musi Palembang	104°43'14.1"E	tala lopis	
	Batanghari River	2°52'56.1"S	Notopterus Notopterus and Chi-	
3	Datairghan Kiver	103°59'15.2"E	tala lopis	
	Kelakar River, Ogan Ko-	3°14'31.6"S	Notopterus Notopterus and Chi-	
4	mering Ilir	104°38'55.4"E	tala lopis	
	Pedamaran (Ogan Komer-	3°27'50.2"S	Notopterus Notopterus and Chi-	
5	ing)	104°49'31.4"E	tala lopis	

III. <u>Results</u>

<u>Chitala</u>

Morphometric Analysis

The result of morphometric analysis in every species is shown in Table 1 Result of Morphometric Analysis of *Chitala*

Code	<i>Chitala borneen- sis</i> in West Borneo	<i>Chitala borneensis</i> in Jambi	<i>Chitala lopis</i> in South Bor- neo	<i>Chitala</i> <i>lopis</i> in Central Borneo	<i>Chitala lopis</i> in Java
------	---	---	--	--	-------------------------------------

Table 3 Morphometric Analysis Result of Chitala

		(KB)	(RIP-JA)	(KS)	(KT)	(TGR)
		SL: 461.31±87.10	SL: 487.60±175.7 7	SL: 300.57±44.55	SL:354. 59±81.1 62	SL:443.8 3
TL	Total length	106.30±4.19	106.80±1.07	105.75±1.33	106.90± 1.22	107.83
HL	Head length	24.17±1.59	23.39±1.21	35.92±2.21	26.15±0. 77	25.94
SNL	Snout length	3.31±0	3.55±0.85	3.61±0.22	3.61±0.2 7	3.37
HW	Head width	7.14±1.11	6.76±1.23	8.37±0.72	7.75±0.8 1	7.49
IW	Interor- bital width	2.84±0.42	3.60±0.94	3.12±0.09	2.97±0.2 0	3.40
HD	Head depth	23.82±	16.93±5.21	24.60±1.96	23.97±2. 21	24.09
UJL	Upper jaw length	9.43±0.93	9.53±0.39	10.38±0.52	9.98±0.8 0	9.92
LJL	Lower jaw length	8.22±1.29	7.23±0.65	9.62±0.46	7.91±1.7 3	9.35
ED	Eye di- ameter	3.30±0.57	3.20±1.02	3.77±0.57	3.56±0.5 2	3.22
BD	Body depth	27.97±2.43	29.19±1.58	28.95±1.93	27.24±2. 17	27.58
BW	Body width	8.54±1.39	8.04±1.09	10.45±0.81	10.20±2. 03	8.90
PDL	Predor- sal length	54.91±7.20	57.91±5.02	54.88±4.74	54.22±3. 37	53.77
PPL	Prepec- toral length	20.24±1.31	20.22±1.01	21.40±0.89	21.24±0. 71	21.17
PVL	Preven- tral length	27.03±1.96	28.13±1.61	28.81±1.84	28.62±1. 39	29.61
PAL	Preanal length	28.26±1.78	29.58±1.93	29.79±2.18	30.09±1. 40	30.16
DFBL	Dorsal fin base length	3.20±0.38	3.31±0.43	3.27±0.27	2.81±0.2 7	3.21

DFL	Dorsal fin length	13.13±1.65	12.82±1.03	11.79±1.06	12.43±1. 02	10.55
VFL	Ventral fin length	1.66±0.55	1.76±0.73	1.81±0.21	1.27±0.2 3	1.25
ABL	Anal fin base length	72.24±7.77	71.51±1.97	69.18±3.51	68.64±1. 71	69.10
AFL	Anal fin length	7.04±1.23	5.84±1.61	5.57±1.59	6.28±1.1 2	4.56
PFL	Pectoral fin length	13.89±1.12	14.95±1.81	11.45±5.69	14.41±0. 42	13.42
PLK	Head Concave Height	7.32±0.96	6.22±3.63	7.66±0.88	7.90±1.2 3	6.16
TLK	Head Con- cave Length	8.92±0.90	6.81±3.87	9.98±0.60	9.35±0.5 7	8.30

The Principal Component Analysis (PCA) was performed on 22 morphometric and meristic characters (Fig 15). All previous morphometric measurements were presented in the form of % head length (only for characters measured on the fish head side) and % standard length for other characters. The first component is mostly structured by upper jaw length, pre-pectoral length, pre-pelvic length, pre-anal length, and pre-dorsal length, while the second component is mostly defined by anterior body depth.

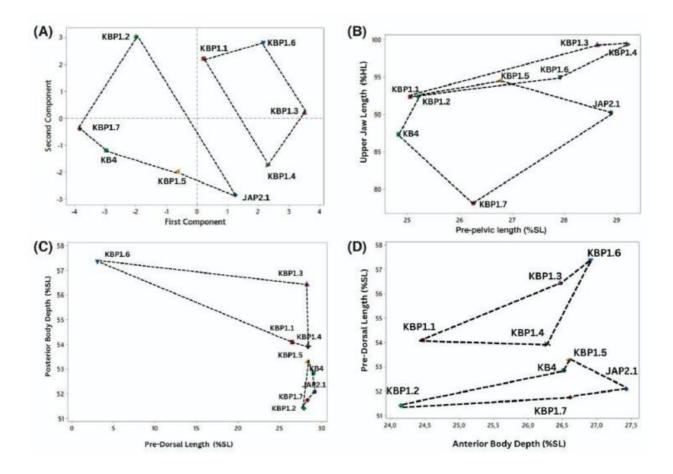


Figure 15 Principle Component Analysis (PCA) of Chitala Characters (pre-anal length, pre-dorsal length and pre-pelvic length)

Two groups corresponding to *C. lopis* (Fig. 15B, right – KBP1.1, KBP1.4, KBP1.6, KBP1.3) and *C. borneensis* (Fig. 15A, left, KBP1.2, KBP1.7, KB4, KBP1.5, JAP2.1) are identified. The two species mostly differ in their Upper jaw length with *C. borneensis* having a shorter jaw (Fig. 15B), the posterior body depth shorter in *C. borneensis* (Fig. 15C) and the pre-dorsal length, shorter in *C. borneensis* (Fig. 15D).

Molecular Analysis

A total of 102 sequences originating from 34 sites in Java, Sumatra and Borneo were produced. Together with 48 sequences mined from BOLD, a total of 150 sequences were assembled. All the newly produced sequences were above 500 bp of length and no stop codons were detected, suggesting that the sequences collected represent functional coding regions. In total, 28 haplotypes were detected among the 150 sequences collected. Figure 3 serves the map of presence of *Chitala*

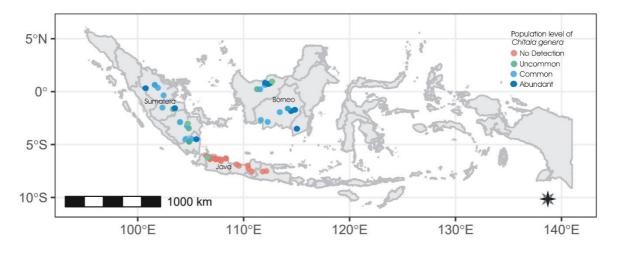


Figure 16 The Presence of Chitala based on Field Survey and Fishermen Information

DNA-based species delimitation methods resulted in congruent delimitation schemes with 6 MOTUs for BIN, ASAP and mPTP, and both mGMYC and sGMYC delimited 7 MOTUs (Figure 17; Table 4).

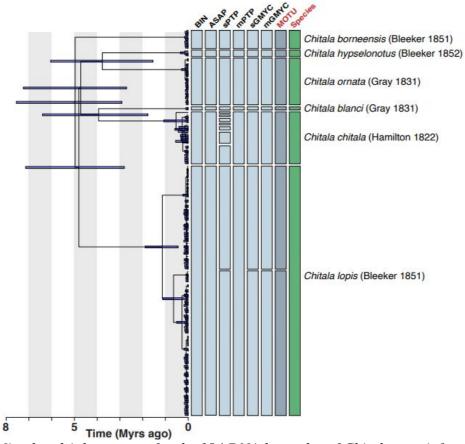


Figure 17 Mitochondrial gene tree for the 184 DNA barcodes of Chitala spp. inferred with Species Tree UCLN

However, sPTP delimited 13 MOTUs partially incongruent MOTUs mostly located in a single clade. The final consensus scheme consisted of 7 MOTUs. In total, 6 species are recognized within the 7 MOTUs. A single conflicting identification is detected between sequences produced here and sequences mined from GenBank for *C. hypselonotus* (BOLD: AEI5739), which were previously assigned to *Chitala Chitala*, a species reported from the inlands of India. The three Indonesian *Chitala* species are represented by distinct MOTUs, and *C. lopis* host two distinct MOTUs. A barcode gap is observed for all species as the maximum intraspecific K2P genetic distances were smaller than the minimum interspecific K2P distances. The maximum intraspecific genetic distance ranged between 0 for *C. borneensis* and 0.0284 for *C. lopis*, and the minimum intraspecific genetic distance ranged between 0.0441 for *C. lopis* and 0.0628 for *C. blanci* (Table 4).

		BIN	K2P Genetic Distance		
Species	Ν		Max.	Min.	
			Intraspecific	Interspecific	
Chitala blanci	1	BOLD:AAJ0132	-	0.0628	
Chitala borneensis	8	BOLD:ADN1667	0	0.0544	
Chitala Chitala	21	BOLD:AAY5141	0.0121	0.0449	
Chitala hypselonotus	4	BOLD:AEI5739	0.0034	0.0449	
Chitala lopis	97	BOLD:AAJ0133	0.0284	0.0441	
Chitala ornata	19	BOLD:AAE9017	0.0059	0.061	

Table 4 Barcode Gap of Chitala Species

The delimitation scheme translates into revised a distribution range for *C. lopis* widely distributed in Java, Sumatra and Borneo and also the most represented species in our sampling (Fig. 18). *C. borneensis* is observed in West Borneo and central Sumatra while *C. hypselonotus* is only observed in Sumatra at its type locality (Musi river).



Figure 18 Distribution Range for C. lopis Widely Distributed in Java, Sumatra and Borneo

The Bayesian gene tree based on the MOTUs recognized here suggests a recent diversification of the Asian *Chitala* species around 5 Myrs (Fig. 17). However, the gene tree does not show evidence of close phylogenetic relationships among Indonesian *Chitala*, *C. hypselonotus* is more closely related to *C. ornata*, while *C. lopis* and *C. borneensis* are placed at the root of the tree. All the mitochondrial divergence events between species observed here predate the Pleistocene.

<u>Notopterus</u>

Morphology Analysis

Featherback fish sampling locations were carried out at various geographic distribution locations of this fish in Indonesia (Robert 1992) (Fig. 19). Fish are caught directly at the sampling location using scoop nets, fishing rods, nets and traditional *kilung* fishing gear or based on fish collections owned by fishermen who are known from the location where they were caught to fully document the presence of the species at the distribution site.

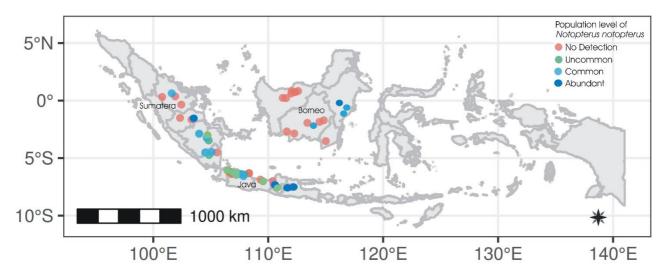
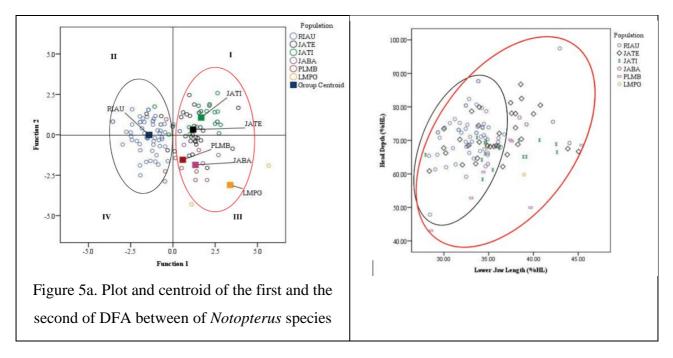


Figure 19 The Presence of Notopterus based on Field Survey and Fishermen Information

Discriminant Fucntion Analysis (DFA) was performed on 21 morphological characters for each specimen , morphometric were measured using dial callipers and data were used with an accuracy of up to 0.1 mm: standard length (SL), from the anterior pre-maxilla to the caudal fin's central base; head length (HL), from the tip of the snout to the occiput's posterior border; snout length (SNL), from the tip of the snout to the anterior front edge of eye; Head Width (HW), from left to right of the jaw; Eye Distance (IW), distance between the left and right eyes; head depth (HD), measured along a line traversing perpendicularly the top of the head just above the eye and passing through the middle of the eye, to the bottom of the lower jaw; upper jaw length (UJL), from the tip of the snout to the posterior edge of the pre-maxilla; lower jaw length (LJL), from the chin's tip to the posterior border of the mandible; eye diameter (ED), from the upper to lower border of the orbital cavity; Body Depth (BD), highest distance between dorsal and ventral; Body Width (BW), from left to right of the pectoral fin anterior; pre-dorsal length (PDL), from the tip of the snout to the bottom of first dorsal fin ray; pre-pelvic length (PPL), from the tip of the snout to the bottom of anterior pelvic fin ray; Pre Ventral Length (PVL), from the tip of the snout to the bottom of anterior ventral fin ray; pre-anal length (PAL), from the tip of the snout to the base of anterior anal fin ray; dorsal base length (DFBL), from first to last dorsal fin ray; dorsal fin length (DFL), from anterior to posterior of dorsal fin aray; Ventral Fin Length (VFL), from anterior to posterior of ventral fin; anal base length (ABL), from first to last anal fin ray; Anal Fin Length (AFL), highest distance of anal fin ray; and pelvic fin length (PFL), from bottom to the top of first pelvic fin ray.

There are six distinguishing characters remaining after stepwise analysis reduces 15 homogenous characters. VFL, DFL, PAL, BW, LJL, and HD are the variant characters. DFA creates two discriminant functions with the characters VFL (DF1) and BW (DF2). The plot and centroid of the two functions are illustrated in Figure 20a. *Notopterus Notopterus* were separated into two groups, i.e., the first group (Riau population) in quadrants II and IV, while the second group, namely the Javanese population: Central Java (JATE), East Java (JATI), and West Java (JABA); Sumatra: Palembang (PLMB) and Lampung (LMPG) in quadrants I and III. The second group had greater jaw and head depth than the first (Figure 20b). Pre-anal length and body width of the second group were also longer than those of the first group (Figure 20c). Meanwhile, Figure 20d reveals that the first group had longer dorsal fins than the second group, whereas the second group had longer ventral fins than the first group.



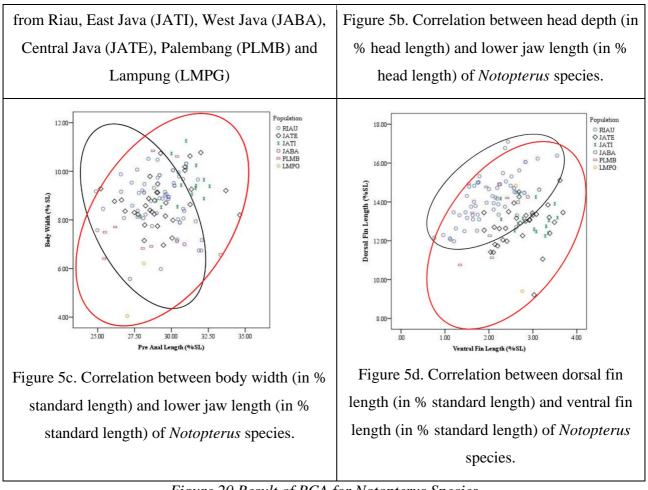


Figure 20 Result of PCA for Notopterus Species

Molecular Analysis

A total of 87 COI sequences were generated from the 28 locations visited in Sumatra, Java and Borneo (Figure 21). All the newly produced sequences were at least 500 bp in length and no stop codons were detected, suggesting that the sequences collected represent functional coding regions. In addition, 30 sequences of *N. notopterus* were mined from GenBank as well as 39 sequences of *N. synurus* and 5 sequences of *Chitala blanci*, *C. lopis*, *C. chitala* and *C. ornata*. In total, 161 COI sequences were analyzed, with 117 sequences belonging to *N. notopterus*. DNA-based species delimitation methods resulted in congruent delimitation schemes with six MOTUs for ASAP, sPTP and mGMYC, three MOTUs delimited by mPTP and five MOTUs delimited by sGMYC (Fig 21). The final consensus scheme consisted of seven MOTUs, including two MOTUs in the genus *Notopterus* and *N. synurus*. The divergence of the mitochondrial lineages of *N. notopterus* and *N. synurus* is dated around 4 Millions years ago (Ma), and the MRCA of *N. notopterus* haplotypes is dated around 0.75 Ma (Fig. 21).

A total of 8 haplotypes is observed among the 117 sequences of *N. notopterus*, and the reconstructed haplotype network displays a balanced structure (Fig. 22) with haplotype VII occupying a central position. Continental Asia and Sumatra host haplotypes scattered across the network, while individuals collected in Java and Borneo display a single haplotype. Both haplotype (Hd) and nucleic (π) diversity are high among samples from Continental Asia (Thailand, Peninsular Malaysia, Myanmar) and Sumatra with π and θ w above 0.008 and 2, respectively. Genetic diversity is particularly low among Java samples as a single haplotype was detected. The Tajima's D tests was significant only for Sumatra with a positive, significant D value, indicative of an excess of rare haplotype.

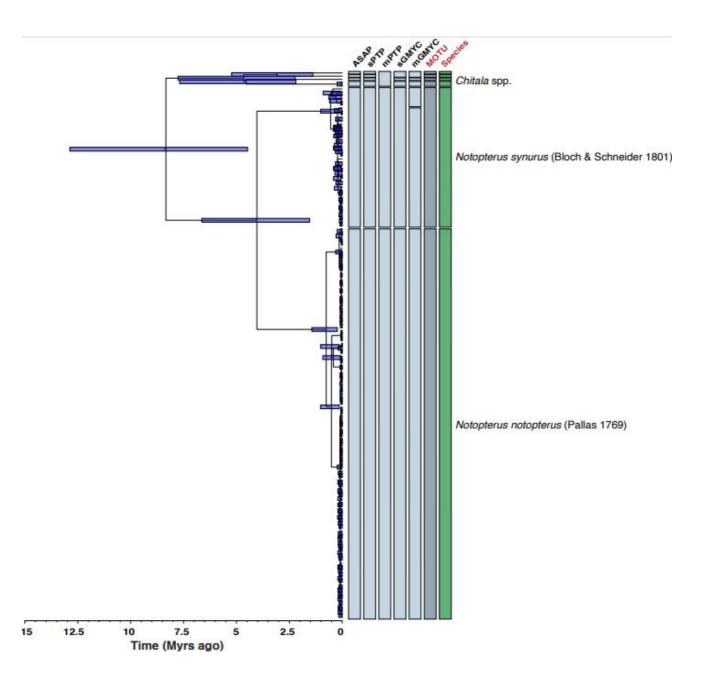


Figure 21 Mitochondrial gene tree for the 161 DNA barcodes of N. notopterus inferred with SpeciesTreeUCLN, including 95% Highest Posterior Density (HPD) interval for node age estimates, genetic species delimitation results for the five methods and their 50% consensus, and species names

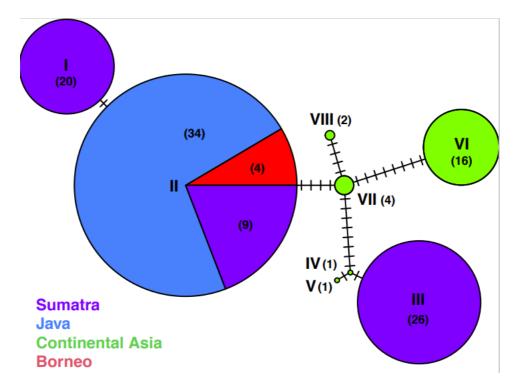


Figure 22 Haplotype network reconstructed based on the 117 sequences available for N. notopterus. Numbers of changes are indicated by small segments on links. Circles represent haplotypes, the size of a circle is proportional to the haplotype frequency. Haplotypes are numbered (I to VIII) and numbers of observation are given in parenthesis.

Environmental DNA survey

The aims of environmental DNA analysis were to detect the existence of Chitala genera in Java waters. We collected water from one location in Cisadane River, Irrigation channel in Cikarang, Citarum river, Ciliwung river, Cimanuk river, Salandarung weir, Kali Babadan, Sragi river, and Rawa Pening. The eDNA survey was carried out to complement the advanced information regarding the existance of Chitala genera in Java from respondens (including fisheries extension, local people, and fishermen, see Appendix III). Based on interview, most of respondens had never found of *Chitala* spesies in their surroundings waters (river, lake, or reservoir) since more than 10 years ago. Similarly, eDNA survey was also still unable to detect the Chitala species in these areas. The result of eDNA survey showed that a total of the other 32 species were detected from 28 genus and 23 different families. The Ambasidae family was the relatively abundance spesies found at all observation sites with the highest percentage being at Sungai Cisadane and Bendungan Salandarung, Compreng. A total of 28 genera were identified from different families, the relative abundance of fish species found from genus level based on reads number at each sampling site.

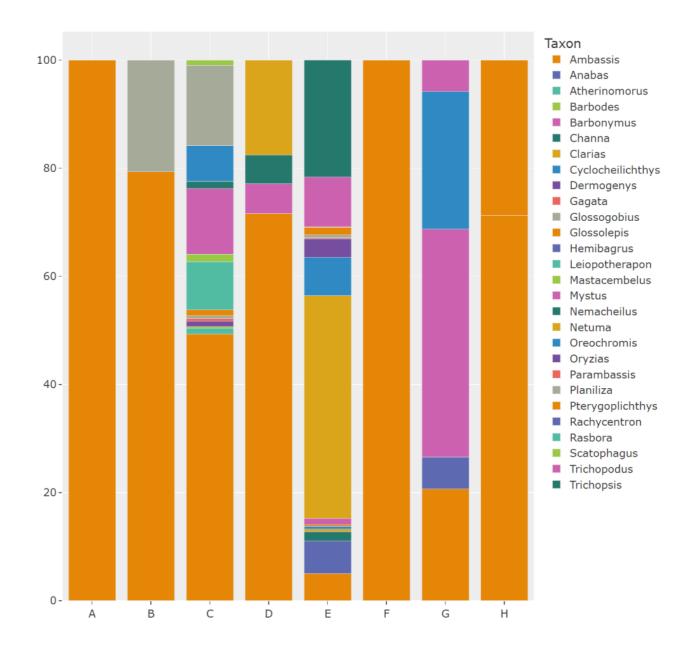


Figure 23 Barplot Relative Abundance of Identified Genus in 9 Sampling Area

The dominating genus composition at each sampling site came from the *Ambassis* followed by the *Babonymus*. Species percentages based on abundance found from all sampling sites were visualized in pie chart reveals that the percentage of occurrence of the three highest species is *Ambassis urotaenia* at 61.2%, *Mytus* sp. at 7.61%, and *Netuma bilineata* at 7.35%. Sungai Cisadae and Bendungan Salandarung, Compreng only detected one species, namely Ambassis urotaenia while locations with Id Sungai Cimanuk, Pabean revealed more species, including *Ambassis urotaenia*, *Anabas testudinatus, Channa strata, Clarias batracus, Clarias gariepinus, Cyclocheilichthys apogon, Glossolepis incises, Mytus sp., Netuma bilineata, Oreochromis niloticus, Oreochromis* sp., *Oryzias* sp., Parambassis ranga, Planiliza macrolepis, Pterygoplichthys disjunctivus, Rachycentron canadum, Trichopodus microlepis, and Trichopodus microlepis.

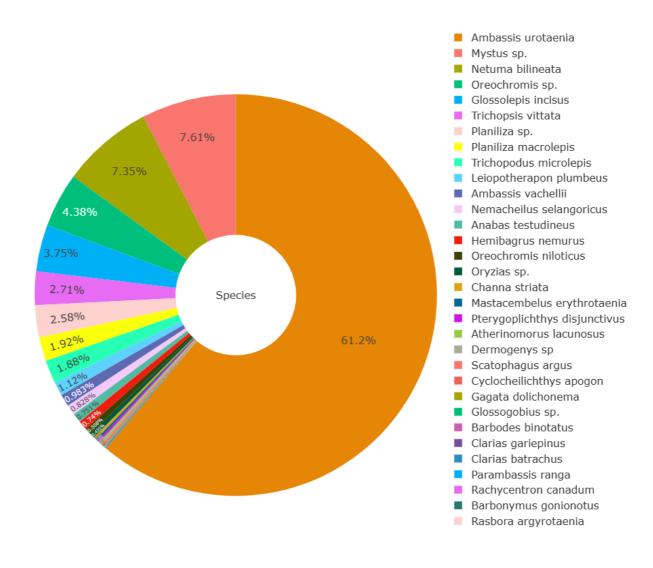


Figure 24 Pie Chart Relative Abundance of Identified Species in 9 Sampling Area

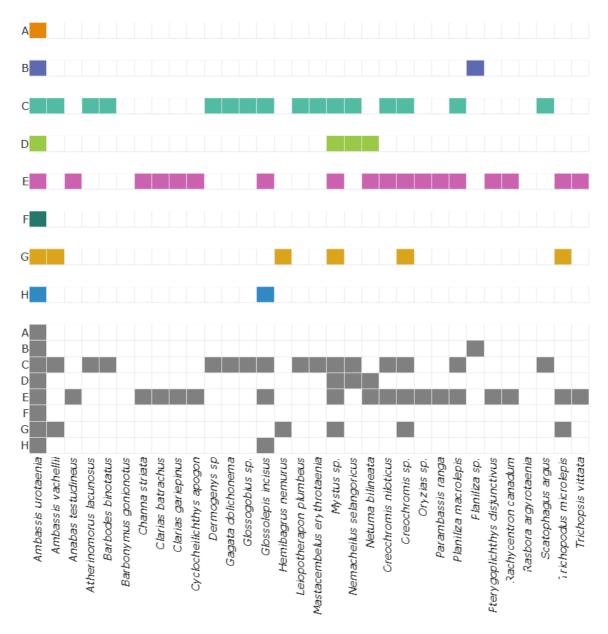


Figure 25 Species Occupancy in 9 Sampling Area

<u>Scale Surface Morphology and Microstructure Examination Using Scanning Electron</u> <u>Microscopy (SEM)</u>

SEM analysis or Scanning Electron Microscopy (SEM) was conducted to complete the data on the structure and taxonomic significance of representative samples from each species. This analysis was conducted to complement the current morphological analysis. The microstructural profiles of *Chitala lopis, Chitala borneensis, and Notopterus notopterus* were determined based on the results of SEM analysis of the scale structure. The results of the SEM indicate that both species have cycloid or circular scales. This type of scales is composed of calcium salts and collagen and has an anterior portion that typically overlaps with the posterior portion. The three species' SEM analyses are presented in the Table below.

Characters	Chitala lopis	Chitala borneensis	Notopterus notopterus
Shape of the scale	Oval	Oval	Oval
Focus of the scale	Present	present	Present
Circuli	Present (regular den-	Present (regular den-	Present (intercircular
	sity)	sity)	more tenous)
Radii	The density is homo-	Density is consistent	In the anterior position,
	geneous and circular	all around, with the ex-	the teeth are less dense,
	around the centre of fo-	ception of the focus's	more distinct, and closer
	cus.	periphery (middle	together.
		width)	

Table 5 The Different Characters Result of SEM Analysis

According to a morphological analysis, the morphological details of the scales of all species were oval with cycloid scales. This type of cycloid scale has no serrations (ctenii) in contrast to the ctenoid type. In each of the three species, the region of the scales behind the central focus has an almost identical shape. All three species have a focus that is not precisely located in the center of the scales. The focus on *Chitala lopis* and *Chitala borneensis* is more similar than the focus on *No-topterus notopterus*. The focus of *Notopterus notopterus* is wider than that of *Chitala lopis* and *Chitala borneensis*. In the posterior area after the focus, there are numerous coarse granules (tubercles) with different shapes and sizes covering a large part of the caudal field. Figure 26 is shown the difference of scale structure among those three species.

In all three species, the structure of the circuli is circular, and the line grows and emerges from encircling the point of focus of the scales. In *Chitala lopis* and *Chitala borneensis*, the intercircular spaces are the same size on the anterior and lateral. The circuli are more compact and regular (dense intercircular) in both species. *Chitala lopis* circulii are still visible up to the centre of focus, whereas *Chitala borneensis* circulii are only visible to the periphery of the focus. *Notopterus notopterus*, on the other hand, has looser circuuli with larger intercircular spaces in the lateral position and tighter circuli in the anterior position. In addition to the circuli, there is also a radii parameter, which refers to grooves that extend radially towards the focus. The spacing between the radii of *Chitala lopis* and *Chitala borneensis* is smaller and more regular in *Chitala lopis* and *Chitala borneensis*. The primary, secondary, and tertiary radii are clearly visible and prominent in both species. The space between

Notopterus notopterus radii appears more distant and distinct. Nonetheless, the radii are more evident at the focus in the anterior centre.

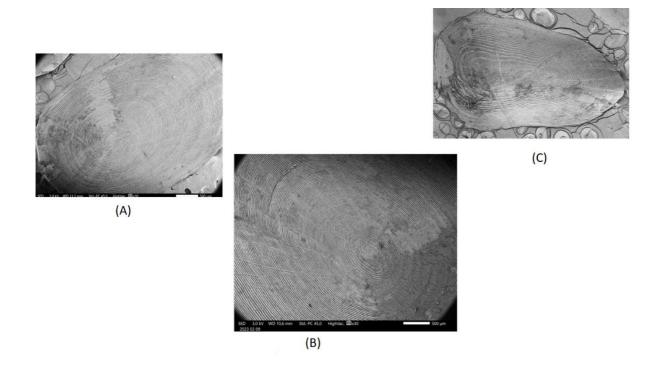


Figure 26 Scanning Microstructure of Scale (A) Chitala lopis; (B) Chitala borneensis; (C) Notopterus notopterus

Updating Specimen Collection for MZB

Temporary results of identification showed that largely specimens obtained from Java is identified as *Notopterus Notopterus*, while further study is needed for some specimens since there is a tendency that the specimens belong to the genus of *Chitala*. Furthermore, the Sumatra region also consisted of two genera (*Chitala* and *Notopterus*). The collected specimens have been sorted based on location and size and stored into the jars (Figure 10). The jar with the volume of 2, 4, and 5 L was used to store the fish specimens. Those specimens have been registered in the catalogue book of Museum Zoologicum Bogoriense (MZB).

Those collections of featherback have been officially registered as scientific collection in MZB and become an important assets for further national or global researches on featherback. These specimens are essential due to the lack of featherback's collection in MZB. The significant contribution of this research through the IFish Project has supported the development of featherbacks study in Indonesia both directly and indirectly through the provision of scientific collections.



Figure 27 Voucher Chitala and Notopterus Specimen Collection

<u>Development of Fish Record Database Fish Occurrence Records of Indonesia (forIDN)</u> <u>Platform</u>

Right now, the platform can be used to store fish databases and is publicly accessible via the URL <u>https://foridn.brin.go.id/belida/input/fish</u>. Currently, the system is still strengthening its security system so that system security can be properly maintained. This platform system is one of the platforms that has provided information such as location, project, collector, distribution status, classification, molecular data, meristic data, and morphological data not only for *Chitala* but also for other types of fish, transforming it into a fish data bank from territory in Indonesia. It will eventually be outfitted with an identification system.

The following shows an example of the display of the reference menu, fish menu, and search menu. Reference Menu (Collector):

	Name	Institution	Email	Created	Updated
1	Kurniawan; Arif Wibowo;Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	2022-12-16 07:58:37	2022-12-16 07:5
2	Arif Wibowo;Kurniawan	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	2022-12-16 07:51:42	2022-12-16 07:5
3	Vitas Atmadi; Hadi Dahruddin;Haryono	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	2022-12-16 07:41:22	2022-12-16 07:4
4	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	2022-12-16 07:41:22	2022-12-16 07:4
5	Yulia Aris Kartika	BRIN	yulia.aris.kartika@gmail.com	2022-11-19 10:35:03	2022-11-19 10:35
6	Pesigrihasta N	BRIN	pesigrihasta@gmail.com	2022-06-28 04:57:27	2022-11-19 10:36
7	N/A - Unknown	N/A - Unknown	N/A - Unknown	2022-06-28 04:57:03	2022-06-28.04

Figure 28 View Menu of "Collector"

Below is a figure for showing display of the contents on the "collector" menu. In this section, there are three main buttons, namely:

- \circ []Create : to add the collector's name (Figure 2 input form)
- \circ []Edit : to edit the collector's name (Figure 2 edit form)
- \circ []Delete : to delete the collector's name (Figure 2 delete)

ahruddin Badan Riset dan Inovasi Nasional (BRIN) yulia_ak(Dahruddin Badan Riset dan Inovasi Nasional (BRIN) yulia_ak@y	Dauan Riser uan movasi Nasional (DRIN) yu
Create Collector × 1k(Edit Collector × k@ 1	Confirmation × ^{yu}
Har: Name Iki Institution ris	tHan Name Yulia Aris Kartika ik@, Institution BRIN rs. k	Celete this data?
Email ina Juli	Email yulia.aris.kartika@gmail.com ihasi Jnkn ✓ Save 💥 Cancel	Ok Cancel N/
Form Input	Form Edit	Delete

Figure 29 Three main buttons on the "collector" menu

Fish Menu

it 🔳 Delete					
ime	Project Date	Collector Name	Collector Institution	Collector Email	Species
g Chitala and Notopteru:	2022-08-15	Vitas Atmadi; Hadi Dah	Badan Riset dan Inovasi Na	yulia_ak@yahoo.com	N/A - Unknown
KALIMANTAN BARA' Sintang Binjai Hulu Sungai Kapuas, Amp nage					
67					

Figure 30 View Menu of "Fish"

Figure 30 is a display of the contents on the "Fish" menu. In this section, there are three main buttons, namely:

- \circ []Create : to add the Fish attribute (Figure 4 input form)
- \circ []Edit : to edit the Fish attribute (Figure 4 edit form)
- \circ [] Delete : to delete the Fish attribute (Figure 4 delete)

Ceneral	Taxonomy DNA Meristic	Color Pattern Morpho >	< General	Taxonomy DNA Meristic Co	lor Pattern Morpho >	Confirmation
roject Name	Enter Project Name		Project Name	Redefining Chitals and Notopterus Genera in Indonesian Fresh	Nater Ecosystem	commutation
roject Date			Project Date	2022-08-15	-	Delete data Redefining Chitala
offector	Choose Collector	~	Collector	Vitas Atmadi; Hadi Dahruddin	×	
rovince	Choose Province	×	Province	61	×	ar I 💛 and Notopterus Genera in
egency.	Choose Regency	v	Regency	6107	×	Indonesian Freshwater
istrict	Choose District	v	District	Binjai Hulu	×	Ecosystem ?
linge	Choose Village	~	Vilage	0000000451	×	
atus Cites	Choose Status Cites	v	Status Cites	N/A - Unknown	v	
atus Conservation	Choose Status Conservation	~	Status Conservation	Dilindungi Penuh	~	Ok Cancel
tatus Distribution	Choose Status Distribution	~	Status Distribution	Endemic	~	OK Cancer

Figure 31 Three main buttons on the "Fish" menu

Search Menu

Figure below is the result of a search page with the keyword "Chordata".

				Please Typ	e Any Keyword and Press Enter	To Search				
			٩	chordata						
									W	
NO.	PROJECT	DATE	COLLECTOR	INSTITUTION	EMAIL	HABITAT	DNA	COLOR	FILUM	ORDO
1	Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem	2022- 08-15	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	freshwater	lst BASE 4605840 01 FishFl F.abl		Chordata	Oste
2	Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem	2022- 08-15	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	freshwater	lst BASE 4605841 02 FishF1 F.ab1		Chordata	Oste
3	Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem	2022- 02-01	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	freshwater	1st BASE 4605842 03 FishF1 F.ab1		Chordata	Oste
4	Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem	2022- 02-01	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	freshwater	lst BASE 4605843 04 FishF1 F.ab1		Chordata	Oste
5	Redefining Chitala and Notopterus Genera in Indonesian Freshwater Ecosystem	2022- 02-01	Vitas Atmadi; Hadi Dahruddin	Badan Riset dan Inovasi Nasional (BRIN)	yulia_ak@yahoo.com	freshwater	lst BASE 4605844 05 FishF1 F.ab1		Chordata	Oste

Figure 32 Search results using the keyword "Chordata"

Figure below illustrates the summary data of the keyword "Chordata".



Figure 33 Example for Summary Result Page for "Chordata" key words

IV. Discussion

<u>Re-Description of Chitala Genera in Indonesia</u>

The rediscovery and comparative analysis of *Chitala lopis* in the Cisadane river in Java, its type locality, has major implications to our knowledge and understanding of *Chitala* diversity and taxonomy in Southeast Asia. By aggregating 150 sequences, largely distributed in Asia and including sequences from specimens caught at type localities, the validity of the three Indonesian species of *Chitala* can be corroborated. DNA-based species delimitation methods agreed on the recognition of all the known *Chitala* species (Eschmeyer et al. 2018; Froese and Pauly 2020), excepting *C. hypselonotus* (BOLD:AEI5735), which sequences in GenBank were initially assigned to *C. Chitala*, a species restricted to India. Although the range distribution of *C. Chitala* might be underestimated due to the difficulties in accurately identifying *Chitala* species in Indonesia, misidentifications are more likely as sequences of the true *C. Chitala* (BOLD: AAY5141) were also included in the present analysis. Besides, sequences of BOLD: AEI5735 originate from the Musi River, the type locality of *C. hypselonotus*.

The family Notopteridae had been revised by Roberts (Roberts 1992), who considered that all indonesian species of Chitala represent variation of a single species, C. lopis, with C. borneensis and C. hypselonotus being different ontogenetic stages of C. lopis. Kottelat and Widjanarti (2005) came to a different conclusion based on the examination of specimens originating from the Danau Sentarum National Park in western Borneo. In particular, they observed that specimens of giant featherback corresponding to the different phases reported by Roberts (Roberts 1992) belonged to similar size classes, hence rejecting the hypothesis of ontogenetic changes in coloration. Following this, Kottelat and Widjanarti (Kottelat 2005) highlighted that until further evidence, C. lopis, C. borneensis and C. hypselonotus should be considered as distinct species. Both genetic and morphological evidence presented here supports the recognition of C. lopis, C. borneensis and C. hypselonotus as distinct species. Several morphological characters, mostly located in the anterior part of the body, enables to distinguish C. lopis and C. borneensis. The jaw, predorsal length and posterior body depth are shorter in C. borneensis but C. lopis can be unambiguously distinguished from C. borneensis in having a black spot at the base of the pectoral fins. This character was consistently observed across C. lopis range distribution as it was present in specimens from the type locality (Fig. 30a) in Java, as well as Sumatra and Borneo (Fig. 30b). That these three sequences were previously identified as C.

Chitala may indicate that *C. hypselonotus* displays a similar coloration pattern consisting of black spot on the posterior part of the body. Interestingly, *C. hypselonotus* is closely related to *C. ornata* from continental Asia (Mekong), a species which also display clear black spot on the posterior part of the body.

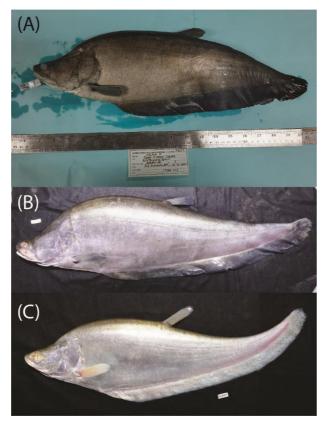


Figure 34 Photograph of selected specimens of Chitala lopis from (A) type locality, Cisadane river, Java (B) Borneo, Sintang, West Kalimantan, (C) C.borneensis from Jambi, Sumatra

The case of *C. hypselonotus* is of particular concern as it was not observed directly in here and is only known by three sequences deposited in Genbank and originating from the Musi River. Unfortunately, the extinction of *C. hypselonotus* cannot be discarded and further exploration is required to confirm its occurrence in other locations in Sundaland. At the moment, our study suggests that *C. hypselonotus* is the *Chitala* species of most urgent concern in Indonesia at the moment.

Strong genetic evidence suggests that *C. hypselonotus* is a valid species. *C. hypselonotus* is only found in Sumatra in its locality type (Musi River and Kampar Kanan River). However, based on the three representative DNA sequences of this species previously identified as *C. Chitala*, it is possible that *C. hypselonotus* has a similar coloration pattern that includes a black spot on the back of its body. *C. hypselonotus* is related to C. ornata, which is found on the Asian (Mekong) continent. The sequence based on complete MtDNA for genetic markers or differentiators that distinguish this species from others is served in below.

	1	2	2	3	3	3	3	4	4	4	4	4	4	5	5	5	6	6
	0	0	4	0	0	2	2	2	2	5	6	7	8	4	5	9	1	6
	9	8	5	1	7	2	5	1	4	7	6	5	1	7	6	5	9	7
C. hypselonotus	G	G	Т	Α	С	С	G	Т	Т	С	С	Т	Α	С	Т	С	С	A
Chitala lopis	A	A	С	G	Т	Α	A	С	С	Т	Т	С	G	Α	С	A	Т	G
C. borneensis	A	A	С	G	Т	Α	Α	С	С	Т	Т	С	G	G	С	Α	Т	G

Figure 35 Complete MtDNA sequences in Chitala

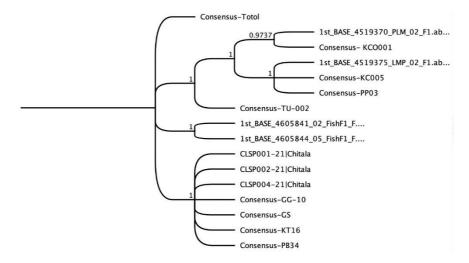


Figure 36 Phylogenetic dendrogram showing the sequences of C. hypselonotus at its locality In terms of conservation, the species *C. hypselonotus* is classified as priority I, requiring immediate conservation action. Taking into account the limited distribution, the alleged small population, and the ongoing threat or pressure (captured for consumption and used as an ornamental fish commodity).

In comparison to *Chitala lopis* and *Chitala borneensis*, the upper head profile of Hypselonotus is relatively shorter. The height is also greater than that of *Chitala lopis* and *Chitala borneensis*. Based on its habitat, this species prefers waters with a low pH/acidity. Furthermore, the black spot at the base of the pectoral fins, which has been used to differentiate *C. lopis* and C. hypselonatus, is no longer relevant, given that we found *C. lopis* with and without black spots that shared genetic similarities (same the species). As a result, the length of the upper jaw in comparison to the eyes is the most important feature for distinguishing *C. lopis* and *C.hypselonatus*.

C. lopis and *C. borneensis* co-occur in Sumatra and Borneo, suggesting common dispersal between populations on each island. Furthermore, shared haplotypes were observed in *C. lopis* between distinct geographic locations in central Sumatra and western Borneo. This suggests that the western parts of Borneo and Sumatra were connected until recently. This observation is in line with the biogeographic history of Sundaland during the Pleistocene. Throughout the Pleistocene, sea-level

first dropped and then fluctuated widely, causing islands of the Sunda Shelf to repeatedly separate and merge (Voris 2000; Woodruff 2010; de Bruyn et al. 2013; Sholihah et al. 2021a). The western part of Borneo was connected to central Sumatra through an ancient river system named North Sunda, and faunal exchanges through this paleodrainage have been previously documented (de Bruyn et al. 2013; Alshari et al. 2021; Sholihah et al. 2021a, 2021b).

Review for IUCN Status of Chitala

The present study clarifies the taxonomic status of the three *Chitala* species in Indonesia and provides the first accurate evidence of their range distribution in the wild. The capture of *C. lopis* in its type-locality after the absence of observations for decades has important implication in our understanding of *Chitala* species distribution, hence conservation status in Indonesia. *C. lopis* was declared extinct by the IUCN (Ng 2022), a decision assuming implicitly that *C. lopis* was an endemic species of Java. Surprinsigly, the present study indicates that *C. lopis* is actually the most widespread *Chitala* species in Sundaland, with a range distribution spreading across Java, Sumatra and Borneo. This information requires reconsidering its IUCN status. Despite being widespread, *C. lopis* is havely harvested as it is an iconic fish with a high economic value, being an important species for food and is the main ingredient of traditional processed fish foods (eg. krupuk, pekpek, lenggang, model, tekwan, burgo *etc*), and the skin is used to produce leather commercial materials such as wallets (Kottelat 2005).

The giant featherback is also targeted by the international ornamental fish trade, as well as other Notopterid species which have altogether become well-known in Asia in particular (Kottelat 2005). For all these reasons, *Chitala* species are under a national regulation by the Indonesian ministry of marine affairs and fisheries. In the case of *C. lopis*, due to its wide geographic distribution, the present study warrant further studies to examine anthropogenic threats at the population level and eventually adopt differentiated regulation according to local uses of *C. lopis*. By contrast, the conservation status *C. borneensis* and *C. hypselonotus* need to be urgently revised. Both are listed as of Least-Concerned by the IUCN, however, our study demonstrates that both are particularly threatened. *C. borneensis* is widely distributed but is at least rare everywhere it was observed during the course of the sampling in the present study. This makes this species particularly vulnerable to further reductions in population size.

Updating Molecular and Morphological Aspect of Notopterus

By aggregating 161 mitochondrial sequences, originating from specimens largely distributed in Southeast Asia, the validity of the species of Notopterus can be corroborated as DNA-based species delimitation methods agreed on the recognition of the Notopterus species (Fricke et al. 2022; Froese and Pauly 2020). Within N. notopterus, three unique haplotypes were detected in Sundaland: (i) haplotype II observed in Java, South Sumatra and South Borneo, (ii) haplotype I restricted to Southern Sumatra, and (iii) haplotype III observed only in the Northern Sumatra. The genetic distance between these haplotypes are low, excepting with haplotype III, which is more closely related to haplotypes observed in Continental Asia, particularly in Peninsular Malaysia. This pattern of haplotype relationships is likely resulting from ancient biogeological processes connecting and disconnecting ancient river system in Sundaland, particularly during the Pleistocene (Dixon, 2015; Hutama et al., 2016; Lohman et al. 2011; de Bruyn et al. 2013; Sholihah et al. 2021b, 2021a). As such, the ancient connection of rivers in Southern Sumatra with rivers on Java Island (Yap, 2002; Hutama et al., 2016; Sholihah et al. 2021b, 2021a) likely explains the co-occurrence of haplotype II in these locations. On the other hand, halotype III from Riau Province in northern Sumatra had more limited ancient connectivity to Java Island, and as such is limited to Northern Sumatra. A similar situation was previously depicted for several fish lineages were detected within species with various distribution ranges in the Sundaland (Dahruddin et al. 2021; Kottelat, 1989; Pouyaud et al., 2009; Sholihah et al. 2021b, 2021a). Similar biogeographic patterns have also been described for invertebrates, where phylogenetic studies on freshwater shrimp (Macrobrachium spp.) also demonstrated the existence of genetic exchange or accumulation in Sundaland (De Bruyn et al., 2004).

Intraspecific variation (genetic variation within the species level) is strongly influenced by the history of changes in the earth and/or environmental conditions and ecological processes that occur in a particular area. The information obtained can provide knowledge about the historical biogeography of the area; however, this history may remain a mystery and has not been revealed if research is focused only at or above the species level (De Bruyn *et al.* 2012). Molecular data can provide an early indication that it is possible to separate and identify the contributions of the various processes driving the evolutionary history of freshwater fauna from geologically complex and biologically rich regions. In particular, the ability to reconstruct past distributions in temporal and spatial contexts based on levels and patterns of genetic diversity and the interrelationships therein can be used to explain current species distribution trends (Avise 1994, 2000).

Differences of the morphological shape from two population groups based on morphometric characters are not clearly visible (individual grouping is mixed), although the six main morphometric characters are potential indicators of difference between the two populations. The low, near-similar morphological variation in the genus *Notopterus* is not surprising considering that the family Notopteridae is known as a group with static morphology. Several species in the family Notopteridae have valid names but are morphologically similar, for example the genus *Chitala* (Lavoué 2020). The genetic and morphological evidence presented in this work support the recognition of a single valid living species of *Notopterus* in Indonesia.

<u>Chitala And Notopterus Genera Prospective Distribution According To Environmental DNA</u> <u>And Their Genetic Links Using SNP Analysis</u>

Based on the results of environmental DNA analysis, specifically water samples, it appears that Chitala and Notopterus were not detected in the Java region during this study. The detected fish species are typically common species of inland water fish found in Indonesian rivers. The largest populations of the genera *Ambasis* and *Babonymus* were discovered in nine sampling locations in the waters of Java. This is consistent with the findings of the interviews and questionnaires, which concluded that, on average, no *Chitala* species have been spotted or discovered in the river for more than ten years. However, this study found that *Notopterus* species were still present in Sawadarma Dam, Sragi River, and Rawa Pening, among other locations. Meanwhile, one species of *Chitala* was discovered in the Cisadane River in Tangerang.

Several factors can affect the disparity between e-DNA analysis and direct species sampling surveys. According to Rourke et al. (2022), the concentration of biomass in e-DNA is affected by two factors: biotic and abiotic factors. Diverse biotic factors influence eDNA concentrations, with biotic and abiotic influences frequently exhibiting interactive effects. Given that the source of eDNA is the biological organism itself, it follows that the movements of these molecules reflect both intrinsic processes and external influences on these intrinsic processes. Despite this, knowledge of combined effects may be sufficient to correct abundance or biomass estimates derived from eDNA concentration measurements. Six key abiotic factors are identified as influencing estimates of abundance or biomass derived from eDNA concentrations. In addition, the vast majority of abiotic factors have complex interactive or confounding effects that encompass influences on the general ecology and biology of species, which in turn affect DNA shedding rates and/or volume. Those six factors are:

- 1. Water flow, flow of water influences the spatial scale over which abundance and/or biomass can be meaningfully estimated, as well as numerous other factors that influence eDNA production, degradation, dilution, and deposition.
- 2. Water temperature, temperature of water such divergent effects reflect, at least in part, species-specific tolerance ranges and metabolic rates that influence shedding, as well as any subsequent eDNA decay after shedding from the fish. Due to the activity levels of the fish, water temperature influences the detection probability and concentration of eDNA in the environment.
- Water depth, clearly, the eDNA sampling depth can bias estimates of fish abundance or biomass, reflecting not only species-specific vertical distributions and habitat preferences, but also the absolute depth of the system.
- 4. Environmental DNA Decay Rate, the decay rate is dependent on whether the eDNA is intraor extracellular and may be influenced by a number of biotic and abiotic factors, such as salinity, water temperature, sunlight, pH, microbial activity, and enzymatic digestion.
- 5. Methods for capturing, extracting, and amplifying e-DNA become factors for biomass concentration.

In conclusion, the effects of abiotic factors such as water flow, depth, and temperature can be partially controlled by collecting detailed knowledge of the hydrodynamics of the sampling site, vertical stratification of the target species, and historical temperature ranges, as well as standardising sampling times to account for tides and seasons. Identifying and partitioning spatio-temporal variation in eDNA concentrations, and ensuring that such variation is accounted for when estimating abundance or biomass, will necessitate hierarchical sampling designs.

Existence of Chitala genera in Indonesia is dispersed across three major islands: Sumatra, Java, and Kalimantan. In Indonesia, only species belonging to the Notopterus genera are known to exist. According to its locality, each of these species has a unique range. *Chitala lopis* species was formerly recognised as a species whose range was restricted to Java or as an extinct endemic Javanese featherback. *Chitala hypselonotus* and *Chitala borneensis* were formerly thought to be widespread in Kalimantan and Sumatra. Based on previous research, *Notopterus notopterus* is only found on the islands of Sumatra and Java. This study demonstrates, contrary to the findings of previous research, that *Chitala lopis* is a species with the widest distribution, found not only in Java, but also in Sumatra and Kalimantan. This study reveals that *Chitala borneensis* is a species with a restricted distribution because it is only found in West Kalimantan and Jambi, Sumatra, whereas *Chitala hypselonotus* is an endemic species that is found only in the Riau and Musi River areas of South Sumatra and is nearly

extinct. In addition to molecular analysis or DNA sequencing, SNP analysis was used to determine the probability of hybridization between *Chitala* species in various regions and *Notopterus notopterus* species in this study. The result of SNP analysis (phylogenic tree) is served in Appendix IV.

According to the results of the SNP analysis (appendix IV), each species (two Chitala sp. and one Notopterus sp.) had distinct habitats. The two large cladograms, in group for Chitala species and out group for Notopterus species, show a clear grouping based on each region, indicating that there was no genetic crossover or hybridization between the two species in Indonesia. In the *Chitala* species group or the primary cladogram (in group), there are four clades, two large clades and two small clades. Clade I is the Chitala borneensis species from West Kalimantan and Jambi, clade II is the Chitala lopis group from Central and South Kalimantan, clade III is the Chitala lopis group from Riau, and clade IV is the Chitala lopis species from Riau, Jambi, Java, South Sumatra, Lampung, and West Kalimantan. In conclusion, there are two large groups of distinct species of Chitala: Chitala borneensis and Chitala lopis, with 5 species of Chitala borneensis from West Kalimantan and Jambi (Cladogram I) and the remaining 64 species of Chitala lopis from Central Kalimantan, West Kalimantan, South Sumatra, South Kalimantan, Jambi, Java, and Riau (Cladogram II, Clad III, and Clad IV). This is consistent with the results of DNA sequencing analysis and morphological analysis in this study, which determined, based on analysis of samples from various regions in Indonesia, that there are two species of Chitala, Chitala borneensis from West Kalimantan and Jambi, and Chitala lopis found elsewhere. This SNP analysis also demonstrates that Chitala lopis has a structured population, as there are pure population groups and mixed population groups. Mixed population groups can be interfered as populations that have genetic diversity or undergone hybridization/genetic interference and have a similar SNP sequence structure. Group of *Chitala lopis* that have not undergone genetic crosses (hybridization) originate from two main regions: the individual group from Central Kalimantan, which shares genetic similarities with the species group from South Kalimantan (clad II), and the individual group from the Riau region (clad III). In the meantime, the population of the *Chitala lopis* or subspecies native to Java joined subspecies from West Kalimantan, South Sumatra, Jambi, and Lampung to form a group (clad IV). This indicates that the Chitala lopis native to Java has a strong genetic similarity with species from regions other than species from Central Kalimantan-South Kalimantan and Riau.

In the outgroup *Chitala* cladogram or *Notopterus* species cladogram, it is divided into two cladograms, with cladogram I of *Notopterus notopterus* species originating from South Sumatra joining Jambi and Java. The grouping of individuals from these various regions demonstrates genetic similarities between Java and South Sumatra-Jambi-originating species. This resemblance may result from hybridization or cross-pollination between species in the three regions. However, in Riau, there are no individuals from other regions included in the cladogram, so it can be concluded that *No-topterus notopterus* in Riau is a pure species, meaning there is no hybridization or interbreeding with species from outside the Riau region.

Based on the results of the conducted analysis, it can be inferred that this study has identified two species of *Chitala: Chitala borneensis* at some locations in West Kalimantan and one location in Jambi. *Chitala lopis* has three distinct localities in the Riau region, including pure species populations, Central and South Kalimantan, and structured species populations or the possibility of hybridization in the regions of South Sumatra, Java, Lampung, and Jambi. These results indicate that conservation of *Chitala* species in the Riau and Central Kalimantan-South Kalimantan regions can be accomplished by restocking species from their respective native areas in order to preserve the genetic integrity of *Chitala lopis* without any genetic mixing or hybridization with individuals from other regions. Similarly, the conservation of the Riau-endemic *Notopterus* species, which has a genetic structure that is uncontaminated by hybridization, requires restocking with parents from the original region of Riau. For the conservation of *Notopterus* species in Java, South Sumatra, Lampung, and Jambi through restocking techniques, it is possible to use parents from all of these regions.

Elaboration Outcomes And Possible Implications Arising From Results

Based on morphological analysis, the results of this study identified four species of belida fish, there are:

A. Notopterus Notopterus (Putak fish) found in Java, South Sumatra, Riau, Jambi

In East Kalimantan, Putak samples were found in Lake Semayang, Kota Bangun, and the Manggar Reservoir in Balikpapan. This information is significant in terms of the geographic distribution of putak fish in Indonesia, which was previously limited to the regions of Java and Sumatera. The distribution of *Notopterus* in Indonesia is provided in Appendix III.

B. Chitala hypselonotus

Chitala hypselonotus originally found in the Musi River - South Sumatra, but based on previous field research (Dr. Arif's tissue sample collection) in 2006, indicates this type of fish was also found in the Kampar Kanan River, Riau, Sumatra.

C. Chitala borneensis

Chitala borneensis is found in the Muaro Jambi- Jambi, Sintang - West Kalimantan and Kapuas hulu regions, West Kalimantan.

D. Chitala lopis

Chitala lopis is found in Riau, Jambi, South Sumatra, Lampung, West Java, West Kalimantan, Central Kalimantan, and South Kalimantan.

The unresolved taxonomy complicates conservation and recovery efforts for belida fish in Indonesia. Uncertainty about the conservation status of *Chitala* species as a result of uncertainty about species identity and distribution in Sundaland can potentially impede the implementation of appropriate conservation and management programs and plans, as well as the determination of appropriate protection status. The genus *Chitala* is found in Indonesia on the islands of Java, Sumatra, and Borneo and is thought to consist of three species: *Chitala* lopis, *Chitala hypselonotus*, and *Chitala borneensis*. Except for *C. lopis*, which is considered extinct, *Chitala* species are "least concerned," according to the International Union for the Conservation of Nature (IUCN). There are no *Chitala* species listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora's Annex (CITES).

The findings of the research lay the groundwork for taxonomic clues, provide the first accurate evidence, and clarify the status of belida fish in Indonesia, as well as the type of locality or the distribution of species in Indonesian, including *C. lopis* discovered at its type location (Java). The discovery of *C. lopis* in its type-locality after an absence of observations for more than 170 years has important implications for our understanding of *Chitala* species distribution. The International Union for the Conservation of Nature (IUCN) declared *Chitala lopis* extinct, implying that *C. lopis* is an endemic species of Java. According to this study, *C. lopis* is the most common species of *Chitala* in Sundaland, with distribution spanning Java, Sumatra, and Borneo.

Furthermore, *C. lopis* and *C.borneensis* are found in Sumatra and Borneo, indicating that populations on each island share a common distribution. Furthermore, haplotype similarities in *C. lopis* were discovered between different geographical locations in central Sumatra and western Kalimantan. This demonstrates that western Kalimantan and Sumatra are still linked today. The available information on *C. lopis* supports a reconsideration of its IUCN status. Furthermore, the IUCN conservation status of *C. borneensis* and *C. hypselonotus* should be revised immediately, while the widespread distribution of *C. lopis* necessitates a new conservation strategy. The findings also provide the first comprehensive DNA barcode reference library for *Chitala* spp., allowing for the

automatic identification of future *Chitala* species, a tool that opens up new avenues for conservation and management.

Based on the findings of this study, the identification of belida in Indonesian can be described as follows:

- 2. A. There are black spots at the base of tip in anal fin to the caudal fin......Chitala borneensisB. There are no black spots at the base of tip in anal fin to the caudal fin......3
- 3. A. The upper jaw extends far beyond the back of the eye (orbital)......Chitala lopisB. The upper jaw is almost bordered/slightly past the back of the eye.....Chitala hypselonotus

Based on the results of this study, there is a change in the determinant character of the belida fish group for *C. borneensis* when compared to *C. lopis* include:

- 1. All genus *Chitala* have black patches at the base of the pectoral fins.
- 2. *Chitala borneensis* has black spots on the tip of the anal fin to the base of the caudal fin while the other *Chitala* is absent.
- 3. The length of the upper jaw of C.borneensis is shorter than C. lopis
- 4. The length before the dorsal fin in C.borneensis is shorter than C. lopis
- 5. The height of the back body in C.borneensis is shorter than C. lopis

The morphology of the four species can be seen in the following figures:



Figure 37 Notopterus Notopterus



Figure 38 Chitala hypselonotus



Figure 39 Chitala lopis



Figure 40 Chitala borneensis

Contribution of External Expert in Research

Some assistance were provided in the form of data exchange, information exchange, and the publication process to support and strengthen the findings in this study. Other researchers who involved in this study include:

- 1. **Prof. Nicholas Hubert** Université Montpellier (UMR), Institut des sciences de l'évolution de Montpellier (ISEM) (IRD, UM, CNRS, EPHE)
 - Providing additional DNA samples of Chitala
 - Assisting in the review of the manuscript of the publication to be published
- 2. Dr. Haraldt Anhalt First Zoological Department, Natural History, Museum Vienna,
 - Assisting in the digital exchange of specimen data
 - Assisting in specimen data analysis
 - Providing reference species of *Chitala* derived from the Vienna Museum
 - Assisting in the review of the manuscript of the publication to be published
- 3. Dr. Meaghan Duncan -Department of Primary Industry NSW, Australia
 - Assisting in the review of the manuscript of the publication to be published
 - Assisting with proofreading in the finalization of publication manuscripts
- 4. Dr. Tedjo Sukmono Department of Biology, University of Jambi
 - Providing additional DNA samples of *Chitala*
 - Providing information related to the distribution of *Chitala* habitats
- **5. Dr. Boby Muslimin -** Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN)
 - Assist in the implementation of field survey / sampling activities
 - Conducting PCA analysis of *Chitala* samples
- **6.** Fathur Rochman Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN)
 - Creating a distribution map of the presence of *Chitala*
 - Making a map for sampling location of *Chitala* and *Notopterus* spesies

V. Conclusion and Recommendation

The present study provides evidence supporting the recognition of three species of *Chitala* in Indonesia, thanks to the rediscovery of *C. lopis* in its type locality, and as such puts an end to two decades to taxonomic confusion in the group. Species range distribution is profoundly revised for each of the three species here, *C. lopis* being the most widespread *Chitala* species in Indonesia. As they are, present results indicates that the IUCN conservation status of *C. borneensis* and *C. hypselonotus* should be urgently revised, while the wide distribution of *C. lopis* calls for locally adapted conservation plans. The present study further provides the first comprehensive DNA barcode

reference library for *Chitala* spp., enabling automated identification of *Chitala* species in the future, a tool which opens new perspectives in terms of conservation and management.

Conclusion

- The results of present study have revealed the rediscovery of *Chitala lopis* in the Cisadane River, Tangerang which shows that *Chitala lopis* still exist, though the IUCN stated that this species has extinct, specifically the population in the river flowing to the North Coast of Java (from Tangerang to Semarang).
- According to the present study, the species of *Chitala lopis*, which was formerly stated as an endemic species in Java Island, particularly along the North Coast of Java, apparently have a wide distribution in Southeast Asia, including in Indonesia (i.e. Java, Sumatra, and Borneo).
- Present study results could be a reference for the certainty of taxonomic status on the Genus *Chitala* which consists of three species, namely *C. lopis*, *C. borneensis*, and *C. hypselonotus*
- *C. borneensis* from the present study was only found in West Borneo and Jambi, while *C. hypselonotus* was only found in the Musi River, South Sumatra Province.
- *Notopterus Notopterus* is found to be single species form with different genetic structure within population of Sumatra, Java, and Borneo.
- Present study has given significant contribution to the continuity of featherback's research (for genus *Chitala* and *Notopterus*) through the addition of specimen collection in the Museum Zoologicum Bogoriense – National Research and Innovation Agency (BRIN). These scientific collections are needed by local or international researchers interested in featherbacks.

Recommendation

- The present study has given the certainty of taxonomic status for featherbacks, especially in Indonesia, which covers three species (*C. lopis*, *C. borneensis*, and *C. hypselonotus*). These information is indispensable for supporting the policy formulation on featherbacks management in Indonesia.
- Featherbacks from three species of the genus *Chitala* are known to have specific location and different extent distribution. However, the population status of these species is yet to be known. Therefore, the stock assessment is needed to be carried out as consideration in formulating policy related the protection of featherbacks.
- The extinction status of *Chitala lopis* which was formerly stated as "extinct" by the IUCN is necessary to be reviewed by the scientific authority in Indonesia.

- It was previously assumed that the distribution of *Chitala hypselonotus* was limited to the Musi River in South Sumatra, but based on the findings of this study, *Chitala hypselonotus* is also found in Riau. As a result, *Chitala hypselonotus* can be classified as a type endemic to Sumatra.
- The taxonomic status of *Notopterus Notopterus* which not showing any other species is needed to be taken into account based on the population status in each region.
- Socialization/dissemination of this present study is urgently needed to spread the information to the stakeholders related to featherbacks management.

VI. <u>References</u>

- Alshari N.F.M.A.H., Lavoué S., Sulaiman M.A.M., Khaironizam M.Z., Nor S.A.M., Aziz F. 2021. Pleistocene paleodrainages explain the phylogeographic structure of Malaysian populations of Asian arowana better than their chromatic variation. Endanger. Species Res. 46:205–214.
- Arida E., Ashari H., Dahruddin H., Fitriana Y.S., Hamidy A., Irham M., Riyanto A., Wiantoro S.,
 Zein M.S.A., Hadiaty R.K. 2021. Exploring the vertebrate fauna of the Bird's Head Peninsula (Indonesia, West Papua) through DNA barcodes. Mol. Ecol. Resour. 21:2369–2387.
- Bermingham E., McCafferty S., Martin A.P. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian isthmus. In: Kocher T.D., Stepien C.A., editors. Molecular systematics of fishes. San Diego: CA Academic Press. p. 113–128.
- Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.H., Xie D., Suchard M.A., Rambaut A., Drummond A.J. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput. Biol. 10:1–6.
- Brown S.D.J., Collins R.A., Boyer S., Lefort C., Malumbres-Olarte J., Vink C.J., Cruickshank R.H. 2012. Spider: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. Mol. Ecol. Resour. 12:562–565.
- de Bruyn M., Rüber L., Nylinder S., Stelbrink B., Lovejoy N.R., Lavoué S., Heok Hui T., Nugroho E., Wowor D., Ng P.K.L., Siti Azizah M.N., Von Rintelen T., Hall R., Carvalho G.R. 2013.
 Paleo-drainage basin connectivity predicts evolutionary relationships across three Southeast asian biodiversity hotspots. Syst. Biol. 62:398–410.
- Dahruddin H., Hutama A., Busson F., Sauri S., Hanner R., Keith P., Hadiaty R., Hubert N. 2017. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Mol. Ecol. Resour. 17:288–299.

Dahruddin H., Sholihah A., Sukmono T., Sauri S., Nurhaman U., Wowor D., Steinke D., Hubert N.

2021. Revisiting the Diversity of Barbonymus (Cypriniformes, Cyprinidae) in Sundaland Using DNA-Based Species Delimitation Methods. Diversity. 13:283.

Eschmeyer W.N., Fricke R., van der Laan R. 2018. Catalog of fishes electronic version. .

Froese R., Pauly D. 2020. Fishbase. Available from http://www.fishbase.org.

- Fujisawa T., Barraclough T.G. 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. Syst. Biol. 62:707–724.
- Ho S.Y.W., Larson G. 2006. Molecular clocks: when timesare a-changin'. TRENDS Genet. 22:79– 83.
- Hubert N., Kadarusman, Wibowo A., Busson F., Caruso D., Sulandari S., Nafiqoh N., Rüber L., Pouyaud L., Avarre J.C., Herder F., Hanner R., Keith P., Hadiaty R.K. 2015. DNA barcoding Indonesian freshwater fishes: challenges and prospects. DNA Barcodes. 3:144–169.
- Hubert N., Lumbantobing D., Sholihah A., Dahruddin H., Delrieu-Trottin E., Busson F., Sauri S., Hadiaty R., Keith P. 2019. Revisiting species boundaries and distribution ranges of Nemacheilus spp. (Cypriniformes: Nemacheilidae) and Rasbora spp. (Cypriniformes: Cyprinidae) in Java, Bali and Lombok through DNA barcodes: implications for conservation in a biodiversity hotspot. Conserv. Genet. 20:517–529.
- Inoue J.G., Kumazawa Y., Miya M., Nishida M. 2009. The historical biogeography of the freshwater knifefishes using mitogenomic approaches: A Mesozoic origin of the Asian notopterids (Actinopterygii: Osteoglossomorpha). Mol. Phylogenet. Evol. 51:486–499.
- Ivanova N. V, Zemlak T.S., Hanner R.H., Hébert P.D.N. 2007. Universal primers cocktails for fish DNA barcoding. Mol. Ecol. Notes. 7:544–548.
- Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A., Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods. 14:587–589.
- Kapli P., S. L., Zhang J., Kobert K., Pavlidis P., Stamatakis A., Flouri T. 2017. Multi-rate Poisson Tree Processes for single-locus species delimitation under Maximum Likelihood and Markov Chain Monte Carlo. Bioinformatics. 33.
- Keith P., Lord C., Darhuddin H., Limmon G., Sukmono T., Hadiaty R., Hubert N. 2017.Schismatogobius (Gobiidae) from Indonesia, with description of four new species. Cybium.41.
- Keith P., Mennesson M., Delrieu-Trottin E., Hubert N. 2020. Giuris (Teleostei: Eleotridae) from Indonesia, with description of a new species. Cybium Rev. Int. d'Ichtyologie. 44:317–329.
- Kekkonen M., Hebert P.D.N. 2014. DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. Mol. Ecol. Resour. 14:706–715.

- Kimura M. 1980. A simple method for estimating evolutionary rate of base subtitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 15:111–120.
- Kottelat M. 2005. The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Borneo Barat, Indonesia. Raffles Bull Zool Suppl. 13:139–173.
- Kottelat M., Whitten A.J., Kartikasari N., Wirjoatmodjo S. 1993. Freshwater Fishes of Western Indonesia and Sulawesi. Periplus Edition (HK) Ltd.
- Lavoué S., Ghazali S.Z., Jamaluddin J.A.F., Nor S.A.M., Zain K.M. 2020. Genetic evidence for the recognition of two allopatric species of Asian bronze featherback *Notopterus* (Teleostei, Osteoglossomorpha, Notopteridae). Zoosystematics Evol. 96:449.
- Luo A., Ling C., Ho S.Y.W., Zhu C.-D. 2018. Comparison of methods for molecular species delimitation across a range of speciation scenarios. Syst. Biol. 67:830–846.
- Mennesson M.I., Keith P., Sauri S., Busson F., Delrieu-Trottin E., Limmon G., Sukmono T., Risdawati R., Dahruddin H., Hubert N. 2021. Eleotris (Teleostei: Eleotridae) from Indonesia with description of three new species within the 'melanosoma'neuromast pattern group. Pacific Sci. 75:469–495.
- Ng H.H. 2022. *Chitala* lopis. The IUCN Red List of Threatened Species 2020: e. T157719927A89815479. .
- Nguyen L.-T., Schmidt H.A., Von Haeseler A., Minh B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32:268–274.
- Ogilvie H.A., Bouckaert R.R., Drummond A.J. 2017. StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. Mol. Biol. Evol. 34:2101–2114.
- Paradis E., Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 35:526–528.
- Pons J., Barraclough T.G., Gomez-Zurita J., Cardoso A., Duran D.P., Hazell S., Kamoun S., Sumlin W.D., Vogler A.P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55:595–606.
- Puillandre N., Brouillet S., Achaz G. 2021. ASAP: assemble species by automatic partitioning. Mol. Ecol. Resour. 21:609–620.
- Rambaut A., Drummond A.J., Xie D., Baele G., Suchard M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67:901–904.
- Ratnasingham S., Hebert P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Notes. 7:355–364.
- Ratnasingham S., Hebert P.D.N. 2013a. A DNA-based registry for all animal species: the barcode

index number (BIN) system. PLoSONE. 8:e66213.

- Ratnasingham S., Hebert P.D.N. 2013b. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS One. 8.
- Roberts T. 1989. The freshwater fishes of western borneo (Borneo Barat, Indonesia). California Academy of sciences.
- Roberts T.R. 1992. Systematic revision of the old world freshwater fish family Notopteridae. Ichthyol. Explor. Freshw. 2:361–383.
- Shen Y., Hubert N., Huang Y., Wang X., Gan X., Peng Z., He S. 2019. DNA barcoding the ichthyofauna of the Yangtze River: insights from the molecular inventory of a mega-diverse temperate fauna. Mol. Ecol. Resour. 19:1278–1291.
- Sholihah A., Delrieu-Trottin E., Condamine F.L., Wowor D., Rüber L., Pouyaud L., Agnèse J.F., Hubert N. 2021a. Impact of Pleistocene Eustatic Fluctuations on Evolutionary Dynamics in Southeast Asian Biodiversity Hotspots. Syst. Biol. 70:940–960.
- Sholihah A., Delrieu-Trottin E., Sukmono T., Dahruddin H., Pouzadoux J., Tilak M.K., Fitriana Y., Agnèse J.F., Condamine F.L., Wowor D., Rüber L., Hubert N. 2021b. Limited dispersal and in situ diversification drive the evolutionary history of Rasborinae fishes in Sundaland. J. Biogeogr. 48:2153–2173.
- Sholihah A., Delrieu-Trottin E., Sukmono T., Dahruddin H., Risdawati R., Elvyra R., Wibowo A., Kustiati K., Busson F., Sauri S., Nurhaman U., Dounias E., Zein M.S.A., Fitriana Y., Utama I.V., Muchlisin Z.A., Agnèse J.F., Hanner R., Wowor D., Steinke D., Keith P., Rüber L., Hubert N. 2020. Disentangling the taxonomy of the subfamily Rasborinae (Cypriniformes, Danionidae) in Sundaland using DNA barcodes. Sci. Rep. 10.
- Trifinopoulos J., Nguyen L.-T., von Haeseler A., Minh B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44:W232–W235.
- Voris H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. J. Biogeogr. 27:1153–1167.
- Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D.N. 2005. DNA barcoding Australia's fish species. Phylosophical Trans. R. Soc. B. 360:1847–1857.
- Woodruff D.S. 2010. Biogeography and conservation in Southeast asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugium-phase biodiversity. Biodivers. Conserv. 19:919–941.
- Zhang J., Kapli P., Pavlidis P., Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics. 29:2869–2876.

- Abbs F., Rehman M.H.U., Ashraf, M & K.J. Iqbal. 2013. Body composition of featherback Notopterus Notopterus and Rita rita Balloki Headworks-Pakistan. Journal of Agr-food and Applied Sciences 1(4): 126-29.
- Avise J.C. 1994. Molecular Markers, Natural History and Evolution. NewYork : Chapman and Hall
- Avise J.C. 2000. *Phylogeography:* The History and Formation of Species . Cambridge, MA : Harvard University Press .
- Casavas I., Doulman D.J., Petr T.O., Padro J. & L. Debas. 1996. *Cambodia Rehabilitation and Development Needs of the Fishery Sector*. FAO, Rome.
- Drummond A.J. *et al.* 2010. Geneious v5.5. Available at <u>http://www.geneious.com</u> (last accessed 12 March 2022).
- Dudgeon D. 2003. The contribution of scientific information to the conservation and management of freshwater biodiversity in tropical Asia. *Hydrobiologia* 500:295–314.
- Dudgeon, D., Arthington A.H. & M.O Gessner et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81:163–82.
- de Bruyn M., Wilson J.C. & P.B. Mather. 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, Macrobrachium rosenbergii. *Molecular Phylogenetics and Evolution* 30: 251 –7.
- de Bruyn M, von Rintelen T., von Rintelen K., Mather P. & G. Carvalho. 2012. Molecular biogeography and phylo-geoography of the freshwater fauna of the Indo-Australian Archipelago. *In: Biotic Evolution and Environ-mental Change in Southeast Asia*. Cambridge University Press. p. 317–47.
- Hebert P.D., Cywinska A., Ball S.L. & J.R. deWaard. 2003. Biological identifications through DNA barcodes. *Proc Biol Sci* 270(1512):313-21. doi: 10.1098/rspb.2002.2218. PMID: 12614582; PMCID: PMC1691236.
- Hausmann A., Haszprunar G. & P.D. Hebert. 2011. DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. *PLoS One* 6(2):e17134. doi: 10.1371/journal.pone.0017134. PMID: 21423340; PMCID: PMC3040642.
- Hoffman M., Hilton-Taylor C., Angulo A., Böhm M., Brooks T.M., Butchart S. H. M., Carpenter K.E., Chanson J., Collen B. & N.A. Cox. 2010. The impact of Conservation on the status of the world's vertebrates. *Science* 330:1503-1509.
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. & A. Drummond. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647-9. doi: 10.1093/bioinformatics/bts199.

- Kottelat M., Whitten A.J., Kartikasari S.N. & S. Wirjoatmodjo. 1993. *Freshwater fishes of western Indonesia and Sulawesi*. Periplus Edition, Hongkong, 259 pp.
- Lavoué S., Zafirah Ghazali S., Amirul Firdaus Jamaluddin J., Azizah Mohd Nor S. & K.M.D. Zain. 2020. Genetic evidence for the recognition of two allopatric species of Asian bronze featherback *Notopterus* (Teleostei, Osteoglossomorpha, Notopteridae). *Zoosystematics and Evolution* 96(2): 449-454. <u>https://doi.org/10.3897/zse.96.51350</u>.
- Liu W.B., Yao Y., Yan C.C., Wang X.H. & X.L. Lin. 2021. A new species of *Polypedilum (Cerobregma)* (Diptera, Chironomidae) from Oriental China. *Zookeys* 1011:139-148. doi: 10.3897/zookeys.1011.59554. PMID: 33568962; PMCID: PMC7847467..
- Ng, H.H., 2010. *Notopterus Notopterus*. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.Downloaded on 07 September 2012).
- Parenti L.R. & K.K.P. Lim. 2005. Fishes of the Rajang Basin, Sarawak, Malaysia. *The Raffles Bulletin of Zoology* 13:175-208.
- Pilipenko V.E., Salmela, J. & E.J. Vesterinen. 2012. Description andDNA barcoding of Tipula (*Pterelachisus*) recondita sp. n. from thePalaearctic region (Diptera, Tipulidae). ZooKeys 192: 51–65.
- Pouyaud L., Sudarto & E. Paradis 2009. The phylogenetic structure of habitat shift and morphological convergence in Asian Clarias (Teleostei, Siluriformes: Clariidae). *Journal of Zoological Systematics and Evolutionary Research* 47: 344-356.
- Roberts T.R. 1992. Systematic revision of the Old World freshwater fish family Notopteridae. *Ich-thyological Freshwater Exploration* 2(4): 361–383.
- Rainboth WJ. 1996. Fishes of the Cambodian Mekong. Rome: FAO.
- Schlick-Steiner B.C., Steiner F.M., Seifert B., Stauffer C., Christian E. & R.H. Crozier. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annu Rev Entomol.* 55:421-38. doi: 10.1146/annurev-ento-112408-085432. PMID: 19737081.
- Stella O. 2019. Discriminant Analysis: An Analysis of Its Predictship Function. *Journal of Education and Practice*. 10(5): 50-57.
- Talwar P.K. & A.G. Jhingran. 1991. Inland fishes of India and adjacent countries. New Delhi: Oxford & IBH Publishing Co(P) Ltd.

Turan, C., Oral, M., Öztürk, B., & Düzgüneş, E. 2006. Morphometric and meristic variation between stocks of Bluefish (Pomatomus saltatrix) in the Black, Marmara, Aegean and northeastern Mediterranean
 Seas. *Fisheries Research*, 79(1-2), 139-147. https://doi.org/10.1016/j.fishres.2006.01.015

- Ward R.D., Zemlak T.S., Innes B.H., Last P.R. & PDN. Hebert. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B-Biological Sciences* 360(1462): 1847–1857. <u>https://doi.org/10.1098/rstb.2005.1716</u>.
- Willis S.C., Winemiller K.O., & Lopez-Fernandez H. 2005. Habitat structural complexity and morphological diversity of fish assemblages in a Neotropical floodplain river. *Community Ecology* 142: 284-295.
- Woodruff D.S. 2010. Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugium-phase biodiversity. *Biodiversity and Conservation* 19: 919-941.

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened Chitala a
 (Deteksi keberadaan ikan belida perdasarkan pengetahuan masyarakat Detecting the occurrence of the threatened Chitala and Notopterus through Fishermen knowledge) (an belida, sinonim Balidra, Pangaju, Belidah, Blidah, lopis, pipih, belido, Lempok
1.	Nama *
2.	Nomor HP *
3.	Nama *
4.	Nomor HP *
5.	Jenis kelamin * Mark only one oval. Laki-laki Perempuan

1/19

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened Chitala a
6.	Alamat (sebutkan Desa dan Kecamatan) *
7.	Kabupaten *
8.	Usia *
9.	Pendidikan *
	Mark only one oval.
	SD
	SMP
	SMA/SMK
	Perguruan Tinggi
	Tidak sekolah
10.	Pekerjaan *
	Check all that apply.
	Nelayan
	Pembudidaya ikan Petani
	Wiraswasta
	Pegawai Negeri
	Pedagang
	Other:

https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-GbiVxp-evZm9e3FnM_-McM/edit?ts=62567dd1

2/19

2/1			

Deteksi keberadaan ikan belida berdasarkan pengelahuan masyarakat (Detecting the occurrence of the threatened Chitala a...

11. Sudah berapa lama pekerjaan utama dilakukan? *

Mark only one oval.

Kurang dari 5 tahun

5-10 tahun

10-15 tahun

_____ 15-20 tahun

> 20 tahun

12. Apakah pekerjaan utama memenuhi kebutuhan hidup?*

Mark only one oval.

Memenuhi kurang dari setengah kebutuhan hidup

📃 Memenuhi lebih dari setengah kebutuhan hidup

Memenuhi semuanya

13. Apakah pekerjaan utama sekarang berpengaruh terhadap kesejahteraan? *

Mark only one oval.

Tidak (Kehidupan sangat sulit jika hanya tergantung pada pekerjaan sekarang)

Cukup (Hanya memenuhi kebutuhan sehari-hari)

Ya (Pekerjaan ini memenuhi semua keperluan hidup, prosfektif, dll)

14. Seberapa sering anda bekerja menangkap/memancing ikan?*

Mark only one oval.

Sangat sering

Sering

Kadang-kadang

🔘 Jarang

Sangat jarang

https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-GbIVxp-evZm9e3FnM_-McM/edit?ts=62567dd1

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threate	ned Chitala a
15.	Bagaimana hasil tangkapan ikan di perairan sungai/danau/waduk akhir-akhir ini?	*
	Mark only one oval.	
	Meningkat	
	Stabil/tetap	
	Berfluktuasi	
	Menurun	
16.	Alat tangkap utama yang digunakan untuk menangkap ikan di	*
	sungai/danau/waduk	
	Mark only one oval.	
	Jala	
	Pancing	
	Bubu	
	Jaring insang	
	Jaring hanyut	
	Other:	
17.	Berapa kali dalam seminggu anggota keluarga mengkonsumsi ikan hasil	*
17.	tangkapan?	
	Mark only one oval.	
	Sangat sering (Setiap hari)	
	Sering (4-6 kali seminggu)	
	Kadang-kadang (1-3 kali seminggu)	
	Jarang (seminggu sekali)	
	Sangat jarang (lebih dari seminggu sekali)	

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened Chitala a
18.	Sebutkan nama perairan dimana anda biasa menangkap ikan air tawar (Misal *
	sungai citarum, sungai bengawan solo, danau rawa pening dll)
19.	Sebutkan jenis-jenis ikan apa saja yang sering saudara tangkap di perairan 🛛 *
	sungai/danau/waduk? Jawaban boleh lebih dari satu, (bila ikan yang
	disebutkan di list tidak ada, bisa ditulis di barisan paling bawah)
	Check all that apply.
	Nila
	Mas
	Lele
	Mujair
	Gurame Patin
	Gabus
	Sepat
	Beunteur/Wadeur
	Nilem
	Tawes
	Lampan/Tengadak
	Baung Betok/Papuyu
	Lais
	Tapah
	Uceng
	Belida
	Other:

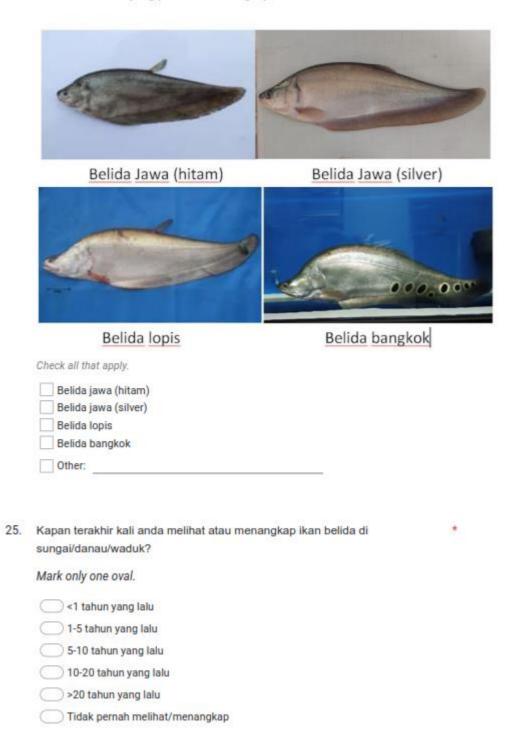
https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-Gb/Vxp-evZm9e3FnM_-McM/edit?ts=62567dd1

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threat	tened Chitala a
20.	Sebutkan jenis ikan yang mulai jarang tertangkap atau menurun populasinya dalam kurun waktu 5 tahun terakhir?	*
21.	Menurut anda, bagaimana populasi/stok sumberdaya ikan di perairan sungai/danau/waduk saat ini? Mark only one oval.	*
	 Populasi ikan semakin meningkat Populasi ikan meningkat Populasi ikan tetap Populasi ikan menurun Populasi ikan sangat menurun 	
22.	Apakah anda mengetahui ikan belida hidup di sungai/danau/waduk ? * Mark only one oval. Tahu Tidak tahu	
23.	Apakah anda pernah menangkap ikan belida? * Mark only one oval. Sangat sering Sering Kadang-kadang Jarang Tidak pernah	

https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-Gb/Vxp-evZm9e3FnM_-McM/edit?ts=62567dd1

Deteksi keberadaan ikan belida berdasarkan pengelahuan masyarakat (Detecting the occurrence of the threatened Chitala a...

24. Ikan belida mana yang pernah anda tangkap?



https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-GbiVxp-evZm9e3FnM_-McM/edil?ts=62567dd1

7/19

2/16/23, 4:20 AM	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened Chitala a.
26.	Dimana ikan belida biasa dapat ditangkap? (sebutkan nama sungai/danaunya)
27.	Di bagian mana ikan belida dapat di tangkap di sungai? *
	Mark only one oval. Bagian hulu sungai Bagian tengah sungai Bagian hilir sungai Muara sungai Semua bagian sungai Tidak tahu
28.	Di bagian mana ikan belida dapat ditangkap di danau/waduk? * Mark only one oval. Bagian tengah Bagian pinggir Bagian yang terdapat tanaman air Disemua bagian Tidak tahu

29. Jenis alat tangkap apa yang sering digunakan untuk menangkap ikan belida?

https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-GbIVxp-evZm9e3FnM_-McM/edit?ts=62567dd1

ð/19

22.14	an 1 1 1 1 1	4.70	
2/1	0/23,	4:20	АΜ

Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened Chitala a...

30. Waktu yang tepat untuk menangkap ikan belida *

Mark only one oval.

C	Malam
C	Siang

- Kedua-duanya
- 🔵 Tidak tahu
- 31. Ikan belida biasanya dapat ditangkap pada musim apa? *

Mark only one oval.

- N		 _	L	an
	D/III	m		an

🔵 Musim kemarau

📃 Tidak dipengaruhi musim

📄 Tidak tahu

32. Menurut saudara bagaimana populasi ikan belida saat ini?*

Mark only one oval.

Punah

Jarang

Kadang-kadang ada

Ada (biasa ditemukan)

Banyak/melimpah (Sering ditemukan)

🔵 Tidak tahu

https://docs.google.com/forms/d/1DcSviHYGD6UTcrt6JrNA-GbfVxp-evZm9e3FnM_-McM/edit?ts=62567dd1

2/16/23, 4:20 AM 64.	Deteksi keberadaan ikan belida berdasarkan pengetahuan masyarakat (Detecting the occurrence of the threatened o Menurut saudara apa penyebab hilang/menurunnya jumlah ikan belida atau * ikan lainnya di sungai/danau/waduk	nitala a
	Check all that apply. Sering ditangkap (Over fishing) Kerusakan habitat sungai Pencemaran limbah (rumah tangga, industri, tambang dll) Ikan asing memangsa ikan lokal Perubahan iklim Habitat terputus karena dibendung Other:	
65.	Bagaimana perhatian pemerintah dalam melindungi ikan yang terancam punah? * Mark only one oval. Sangat memperhatikan Sangat memperhatikan Biasa saja (Netral) Tidak memperhatikan Sangat tidak memperhatikan	
66.	Saran	

This content is neither created nor endorsed by Google.

Google Forms

https://docs.google.com/forms/d/1DcSvIHYGD6UTcrt6JrNA-GbiVxp-evZm9e3FnM_-McM/edit?ts=62567dd1

19/19

VIII. Appendix II List of Catalog Number MZB Labelling on Sample

Catalog No.	Species	Location/ Habitats	Collection	Collector(s)	Quan-
			Date		tity
MZB.26497	Notopterus Notopterus	JT-1 B1, Rawa Pening, Ds. Kerungu, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26498	Notopterus Notopterus	JT-1 B2, Rawa Pening, Ds. Pesongok, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26499	Notopterus Notopterus	JT-1 B3, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26500	Notopterus Notopterus	JT-1 B4, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26501	Notopterus Notopterus	JT-1 B5, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26502	Notopterus Notopterus	JT-1 B6, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26503	Notopterus Notopterus	JT-1 B7, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26504	Notopterus Notopterus	JT-1 B8-A, Rawa Pening, Ds. Kesongo, Kec. Tuntang, Kab. Semarang, Jawa Ten-	11 Maret 2022	Arif & Kurniawan	8
		gah.			
MZB.26505	Notopterus Notopterus	JT-1 B8-B, Rawa Pening, Ds. Kesongo, Kec. Tuntang, Kab. Semarang, Jawa Ten-	11 Maret 2022	Arif & Kurniawan	8
		gah.			
MZB.26506	Notopterus Notopterus	JT-1 B8-C, Rawa Pening, Ds. Kesongo, Kec. Tuntang, Kab. Semarang, Jawa Ten-	11 Maret 2022	Arif & Kurniawan	8
		gah.			
MZB.26507	Notopterus Notopterus	JT-1 B9, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26508	Notopterus Notopterus	JT-1 B10, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Ten-	11 Maret 2022	Arif & Kurniawan	1
		gah.			
MZB.26509	Notopterus Notopterus	JT-2 B1, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa	13 Maret 2022	Arif & Kurniawan	1
		Timur.			
MZB.26510	Notopterus Notopterus	JT-2 B2, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa	13 Maret 2022	Arif & Kurniawan	1
		Timur.			
MZB.26511	Notopterus Notopterus	JT-2 B4, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa	13 Maret 2022	Arif & Kurniawan	1
		Timur.			
MZB.26512	Notopterus Notopterus	JT-2 B5, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa	13 Maret 2022	Arif & Kurniawan	1
		Timur.			
MZB.26513	Notopterus Notopterus	JT-2 B8, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa	13 Maret 2022	Arif & Kurniawan	1
		Timur.			
MZB.26514	Notopterus Notopterus	JT-2, Waduk Bening/Widas, Ds. Pajaran, Kec. Saratan, Kab. Madiun, Jawa Timur.	13 Maret 2022	Arif & Kurniawan	10

MZB.26515	Notopterus Notopterus	ST. JB3, Soburon irigasi, Ds. Sukamandi, Kec. Patok Besi, Kab. Subang, Jawa	13 Maret 2022	Haryono, Vitas, &	4
		Barat.		hadi	
MZB.26516	Notopterus Notopterus	JT-1 B8, Rawa Pening, Ds. Pajajaran, Kec. Tuntang, Kab. Semarang, Jawa Tengah.	11 Maret 2022	Arif & Kurniawan	1
MZB.26517	Notopterus Notopterus	PLM.01, (Putak), Kota Palembang, Musi II, Palembang, Sumatera selatan	06-Apr-22	Arif & Kurniawan	1
MZB.26518	Notopterus Notopterus	PLM.02, Kota Palembang, Musi II, Palembang, Sumatera selatan	06-Apr-22	Arif & Kurniawan	1
MZB.26519	Notopterus Notopterus	PLM.03, Kota Palembang, Musi II, Palembang, Sumatera selatan	06-Apr-22	Arif & Kurniawan	1
MZB.26520	Notopterus Notopterus	PLM.04, Kota Palembang, Musi II, Palembang, Sumatera selatan	06-Apr-22	Arif & Kurniawan	1
MZB.26521	Notopterus Notopterus	PLM.05, Kota Palembang, Musi II, Palembang, Sumatera selatan	06-Apr-22	Arif & Kurniawan	1
MZB.26522	Notopterus Notopterus	PLM.01, OKI, (Belida), Ds. Serinanti, Kec. Padamaran, Palembang, Sumatera Se- latan	08-Apr-22	Arif & Kurniawan	1
MZB.26523	Notopterus Notopterus	PLM.02, OKI, (Putak), Ds. Serinanti, Kec. Padamaran, Palembang, Sumatera Selatan	08-Apr-22	Arif & Kurniawan	1
MZB.26524	Notopterus Notopterus	PLM.03, OKI, (Putak), Ds. Serinanti, Kec. Padamaran, Palembang, Sumatera Selatan	08-Apr-22	Arif & Kurniawan	1
MZB.26525	Notopterus Notopterus	PLM.04, OKI, (Belida), Ds. Serinanti, Kec. Padamaran, Palembang, Sumatera Se- latan	08-Apr-22	Arif & Kurniawan	1
MZB.26526	Notopterus Notopterus	LPM.01, Way Kanan, Kec. Blambangan, Umpu, Kab. Way Kanan, Lampung	10-Apr-22	Arif & Kurniawan	1
MZB.26527	Notopterus Notopterus	LPM.02, (Belida), Way Kanan, Kec. Blambangan, Umpu, Kab. Way Kanan, Lampung	10-Apr-22	Arif & Kurniawan	1
MZB. 26555	Chitala lopis	Pasar Taratak Buluh, Pekanbaru, Riau. (Ri pi)	5 Juni 2022	Haryono, Hadi, dan Vitas	3
MZB. 26556	Notopterus Notopterus	Pasar Taratak Buluh, Pekanbaru, Riau. (Ri p2)	5 Juni 2022	Haryono, Hadi, dan Vitas	14
MZB. 26557	Notopterus Notopterus	Pasar Taratak Buluh, Pekanbaru, Riau. (Ri p3)	5 Juni 2022	Haryono, Hadi, dan Vitas	27
MZB. 26558	Chitala lopis	Pasar Taratak Buluh, Pekanbaru, Riau. (Ri p4)	5 Juni 2022	Haryono, Hadi, dan Vitas	3
MZB. 26559	Notopterus Notopterus	Pasar Taratak Buluh, Pekanbaru, Riau. (Ri p5)	5 Juni 2022	Haryono, Hadi, dan Vitas	22
MZB. 26560	Notopterus Notopterus	Sungai Siak, Desa Tualang, Kec. Tualang, Kab. Siak (St. 2)	5 Juni 2022	Haryono, Hadi, dan Vitas	7

MZB. 26561	Chitala lopis	Waduk Kota Panjang, Desa Pangkal, Kec XIII, Kota Kampar, Kab Kampar. Riau	6 Juni 2022	Haryono, Hadi, dan Vitas	2
MZB. 26562	Chitala lopis	Desa Muara Tobo, Kec Tobo Tengah, Kab Tobo, Prov Jambi	8 Juni 2022	Haryono, Hadi, dan Vitas	1
MZB. 26563	Chitala borneensis andd Chitala lopis	Sungai Pijoan, Desa Pijoan, Kec Jambi Luar Kota, Kab Muara Jambi	9 Juni 2022	Haryono, Hadi, dan Vitas	2
MZB. 26564	Notopterus Notopterus	Desa Muara Tobo, Kec Tobo Tengah, Kab Tobo, Prov Jambi	8 Juni 2022	Haryono, Hadi, dan Vitas	3
MZB. 26611	Chitala lopis and Chitala borneensis	Pasar Junjung Buih, Ds. Tanjung Kuri, Kec. Sintang, Kab. Sintang, Kalimantan Barat.	15 agustus 2022	Vitas & Hadi	2
MZB. 26612	Chitala lopis and Chitala borneensis	Pasar Junjung Buih, Ds. Tanjung Kuri, Kec. Sintang, Kab. Sintang, Kalimantan Barat.	15 agustus 2022	Vitas & Hadi	3
MZB. 26613	Chitala lopis and Chitala borneensis	Pasar Junjung Buih, Ds. Tanjung Kuri, Kec. Sintang, Kab. Sintang, Kalimantan Barat.	15 agustus 2022	Vitas & Hadi	2
MZB. 26614	Chitala borneensis	Sungai Kapuas, Ds. Nanga Embaloh, Kec. Embaloh hilir, Kab. Kapuas hulu, Kalimantan Barat.	17 Agustus 2022	Vitas & Hadi	1
MZB. 26615	Chitala lopis	Sungai Cisadane, Cikokol, Kota Tangerang, Banten.	25 September 2022	Vitas & Hadi	1
MZB. 26616	Chitala lopis	TPI Djongkong (asal ikan: Sungai Nibung, Kecamatan Selimau, Kabupaten Kapuas Hulu, Kalimantan Barat)	17 Agustus 2022	Vitas & Hadi	2
MZB. 26617	Chitala lopis	Danau Lindung Empangau, Desa Empangau, Kecamatan Bunut Hilir, Kabupaten Kapuas Hulu, Kalimantan Barat	16 Agustus 2022	Vitas & Hadi	2
MZB. 26618	Chitala lopis	Danau Tempurau, Desa Tempurau, Kecamatan Selimbau, Kapubaten Kapuas Hulu, Kalimantan Barat	16 Agustus 2022	Vitas & Hadi	2
MZB. 26619	Chitala lopis	Sungai Belitung, Dusun Kenasau, Desa Djongkong Kiri Hilir, Kecamatan Djongkong, Kabupaten Kapuas Hulu, Kalimantan Barat	16 Agustus 2022	Vitas & Hadi	2
MZB. 26620	Chitala lopis	Soehait, Kabupaten Kapuas Hulu, Kalimantan Barat	17 Agustus 2022	Vitas & Hadi	2
MZB. 26621	Chitala lopis	Sungai Embaloh, Desa Ulak Pauk, Kecamatan Embaloh Hulu, Kabupaten Kapuas Hulu, Kalimantan Barat	18 Agustus 2022	Vitas & Hadi	2
MZB. 26622	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1

MZB. 26623	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26624	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26625	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26626	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26627	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26628	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26629	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26630	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26631	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26632	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26633	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26634	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26635	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26636	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26637	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1
MZB. 26638	Chitala lopis	Riam kanan, kec Aronia, Kab. Banjar, Kalimantan Selatan	13 Agustus 2022	Arif & Kurniawan	1

MZB. 26639	Chitala lopis	Buntok, Barito Selatan	14 Agustus	Arif & Kurniawan	1
			2022		
MZB. 26640	Chitala lopis	Buntok, Barito Selatan	14 Agustus 2022	Arif & Kurniawan	1
MZB. 26641	Chitala lopis	Buntok, Barito Selatan	14 Agustus 2022	Arif & Kurniawan	1
MZB. 26642	Chitala lopis	Buntok, Barito Selatan	14 Agustus 2022	Arif & Kurniawan	1
MZB. 26643	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26644	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26645	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26646	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26647	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26648	Chitala lopis	Ds. Taruk Timpah, Kec Timpah, Kab. Kapuas	15 Agustus 2022	Arif & Kurniawan	1
MZB. 26649	Chitala lopis	Katingan, Ds. Tumbang Lintang, Katingan	16 Agustus 2022	Arif & Kurniawan	1
MZB. 26650	Chitala lopis	Katingan, Ds. Tumbang Lintang, Katingan	16 Agustus 2022	Arif & Kurniawan	1
MZB. 26651	Chitala lopis	Katingan, Ds. Tumbang Lintang, Katingan	16 Agustus 2022	Arif & Kurniawan	1
MZB. 26652	Chitala lopis	Seruyan, Telaga Pulang, Kec. Danau Sembuluh, Kab. Seruyan	14 Agustus 2022	Arif & Kurniawan	1
MZB. 26653	Chitala lopis	Seruyan, Telaga Pulang, Kec. Danau Sembuluh, Kab. Seruyan	17 Agustus 2022	Arif & Kurniawan	1
MZB. 26654	Chitala lopis	Seruyan, Telaga Pulang, Kec. Danau Sembuluh, Kab. Seruyan	17 Agustus 2022	Arif & Kurniawan	1

MZB. 26655	Chitala lopis	Seruyan, Telaga Pulang, Kec. Danau Sembuluh, Kab. Seruyan	17 Agustus 2022	Arif & Kurniawan	1
MZB. 26698	Chitala lopis	Seruyan, Telaga Pulang, Kec. Danau Sembuluh, Kab. Seruyan	17 Agustus 2022	Arif & Kurniawan	1
MZB. 26699	Chitala lopis	Kota Wangun Barat, Kec. Arot Selatan, Kab, Kota Wangun Barat	18 Agustus 2022	Arif & Kurniawan	1
MZB. 26700	Chitala lopis	Kota Wangun Barat, Kec. Arot Selatan, Kab, Kota Wangun Barat	18 Agustus 2022	Arif & Kurniawan	1
MZB. 26701	Chitala lopis	Kota Wangun Barat, Kec. Arot Selatan, Kab, Kota Wangun Barat	18 Agustus 2022	Arif & Kurniawan	1

IX. Appendix III Distribution and Abundance of Chitala Genera based on local communities respondent information (Questionnaire online)

No	Water name	Site address	Species	Popula- tion level (interview)	Popula- tion Status
1	Rawa Pening, Semarang	7°17'22.4"S 110°27'10.6"E	Notopterus Notopterus	4	Abundant
2	White Elephant River (Bengawan Solo), Solo	7°32'47.4"S 110°47'01.4"E	Notopterus No- topterus	1	Uncommon
3	Bening Reservoir/Widas Reservoir, Nganjuk	7°32'54.4"S 111°47'36.8"E	Notopterus Notopterus	4	Abundant
4	Brantas River, Nganjuk	7°30'21.1"S 112°08'59.6"E	Notopterus Notopterus	4	Abundant
5	Pekalongan River	7°00'22.8"S 109°32'57.1"E	Notopterus Notopterus	1	Uncommon
6	West Canal Flood, Semarang	6°59'35.4"S 110°24'08.1"E	Notopterus Notopterus	0	None
7	Kali Babadan, Tegal, Central Java	6°52'13.2"S 109°19'31.7"E	Notopterus Notopterus	0	None
8	New Cimanuk, Indramayu, West Java	6°19'00.8"S 108°19'29.9"E	Notopterus Notopterus	0	None
9	Karangsong, Indramayu, West Java	6°19'09.8"S 108°19'39.6"E	Notopterus Notopterus	0	None
10	Cimanuk Lama, Indramayu, West Java	6°18'09.4"S 108°20'09.3"E	Notopterus Notopterus	0	None
11	Salamdarma Reservoir, Subang, West Java	6°25'11.0"S 107°53'37.0"E	Notopterus Notopterus	2	Present
12	Cipunagara, Subang, West Java	6°34'16.3"S 107°51'08.1"E	Notopterus Notopterus	3	Common
13	Irrigation canal of BDA Sukamandi (connected to East Tarum River), Subang, West Java	6°22'08.5"S 107°37'21.8"E	Notopterus Notopterus	3	Common
14	Sukaluyu (Citarum River), Karawang, West Java	6°19'10.3"S 107°16'38.5"E	Notopterus Notopterus	1	Uncommon
15	Walahar Reservoir, Karawang, West Java	6°23'08.0"S 107°21'43.3"E	Notopterus Notopterus	1	Uncommon
16	Leuwi Goong, Karawang, West Java	6°24'24.9"S 107°21'46.3"E	Notopterus Notopterus	1	Uncommon
17	Lontar Village (Cimanceuri River), Tangerang, Banten	6°03'59.8"S 106°27'05.8"E	Notopterus Notopterus	1	Uncommon
18	Irrigation canal of Cisadane River, Tangerang, Banten	6°08'12.4"S 106°35'15.8"E	Notopterus Notopterus	0	None
19	Rawa Total, Tangerang, Banten	6°09'23.6"S 106°35'47.3"E	Notopterus Notopterus	0	None

20	Cipayung Village (Irrigation canal connected to Kalimalang River), Bekasi Regency, West Java	6°18'09.3"S 107°13'31.5"E	Notopterus Notopterus	1	Uncommon
21	Ciliwung River (Cikoko), Jakarta	6°14'43.4"S 106°51'43.2"E	Notopterus Notopterus	1	Uncommon
22	Ciliwung River (River School), Jakarta	6°21'09.6"S 106°50'08.3"E	Notopterus Notopterus	0	None
23	Great Swamp Lake, Depok, West Java	6°23'31.4"S 106°49'01.8"E	Notopterus Notopterus	0	None
24	Cikokol, Cisadane River, Tangerang, Banten	6°12'54.7"S 106°37'36.0"E	Chitala lopis	3	Common
25	Muaro Belida River, South Sumatra	2°52'21.9"S 104°00'12.8"E	Notopterus Notopterus and Chitala lopis	3	Common
26	Musi Palembang, South Sumatra	3°00'59.9"S 104°43'14.1"E	Notopterus Notopterus and Chitala lopis	1	Uncommon
27	Batanghari River, South Sumatra	2°52'56.1"S 103°59'15.2"E	Notopterus Notopterus and Chitala lopis	2	Present
28	Kelakar River, Ogan Komering Ilir, South Sumatra	3°14'31.6"S 104°38'55.4"E	<i>Notopterus Notopterus</i> and <i>Chitala</i> lopis	3	Common
29	Pedamaran (OIC), South Sumatra	3°27'50.2"S 104°49'31.4"E	<i>Notopterus Notopterus</i> and <i>Chitala</i> lopis	2	Present
30	Way Left River, West Onion Bone, Lampung	4°28'12.9"S 105°04'55.2"E	Notopterus Notopterus and Chitala lopis	3	Common
31	Tirta Sinta Dam, Kotabumi, Lampung	4°44'03.1"S 104°51'15.3"E	Notopterus Notopterus	2	Present
32	Way Kanan River, Lampung	4°30'17.6"S 104°31'27.9"E	Notopterus Notopterus and Chitala lopis	3	Common
33	Kuto Panjang Reservoir, Kampar Regency, Riau	0°18'55.7"N 100°46'39.7"E	Chitala lopis	4	Abundant
34	Siak River, Riau	0°38'09.9"N 101°36'31.1"E	Notopterus Notopterus and Chitala lopis	3	Common
35	Pelalawan River, Riau	0°21'40.0"N 101°54'33.1"E	Chitala lopis	3	Common
36	Indragiri River, Riau	0°20'53.1"S 102°26'39.4"E	Chitala lopis	3	Common
37	Batanghari Regenci, Jambi	1°39'01.9"S 103°19'45.4"E	Chitala lopis	2	Present
38	Tebo Regency, Jambi	1°30'23.3"S 102°19'59.7"E	Chitala lopis	3	Common
39	Muaro Jambi Regency, Jambi	1°34'33.5"S 103°30'48.3"E	Notopterus Notopterus and Chitala borneensis	4	Abundant
40	Riam Kanan Reservoir Weir, Aranio District, Banjar Regency South Kalimantan	3°31'12.2"S 115°00'35.1"E	Chitala lopis	4	Abundant

41	Barito River, Buntok, South Barito, Central Kalimantan	1°42'50.5"S 114°50'17.7"E	Chitala lopis	4	Abundant
42	Haleung Lake, Aruk Village, Timpah District, Kapuas Regency, Central Kalimantan	1°51'04.4"S 114°28'02.0"E	Chitala lopis	4	Abundant
43	Tanjung Tasik Lake, Tumbang Liting Village, Katingan Regency, Central Kalimantan	1°56'09.0"S 113°24'30.3"E	Chitala lopis	3	Common
44	Seruyan River, Telaga Pulang District, Seruyan Regency, Central Kalimantan	2°51'41.0"S 112°15'58.4"E	Chitala lopis	3	Common
45	Arut River, West Kotawaringin Regency, Central Kalimantan	2°40'46.5"S 111°37'18.9"E	Chitala lopis	3	Common
46	Sentuntung, Belitang River, Sekada, West Kalimantan	0°13'55.4"N 111°15'56.6"E	Chitala lopis	2	Present
47	Mensiku River, Binjai Hulu, Sintang Regency, West Kalimantan	0°13'07.7"N 111°33'24.5"E	Chitala borneensis	3	Common
48	Empangau, Sintang, West Kalimantan	0°44'26.5"N 112°22'53.3"E	Chitala borneensis	4	Abundant
49	Belitung River, Jongkong Kiri Hilir, West Kalimantan	0°43'57.8"N 112°16'09.7"E	Chitala lopis	4	Abundant
50	Lake Sentarum, Kapuas, West Kalimantan	0°49'07.3"N 112°02'33.6"E	Chitala lopis	4	Abundant
51	Mobile Lake, West Kalimantan	0°37'20.4"N 112°00'29.7"E	Chitala lopis	3	Common
52	New Lake, Nangah Embaloh Village, Kapuas Hulu Regency, West Kalimantan	0°49'50.3"N 112°36'01.1"E	Chitala borneensis	1	Uncommon
53	Mataram Udik, Bandar Mataram, Central Lampung Regency	4°30'29.1"S+105°31'40.5"E	Chitala lopis	4	Abundant
<mark>54</mark>	Musi River, South Sumatra	-	Chitala hypselonotus	1	Uncommon
<mark>55</mark>	Kampar River, Riau	-	Chitala hypselonotus	1	Uncommon

Information:

Population level	English
4	Abundant
3	Common
2	Present
1	Uncommon
0	None

