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Rediscovery of the giant featherback *Chitala lopis* (Notopteridae) in its type locality resolves decades of taxonomic confusion

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ABSTRACT: Unresolved taxonomy poses a significant challenge for conservation and recovery efforts of freshwater fishes in Indonesia. Asian featherbacks of the genus *Chitala* are found in Java, Sumatra and Borneo, and currently thought to comprise 3 of 6 species: *C. lopis*, *C. hypselonotus*, and *C. borneensis*. According to the IUCN, *Chitala* species are of Least Concern in Indonesia, except for *C. lopis*, which is considered Extinct. However, the taxonomy of *Chitala* species is unclear, with 3 nominal species (*C. lopis*, *C. hypselonotus* and *C. borneensis*) historically synonymized under a single name (*C. lopis*), but more recently tentatively considered as a valid species. The recent rediscovery of *C. lopis* in its type locality (Java) since last recorded in 1851 enabled a comprehensive genetic and morphological study of the 3 nominal species to clarify their status. We examined 151 mitochondrial sequences from all known species of *Chitala*, including sequences from the type localities of the 3 taxa in question. We identified 3 well-supported clades corresponding to *C. lopis*, *C. hypselonotus*, and *C. borneensis.* The analyses of 22 measurements identified several diagnostic characters between *C. lopis* and *C. borneensis*. We provide evidence that *C. lopis* is not extinct and is widespread across Java, Sumatra and Borneo. In contrast, *C. hypselonotus* has a more restricted distribution to Central Sumatra and may be at risk of extinction given it has not been collected from the Musi River since 2015. We argue for an urgent revision of the IUCN conservation status of the 3 species and recommend an expansion of molecular-based inventories to all freshwater fishes in Indonesia.

KEY WORDS: DNA barcodes · Morphometrics · Sequence-based species delimitation · Southeast Asia · Taxonomy

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1. INTRODUCTION

Commonly known as featherback, knife fish, or belida, the genus *Chitala* belongs to the family Notopteridae, an iconic group of tropical freshwater fishes inhabiting the lowlands of Africa, India and Asia. The family is composed of 4 genera, with *Chitala* and *Notopterus* occurring from India to Southeast Asia and *Xenomystus* and *Papyrocranus* occurring in West and Central Africa (Roberts 1992, Inoue et al. 2009, Froese & Pauly 2023). The family Notopteridae is among the most ancient of extant freshwater fish families, with its origin tracing back to the Early Cretaceous when the African (*Papyrocranus* and *Xenomystus*) and Asian (*Notopterus*, *Chitala*) clades split (Inoue et al. 2009). This ancient origin, confinement to freshwater ecosystems and restricted distribution to the tropics of Africa, India and Southeast Asia, suggest its current distribution is refugial. The 4 genera comprise only a handful of species including 1 in *Xenomystus*, 2 in *Notopterus* and *Papyrocranus* and 6 in *Chitala* (Lavoué et al. 2020, Fricke et al. 2023, Froese & Pauly 2023). Despite the low diversity in this family, taxonomic confusion has persisted for decades, particularly within the Southeast Asian lineage (Roberts 1992, Kottelat et al. 1993, Kottelat 2005, Lavoué et al. 2020).

Two featherback genera occur in Indonesia, namely *Chitala* and *Notopterus*. Both genera are widely distributed in Sundaland, in the islands of Sumatra, Java and Borneo (Kottelat et al. 1993, Hubert et al. 2015) and are represented by 4 species: *C. lopis, C. hypselonotus, C. borneensis*, and *N. notopterus*. Following to the IUCN Red List (IUCN 2019), *Chitala* species are of Least Concern in Indonesia, excepting *C. lopis*, which is considered Extinct. No *Chitala* species is listed in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). However, a rapid decline in abundance and distribution has been observed in Sumatra and Java during the last 2 decades (Hubert et al. 2015, Dahruddin et al. 2017). Consequently, all Notopterid species are currently protected by the Ministry of Marine Affairs and Fisheries (MMAF) to prevent their extinction. *C. lopis* is only known in Java from its type locality, where it has not been observed since 1851 (Ng 2022).

Uncertainties about the conservation status of *Chitala* species are linked to knowledge deficiencies of their evolutionary boundaries and range distribution in Sundaland, which hinder the formulation of appropriate conservation and management plans or determining an appropriate protection status. Roberts (1989) stated that Asian notopterids are not widely known and the entire group must be revised before species can be determined. Later, Roberts (1992) stated that Indonesia likely hosts a single species, *C. lopis* (commonly known as the giant featherback), and other species represent distinct life-stages of *C. lopis*, varying only in subtle color differences and size. This synonymizing of *C. borneensis* and *C. hypselonotus* with *C. lopis* by Roberts (1992) was later challenged by Kottelat & Widjanarti (2005), who pointed out inconsistencies in the association between coloration patterns and size. They concluded that until further evidence was obtained, the 3 species should be considered as valid. This debate casts doubt on whether *C. borneensis* and *C. hypselonotus* are valid species, and whether *C. lopis* is actually extinct, a confusion which has persisted to date. The type localities of *C. borneensis* (Sambas, Western Borneo), *C. hypselonotus* (Musi River, Sumatra) and *C. lopis* (Cisadane River, Java) are all in Sundaland. However, the historical lack of observations of *C. lopis* in the Cisadane River and the recent apparent disappearance of *Chitala* spp. from the Musi River due to overharvesting (Hubert et al. 2015, Dahruddin et al. 2017) has prevented the comparison of specimens from type localities and prolonged taxonomic uncertainties.

One line of evidence for investigating and guiding taxonomic assessments is through the use of DNA barcoding to enable morphologically similar species to be delimited and further identified. For example, the mitochondrial cytochrome oxidase I (COI) gene was recently used to resolve similar uncertainties in other problematic fish groups in Sundaland, such as the subfamily Rasborinae (Sholihah et al. 2020) and the genus *Barbonymus* (Dahruddin et al. 2021). By sequencing the COI gene region for multiple individuals and populations range-wide and applying DNA-based species delimitation, the boundaries 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).

Recently, one individual of *C. lopis* was captured in the Cisadane River, the type locality of the species, revealing the species was not extinct. This presented an opportunity to reassess the taxonomy of the *Chitala* species in Indonesia. We collected *Chitala* samples from across their distribution in Sundaland and collated existing genetic sequences from previous studies. We used sequence data combined with morphometric and meristic analyses to clarify the boundaries and distribution of this species. *Chitala* species clari-

fication was achieved by first delimitating molecular operational taxonomic units (MOTUs) using species delimitation algorithms, then characterizing morphomeristic variability of each MOTU to confirm their species status and assigning them to a species name by comparison with type-specimens and original descriptions. Species range distribution and genetic patterns were further interpreted in the light of past river systems of the Pleistocene related to sea level changes and emerged lands in the area (Sholihah et al. 2021a,b). We then discussed the implications of the findings for the management of *Chitala* in Indonesia as a demonstration for the work required to protect freshwater biodiversity in biodiversity hotspots under increasing threat globally.

2. MATERIALS AND METHODS

2.1. Sampling and collection management

Sampling was conducted between November 2015 and September 2022 at 34 locations throughout the range of *Chitala* in Borneo (17 locations), Java (1 location) and Sumatra (16 locations) (Kottelat & Widjanarti 2005, Hubert et al. 2015) (Fig. 1). Specimens were collected using an assortment of sampling gear including fishing rods, nets, traps, gill nets and cast nets. The specimens were photographed and individually labelled and geographic information was recorded including geocoordinates. For the purpose of genetic analysis, a muscle biopsy of about 1 cm^3 of muscle tissue was taken 1 to 2 cm below the dorsal fin on the right side and preserved in a 1.5 ml tube containing 96% ethanol. Specimens were then soaked in formalin (5% formalin for sizes ≤15 cm and 10% formalin for sizes >15 cm) during transportation from the fishing area to the laboratory (1–7 d, depending on the distance). In the laboratory, the specimens were washed and soaked in running water for 4 h, sorted, and preserved in 70% alcohol before being measured and given a catalogue number of the Bogor Zoological Museum (MZB). Tissue samples were deposited at the Research Center for the Conservation of Marine and Inland Water Resources, National Research and Innovation Agency (BRIN).

2.2. Sequencing and international repositories

Genomic DNA was extracted from the muscle tissue samples using a Qiagen DNeasy Blood and Tissue Kit

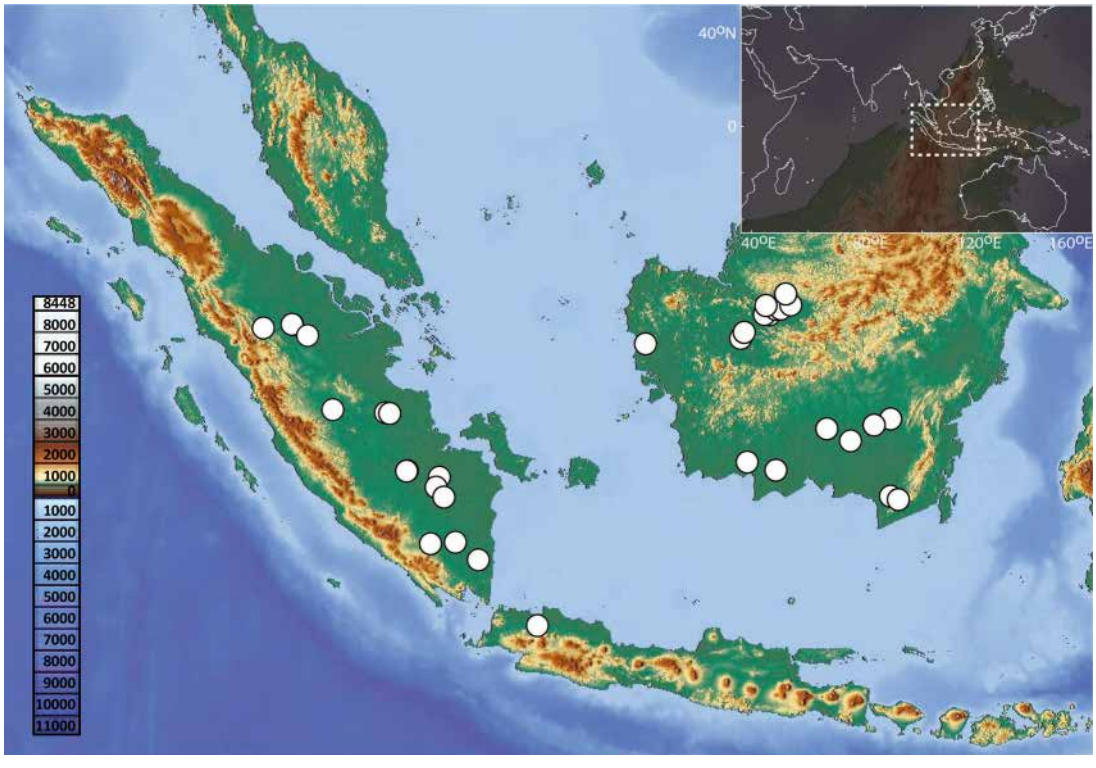

Fig. 1. Collecting sites in Sundaland for the 102 newly produced DNA barcode records of *Chitala* analyzed in this study. Color gradient refers to depth and altitude (m)

following the manufacturer's instructions. A 652 bp segment from the 5′ region of the COI gene was amplified using the primer pair FishF1/FishR1 (Ward et al. 2005) or the primer cocktail C_FishF1t1–C_FishR1t1 (Ivanova et al. 2007) for proper amplification. The PCR reactions for the primer cocktail had a final volume of 10.0 µl, containing 5.0 µl Buffer 2X, 3.3 µl ultrapure water, 1.0 µl of each primer (10 µM), 0.2 µl enzyme Phire Hot Start II DNA polymerase (5 U) and 0.5 µl of DNA template (~50 ng). PCR amplifications with the primer pair FishF1/FishR1 had a final volume of 25.0 µl, containing 12.5 µl of Taq ready mix, 9.5 µl ultrapure water, 1.0 µl of each primer (10 µM) and 1 µl of DNA template. PCR amplifications for both sets of primers were conducted on a Veriti 96-well Fast thermocycler (ABI-AppliedBiosystems). The thermal cycling conditions for the primer cocktail consisted of an initial denaturation at 98°C for 5 min followed by 30 cycles denaturation at 98°C for 5 s, annealing at 56°C for 20 s and extension at 72°C for 30 s, followed by a final extension step at 72°C for 5 min. The thermal cycling conditions for the primer pair FishF1/FishR1 consisted of an initial denaturation at 95°C for 10 min followed by 35 cycles denaturation at 94°C for 60 s, annealing at 48°C for 60 s and extension at 72°C for 20s, followed by a final extension step at 72°C for 7 min. PCR products were purified with ExoSap-IT (USB Corporation) and sequenced in both directions. Sequences and collateral information were deposited in BOLD (Ratnasingham & Hebert 2007) and GenBank. The number of samples caught per species and the number of obtained sample sequences are provided in Table S1 in the Supplement at www.int-res.com/articles/suppl/n052p285_supp.xls.

2.3. Genetic species delimitation and phylogenetic inferences

Several methods for species delineation based on DNA sequences have been proposed (Pons et al. 2006, Ratnasingham & Hebert 2013, Kapli et al. 2017, Puillandre et al. 2021). Each of these have 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 being increasingly used to overcome potential pitfalls arising from uneven sampling (Kekkonen & Hebert 2014, Shen et al. 2019, Sholihah et al. 2020, Arida et al. 2021). We used 6 different sequence-based methods of species delimitation to identify MOTUs: (1) refined single linkage (RESL) as implemented in BOLD and used to generate barcode index numbers (BIN) (Ratnasingham & Hebert 2013); (2) assemble species by automated partitioning (ASAP) (Puillandre et al. 2021), available at https://bioinfo.mnhn.fr/abi/public/asap/; (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 & Barraclough 2013). Both the mPTP algorithm and the GMYC 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 http://iqtree.cibiv.univie.ac.at (Trifinopoulos et al. 2016). For the GMYC 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 ALTER online portal (www.sing-group.org/ALTER/) and Bayesian reconstructions were based on a strict-clock prior of 1.2% per million yr (Myr) (Bermingham et al. 1997). Two Markov chains of 20 million each were run independently using Yule pure birth and GTR+I+Γ substitution models. Trees were sampled every 5000 states after an initial burnin period of 5 million. Both runs were examined using Tracer 1.7.1 (Rambaut et al. 2018) (effective sample size > 200), combined using LogCombiner 2.6.2, and the maximum credibility tree was constructed using TreeAnnotator 2.6.2 (Bouckaert et al. 2014). Once obtained, results of the 6 delimitation analyses were combined and a final consensus scheme was established based on a majority-rule consensus.

For a visual examination of MOTU divergence and boundaries, a COI gene tree was reconstructed using the SpeciesTreeUCLN algorithm of the StarBEAST2 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 & Larson 2006). SpeciesTreeUCLN 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 SpeciesTreeUCLN analysis was performed with the same parameters as men-

tioned above. For the most common and widespread species in Sundaland, a haplotype tree was reconstructed with BEAST 2.6.2 using a strict clock model with 1.2% of genetic divergence per Myr and a coalescent model. Other parameters were set as mentioned above. Individual species haplotypes were extracted from the alignment obtained after collapsing sequences with ALTER.

Kimura 2-parameter (K2P) (Kimura 1980) pairwise genetic distances were calculated using the R package Ape 5.4 (Paradis & 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).

2.4. Morphometric and meristic analysis

Five specimens of *C. borneensis* (2 adults and 3 juveniles), 53 of *C. lopis* (7 adults and 46 juveniles) and 2 specimens of *C. hypselonotus* (2 adults) from Java, Sumatra and Borneo were compared to assess possible morphometric and meristic differences of these species. However, the 2 specimens of *C. hypselonotus* were collected prior to this study and only meristic counts were recorded and voucher specimens were not preserved. As such, they were only included in the meristic comparisons. We define the specimens ≥500 mm standard length (SL) as adult and <500 mm SL as juvenile, following Kottelat & Widjanarti (2005). For a first morphological approach we used specimens (1) from a region where species co-occur and (2) just from running waters to avoid possible differences in body shape induced by varying hydrodynamics. Bleeker based the original descriptions of *C. Borneensis*, *C. hypselonotus* and *C. lopis* on juveniles, of a size between 235 mm total length (TL) and 372 mm TL (Table 1). As the measurements of Bleeker are partly hard to transfer into modern standards because they are repeatedly given as 'circiter' (latin for 'about'), we only give the counts of the type specimens (Table 1).

For each specimen, 22 morphometric measurements (all in mm) were recorded using a dial caliper as follows: SL, from the tip of the snout to the caudal fin central base; head length (HL), from the tip of the snout to the posterior border of the opercle; head depth (HD), measured along a line traversing perpendicularly to the border of the opercle; upper jaw length (UJL), from the tip of the snout to the posterior edge of the maxilla; lower jaw length (LJL), from the chin's tip to the posterior border of the mandible at the retroarticular; anterior snout length (ASNL), from the tip of the snout to the posterior nostril; snout length (SNL), from the tip of the snout to the anterior edge of the orbit; eye diameter (ED), from the upper to lower border of the orbit; pre-pectoral length (PPEL), from the tip of the snout to the base of the 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 first pelvic fin ray to the base of the first anal fin ray; posterior body depth (PBD), measured vertically at the base of the first pectoral; anterior body depth (ABD), maximal value measured vertically from the abdominal region to the dorsal surface in front of the

Table 1. Meristics of the types of *Chitala borneensis*, *C. lopis* , and *C. hypselonotus* based on the original descriptions. Distinct differences to the values of our study are highlighted in grey

	C. borneensis[a]	*C. lopis*[b]	*C. hypselonotus*
Type status	Holotype (unique)	Syntypes	Holotype (unique)
Type locality	Sambas, western Borneo	Jakarta, Java	Musi River, Sumatra
Total length (mm)	235	275	372
Dorsal fin rays (no.)	10	10	11
Anal fin rays (no.)	124	128	125
Pectoral fin rays (no.)	17	16	16
Scale rows on preoperculum (no.)	12	15	20
Scales in lateral series (no.)	200	170	220
Abdominal scutes (no.)	37[c]	45	42

[a]Photos and an x-ray of *C. borneensis* available from the Natural History Museum, London, BMNH 1867.11.28.2, at https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/2599845 (accessed at 04-08-2023)

[b]Photos of *C. lopis* available from the Natural History Museum, London, BMNH 1867.11.28.5, at https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/3100045. Accessed at 04-08-2023

[c]43 from the x-ray of the holotype

pelvic fin base; pectoral fin length (PEFL), from base to the tip of first pectoral fin ray; pelvic fin length (PFL), from the base to the tip of first pelvic fin ray; length of anal fin base (AFL), from the base of the first to the base of the last anal fin ray; length of the dorsal fin base (DFL), from the base of the first to the base of the 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.

Measurements on the lateral side of the body as well as the length of the head are presented as a percentage of SL. All other measurements of the head are presented as a percentage of HL. Meristic counts of 5 specimens of *C. borneensis* (2 adults and 3 juvenile), 53 of *C. lopis* (7 adults and 46 juveniles), and 2 specimens of *C. hypselonotus* (2 adults) include the number of dorsal fin rays, pectoral fin rays, anal fin rays, the number of scale rows on the preoperculum, the presence/absence of black spots at the pectoral fin base, the number of scales at the lateral line, the number of scales between linea lateralis and dorsal fin, and the number of abdominal scutes. All morphometric data were analyzed by principal component analysis (PCA) using MINITAB Statistical Software version 17 package. All measurements were log-transformed.

3. RESULTS

3.1. Species delimitation and phylogenetic analysis

A total of 103 COI sequences originating from 35 sites in Java, Sumatra and Borneo were produced (Table S1). Together with 48 sequences mined from BOLD, a total of 151 sequences were included in the data analyses (Table S1). 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 total, 28 haplotypes were detected among the 151 sequences collected. DNA-based species delimitation methods resulted in congruent delimitation schemes with 6 MOTUs for BIN, ASAP and mPTP, and 7 MOTUs delimited by mGMYC and sGMYC, and 8 MOTUs by sPTP (Fig. 2, Table S1). The final consensus scheme consisted of 7 MOTUs, and the 4 MOTUs observed in Sundaland were assigned to 3 nominal species according to their morpho-meristic attributes, 2 MOTUs being recognized within *Chitala lopis*. The morphological identification of *C. lopis* was further corroborated by the placement within 1 of the 2 *C. lopis* MOTUs of the sequence from the specimen collected in the Cisadane River in Java. A single conflicting identification was detected between sequences produced here and sequences mined from GenBank for *C. hypselonotus* (BOLD:AEI5739), which were originally assigned to *C. chitala*, a species reported from the inlands of India. A barcode gap was 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 2). The delimitation scheme translated into a revised distribution range for *C. lopis*, which is widely distributed in Java, Sumatra and Borneo and also the most represented species in our sampling (Fig. 3). *C. borneensis* was observed in West Borneo and central Sumatra, while *C. hypselonotus* was observed in Sumatra at 2 localities, including its type locality (Musi River).

The Bayesian gene tree based on the MOTUs recognized here suggests a recent diversification of the Asian *Chitala* species around 5 Myr ago (Fig. 2). However, the gene tree does not show evidence of close phylogenetic relationships among Indonesian *Chitala*, given *C. hypselonotus* is more closely related to *C. ornata*, while *C. lopis* and *C. borneensis* are closely related and placed at the root of the tree. All the mitochondrial divergence events between species observed here predate the Pleistocene. The Bayesian reconstruction of the haplotype tree within *C. lopis* indicates 3 main lineages with a most recent common ancestor (MRCA) dated around 1.2 Myr ago (Fig. 4). Among the 12 haplotypes recognized within *C. lopis*, 7 are endemic of Borneo, 1 is endemic of Sumatra, 1 is shared between Sumatra and Java, and 2 are shared between Sumatra and Borneo (Fig. 4). Lineage I is endemic of the North Sunda ancient river system, while lineage II is restricted to the East Sunda river system, and lineage II is present in both (Fig. 4). The haplotype collected in Java is one of the most abundant in lineage III, with 17 individual sequences. These 3 lineages were varyingly delimited by delimitation analyses as only sPTP delimited them (Table S1).

3.2. Morphometric and meristic analysis

The PCA was performed on 22 log-transformed morphometric characters (Fig. 5A). All previous mor-

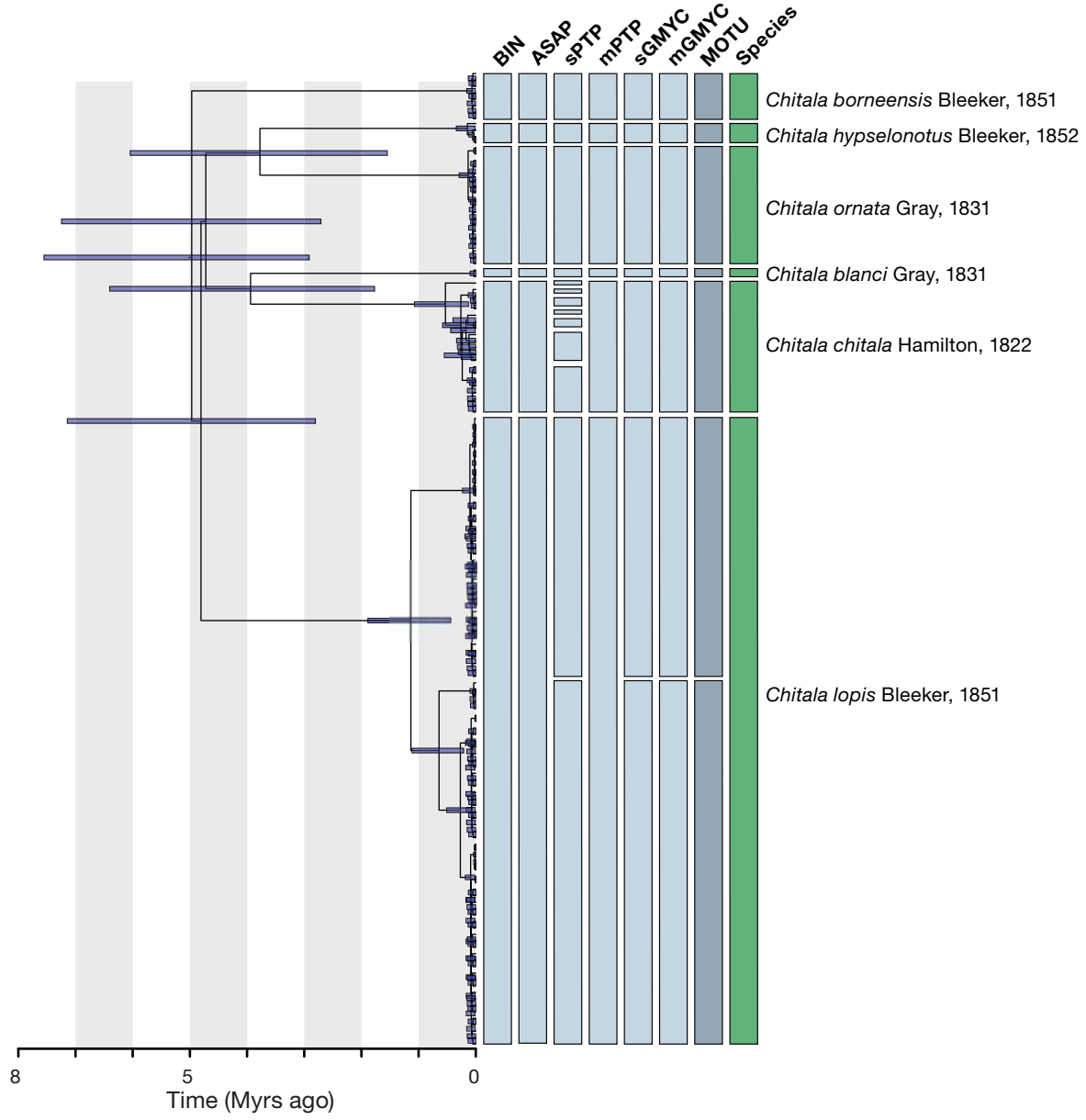

Fig. 2. Mitochondrial gene tree for the 150 DNA barcodes of *Chitala* inferred with SpeciesTreeUCLN, including 95% highest posterior density (HPD) interval for node age estimates, genetic species delimitation results for the 6 methods and their 50% consensus, and species names. BIN: barcode index numbers; ASAP: assemble species by automated partitioning; sPTP: single Poisson tree process; mPTP: multiple Poisson tree process; sGMYC: single general mixed Yule-coalescent; mGMYC: multiple general mixed Yule-coalescent; MOTU: molecular operational taxonomic unit

phometric measurements were presented in the form of % HL (only for characters measured on the head) and % SL for other characters (Fig. 5B–D). The first component is mostly structured by UJL, PPEL, PPL, PAL, and PDL, while the second component is mostly defined by ABD. Two groups corresponding to *C. lopis* (Fig. 5A, right, KBP1.1, KBP1.4, KBP1.6, KBP1.3) and *C. borneensis* (Fig. 5A, left, KBP1.2, KBP1.7, KB4, KBP1.5, JAP2.1) are identified. The 2 species mostly differ in their UJL, with *C. borneensis* having a shorter jaw (Fig. 5B); the PBD was lower in *C. borneensis* (Fig. 5C) and the PDL shorter in *C. borneensis* (Fig. 5D).

There is no effect of the size or of the ontogenetic stages on meristic characters of *C. borneensis* and *C. lopis*, possibly because of a high variability in these characters (Table 3). The mean number of dorsal fin rays was 8.67 in juvenile and 10 in adult

Table 2. Summary of genetic distances and MOTUs including species names, number of individuals analyzed, BOLD barcode index number (BIN), maximum intraspecific and minimum interspecific K2P genetic distances

Species	N	BIN	K2P genetic distance	
			Max. intraspecific	Min. interspecific
Chitala blanci	1	BOLD:AAJ0132	–	0.0628
Chitala borneensis	8	BOLD:and1667	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

C. borneensis, 7.91 in juvenile and 8.57 in adult *C. lopis*. The mean number of pectoral fin rays was 14 for juvenile and 13.5 for adult *C. borneensis*, and 13.39 for juvenile and 13.57 for adult *C. lopis*. The mean number of anal fin rays was 121 for adult and 128 for juvenile *C. borneensis*, and 125.14 for adult and 128.1 for juvenile specimens of *C. lopis*, respectively. The mean number of scale rows on the preoperculum was 31.5 in adult and 21.0 in juvenile specimens of *C.*

Fig. 3. Revised range distribution and type localities (white-edged circle) for: (A) *Chitala lopis*, (B) *C. borneensis*, (C) *C. hypselonotus*

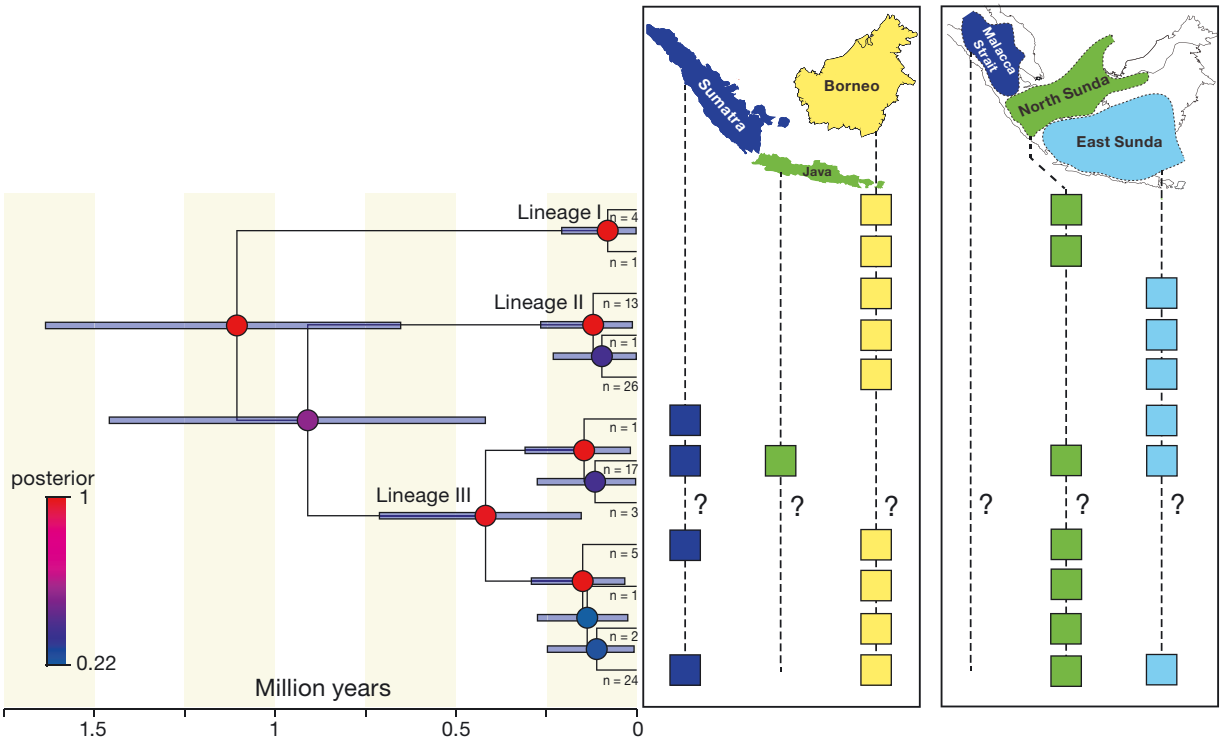

Fig. 4. Bayesian tree inferred with BEAST 2.6.2 based on the 12 haplotypes of *Chitala lopis* with a GTR+I+Γ substitution model, a lognormal clock prior of 1.2% per million yr and a coalescent model. Node circles illustrate posterior probabilities, and node bars represent 95% highest posterior density. Squares illustrate the distribution of haplotypes across islands and paleorivers. Unknown occurrences are represented by a question mark

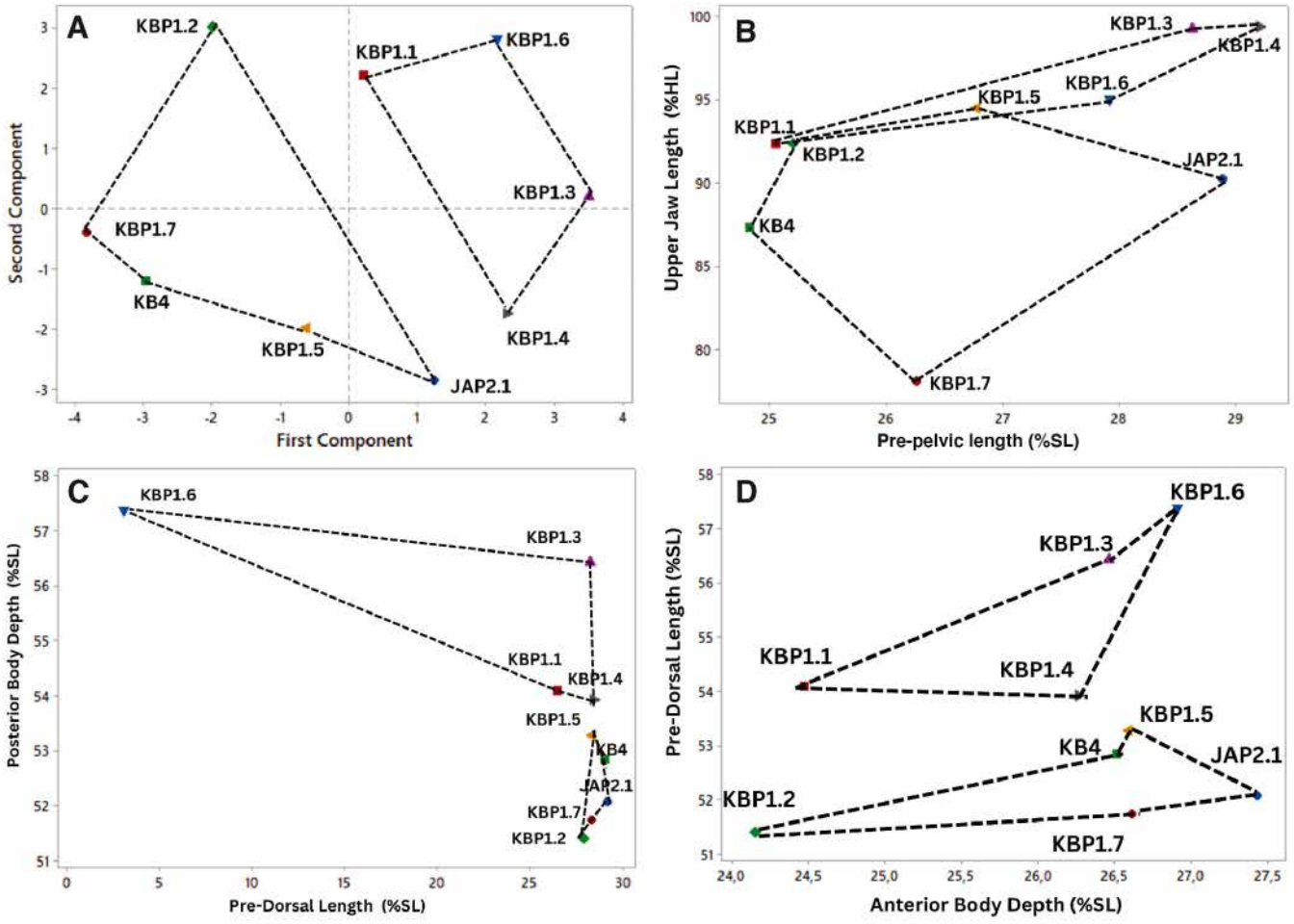

Fig. 5. Morphometric variability for *Chitala lopis* (A, right, individual fish: KBP1.1, KBP1.4, KBP1.6, KBP1.3) and *C. borneensis* (A, left, individual fish: KBP1.2, KBP1.7, KB4, KBP1.5, JAP2.1) including (A) principal component analysis projections for the first and second component, (B) plots of upper jaw and pre-pelvic length, (C) plots of posterior body depth and pre-dorsal length, (D) plots of pre-dorsal length and anterior body depth. HL: head length; SL: standard length

borneensi. In *C. lopis*, the mean number of preopercular scale rows was 27.71 for adults and 25.93 for juveniles. The mean number of lateral line scales was 244.67 in juvenile and 298.5 in the adult *C. borneensis*, and 224.60 in juvenile and 241.28 in adult *C. lopis*. The mean number of transverse scales rows was 27 for adult and 26.33 for juvenile *C. borneensis*, and 25 for adult and 25.21 for juvenile *C. lopis*. The mean number of abdominal scutes was 46.67 for juvenile *C. borneensis* and 48 for adult *C. borneensis*, and 43.85 for juvenile and 41.28 for adult *C. lopis*. Several meristic characters display differences among species; however, distributions overlap. For example, the average number of anal fin rays is higher in *C. hypselonotus* (142) than in *C. borneensis* (125) and *C. lopis* (127.9), but ranges overlap between *C. lopis* (113–153) and *C. hypselonotus* (140–144) (Table 3). The same was observed for the number of pectoral fin rays, with 10 rays on average in *C. hypselonotus* and 13.8 and 13.4 in *C. borneensis* and *C. lopis*, respectively, but ranges overlap. Likewise, the number of scale rows on the preoperculum is 30 (28–31) scales in *C. hypselonotus* against 25.2 (13–36) and 26.24 (19–39) in *C. borneensis* and *C. lopis*, respectively, or the number of scales in dorsal transverse of 29 (28–30) in *C. hypselonotus* against 26.6 (23–31) and 25.26 (21–33) in *C. borneensis* and *C. lopis*, respectively, or the number of abdominal scutes of 37 (36–38) in *C. hypselonotus* against 47.2 (42–52) in *C. borneensis* and 43.62 (34–57) in *C. lopis*.

In terms of coloration pattern, almost all the specimens examined displayed a black spot at the basis of the pectoral fin, except 2 juveniles *C. borneensis*, 4 adult and one juvenile *C. lopis*. This indicates that the black spot at the basis of pectoral fin cannot be

Table 3. Meristics of *Chitala borneensis*, *C. lopis* and *C. hypselonotus*. Values are range (mean). SL: standard length

Species	*C. borneensis*	*C. lopis*	*C. hypselonotus*
Sample size	n = 5	n = 53	n = 2
SL (mm)	346.45–570.02 (466.02)	188.60–658.04 (377.345)	456.76–639.64 (548.20)
Dorsal fin rays (no.)	8–10 (9.20)	7–10 (8)	8 (8)
Anal fin rays (no.)	115–129 (125.20)	113–153 (127.90)	140–144 (142)
Pectoral fin rays (no.)	13–14 (13.80)	11–15 (13.41)	9–11 (10)
Scale rows on preoperculum (no.)	13–36 (25.20)	19–39 (26.24)	28–32 (30)
Scales in lateral series/linea lateralis scales (no.)	226–305 (266.20)	122–289 (227.56)	285–288 (286.5)
Scales in dorsal transverse series/scales between lateral line and dorsal fin (no.)	23–31 (26.60)	21–33 (25.26)	28–30 (29)
Abdominal scutes (no.)	42–52 (47.20)	34–57 (43.62)	36–38 (37)
Specimen <500 mm SL (juvenile)			
Sample size	n = 3	n = 46	n = 0
SL (mm)	346.45–487.66 (413.51)	188.6–494.35 (343.82)	–
Dorsal fin rays (no.)	8–10 (8.67)	8–10 (7.91)	–
Anal fin rays (no.)	127–129 (128)	105–153 (128.1)	–
Pectoral fin rays (no.)	14 (14)	11–15 (13.39)	–
Scale rows on preoperculum (no.)	14–36 (21)	19–39 (25.93)	–
Scales in lateral series/linea lateralis scales (no.)	226–276 (244.67)	122–289 (224.60)	–
Scales in dorsal transverse series/scales between lateral line and dorsal fin (no.)	25–31 (26.33)	21–33 (25.21)	–
Abdominal scutes (no.)	42–52 (46.67)	34–57 (43.85)	–
Specimen >500 mm SL (adult)			
Sample size	n = 2	n = 7	n = 2
SL (mm)	519.56–570.02 (525.75)	523.95–658.04 (564.55)	456.76–639.64 (548.20)
Dorsal fin rays (no.)	10 (10)	8–9 (8.57)	8 (8)
Anal fin rays (no.)	115–127 (121)	115–130 (125.14)	140–144 (142)
Pectoral fin rays (no.)	13–14 (13.5)	11–15 (13.57)	9–11 (10)
Scale rows on preoperculum (no.)	29–34 (31.5)	22–35 (27.71)	28–32 (30)
Scales in lateral series/linea lateralis scales (no.)	292–305 (298.5)	221–273 (241.28)	285–288 (286.5)
Scales in dorsal transverse series/scales between lateral line and dorsal fin (no.)	27 (27)	22–26 (25)	28–30 (29)
Abdominal scutes (no.)	45–51 (48)	36–48 (41.28)	36–38 (37)

used as a diagnostic characteristic to distinguish *C. lopis* from *C. borneensis*.

4. DISCUSSION

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 151 sequences, largely distributed in Asia, and including sequences from specimens caught at type localities, the validity of the 3 Indonesian species of *Chitala* can be corroborated. DNA-based species delimitation methods agreed on the recognition of all the known *Chitala* species (Fricke et al. 2023, Froese & Pauly 2023), excepting *C. hypselonotus* (BOLD:AEI5735), whose 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 (1) sequences of the true *C. chitala* (BOLD:AAY5141) were also included in the present analysis and belong to a distinct lineage, (2) the sequence of a *C. hypselonotus* specimen originating from central Sumatra was included and belongs to the same MOTU (BOLD:AEI5735) and (3) sequences of BOLD:AEI5735 from GenBank originate from the Musi River, the type locality of *C. hypselonotus*.

The family Notopteridae had been revised based on morphological characters by 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 onto-

genetic stages of *C. lopis*. Kottelat & Widjanarti (2005) rejected the hypothesis of ontogenetic changes in coloration and suggested that the 3 species be considered as distinct. Nevertheless, no detailed characters besides color and size were discussed by these authors. Additionally, Kottelat & Widjanarti (2005) also mentioned the possibility that *C. hypselonotus* might be a junior synonym of *C. lopis*, which would reduce the number of species of *Chitala* in Indonesia to 2, *C. borneensis* and *C. lopis*. By combining genetic and morphological data, we were able to solve this taxonomic confusion and to recognize *C. lopis*, *C. borneensis* and *C. hypselonotus* as distinct species.

Both genetic and morphological evidence presented here supports the recognition of *C. lopis* and *C. borneensis*. Several morphological characters, mostly located in the anterior part of the body, differentiate between these species. The UJL, PDL and PBD are shorter in *C. borneensis*. The black spot at the base of the pectoral fin is not a suitable characteristic for unambiguously distinguishing *C. lopis* from *C. borneensis* or from *C. hypselonotus*. This characteristic was consistently observed across *C. lopis* range distribution as it was present in specimens from the type locality (Fig. 6A) in Java, as well as Sumatra and Borneo (Fig. 6B). Nevertheless, this trait is seemingly variable and, at least partly, dependent of the water quality. In muddy waters, specimens of *C. lopis* are often pallid and the spot is faint (Roberts 1992). This was the case in specimens of *C. lopis* from a locality in Borneo, where this species occurred sympatrically with *C. borneensis*. A variable expression of the spot at the pectoral fin base was also found in *C. borneensis* from Borneo and Sumatra, with specimens having a faint spot (Fig. 7A) or a distinct spot (Fig. 7B,C). A black spot at the pectoral fin base was observed in the 3 specimens of *C. hypselonotus* examined (Fig. 8), including the specimen associated to the previously published sequence BOLD:CLSP003-21 (Fig. 8A, courtesy of Yulianti Anjarsari, Sriwijaya University).

Unfortunately, morphological comparisons at the 22 morphometric measurements with *C. hypselonotus* were not possible, given that the 2 individuals examined correspond to ancient captures and neither morphometric measurements were recorded nor voucher specimens preserved. Despite this, the genetic evidence is strong that *C. hypselonotus* is a valid species and meristic counts agree with this hypothesis. Subtle differences were found between *C. borneensis* and *C. lopis* especially in the number of lateral line scales, abdominal scutes, and dorsal fin rays. The average number of those characters are higher in *C. borneensis* compared to *C. lopis*. However, those ranges overlap between each species (Table 3). On the other hand, according to the size, most of the measured characteristics have higher average number in adult size than juvenile size for both species. Consequently, confident morphological differentiation of the 3 species is with our current knowledge not possible in areas where they could potentially co-occur.

C. lopis and *C. borneensis* co-occur in Sumatra and Borneo (Fig. 3), 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,b).

The present study clarifies the taxonomic status of the 3 *Chitala* species in Indonesia and provides the first accurate evidence of their range distribution in the wild. The rediscovery of *C. lopis* in its type-locality after the absence of observations for more than 170 yr has important implication in our understanding of *Chitala* species distribution. Although recent translocations may explain this new observation of *C. lopis* in its type-locality after decades, it is unlikely as no national program of translocation have been conducted to date for *Chitala* species and the haplotype detected in Java is shared with populations from South and Central Sumatra (North and East Sunda ancient river systems, Fig. 4), a pattern previously observed in multiple fish taxa (Sholihah et al. 2020, 2021a,b, Dahruddin et al. 2021). *C. lopis* was declared Extinct by the IUCN (Ng 2022), a decision assuming implicitly that *C. lopis* was an endemic species of Java. Surprisingly, 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 reconsideration of its IUCN status. Despite being widespread, *C. lopis* is heavily harvested as it is an iconic fish with a high economic value, being an important species for food. It is the main ingredient of traditional processed fish foods (e.g. krupuk, pempek,

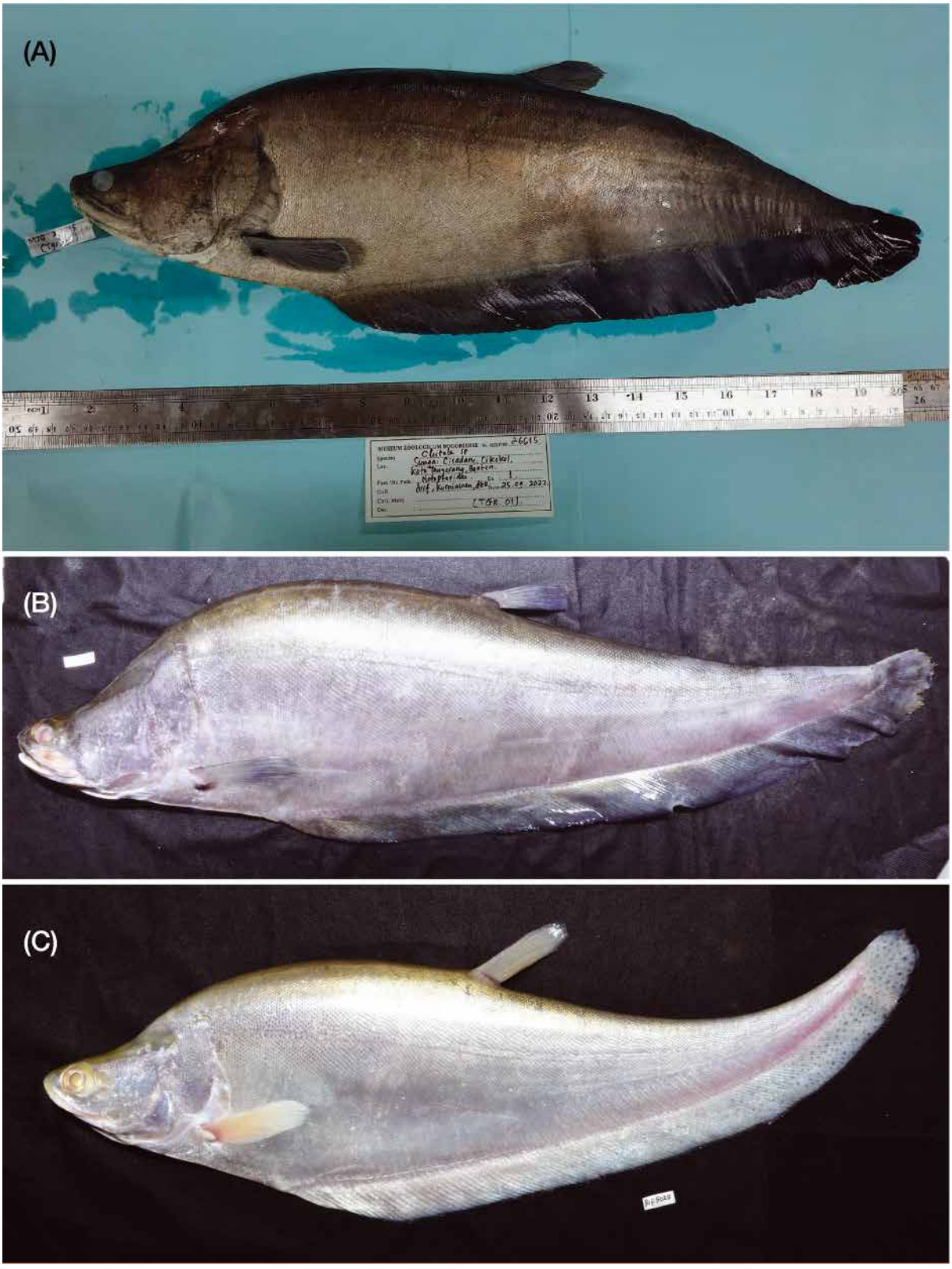

Fig. 6. Selected specimens of *Chitala lopis* from (A) type locality, Cisadane River (specimen TGR01), (B) Sintang, West Borneo (specimen BIF7622, standard length: 535 mm), and *C. borneensis* from (C) from Jambi, Sumatra (specimen BIF5025, standard length: 379 mm)

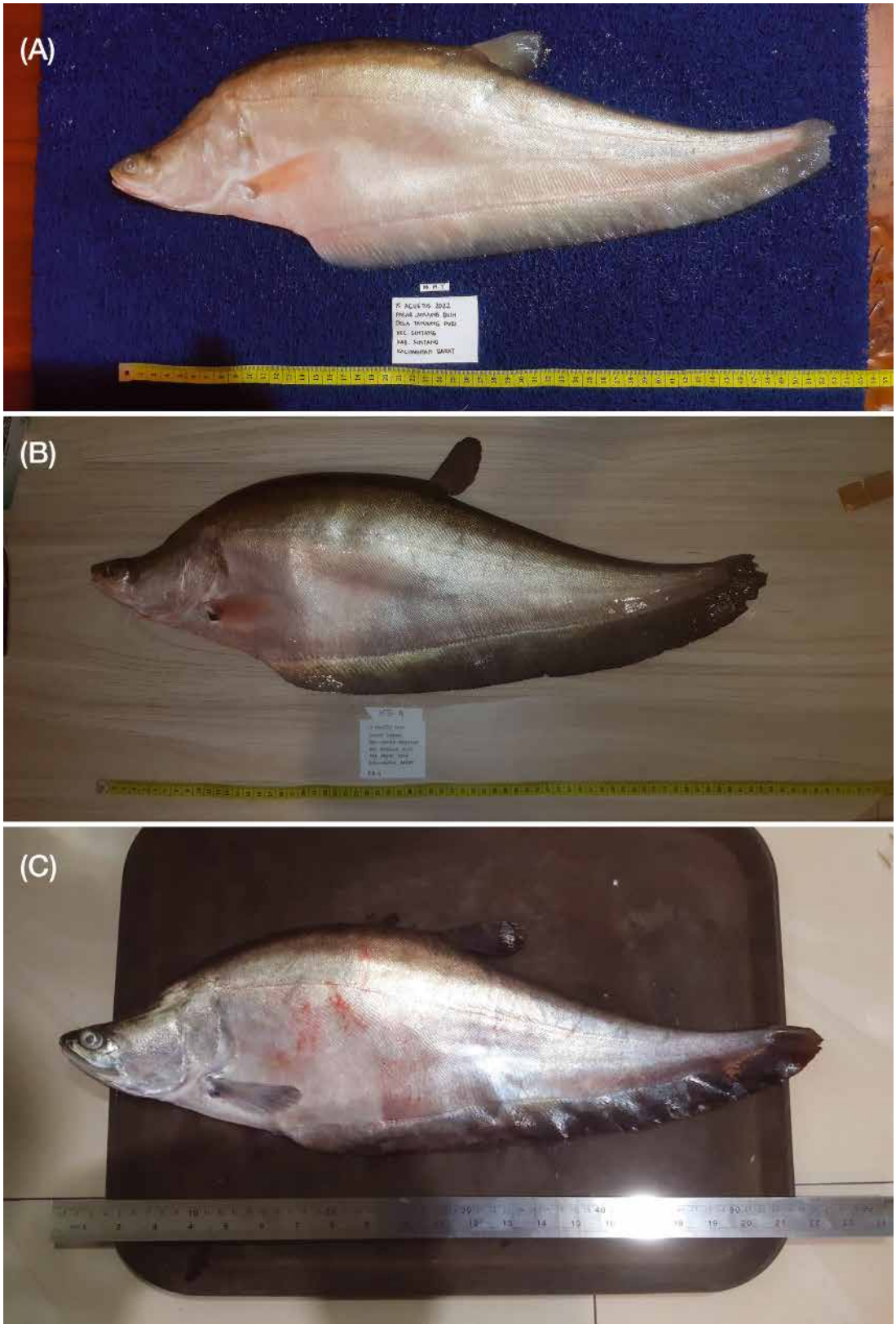

Fig. 7. *Chitala borneensis* with black spot at the base of the pectoral fin. (A) Indistinct, faint spot, Borneo; (B) distinct spot, Borneo; (C) distinct spot, Sumatra. All the specimens are freshly dead

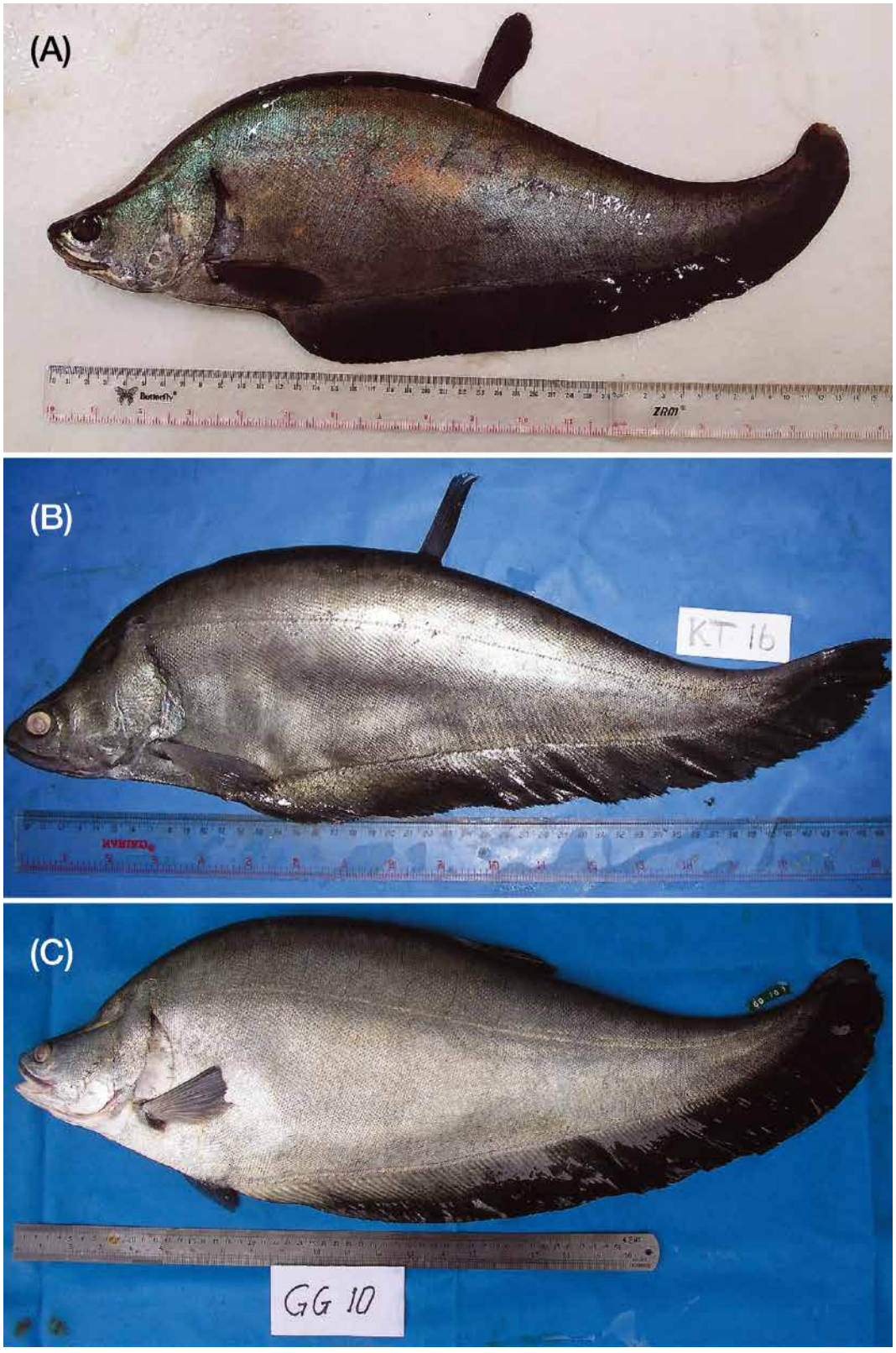

Fig. 8. *Chitala hypselonotus* from (A) Musi River and corresponding to previously published sequenced BOLD:CLSP003-21 (Courtesy of Yulianti Anjarsari), (B) Kampar River, Riau province (specimen KT16, standard length: 456 mm), (C) Kampar River, Riau province (specimen BIF6594, standard length: 639 mm)

lenggang, model, tekwan, burgo, etc.), and the skin is used to produce leather for commercial products such as wallets (Kottelat & Widjanarti 2005). *C. lopis* is also targeted by the international ornamental fish trade, as well as other notopterid species (Kottelat & Widjanarti 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 warrants further studies to examine anthropogenic threats at the population level to enable appropriate fishing regulations to be put in place. In contrast, the conservation status of *C. borneensis* and *C. hypselonotus* need to be urgently revised. Both are listed as of Least Concern by the IUCN; however, our study demonstrates that both are likely to be at risk of extinction. *C. borneensis* is widely distributed, but we observed it to be very rare during the course of the present study. This makes this species particularly vulnerable. Of greater concern is the scarcity of *C. hypselonotus*, given it was not captured during this study despite considerable effort. The 2 specimens included here correspond to ancient captures in the 2010s. *C. hypselonotus* is currently only known by 3 sequences deposited in GenBank and originating from the Musi River, and a fourth presented here and originating from Central Sumatra. Interestingly, all *C. hypselonotus* records discussed in the present study originate from peat-swamp areas in Sumatra, including the 3 previously published sequences (Y. Anjarsari pers. comm.). Peat-swamps are usually restricted to remote forest areas, which have dramatically decreased in Sumatra during the last decades as a consequence of deforestation (Laumonier et al. 2010). This probably accounts for the lack of recent observation of *C. hypselonotus*. We strongly suggest that searches for this species be carried out in the peat-swamps of the Musi River and surrounding watersheds to confirm whether other populations occur in Sumatra.

5. CONCLUSIONS

Our study has provided additional evidence supporting the recognition of 3 species of *Chitala* in Indonesia. The rediscovery of *C. lopis* puts an end to 2 decades of taxonomic confusion in this group. Species ranges are revised for each of the 3 species, *C. lopis* being the most widespread *Chitala* species in Indonesia. Our results suggest that the IUCN conservation status of *C. borneensis* and *C. hypselonotus* should be urgently revised, while the wide distribution of *C. lopis* calls for revision of the current 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.

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LITERATURE CITED

Alshari NFMAH, Lavoué S, Sulaiman MAM, Khaironizam MZ, Nor SAM, Aziz F (2021) Pleistocene paleodrainages explain the phylogeographic structure of Malaysian populations of Asian arowana better than their chromatic variation. Endang Species Res 46:205–214

Arida E, Ashari H, Dahruddin H, Fitriana YS and others (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 AP (1997) Fish biogeography and molecular clocks: perspectives from the Panamanian isthmus. In: Kocher TD, Stepien CA (eds) Molecular systematics of fishes. Academic Press, San Diego, CA, p 113–128

Bouckaert R, Heled J, Kühnert D, Vaughan T and others (2014) BEAST 2: a software patform for Bayesian evolutionary analysis. PLOS Comput Biol 10:e1003537

Brown SDJ, Collins RA, Boyer S, Lefort C, Malumbres-Olarte J, Vink CJ, Cruickshank RH (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

Dahruddin H, Hutama A, Busson F, Sauri S and others (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 and others (2021) Revisiting the diversity of *Barbonymus* (Cypriniformes, Cyprinidae) in Sundaland using DNA-based species delimitation methods. Diversity 13:283

de Bruyn M, Rüber L, Nylinder S, Stelbrink B and others (2013) Paleo-drainage basin connectivity predicts evolutionary relationships across three Southeast Asian biodiversity hotspots. Syst Biol 62:398–410

Fricke R, Eschmeyer WN, Van der Laan R (eds) (2023) Eschmeyer's catalog of fishes: genera, species, references. https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Froese R, Pauly D (2023) Fishbase. www.fishbase.org

Fujisawa T, Barraclough TG (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 SYW, Larson G (2006) Molecular clocks: when times are a-changin'. Trends Genet 22:79–83

Hubert N, Kadarusman, Wibowo A, Busson F and others (2015) DNA barcoding Indonesian freshwater fishes: challenges and prospects. DNA Barcodes 3:144–169

Hubert N, Lumbantobing D, Sholihah A, Dahruddin H and others (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 JG, 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

IUCN (2019) The IUCN Red List of Threatened Species. https://www.iucnredlist.org

Ivanova NV, Zemlak TS, Hanner RH, Hébert PDN (2007) Universal primers cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589

Kapli P, Lutteropp S, 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 33:1630–1638

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 41:195–211

Keith P, Mennesson M, Delrieu-Trottin E, Hubert N (2020) *Giuris* (Teleostei: Eleotridae) from Indonesia, with description of a new species. Cybium 44:317–329

Kekkonen M, Hebert PDN (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 16:111–120

Kottelat M (2005) The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Kalimantan Barat, Indonesia. Raffles Bull Zool Suppl 13:139–173

Kottelat M, Widjanarti E (2005) The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Kalimantan Barat, Indonesia. Raffles Bull Zool Suppl 13:139–173

Kottelat M, Whitten AJ, Kartikasari N, Wirjoatmodjo S (1993) Freshwater fishes of Western Indonesia and Sulawesi. Periplu, Hong Kong

Laumonier Y, Uryu Y, Stüwe M, Budiman A, Setiabudi B, Hadian O (2010) Eco-floristic sectors and deforestation threats in Sumatra: identifying new conservation area network priorities for ecosystem-based land use planning. Biodivers Conserv 19:1153–1174

Lavoué S, Ghazali SZ, Jamaluddin JAF, Nor SAM, Zain KM (2020) Genetic evidence for the recognition of two allopatric species of Asian bronze featherback *Notopterus* (Teleostei, Osteoglossomorpha, Notopteridae). Zoosyst Evol 96:449

Luo A, Ling C, Ho SYW, Zhu CD (2018) Comparison of methods for molecular species delimitation across a range of speciation scenarios. Syst Biol 67:830–846

Mennesson MI, Keith P, Sauri S, Busson F and others (2021) *Eleotris* (Teleostei: Eleotridae) from Indonesia with description of three new species within the '*melanosoma*' neuromast pattern group. Pac Sci 75:469–495

Ng HH (2022) *Chitala lopis*. IUCN Red List of Threat Species 2020:e. T157719927A89815479. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T157719927A89815479.en

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274

Ogilvie HA, Bouckaert RR, Drummond AJ (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 TG, Gomez-Zurita J, Cardoso A and others (2006) Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst Biol 55: 595–609

Puillandre N, Brouillet S, Achaz G (2021) ASAP: assemble species by automatic partitioning. Mol Ecol Resour 21: 609–620

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol 67:901–904

Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system (www.barcodinglife.org). Mol Ecol Notes 7:355–364

Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: the barcode index number (BIN) system. PLOS ONE 8:e66213

Roberts T (1989) The freshwater fishes of western Borneo (Kalimantan Barat, Indonesia). California Academy of Sciences, San Francisco, CA

Roberts TR (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, Sukmono T, Dahruddin H and others (2020) Disentangling the taxonomy of the subfamily Rasborinae (Cypriniformes, Danionidae) in Sundaland using DNA barcodes. Sci Rep 10:2818
Sholihah A, Delrieu-Trottin E, Condamine FL, Wowor D and others (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 and others (2021b) Limited dispersal and in situ diversification drive the evolutionary history of Rasborinae fishes in Sundaland. J Biogeogr 48:2153–2173
Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res 44:W232–W235
Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27:1153–1167
Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 360:1847–1857
Woodruff DS (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

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