

Genetic evidence for the recognition of two allopatric species of Asian bronze featherback *Notopterus* (Teleostei, Osteoglossomorpha, Notopteridae)

Sébastien Lavoué¹, Siti Zafirah Ghazali^{1,2}, Jamsari Amirul Firdaus Jamaluddin¹, Siti Azizah Mohd Nor², Khaironizam Md. Zain¹

¹ School of Biological Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia

² Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Nerus, 21030, Malaysia

<http://zoobank.org/64287A82-6E8C-4AD0-9754-6C687D2AF15F>

Corresponding author: Sébastien Lavoué (microceb@hotmail.com)

Academic editor: Nicolas Hubert ♦ Received 22 February 2020 ♦ Accepted 20 April 2020 ♦ Published 1 July 2020

Abstract

The fish genus *Notopterus* Lacepède, 1800 (Notopteridae) currently includes only one species, the Asian bronze featherback *Notopterus notopterus* (Pallas, 1769). This common freshwater species is widely distributed in the Oriental region, from the Indus basin in the west, the Mekong basin in the east and Java Island in the south. To examine the phylogeographic structure of *N. notopterus* across its range, we analysed 74 publicly available cytochrome oxidase I (COI) sequences, 72 of them determined from known-origin specimens, along with four newly-determined sequences from Peninsular Malaysian specimens. We found that *N. notopterus* is a complex of two allopatric species that diverge from each other by 7.5% mean *p*-distance. The first species is endemic to South Asia (from Indus basin to Ganga-Brahmaputra system), whereas the distribution of the second species is restricted to Southeast Asia. The exact limit between the distributions of these two species is not known, but it should fall somewhere between the Ganga-Brahmaputra and Salween basins, a region already identified as a major faunal boundary in the Oriental region. The name *N. notopterus* is retained for the Southeast Asian species, while the name *Notopterus synurus* (Bloch & Schneider, 1801) should be applied to the South Asian species. A comparative morphological study is needed to reveal the degree of morphological differentiation between the two species.

Key Words

Orient, molecular taxonomy, biogeography, biodiversity, cryptic species

Introduction

The complex geological history of the Oriental region caused a high degree of geographical genetic structure within freshwater organisms and species, once considered widely distributed in this region, are often formed by distinct genetic lineages (e.g. de Bruyn et al. 2013; Dahrudin et al. 2017; Jamaluddin et al. 2019; Rüber et al. 2020). The bronze featherback fish *Notopterus notopterus*, currently the only valid species of the genus *Notopterus*, is one of such widely-distributed species, occurring from the Indus basin (Pakistan and India) in

the west, to the Mekong region – slightly extending east to the Annamite Range – (Cambodia, Laos, Thailand and Vietnam) in the east and to Java (Indonesia) in the south. Intriguingly, *N. notopterus* has not yet been recorded in Borneo where it is most likely absent (Roberts 1989; Christensen 1992; Roberts 1992; Kottelat 1995; Kottelat and Widjanarti 2005; Parenti and Lim 2005).

Specimens of *N. notopterus* are identifiable from all other Oriental freshwater fishes based on (amongst other features) their distinct tapered tail and the corners of their mouth below eye (not behind as in the other Oriental notopterid genus *Chitala* Fowler, 1934) (Fig. 1A). It is

reported that this species reaches up to 60 cm in standard length (Roberts 1992). A wealth of data is available for this species, documenting its reproductive behaviour and embryonic development (Yanwirsal et al. 2017), cytogenetics (Barby et al. 2019) and phylogeography (Gupta et al. 2013; Takagi et al. 2010). However, none of these studies simultaneously examined specimens sampled across the whole distributional range of *N. notopterus*.

Inoue et al. (2009) reconstructed the molecular phylogeny of the family Notopteridae to discuss its evolution and biogeography. These authors sequenced the complete mitogenomes of two specimens of *N. notopterus*, one from India and the other from Thailand. The comparison of these two mitogenomic sequences revealed that these two specimens diverged from each other about 25 million years ago. This result was unexpected because no consistent morphological variation was previously reported within this species (see Roberts 1992).

We herein investigated the genetic diversity within the genus *Notopterus* across its full range. For that, we analysed a dataset comprising 72 publicly available sequences of the standard barcoding fragment (655 base pairs) of the cytochrome oxidase I (COI) gene that were determined from specimens of *Notopterus* with precise information on their geographical collection plus two COI sequences extracted from two complete mitogenomes from two specimens of *Notopterus* without precise localities (Inoue et al. 2009) and four sequences we newly determined from specimens collected in Peninsular Malaysia.

Material and methods

COI sequences mining and selection

Using the NCBI GenBank nucleotide database (<https://www.ncbi.nlm.nih.gov/>), we searched for available cytochrome oxidase subunit I sequences of *N. notopterus* (search made on 02/12/2019) using the terms “Notopterus” and “oxidase.” This search retrieved 88 mitochondrial entries (excluding three entries related to whole mitogenomes), from which we selected only those with either latitude-longitude coordinates or geographic localities sufficiently precise that we can confidently estimate their latitude-longitude coordinates. After this initial screening, the dataset included partial COI sequences of 77 specimens of *Notopterus*. We then checked the length and characteristics of these sequences using the software Mesquite version 3.31 (Maddison and Maddison 2017). Their quality was assessed in searching for stop codons, indels and/or the presence of relatively higher number of autapomorphic changes as indication of possible either pseudogenes or sequencing/editing errors. Five out of 77 sequences were found below standards and were excluded (all 16 excluded sequences and the respective reasons to exclude them are listed in Suppl. material 1: Table S1). After this screening, the dataset comprised 72 COI sequences of specimens collected in India (40 specimens),

Bangladesh (two), Peninsular Malaysia (two), Indonesia (six), Myanmar (16) and Thailand (six) (localities are mapped in Fig. 1A and listed in the Suppl. material 1: Table S1). We added to this dataset two COI sequences of specimens (without precise locality) examined in Inoue et al. (2009) along with the four sequences we newly determined from specimens collected in Peninsular Malaysia, two from Kerian River (Penang State) and two from Bera lake (Pahang State). We deposited these voucher specimens in the ichthyological collection of the School of Biological Sciences, Universiti Sains Malaysia under accession numbers USMFC (3) 00002–3.

DNA extraction, amplification and sequencing

To determine the COI sequences of these specimens, we used the PCR technique to amplify them using the following PCR primer pair: forward FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and reverse FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al. 2005). Reactions were carried out in 25 µl reaction volume containing 15.75 µl of sterile distilled H₂O, 5.5 µl of 5× MyTaq Red reaction buffer (Bioline), 0.5 µl of each primer (10 µM), 0.25 µl of iTaq DNA polymerase (INtRON Biotechnology) and 2.5 µl of template containing approximately 5 ng DNA. The thermal cycle profile consisted of an initial 94 °C denaturation step for 4 min, 35 cycles of 94 °C for 30 sec, annealing for 50 sec at 47.9 °C, extension at 72 °C for 1 min, followed by a final extension at 72 °C for 7 min. PCR products were sent to First Base Sdn. Bhd. for sequencing analysis by the standard Sanger methodology. Chromatograms were edited with the Molecular Evolutionary Genetics Analysis X (MEGA X) (Stecher et al. 2020). Sequences are deposited in GenBank under accession numbers MT328860-3.

Comparative analysis

The alignment of the 78 COI nucleotide sequences was done by eye. Some sequences were shorter than others (at the 5' and 3' ends) and the overall proportion of missing data in the alignment was 4.6%. The alignment comprised 655 positions of which 62 were parsimony-informative. The alignment in Phylip format is provided as Suppl. material 2: (“Notopterus_align_78COI.phy”). We did not use an outgroup and we present only an unrooted haplotype network. Relationships amongst COI haplotypes were inferred with an unrooted network constructed with the programme PopArt (Leigh and Bryant 2015) using a median-joining algorithm (Bandelt et al. 1999) and default settings. Uncorrected pairwise genetic distances and numbers of nucleotide difference within and amongst groups along with their respective standard errors (SE) were calculated with MEGA X. SE were obtained by a bootstrap procedure (500 replicates).

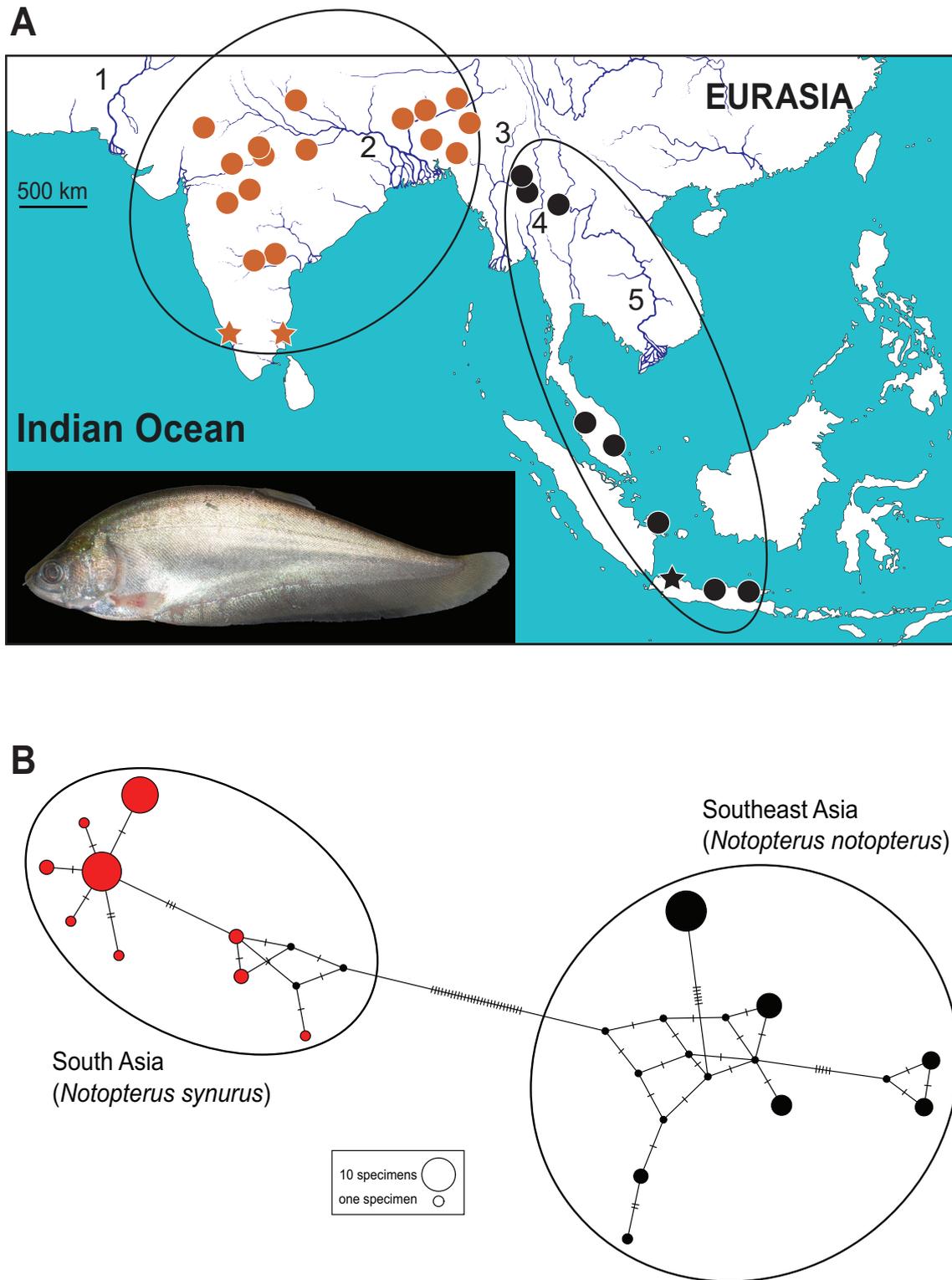


Figure 1. **A** Map covering the Oriental biogeographic region and showing the distribution of localities (black and red circles) of specimens of *Notopterus* examined in this study. Black star indicates the estimated type locality of *Notopterus notopterus* in Java (i.e. Jakarta region) and red stars indicate the likely origin localities of the two syntypes of *Notopterus synurus* (i.e. coast of Malabar and Coromandel coast at Tharangambadi [formerly Tranquebar]). Main Oriental river basins from West to East: **1.** Indus basin; **2.** Ganga-Brahmaputra river system; **3.** Irrawaddy basin; **4.** Salween basin, **5.** Mekong basin. Insert shows the left lateral view of a specimen of *Notopterus notopterus* (Penang State, west Peninsular Malaysia; 20 cm in standard length, voucher specimen number USMFC (3) 00002, NO_1). **B** The unrooted network constructed with the software PopArt and a median-joining algorithm showing the COI haplotype relationships within the genus *Notopterus*. Branch lengths are not proportional to the number of changes. Red circles indicated haplotypes of *Notopterus synurus* (South Asia) and black circles indicated haplotypes of *Notopterus notopterus* (Southeast Asia).

Results

The haplotype network is shown Fig. 1B. Haplotypes segregate into two main groups having distinct distributions. The first group includes all haplotypes from India and Bangladesh (South Asia group). The second group includes all haplotypes from Peninsular Malaysia, Thailand (Mekong River), Indonesia (Sumatra and Java) and Myanmar (Lake Inle, Salween basin) (Southeast Asia group). These two main groups diverge by 7.5% *p*-genetic distance [min-max = 7.0–8.8%; SE = 1%] that represents, on average, 45 differences [SE = 5.8] between any combination of two specimens sampled from different groups. In contrast, each of these two groups is genetically uniform with intra-group differentiation that does not exceed 1% (within the South Asia group, mean *p*-distance = 0.4%; min-max = 0.0–1.6%; SE = 0.1%; and within the Southeast Asia group, mean *p*-distance = 1%; min-max = 0.0–2.5%; SE = 0.2%). This represents six nucleotide differences [SE = 1.5] on average within the Southeast Asian group and only two nucleotide differences [SE = 0.6] on average within the South Asian group. Furthermore, the two specimens examined by Inoue et al. (2009) fell in their corresponding geographic origin groups.

Discussion

The minimal genetic distance separating the genus *Notopterus* into two main groups is well above 3% (using COI marker) which is considered as a conservative threshold between population and species levels in vertebrates (Ward et al. 2009). Furthermore, the existence of a so-called barcode gap (= intergroup distance/intragroup distance) of magnitude ~7X between these two groups, along with the fixation of more than 40 diagnostic nucleotide changes in COI, strongly indicate that *Notopterus* is formed by two species (Meyer and Paulay 2005; Meier et al. 2008). One species is distributed in South Asia (from the Indus basin to Ganga-Brahmaputra basin) and the other in Southeast Asia (from the Salween basin to Mekong basin plus Malay Peninsula, Sumatra and Java).

Roberts (1992) examined the variation of several meristic characters of *N. notopterus* throughout its entire range. He did not report any significant intraspecific variability that could be additional evidence for the recognition of more than one species in the genus *Notopterus*. The low amount of morphological variability within the genus *Notopterus*, however, is not surprising given that the family Notopteridae is known for its morphological stasis. Several valid species of Notopteridae are morphologically similar. For example, species of the genus *Chitala* are only distinguishable based on their colour pattern (Roberts 1992) and more than one species is suspected to occur within *Chitala lopis* (Bleeker, 1851) (Kottelat and Widjanarti 2005; personal observation). Barby et al. (2019) and earlier cytogenetic studies cited in this work, reported the same karyotype formula in *N. notopterus*,

regardless to their origins, with $2n = 42$ and all chromosomes acrocentric.

Our COI-based results strongly support the presence of two allopatric species of *Notopterus* which need names. There are several nominal species of *Notopterus* which have been described from Southeast Asia and South Asia and several of these names are available. To determine which name should be applied to each of our two species, we examined the synonym lists of Fricke et al. (2020) and Kottelat (2013) and checked the date of description and type locality of these synonyms.

Notopterus notopterus was described and illustrated by Pallas in 1769 as *Gymnotus notopterus* from a specimen said to have been collected nearby Ambon [Ambon Island], Indian Ocean, a region where this species has never been recorded since. Kottelat (2013) and Fricke et al. (2020) suggested that the type locality given in the description of Pallas (1769) is an error because this species does not occur on the Island of Ambon which lies east of the Wallace Line, in a different biogeographical region. Furthermore, the local vernacular name of this species “Ikan Pangaio”, reported by Pallas, is in the Malay language which was not used in Ambon at that time. For these reasons, Kottelat (2013) suggested that the specimen used by Pallas (1769) for the description of *N. notopterus* should have been collected in Java where the Dutch established their main colony in Indonesia. Consequently, the name *N. notopterus* should be retained for the species occurring in Southeast Asia with Java as its type locality. According to Fricke et al. (2020), there is no type specimen known.

Bloch and Schneider (1801) described *Clupea synnura* Bloch & Schneider, 1801 from two syntypes, a name which was soon considered as a junior synonym of *N. notopterus* (e.g. as in Cuvier and Valenciennes 1847). Bloch and Schneider (1801) first indicated that these specimens are from the coast of Malabar, India (“*Habitat ad oram Malabaricam*” in Bloch and Schneider [1801]). However, in a following remark signed only by J.G. Schneider, China and Tranquebar (now known as Tharangambadi, India) were listed as the localities of the syntypes. *Notopterus*, however, does not seem to occur in China and Paepke (1999) found convincing explanations why Schneider could have mistaken China with the coast of Malabar, India (see Paepke 1999; Kottelat 2013; Fricke et al. 2020). According to Paepke (1999), the localities of the dry right skins of the two syntypes of *Clupea synnura* that are housed in the Berlin Museum under catalogue numbers ZMB 8806 and ZMB 32057 are coast of Malabar and Tranquebar, respectively. We suggest the revalidation of *Clupea synnura* for the species of *Notopterus* occurring in South Asia which should be recognised as *Notopterus synnurus*.

Whereas the genetic evidence presented in this work supports the recognition of two valid living species of *Notopterus*, a detailed morphological comparison of the two species is lacking. Such morphological study is needed to identify possible diagnostic characters (in addition to the molecular diagnostic characters presented in this study)

and to document the early diversification of the genus *Notopterus* in the Orient. In this respect, a fossil of *Notopterus*, morphological similar to living species, indicates that this genus was already present in Sumatra at least 33 million years ago (the Eocene-Oligocene boundary) (Sanders 1934). In addition, because of the cryptic diversity occurring in the genus *Notopterus* and the difficulty to identify the type localities of species of this genus, it will be important to designate a neotype for *N. notopterus* and a lectotype for *N. synurus*. Finally, the geographic coverage needs to be expanded with the study of specimens collected from the Mekong basin, Indus basin and, especially, from the region comprising the Irrawaddy basin situated between the Ganga-Brahmaputra river system and the Salween basin to determine the location of the exact distributional limit between these two species.

Acknowledgements

We conducted fieldwork in Tasek Bera (Pahang, Peninsular Malaysia) under permit reference number 100-34/1.24Jld14(67) delivered by the Department of Wildlife and National Parks (DWNP). We thank the staff of Tasek Bera Reserve and Siti Zuliana Ahmad (USM) for their assistance in the field. We express our gratitude to Richard van der Laan and Ron Fricke (Eschmeyer's Catalog of Fishes) for helpful discussions on the nomenclature of these fishes. We also thank Nicolas Hubert (editor) for handling our manuscript and Erick Guimarães and Rupert Collins (reviewers) for their constructive comments on the submitted version of this work. This study was supported by a short-term research grant 304/PBIOLOGI/6315400 from Universiti Sains Malaysia to SL.

References

- Bandelt HJ, Forster P, Rohlf A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16(1): 37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>
- Barby FF, Ráb P, Lavoué S, Ezaz T, Bertollo LAC, Kilian A, Maruyama SR, de Oliveira EA, Artoni RF, Santos MH, Jegede OI, Hatanaka T, Tanomtung A, Liehr T, Cioffi MB (2018) From chromosomes to genome: Insights into the evolutionary relationships and biogeography of Old World knifefishes (Notopteridae; Osteoglossiformes). *Genes* 9(6): e306. <https://doi.org/10.3390/genes9060306>
- Barby FF, Bertollo LAC, de Oliveira EA, Yano CF, Hatanaka T, Ráb P, Sember A, Ezaz T, Artoni RF, Liehr T, Al-Rikabi ABH, Trifonov V, de oliveira EHC, Molina WF, Jegede OI, Tanomtung A, Cioffi MB (2019) Emerging patterns of genome organization in Notopteridae species (Teleostei, Osteoglossiformes) as revealed by Zoo-FISH and Comparative Genomic Hybridization (CGH). *Scientific Reports* 9: e1112. <https://doi.org/10.1038/s41598-019-38617-4>
- Barman AS, Singh M, Singh SK, Saha H, Singh YJ, Laishram M, Pandey PK (2018) DNA Barcoding of freshwater fishes of Indo-Myanmar biodiversity hotspot. *Scientific Reports* 8: e8579. <https://doi.org/10.1038/s41598-018-26976-3>
- Bloch ME, Schneider JG (1801) M. E. Blochii, Systema Ichthyologiae Iconibus ex Illustratum. Post obitum auctoris opus inchoatum absolvit, correxit, interpolavit Jo. Gottlob Schneider, Saxo. Berolini. Sumtibus Auctoris Impressum et Bibliopolio Sanderiano Commisum. 584 pp. <https://doi.org/10.5962/bhl.title.5750>
- Christensen MS (1992) Investigations on the ecology and fish fauna of the Mahakam River in East Kalimantan (Borneo), Indonesia. *Internationale Revue ges. Hydrobiologie* 77(4): 593–608. <https://doi.org/10.1002/iroh.19920770405>
- Cuvier G, Valenciennes A (1847) Histoire naturelle des poissons. Tome vingtième. Livre vingt et unième. De la famille des Clupéoïdes. 472 pp.
- Dahrudin 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. *Molecular Ecology Resources* 17(2): 288–299. <https://doi.org/10.1111/1755-0998.12528>
- de Bruyn M, Rüber L, Nylinder S, Stelbrink B, Lovejoy NR, Lavoué S, Tan HH, Nugroho E., Wowor D, Ng PKL, Siti Azizah MN, von Rintelen T, Hall R, Carvalho GR (2013) Paleo-drainage basins delineate priority areas for freshwater biodiversity conservation. *Systematic Biology* 62(3): 398–410. <https://doi.org/10.1093/sysbio/syt007>
- Fricke R, Eschmeyer WN, Van der Laan R [Eds] (2020) Eschmeyer's Catalog of Fishes: Genera, Species, References. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [Electronic version accessed 17 Jan 2020]
- Gupta A, Lal KK, Mohindra V, Singh RK, Punia P, Dwivedi AK, Gupta BK, Luhariya RK, Masih P, Mishra RM, Jena JK (2013) Genetic divergence in natural populations of bronze featherback, *Notopterus notopterus* (Osteoglossiformes: Notopteridae) from five Indian rivers, analyzed through mtDNA ATPase6/8 regions. *Meta Gene* 1: 50–57. <https://doi.org/10.1016/j.mgene.2013.10.007>
- 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). *Molecular Phylogenetics Evolution* 51(3): 486–499. <https://doi.org/10.1016/j.ympev.2009.01.020>
- Jamaluddin JAF, So N, Tam BM, Ahmad A, Grudpan C, Page LM, Khaironizam Z, Nor SAM (2019) Genetic variation, demographic history and phylogeography of tire track eel, *Mastacembelus favus* (Synbranchiformes: Mastacembelidae) in Southeast Asia. *Hydrobiologia* 838: 163–182. <https://doi.org/10.1007/s10750-019-03987-3>
- Kottelat M (1995) The fishes of the Mahakam River, East Borneo: an example of the limitations of zoogeographic analyses and the need for extensive fish surveys in Indonesia. *Tropical diversity* 2(3): 401–426.
- Kottelat M (2013) The fishes of the inland waters of Southeast Asia: A catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bulletin of Zoology Supplement* 27: 1–633.
- Kottelat M, Widjanarti E (2005) The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Kalimantan Barat, Indonesia. *Raffles Bulletin of Zoology Supplement* 13: 139–173.
- Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6(9): 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Maddison WP, Maddison DR (2017) Mesquite: A modular system for evolutionary analysis. Version 3.31. <http://mesquiteproject.org>
- Meier R, Zhang G, Ali F (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap”

- and leads to misidentification. *Systematic Biology* 57(5): 809–813. <https://doi.org/10.1080/10635150802406343>
- Meyer CP, Paulay G (2005) DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biology* 3(12): e422. <https://doi.org/10.1371/journal.pbio.0030422>
- Paepke HJ (1999) Bloch's fish collection in the Museum für Naturkunde der Humboldt Universität zu Berlin: an illustrated catalog and historical account. Ruggell (Liechtenstein). *Theses Zoologicae* 32: 1–216. [32 unpaginated plates.]
- Pallas PS (1769) *Spicilegium Zoologicum quibus novae imprimis et obscurae animalium species iconibus, descriptionibus atque commentariis illustrantur*. Berolini, Gottl. August. Lange 1(7): 1–42. <https://doi.org/10.5962/bhl.title.39832>
- Parenti LR, Lim KKP (2005) Fishes of the Rajang basin, Sarawak, Malaysia. *Raffles Bulletin of Zoology Supplement* 13: 175–208.
- Roberts TR (1989) The freshwater fishes of western Borneo (Kalimantan Barat, Indonesia). *Memoirs of the California Academy of Sciences* 14: 1–210.
- Roberts TR (1992) Systematic revision of the Old World freshwater fish family Notopteridae. *Ichthyological Freshwater Exploration* 2(4): 361–383.
- Rüber L, Tan HH, Britz R (2020) Snakehead (Teleostei: Channidae) diversity and the Eastern Himalaya biodiversity hotspot. *Journal of Zoological Systematics and Evolutionary Research* 58(1): 356–386. <https://doi.org/10.1111/jzs.12324>
- Stecher G, Tamura K, Kumar S (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution* 37(4): 1237–1239. <https://doi.org/10.1093/molbev/msz312>
- Takagi AP, Ishikawa S, Nao T, Song SL, Hort S, Thammavong K, Saphakdy B, Phomsouvanh A, Nishida M, Kurokura H (2010) Genetic differentiation and distribution routes of the bronze featherback *Notopterus notopterus* (Osteoglossiformes: Notopteridae) in Indochina. *Biological Journal of the Linnean Society* 101(3): 575–582. <https://doi.org/10.1111/j.1095-8312.2010.01514.x>
- Yanwirsal H, Bartsch P, Kirschbaum F (2017) Reproduction and development of the Asian bronze featherback *Notopterus notopterus* (Pallas, 1769) (Osteoglossiformes, Notopteridae) in captivity. *Zoosystematics and Evolution* 93(2): 299–324. <https://doi.org/10.3897/zse.93.13341>
- Ward RD, Hanner R, Hebert PDN (2009) The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* 74(2): 329–356. <https://doi.org/10.1111/j.1095-8649.2008.02080.x>
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B-Biological Sciences* 360(1462): 1847–1857. <https://doi.org/10.1098/rstb.2005.1716>

Supplementary material 1

Table S1

Authors: Sébastien Lavoué, Siti Zafirah Ghazali, Jamsari Amirul Firdaus Jamaluddin, Siti Azizah Mohd Nor, Khaironizam Md. Zain

Data type: Genetic data information

Explanation note: List of COI sequences of specimens of *Notopterus* mined from Genbank and examined in this study along with their Genbank accession numbers, the geographical origins with geographical coordinates (in Decimal Degrees) of specimens and the published references where sequences were published.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.96.51350.suppl1>

Supplementary material 2

DNA Data matrix

Authors: Sébastien Lavoué, Siti Zafirah Ghazali, Jamsari Amirul Firdaus Jamaluddin, Siti Azizah Mohd Nor, Khaironizam Md. Zain

Data type: Phylogenetic

Explanation note: Phylogenetic matrices used in the comparative part of this work.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zse.96.51350.suppl2>