

Beyond Names: Phylogeographic Insights into the Mahseer (Teleostei: Cyprinidae: *Tor*) of Sarawak, Malaysia Using the Mitochondrial COI Gene

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ABSTRACT

The mahseer (Teleostei: Cyprinidae: *Tor*) are highly valued freshwater fishes in Southeast Asia, with ecological, fisheries, and cultural importance. However, their taxonomy and biogeography remain unresolved, especially in Sarawak, Malaysia. This study assessed genetic divergence and phylogeographic structure of Sarawak *Tor* using the mitochondrial cytochrome oxidase subunit I (COI) gene. Ninety-four (94) COI sequences were analysed, comprising 19 newly determined sequences from specimens referable to *T. tambroides* and *T. douronensis* from North and South Sarawak and 75 GenBank sequences. Mean interspecific divergence between the nominal taxa was 3.62%, compared with intraspecific divergence of 2.24% for *T. douronensis* and 1.16% for *T. tambroides*, yielding a narrow difference of 1.38% between interspecific and the higher intraspecific divergence. Geographic differentiation was clearer, with 1.93% divergence between North and South Sarawak populations, compared with 0.04% and 0.62% within regions. Phylogenetic analyses recovered two major regional lineages, with the Sarawak specimens forming a distinct lineage subdivided into northern and southern subclades. The nominal taxa were not reciprocally monophyletic, and *T. tambroides* from Baram was nested within the North Sarawak *T. douronensis* lineage. These results indicate that mitochondrial variation is structured more strongly by geography than by nominal species designation and support interpreting Sarawak *T. tambroides* and *T. douronensis* as an unresolved, geographically structured species complex. Historical isolation with incomplete lineage sorting provides the most parsimonious explanation, although introgression and reference misidentification cannot be excluded. Broader sampling, nuclear or genomic data, and integrated morphological analyses are required to resolve their taxonomy and support provenance-based conservation.

Keywords: Biodiversity conservation, Cyprinidae, DNA barcoding, Freshwater fish, Mitochondrial COI, Species delimitation

INTRODUCTION

Malaysia's freshwater ecosystems are home to an exceptionally rich ichthyofauna (Dudgeon, 2000; Dudgeon *et al.*, 2006; Yusoff *et al.*, 2006; Nyanti *et al.*, 2019; Mohd Izam *et al.*, 2021; Soo *et al.*, 2021; Asif *et al.*, 2022). Among this diverse assemblage are the mahseers, large-bodied cyprinids that inhabit clear, fast-flowing rivers (Barbhuiya *et al.*, 2022; Muchlisin *et al.*,

2022a). The highly valued genus *Tor* (Gray, 1834) is a flagship taxon for biodiversity conservation, aquaculture, and biological monitoring (Shaefe and Kamaruddin, 2020; Ng *et al.*, 2022; Jaapar *et al.*, 2023; Lad, 2023; Indra *et al.*, 2024; Iskandar *et al.*, 2024). In Sarawak, *Tor* species are known as 'Ikan Empurau' and 'Ikan Semah,' generally taken to correspond to *Tor tambroides* (Bleeker, 1854) and *Tor douronensis* (Cuvier and Valenciennes, 1842) respectively, as reported by multiple previous

studies (Parenti and Lim, 2005; Esa *et al.*, 2006; Chew *et al.*, 2021; Jaafar *et al.*, 2021; Lim, 2022; Jaapar *et al.*, 2023; Redhwan *et al.*, 2023; Indra *et al.*, 2024). Yet, relying on these vernacular names for formal identification risks obscuring true evolutionary boundaries.

Tor taxonomy in Sarawak is notoriously unstable and subject to frequent revision. A primary driver of this instability is the genus's high intraspecific morphological plasticity, which frequently obscures interspecific boundaries (Roberts, 1993; 1999; Roberts and Khaironizam, 2008; Khaironizam *et al.*, 2015; Walton *et al.*, 2016; Jaafar *et al.*, 2021). Sarawak's historical geology compounds this: river basins now isolated were interconnected during the Pleistocene, leaving present-day populations with a shared, complex history (Rainboth, 1996; Lambeck *et al.*, 2002; McConnell, 2004; de Bruyn *et al.*, 2014) that can mask natural hybridization and cryptic diversity (Parenti and Lim, 2005; Hoang *et al.*, 2015; Jaafar *et al.*, 2021). Pinder *et al.* (2019), based on historically vague and inconsistent species descriptions, longstanding taxonomic debate, and emerging genetic evidence of widespread misidentification of *T. tambroides* as *T. tambra* across Southeast Asia, proposed designating *T. douronensis* as a junior synonym of *T. tambra*. This classification is now reflected in major repositories like FishBase (Froese and Pauly, 2025) and Eschmeyer's Catalog of Fishes (Fricke *et al.*, 2026). However, the historical usage of *T. douronensis* remains widespread in regional literature, museum records, GenBank accessions, and local fisheries documentation (Firman *et al.*, 2020; Biun *et al.*, 2021; Rosali and Adhiyasa, 2021; Akmal *et al.*, 2022; Dwirastina and Wibowo, 2022; Muchlisin *et al.*, 2022a; Muchlisin *et al.*, 2022b; Endryeni *et al.*, 2023; Febri *et al.*, 2023; Rochmatika *et al.*, 2024; Arifn *et al.*, 2025). It thus remains unclear whether the fishes locally recognized as 'Ikan Empurau' (nominally *T. tambroides*) and 'Ikan Semah' (nominally *T. douronensis*) represent distinct evolutionary lineages or merely geographical variants of a broader species complex. We therefore retain the historical nomenclature of *T. douronensis* and *T. tambroides* consistent with specimen identification, publicly available reference sequences,

and previous regional studies, while acknowledging that their taxonomic status is still under revision. Substantial uncertainty therefore remains regarding the evolutionary boundaries of *Tor* in Sarawak, and resolving it is essential for taxonomic stability, regional biodiversity assessment, and conservation planning grounded in biologically meaningful units.

Molecular tools in systematics have become increasingly important in addressing these challenges (Haslawati *et al.*, 2023; Li *et al.*, 2023a; Zhang *et al.*, 2023; Pattarach *et al.*, 2025). The mitochondrial cytochrome c oxidase subunit I (COI) gene is widely used as the standard DNA barcode because it frequently captures interspecific variation while remaining relatively conserved within species (Ward *et al.*, 2005). Despite limited resolution in recently diverged or hybridizing groups, COI has been useful for preliminary species identification, detecting cryptic lineages, and assessing phylogenetic patterns in freshwater fishes (Carugati *et al.*, 2022; da Silva *et al.*, 2023; Zheng *et al.*, 2024), including cyprinids (Sherzada *et al.*, 2020; Dahrudin *et al.*, 2021; Tadmor-Levi *et al.*, 2023). Accordingly, COI has been widely applied to investigate the systematics and genetic diversity of *Tor* populations across Southeast Asia, including Peninsular Malaysia, Sumatra, and parts of Indochina (Nadiatul *et al.*, 2011; Esa and Abdul Rahim, 2013; Azhar and Hassan, 2015; Roesma *et al.*, 2018; Lim *et al.*, 2021; Muchlisin *et al.*, 2022b; Wu *et al.*, 2024). Populations from Sarawak remain conspicuously underrepresented in public repositories such as GenBank and BOLD (Esa *et al.*, 2008; Azhar and Hassan, 2015). This underrepresentation limits current understanding of the genus's genetic diversity and evolutionary biogeographic structure.

Regional studies integrating molecular data with spatial distribution patterns are essential for taxonomic clarity and conservation planning (Guillot *et al.*, 2012; Geiger *et al.*, 2014; Jamu *et al.*, 2023). Given the ecological vulnerability and high market demand for *Tor*, resolving its phylogeographic and genetic structure in Sarawak is critical for taxonomic clarity, distribution mapping, and conservation planning (Piorski *et al.*, 2008; Olden *et al.*, 2010; Hammer *et al.*, 2025). Accordingly, the nominal *T. douronensis* is retained

in the present framework strictly as an operational taxonomic unit (OTU), ensuring data consistency with the vast majority of archived GenBank reference sequences, and to provide a baseline for testing whether historical, morphologically based field identifications correspond to distinct genetic structures (Stach *et al.*, 2003; Galimberti *et al.*, 2012; Hleap *et al.*, 2021; Marnis *et al.*, 2024). This usage does not assert taxonomic validity; it ensures interpretation rests on underlying genetic patterns rather than shifting nomenclature.

This study aims to examine the molecular taxonomy and phylogeography of the genus *Tor* in Sarawak using the mitochondrial COI marker. We seek to provide taxonomic and phylogeographic insights into Sarawak mahseer which will be discussed in a larger geographical context by integrating GenBank reference data. The findings provide new insights into the evolutionary history and taxonomic status of the genus *Tor* in Sarawak while contributing molecular evidence to support biodiversity assessment and conservation planning (Giangrande, 2003; Loon, 2023).

MATERIALS AND METHODS

Sample collection

All *Tor* fish specimens were collected using cast and pole nets from river systems located in both northern and southern Sarawak (Figure 1). Samples were initially identified based on vernacular names provided by local fishermen ('Ikan Empurau' for *T. tambroides* versus 'Ikan Semah' for *T. douaronensis*) (Parenti and Lim, 2005; Jaafar *et al.*, 2021). As outlined previously, these historical species names are retained strictly as operational taxonomic units (OTUs) to ensure comparability with existing literature and publicly available reference datasets (Stach *et al.*, 2003; Hleap *et al.*, 2021; Marnis *et al.*, 2024). These field identifications were subsequently evaluated based on morphological characteristics to confirm assignment to the genus *Tor*, following published taxonomic keys, while recognizing the limitations of morphology in resolving species-level boundaries (Cuvier and Valenciennes, 1842; Bleeker, 1854; Weber and de Beaufort, 1916; Mohsin and Ambak,

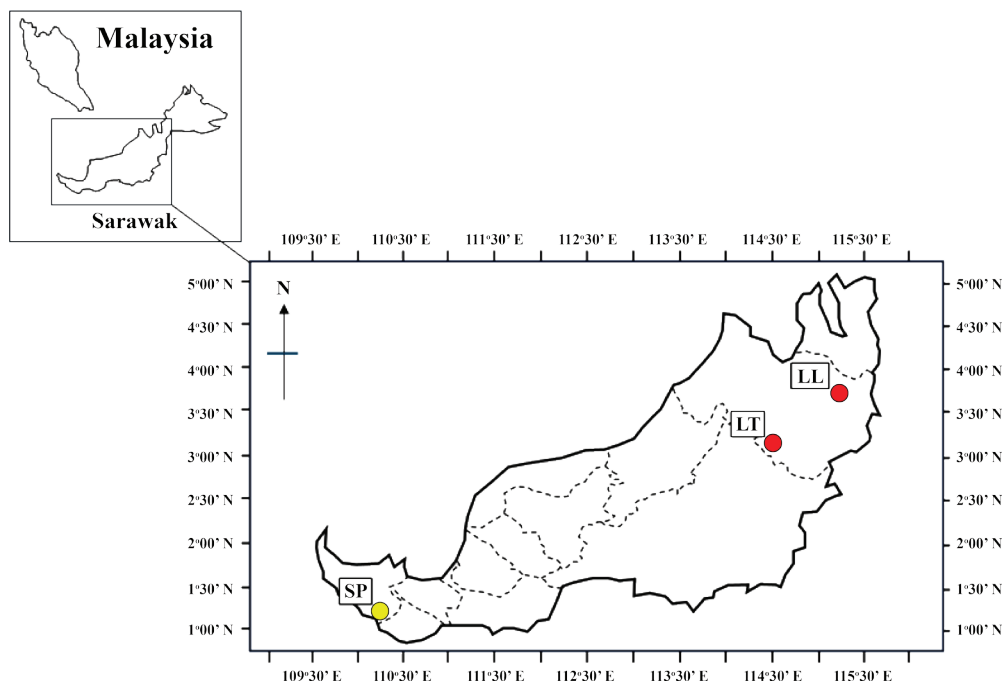


Figure 1. Locations where the specimens of *Tor* were collected (indicated by circles): Long Lellang, Baram (code: LL), Long Ta'ah, Baram (code: LT) and Kampung Sapih, Padawan (code: SP) in Sarawak, Malaysia. Red circles are within the northern region and yellow circle is within the southern region.

1983; Kottelat *et al.*, 1993; Roberts, 1993, 1999; Kottelat and Whitten, 1996; Martin-Smith and Tan, 1998; Kottelat, 2001; Tan and Lim, 2004; Parenti and Lim, 2005; Haryono, 2006; Hoang *et al.*, 2015; Walton *et al.*, 2016; Wilkinson and Tan, 2018; Jaafar *et al.*, 2021; Taki *et al.*, 2021).

Approximately 1 g of muscle tissue was excised and collected from the right lateral side of each specimen immediately below the dorsal fin and preserved in 95% ethanol in labeled specimen tubes, while the remaining specimen body was immediately chilled in ice and transported to the Aquatic Molecular Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). Tissue samples were stored at -20 °C until DNA extraction. Voucher specimens were fixed with 7–10% formalin (Saha *et al.*, 2019), rinsed in tap water for 2–3 days, and preserved in 70% ethanol at the Aquatic Science Museum, UNIMAS.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from muscle tissue using cetyltrimethylammonium bromide (CTAB) method (Doyle and Dickson, 1987). The mitochondrial COI fragment was amplified using primers Fish F1 (5-TCAACCAACCACAAAGA CATTGGCAC-3) and Fish R1 (5-TAGACTTCTG GGTGGCCAAAGAATCA-3) (Ward *et al.*, 2005) in a Bio-Rad MyCycler™ Thermal Cycler. Each 25 µL PCR reaction contained deionized water (14.6 µL), 5× PCR buffer (Promega, Madison, Wisconsin, USA) (5.0 µL), 2.0 mM magnesium chloride (MgCl₂) (2.0 µL), 0.08 mM dNTPs (Promega, Madison, Wisconsin, USA) (0.2 µL), 0.5 µM of each primer (Integrated DNA Technologies, Coralville, Iowa, USA) (0.5 µL), DNA template (2 µL), and 0.04 U of Taq DNA polymerase (Promega, Madison, Wisconsin, USA) (0.2 µL). The PCR protocol followed Ward *et al.* (2005) and included a pre-denaturation step at 95 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min, after which the reaction was held at 4 °C for short-term storage prior to downstream processing. PCR products were purified and

sequenced bidirectionally by Bio Basic Asia Pacific Pte Ltd (Singapore).

Genetic analysis

Chromatograms from both forward and reverse reads were visualized using the CHROMAS software (version 2.6.6) (Technelysium Pty Ltd., 2019) and manually inspected and edited to obtain consensus sequences. Alignments were performed in MEGA software (version 12.1) (Kumar *et al.*, 2024) using the MUSCLE algorithm, with further manual adjustments to remove ambiguous regions (Yang *et al.*, 2019), followed by translation to amino acids to ensure accurate alignment and detect the presence of stop codons. Newly generated sequences from this study were deposited in GenBank (accession numbers: PV701035–PV701036; PV701164–PV701167; PV701112–PV701120; PV701180–PV701183). All specimens used in this study are listed in Table 1, and the dataset was supplemented with 73 COI sequences of *T. tambroides* and *T. douronensis* retrieved from GenBank, representing multiple geographic regions.

Genetic diversity parameters including conserved and variable sites, parsimony informative sites, transition/transversion counts, and nucleotide frequencies were calculated in MEGA software (version 12.1) (Kumar *et al.*, 2024). Pairwise genetic distances were estimated using Tamura–Nei model (Tamura and Nei, 1993) with a gamma distribution (Yang, 1994) (TN93+G) to compare intraspecific and interspecific divergences across species and geographic regions.

Phylogenetic analysis

Phylogenetic relationships were inferred using Maximum Parsimony (MP) (Fitch, 1971), and Maximum Likelihood (ML) (Felsenstein, 1981), both conducted in MEGA software (version 12.1) (Kumar *et al.*, 2024). The best-fitting nucleotide substitution model for the ML analysis was determined using jModelTest (Posada, 2008). *Neolissochilus soroides* (accession number OM 203154) and *Neolissochilus stracheyi* (accession number OM203155) sequences were used as outgroups to root the phylogenetic trees, as

Table 1. Nominal species, sample size (n), sampling locality, and COI accession numbers of the *Tor* specimens included in this study.

Nominal species	Sample size (n)	Locality	Accession number
<i>T. douronensis</i>	4	Long Ta'ah, Baram, North Sarawak, Malaysia	PV701180–PV701183
	4	Long Lellang, Baram, North Sarawak, Malaysia	PV701164–PV701167
	9	Kampung Sapit, Padawan, South Sarawak, Malaysia	PV701112–PV701120
	2	Sandakan, Sabah, Malaysia	JN646100, KT799805
	1	Interior, Sabah, Malaysia	KF240784
	1	Xishuangbanna, Yunnan, China	KP712053
	1	Indonesia	OR288574
	1	Malaysia	KJ880045
<i>T. tambroides</i>	2	Long Ta'ah, Baram, North Sarawak, Malaysia	PV701035–PV701036
	2	Sandakan, Sabah, Malaysia	JN646101, KT799815
	1	Interior, Sabah, Malaysia	KF240785
	24	Tarusan, West Sumatra, Indonesia	KC905001–KC905024
	37	South Sumatra, Indonesia	JQ665787–JQ665823
	1	Malaysia	KT001033
	1	China	HM536923
	1	Unknown	AP011372
<i>Neolissochilus soroides</i>	1	China	OM203154
<i>Neolissochilus stracheyi</i>	1	China	OM203155

Neolissochilus is the closest sister lineage to *Tor* (Wu *et al.*, 2024). Node robustness was estimated by bootstrapping (Felsenstein, 1985) with 1,000 replicate data sets. Only nodes supported by bootstrap proportion (BP) >70% were considered robust whereas those with 50%<BP<70% were considered as weakly supported (Hillis and Bull, 1993).

RESULTS AND DISCUSSION

A total of 19 *Tor* specimens were collected from northern and southern river systems in Sarawak. Preliminary identification, guided by local ethnobiological knowledge, assigned two individuals as Ikan Empurau (*T. tambroides*) and 17 specimens as Ikan Semah (*T. douronensis*). Identification was supported by the median lobe of the lower lip, which is prominent and elongated in *T. tambroides* but short, indistinct, or absent in *T. douronensis* (Kottelat *et al.*, 1993; Kottelat and Whitten, 1996; Rainboth, 1996; Jaafar *et al.*, 2021). Coloration was used as a supplementary

character (Ng, 2004; Jaafar *et al.*, 2021; Surachat *et al.*, 2022). Diagnostic features are illustrated in Figure 2, and preliminary identifications are summarized in Table 2.

All 94 COI sequences were trimmed and aligned to a final length of 561 base pairs (bp). The alignment showed a typical vertebrate mitochondrial base composition, with higher AT content (T = 28.8%, C = 27.9%, A = 26.6%, and G = 16.6%), consistent with previous cyprinid studies (Lin *et al.*, 2014; Yu *et al.*, 2023). Of 561 sites, 484 (86.3%) were conserved and 77 (13.7%) variables, of which 61 (79.2%) were parsimony-informative and 16 (20.8%) were singleton. Transitions (si = 11) outnumbered transversions (sv = 1), giving a transition/transversion ratio of 17.5, concentrated at the third codon position, and consistent with typical teleost mitochondrial mutation patterns (Inoue *et al.*, 2001; Sherzada *et al.*, 2022). Given this unequal base composition and strong ts/tv bias, genetic distances were estimated under TN93+G model (Tamura and Nei, 1993; Yang, 1994), and are summarized at the species level in Table 3 and at the regional level in Table 4.

Based on the genetic distance between species, the mean interspecific divergence between the nominal *T. douronensis* and *T. tambroides* (3.62%) exceeded the intraspecific divergence within *T. douronensis* (2.24%) by only 1.38% (Table 3).

COI studies of cyprinids have successfully discriminated species where a clear barcode gap separates intra- and interspecific variation (Hebert *et al.*, 2003; Hubert *et al.*, 2008; Lakra *et al.*, 2016). For example, Adeoba *et al.* (2018) reported 79.4–

Table 2. Summary of preliminary identification of *Tor* specimens collected from Sarawak based on vernacular classification and diagnostic morphological characters.

Nominal species	Vernacular classification	Sample size (n)	Sampling locality (Region)	Diagnostic morphological characters
<i>T. douronensis</i>	Ikan Semah	4	Long Ta'ah, Baram (North Sarawak)	Median lobe short to indistinct; elongated fleshy projection absent
		4	Long Lellang, Baram (North Sarawak)	Median lobe variable: absent to short or indistinct; elongated fleshy projection absent; reddish to yellowish colour morphs present
		9	Kampung Sapit, Padawan (South Sarawak)	Median lobe absent to indistinct; elongated fleshy projection absent
<i>T. tambroides</i>	Ikan Empurau	2	Long Ta'ah, Baram (North Sarawak)	Median lobe prominent and well-developed, forming an elongated fleshy projection on the lower lip

Table 3. Mean±SD pairwise genetic distances (TN93+G) within and between *Tor* species across all sampled populations. Genetic distances are presented as percentages (%) with intraspecific distances highlighted in bold.

Species	Sample size (n)	<i>T. douronensis</i>	<i>T. tambroides</i>
<i>T. douronensis</i>	23	2.24±0.39	
<i>T. tambroides</i>	69	3.62±0.69	1.16±0.22
<i>Neolissochilus soroides</i> &	1	4.99±0.88	5.36±1.01
<i>Neolissochilus stracheyi</i> (Outgroups)	1		

Table 4. Mean±SD pairwise genetic distances (TN93+G) within and between geographical populations of *Tor*. Genetic distances are presented as percentages (%) with within-population distances highlighted in bold.

Population/Locality	Species included	Sample size (n)	North Sarawak (Malaysia)	South Sarawak (Malaysia)	Non-Sarawak populations
North Sarawak (Malaysia)	<i>T. douronensis</i>	8	0.04±0.04		
	<i>T. tambroides</i>	2			
South Sarawak (Malaysia)	<i>T. douronensis</i>	9	1.93±0.58	0.62±0.20	
Non-Sarawak populations	<i>T. douronensis</i>	6	2.99 ± 0.73	4.00 ± 0.63	2.13 ± 1.62
	<i>T. tambroides</i>	67			
Outgroup	<i>Neolissochilus soroides</i>	1	4.57±0.92	4.92±0.95	5.39 ± 0.55
	<i>Neolissochilus stracheyi</i>	1			

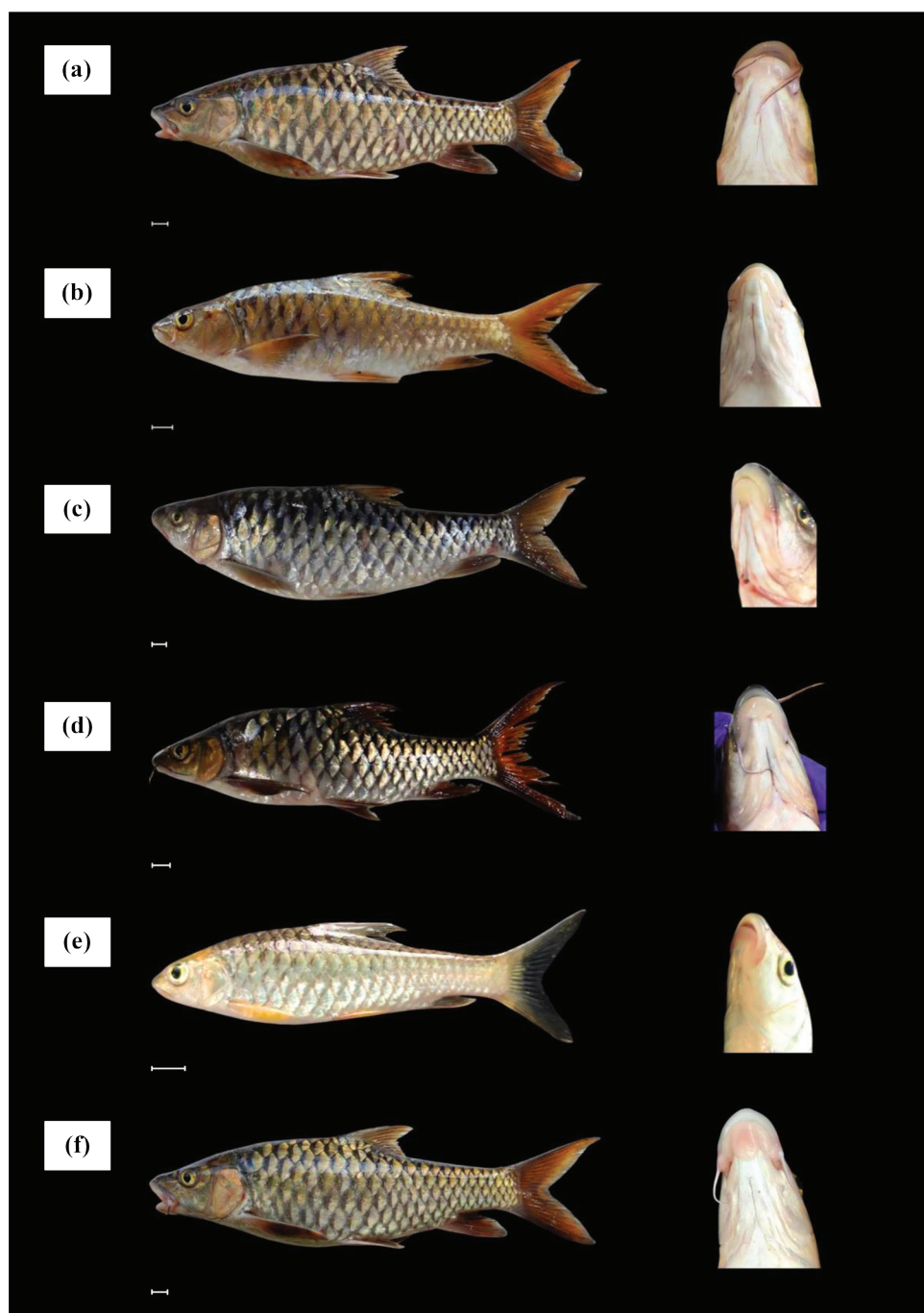


Figure 2. Representative *Tor* specimens collected from Sarawak, Malaysia. (a–f) Lateral views (left) and corresponding close-up views of the lower lip (right) of specimens preliminarily identified as *T. douronensis* from (a) Long Ta'ah, (b–d) Long Lellang, and (e) Kampung Sapit, illustrating variation in body coloration and median lobe morphology. (f) Lateral view (left) and corresponding close-up view of the lower lip (right) of a specimen preliminarily identified as *T. tambroides* from Long Ta'ah, showing the characteristic elongated fleshy median lobe. Scale bars = 1 cm (lateral views).

92.1% identification success in African Cyprinidae, and Shen *et al.* (2016) found complete separation between intra- and interspecific genetic distances in Yangtze cyprinids. By contrast, the narrow margin observed here indicates no distinct barcode gap and limited discriminatory power of COI between the nominal *T. douronensis* and *T. tambroides*: the marker characterizes mitochondrial variation within *Tor* well, but genetic distance alone cannot resolve species boundaries in these populations.

The intraspecific divergence observed within nominal *T. douronensis* group (2.24%) is itself noteworthy, as COI divergence within cyprinid species is generally low (Hebert *et al.*, 2003; Lakra *et al.*, 2016). Rather than ordinary within-species variation, this level is consistent with genetic structuring among geographically separated populations: because *Tor* is largely confined to fast-flowing upstream habitats, dispersal among isolated catchments is restricted, limiting maternal gene flow and allowing mitochondrial lineages to diverge regionally over time (Thiessen, 2012; Brodie *et al.*, 2025). In Sarawak, historical features such as the Lupar Line are recognized as important biogeographical barriers that restrict freshwater dispersal and promote genetic differentiation among riverine taxa (Zainudin *et al.*, 2010; Zainudin and Naim, 2018), and likely contributed to the north–south structuring recovered here.

Comparable patterns have been reported in other freshwater fishes. A study by Aminan *et al.* (2020) documented high intraspecific divergence in *Rasbora caudimaculata* and *R. sumatrana* (up to 8.8% and 3.5%), alongside relatively low interspecific divergence (0.8–3.3%), leaving phylogenetic relationships unresolved despite morphological differences; Alshari *et al.* (2021) similarly found low COI divergence (1.3–1.5%) among geographically structured lineages of *Scleropages* spp. and concluded that morphologically distinct nominal species were in fact closely related lineages rather than fully separate species. Together with the present data, these studies show that elevated intraspecific divergence commonly reflects historical isolation, geographic structuring, and incomplete lineage sorting rather than clear species boundaries (Johnson *et al.*, 2023; Phanklam *et al.*, 2026).

Geographic comparisons showed clearer structuring than the species-level comparisons (Table 4). Divergence between North and South Sarawak (1.93%) substantially exceeded within-region divergence observed in North Sarawak (0.04%) and South Sarawak (0.62%), indicating that mitochondrial differentiation tracks geography more closely than current species classification.

This is consistent with Sarawak's biogeography: *Tor* is confined to fast-flowing upland rivers with limited dispersal between catchments (Barbhuiya *et al.*, 2022; Renjithkumar *et al.*, 2022; Singh *et al.*, 2022; Kaur *et al.*, 2025), and repeated Pleistocene sea-level fluctuations periodically altered drainage connectivity across multiple basins separated by watershed barriers, producing cycles of isolation and reconnection (Seehausen and Wagner, 2014; Biun *et al.*, 2021; Sudasinghe *et al.*, 2021; He *et al.*, 2024). These processes plausibly restricted maternal gene flow enough to allow regional mitochondrial divergence while populations retained broadly similar external morphology (Thiessen, 2012; Brodie *et al.*, 2025). Notably, North Sarawak contains both nominal species while South Sarawak contains only nominal *T. douronensis*, a pattern that itself points to geography, rather than nominal classification, as the stronger organizing factor.

Phylogenetic trees were built for Maximum Likelihood (ML) (Felsenstein, 1981) using the TN93+G model (Tamura and Nei, 1993; Yang, 1994). jModelTest (Posada, 2008) identified TPM2uf+G (Belyaeva and Taylor, 2009) as the best-fit model under AICc, but as this model is unavailable in MEGA v.12.1 (Kumar *et al.*, 2024), TN93+G was applied instead as the closest approximation, consistent with MEGA's own model selection. Maximum Parsimony (MP) analysis (Fitch, 1971) was conducted using equally weighted characters. Both methods recovered broadly similar topologies (Figures 3–4), identifying two major *Tor* lineages clearly distinct from the *Neolissochilus* outgroups (bootstrap >95%), but neither recovered the nominal *T. douronensis* and *T. tambroides* as reciprocally monophyletic.

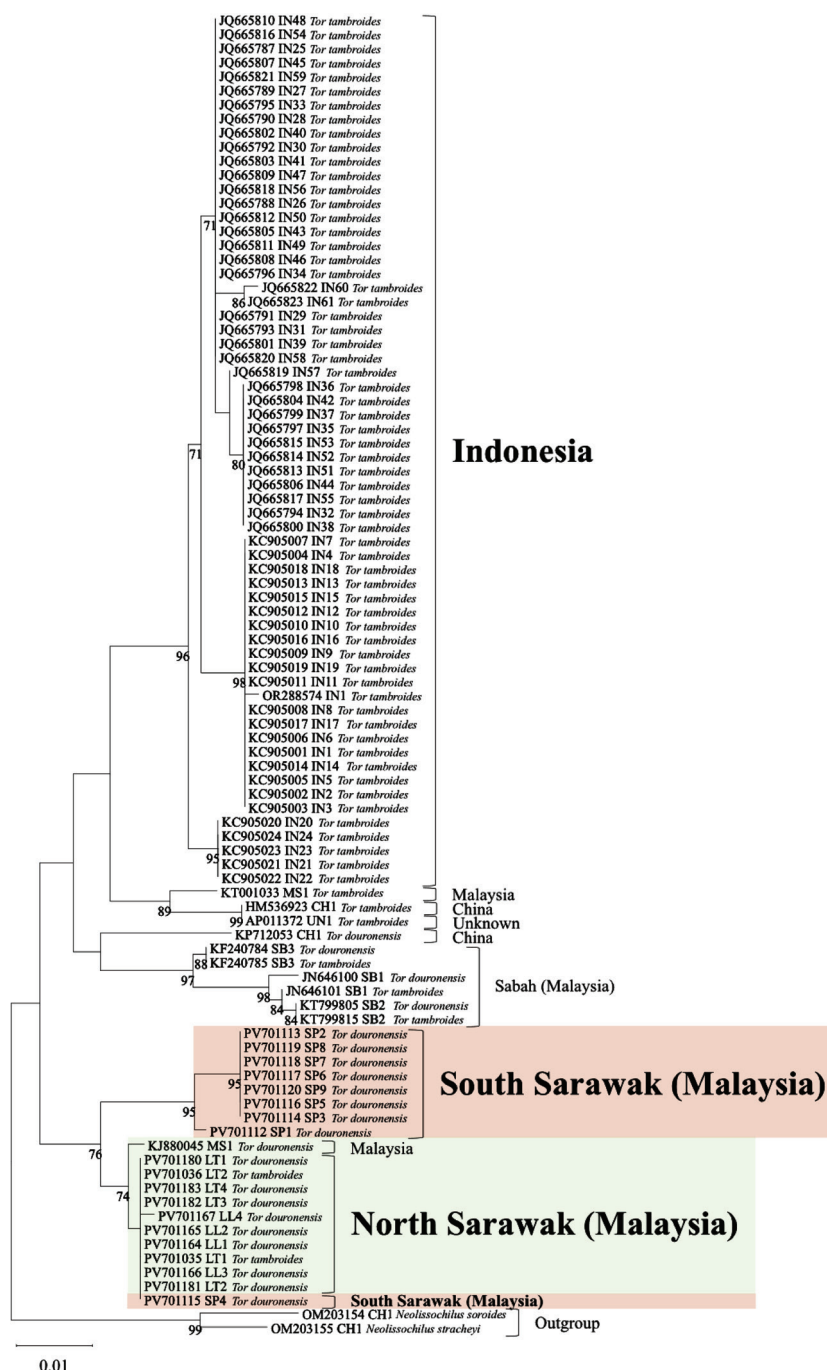


Figure 3. Maximum Likelihood tree of *Tor* spp. based on partial COI gene sequences, constructed using the TN93+G model (Tamura and Nei, 1993; Yang, 1994). LT = Long Ta'ah, LL = Long Lellang, SP = Kampung Sapit, SB = Sabah, MS = Malaysia, CH = China, IN = Indonesia, and UN = Unknown. Sequences obtained in the present study are in bold letters. Numbers above branches indicate bootstrap values greater than 70.

Note: Green and peach shading denote the North and South Sarawak clades, respectively, with PV701115 representing the only South Sarawak specimen clustering within the North Sarawak clade.

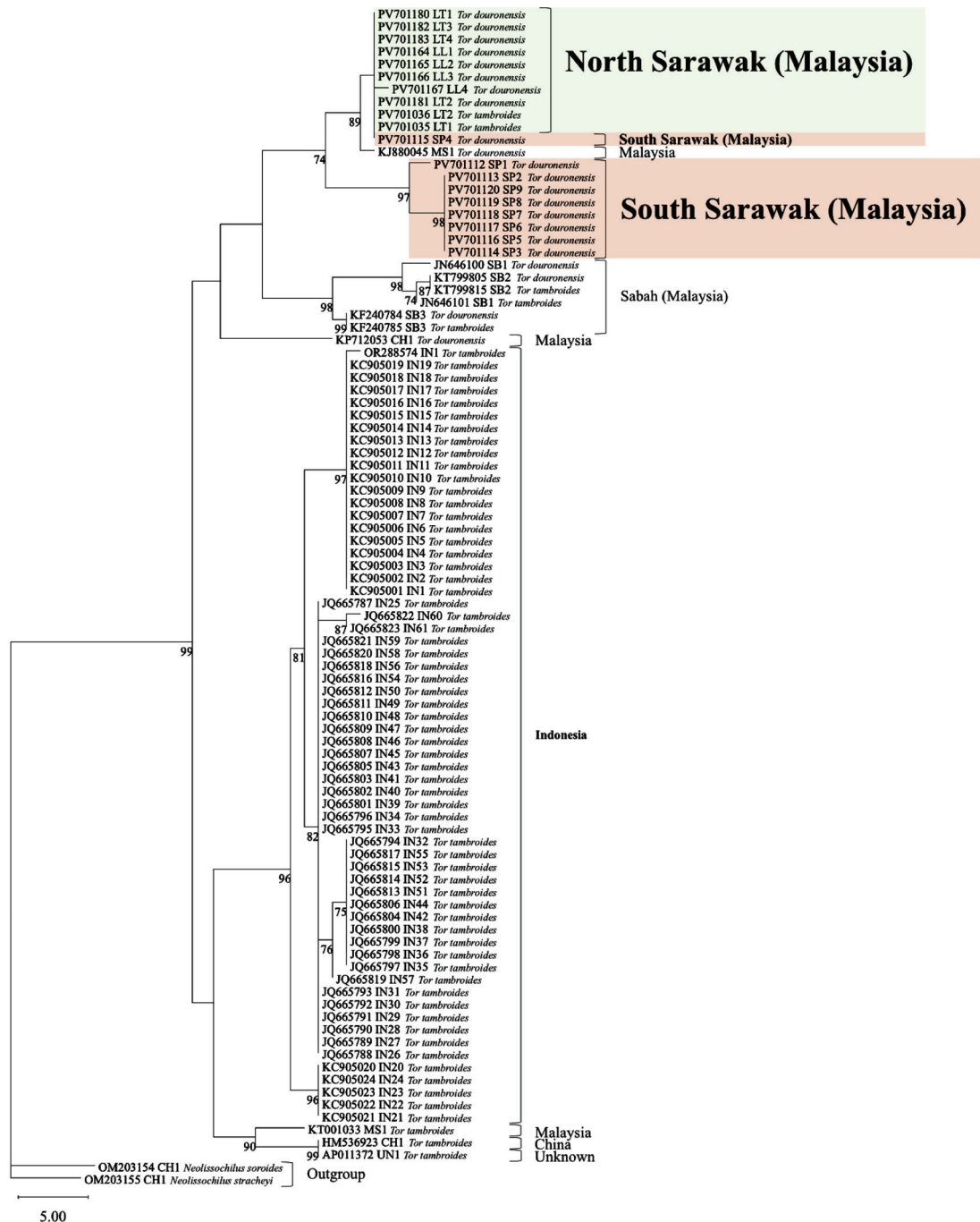


Figure 4. Maximum Parsimony tree of *Tor* spp. based on partial COI gene sequences, constructed using the Subtree-Pruning-Regrafting (SPR) heuristic search method. LT = Long Ta'ah, LL = Long Lellang, SP = Kampung Sapit, SB = Sabah, MS = Malaysia, CH = China, IN = Indonesia, and UN = Unknown. Sequences obtained in the present study are in bold letters. Numbers above branches indicate bootstrap values greater than 70.

Note: Green and peach shading denote the North and South Sarawak clades, respectively, with PV701115 representing the only South Sarawak specimen clustering within the North Sarawak clade.

The predominantly *T. douronensis* clade (ML = 76%) included all newly generated Sarawak sequences plus GenBank sequences from another Malaysian locality. Within this clade, Sarawak samples formed two geographically distinct subclades corresponding to South and North Sarawak. The South Sarawak subclade, consisting primarily of specimens from Kampung Sapit, was consistently recovered with high bootstrap support (ML = 95%; MP = 97%), and North Sarawak subclade, comprising specimens from Long Ta'ah and Long Lellang specimens plus one Kampung Sapit specimen (PV701115) (ML = 74%; MP = 89%). The North Sarawak subclade also included one Malaysian GenBank sequence (KJ880045), and notably, the two nominal *T. tambroides* specimens from Long Ta'ah (PV701035–PV701036) were nested within it rather than forming an independent lineage. The predominantly *T. tambroides* clade outside Sarawak was more weakly resolved (ML = 52%; MP = 47%) and included intermixed non-Sarawak *T. douronensis* sequences; within it, only one exclusively Indonesian subclade was strongly supported by both methods (96%), while the rest were weak in ML (41–57%) but strong in MP (90%).

Across both the distance and phylogenetic analyses, geographic origin explains the mitochondrial data more consistently than the current nominal species classification. Sarawak specimens formed well-supported North and South subclades (ML = 74–95%; MP = 89–97%) matching the regional divergence values above (0.04–1.93%), while the nominal *T. tambroides* assemblage outside this structure remained weakly resolved (ML = 52%; MP = 47%) and intermixed with *T. douronensis*. The intermixing of the two nominal taxa, the absence of reciprocal monophyly, and the narrow interspecific–intraspecific divergence gap together are best explained by incomplete lineage sorting following historical geographic isolation and restricted maternal gene flow (Thiessen, 2012; Johnson *et al.*, 2023; Brodie *et al.*, 2025; Phanklam *et al.*, 2026): isolated river populations would have retained ancestral haplotypes while slowly accumulating regional divergence, producing geographically structured lineages not fully

concordant with morphologically defined species (Fang *et al.*, 2022; Wei *et al.*, 2023). Under this reading, the nominal *T. tambroides* and *T. douronensis* in Sarawak have not achieved complete mitochondrial differentiation and are better treated as closely related lineages sharing ancestral variation than as two fully separated species (Avise, 2000; Durand *et al.*, 2000; Funk and Omland, 2003; Nguyen *et al.*, 2006; Esa and Abdul Rahim, 2013; Walton *et al.*, 2016; Karnasuta, 2017; Roesma *et al.*, 2018; Hassan and Mustapah, 2019; Pinder *et al.*, 2019; Chew *et al.*, 2021), a pattern paralleled elsewhere in Southeast Asian freshwater fishes where historical drainage isolation drove strong phylogeographic divergence despite limited species-level mitochondrial differentiation (Dodson *et al.*, 1995; De Bruyn *et al.*, 2013; Chen *et al.*, 2017; Thomaz *et al.*, 2017; Sholihah *et al.*, 2020; 2021; Waters *et al.*, 2020; Sudasinghe *et al.*, 2021; Li *et al.*, 2023b).

Incomplete lineage sorting is the most parsimonious explanation, but it is not the only possible one. Historical introgressive hybridization could similarly produce shared mitochondrial haplotypes between morphologically distinct taxa, and misidentification of historical museum vouchers or GenBank reference sequences could also blur the signal among morphologically similar taxa (Wibowo *et al.*, 2013; Azhar and Hassan, 2015; Walton *et al.*, 2016; Priyadarshini, 2017; Biun *et al.*, 2021; Johnson *et al.*, 2023; Pongsanarm *et al.*, 2025). It also remains possible that the nominal *T. tambroides* and *T. douronensis* are incompletely resolved or partially synonymous taxonomic entities, consistent with earlier morphological and molecular work questioning their separation (Roberts, 1999; Esa and Abdul Rahim, 2013; Walton *et al.*, 2016). The present COI data cannot distinguish definitively among these scenarios (Priyadarshini, 2017; Johnson *et al.*, 2023; Pongsanarm *et al.*, 2025; Phanklam *et al.*, 2026), underscoring that species delimitation in this group requires a broader evolutionary and taxonomic framework than a single marker can provide.

Two limitations should be noted. First, the newly collected sample was small, especially for nominal *T. tambroides* (n = 2, both from Long

Ta'ah); despite targeted effort, none were found at Long Lellang or Kampung Sapit, and local fishermen described it as rare to absent at these sites. The apparent scarcity of *T. tambroides* is consistent with previous reports of declining wild *Tor* populations from habitat degradation, overexploitation, and other anthropogenic pressures (Ingram *et al.*, 2005; De Silva *et al.*, 2004; Chew *et al.*, 2021) though these accounts alone cannot establish abundance; remote access, weather, time, and budget further limited geographic coverage, so the dataset may underrepresent Sarawak's mitochondrial diversity, particularly for *T. tambroides*. Second, the study relied on a single maternally inherited marker: COI characterized broad geographic structuring well but cannot alone distinguish incomplete lineage sorting from introgression or unresolved taxonomy (Priyadarshini, 2017; Johnson *et al.*, 2023; Pongsanarm *et al.*, 2025; Phanklam *et al.*, 2026). The GenBank sequences used to broaden coverage may also carry uncertain identifications or uneven sampling. The conclusion that geography structures Sarawak *Tor* more than nominal classification is therefore well-supported but provisional, pending broader sampling and independent nuclear or genomic evidence.

Future studies include expanded sampling across additional river basins, especially targeting *T. tambroides* and sampling both nominal taxa at the same localities, extending north to Ba'kelalan and Limbang and centrally to Kapit and Belaga, to test whether the North–South pattern reflects a gradual transition, independent basin populations, or a sharper phylogeographic discontinuity. Additional mitochondrial markers (*Cytb*, ND2) alongside nuclear markers (RAG1, S7 intron) or genome-wide SNP data, combined with morphometric and meristic assessment of verified vouchers, would help separate incomplete lineage sorting from introgression and provide a firmer basis for resolving the taxonomic status of *T. tambroides* and *T. douronensis*.

These findings bear directly on conservation and fisheries management. The 1.93% divergence between North and South Sarawak reflects

historically restricted maternal gene flow (Thiessen, 2012; Brodie *et al.*, 2025). While insufficient alone to define separate species or formal evolutionarily significant units, this supports treating the two regions as precautionary management units pending further evidence (Binashikhbubkr *et al.*, 2023; Prawira *et al.*, 2025; Suwarsito *et al.*, 2026), which is important regardless of the taxa's eventual species status. This bears directly on aquaculture, captive propagation, and stocking programmes, including Department of Agriculture Sarawak's inland-fisheries facility at Tarat (DayakDaily, 2021; Department of Agriculture Sarawak, 2022a; 2025). Broodstock mixing or stocking across regions should be avoided to prevent homogenizing historically distinct gene pools (Masuda *et al.*, 2024; Euclide *et al.*, 2025). Broodstock should be sourced from the same region, ideally the same river basin, as the release sites (Yan *et al.*, 2026), or the nearest genetically characterised population where local stocks is unavailable, rather than by nominal species name alone (Buckley *et al.*, 2024; Pongsanarm *et al.*, 2025). Hatcheries should keep broodstock lines separated by provenance, document founder locality, retain breeding records, and log release numbers and origins (Fitzpatrick *et al.*, 2023; Horn *et al.*, 2023). Periodic genetic screening would further help detect unintended mixing or loss of naturally occurring lineages (Li *et al.*, 2020; Attard *et al.*, 2022).

The apparent scarcity of wild *T. tambroides*, consistent with earlier reports of decline (De Silva *et al.*, 2004; Ingram *et al.*, 2005; Chew *et al.*, 2021), warrants targeted population surveys, harvest monitoring, and habitat assessment in northern Sarawak (Nunn *et al.*, 2023; Sayer *et al.*, 2025), extending management beyond restocking to protecting self-sustaining wild populations, potentially through the community-based Sarawak Tagang system, already used to regulate harvest in rivers such as Terbat Mawang and Long Lidong (Ansley *et al.*, 2018; Keling *et al.*, 2021; Department of Agriculture Sarawak, 2022b), complemented by catchment protection under the Heart of Borneo programme, including the Upper Baleh headwaters (Brander *et al.*, 2018; World Wide Fund (WWF), 2019; Forest Department Sarawak, 2024).

CONCLUSIONS

Mitochondrial COI data do not support the nominal *Tor tambroides* and *T. douronensis* in Sarawak as two clearly differentiated species. The two nominal taxa were frequently intermixed and were not recovered as reciprocally monophyletic, with only a narrow gap between mean interspecific (3.62%) and intraspecific (2.24%) divergence, which is instead consistent with a single, geographically structured mitochondrial assemblage retaining shared ancestral variation. By contrast, both genetic distance and phylogenetic analyses consistently recovered distinct North and South Sarawak lineages (1.93% divergence between regions, versus 0.04–0.62% within regions), indicating that historical isolation among river systems, rather than current nominal classification, is the primary driver of mitochondrial diversification in Sarawak *Tor*. These conclusions rest on a single maternally inherited marker and limited sampling of nominal *T. tambroides*, and should be treated as a provisional hypothesis pending broader geographic sampling and independent nuclear or genomic evidence; in the meantime, the North and South Sarawak populations should be managed as precautionary geographic units, with broodstock provenance tracked and translocation between regions avoided, to preserve the genetic and evolutionary diversity of these culturally and economically important fishes while their taxonomy is resolved.

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