



Morphometric Analysis and Genetic Relationship of *Rasbora* spp. in Sarawak, Malaysia

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Highlights

- Total of 23 morphometric parameters were used to differentiate the 103 *Rasbora* samples from Sarawak.
- *Cytochrome oxidase subunit I (COI)* gene was utilised to further distinguish these fishes through phylogenetic analysis.
- Pre-anal length identified as strongest morphometric discriminant (100%) and all eight *Rasbora* species tested are monophyletic except for *R. sumatrana* and *R. caudimaculata*.

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Abstract: The genus *Rasbora* is one of the most species-rich genus among the freshwater fishes and cryptic diversity has been a major hindrance in species identification in the past four decades due to their high similarities in terms of morphology. This study aimed to investigate this issue both morphologically and molecularly. In this study, a total of 23 morphometric parameters were used to differentiate the 103 *Rasbora* fish samples harvested from different regions of Sarawak state of Malaysia via Multivariate Stepwise Discriminant Function Analysis (SDFA). Then, *cytochrome oxidase subunit I (COI)* gene was utilised to further distinguish 33 of these fishes, followed by sequence and phylogenetic analysis. Our results unravelled pre-anal length as strongest morphometric discriminant (100%) and that all eight *Rasbora* species tested are monophyletic except for *R. sumatrana* and *R. caudimaculata*, revealing possible cryptic *Rasbora* species. Further investigations are vital to enrich the data from this study for *Rasbora* cryptic diversity and conservation studies in future.

Keywords: Cryptic Diversity, *COI*, Morphometric, Molecular, *Rasbora* spp.

Abstrak: Genus *Rasbora* adalah salah satu daripada genus yang memiliki keragaman spesies yang paling tinggi dalam kalangan ikan air tawar dan kepelbagaian krip telah menjadi penghalang utama dalam pengecaman spesies sepanjang empat dekad disebabkan persamaannya yang tinggi dari segi morfologi. Kajian ini bertujuan untuk mengkaji isu-isu morfologi dan molekular. Dalam kajian ini, 23 parameter morfometrik telah digunakan untuk membezakan 103 sampel ikan *Rasbora* yang dikutip daripada daerah berbeza di negeri Sarawak, Malaysia menggunakan *Multivariate Stepwise Discriminant Function Analysis* (SDFA). Kemudian, gen *cytochrome oxidase subunit I (COI)* digunakan

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untuk membezakan 33 jenis ikan ini, diikuti dengan analisis jujukan dan analisis filogenetik. Keputusan menunjukkan panjang pra-anal sebagai morfometrik diskriminan terkuat (100%) dan kesemua lapan spesies *Rasbora* yang diuji adalah monofiletik kecuali *R. sumatrana* and *R. caudimaculata*, dan mendedahkan spesies *Rasbora* yang mungkin krip. Penyelidikan lebih lanjut adalah sangat penting untuk memperkayakan data dari kajian ini untuk kepelbagaian dan pemuliharaan krip di masa akan datang.

Kata kunci: Kepelbagaian Krip, COI, Morfometrik, Molekular, *Rasbora* spp.

INTRODUCTION

The *Rasbora* fish are from the family of Cyprinidae and they are small to moderate in size, inhabiting the Asian region. The *Rasbora* genus is the most species-rich genus in the cyprinid Danioninae subfamily (Lumbantobing 2014). Currently, a total of 150 *Rasbora* species had been discovered where 39 species are distributed across Borneo and six are distributed across Sarawak (Fricke et al. 2019). There are a total of eight *Rasbora* groups identified by Brittan (1984), which are *R. trifasciata*, *R. argyrotaenia*, *R. einthovenii*, *R. daniconius*, *R. lateristriata*, *R. caudimaculata*, *R. sumatrana-elegans* and *R. pauciperforata* comprises of several species that are closely related according to their similar obvious features and linked evolutionarily. Taxonomically, *Rasbora* genus is known as catch all group due to insufficient unique diagnostic characteristics per species and morphological difficulty in characterisation (Muchlisin et al. 2012). This explains why the genus experiences cryptic diversity which this term translates as comprising more than one species that are morphologically indistinguishable and thus being characterised as a single species.

According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, there are currently ten *Rasbora* species being categorised as “Near Threatened” and above, with two being labelled critically endangered, two labelled endangered, four labelled vulnerable and the remaining four labelled near threatened (IUCN 2019). These fishes, discovered to inhabit river streams, waterfalls and peat swamps, are deemed one of the important contributors towards the diversity of the peat swamp ecosystem (Sule et al. 2018). However, they are mostly threatened by residential and commercial development, natural system modifications, agriculture and aquaculture, invasive and other problematic species, genes and diseases, energy production and mining as well as pollution (IUCN 2019). For instance, *R. tawarensis* is one of the IUCN Red List’s critically endangered *Rasbora* species only found in Lake Laut Tawar, Aceh, Indonesia that is severely affected by fishing and pollution (Lumbantobing 2019). Conservation efforts such as gill nets size regulation as well as pesticide and chemical fertilisers ban have been implemented for conservation purposes (Lumbantobing 2019). In the case of the Sarawak state of Malaysia, an investigation on water quality in downstream of Bakun Dam revealed the impact of water quality on fish diversity and the *R. caudimaculata*, *R. borneensis*, *R. dusonensis* and *R. volzi* are some of