

DNA barcoding of an endemic species *Rasbora philippina* (Günther, 1880) found in Lake Wood, Zamboanga del Sur, Philippines

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Abstract

Rasbora species are a diverse group of freshwater fishes in Southeast Asia whose morphological similarity often complicates species delimitation. In the Philippines, six to seven species are recognized, including the endemic *Rasbora philippina*, which contributes to freshwater food webs and supports subsistence fisheries in Mindanao. Species identification is quite challenging, as ambiguous forms are variably identified as *R. philippina*, *Rasbora argyrotaenia everetti*, or *Rasbora* sp., reflecting its previous placement within the morphologically variable *R. argyrotaenia* complex. To clarify its taxonomy, we generated the first mitochondrial cytochrome c oxidase subunit I (COI) DNA barcodes for *R. philippina* using specimens from Lake Wood, Mindanao. Phylogenetic analyses revealed that *R. philippina* forms a distinct clade, clearly separated from other *Rasbora* species, including those from the *R. argyrotaenia* complex. These results establish the first genetic reference for *R. philippina*, resolving taxonomic uncertainties and providing a baseline for accurate identification, biodiversity monitoring, and conservation.

Keywords: Cytochrome c oxidase subunit I (COI), Lake Wood, *Rasbora*, Porang, Cypriniformes

Introduction

The Cypriniformes are the most diverse group of freshwater fishes, with the family Cyprinidae sensu lato comprising more than 3,000 species in over 220 genera (Liao et al., 2010). Recent revisions have divided Cyprinidae sensu lato into 12 families, with subfamily Rasborinae now placed under Danionidae (Tan & Armbruster, 2018). Within this group, *Rasbora* is one of the most species-rich

genera, with over 80 recognized species widely distributed from India to the Philippines (Froese & Pauly, 2026; Günther, 1880; Kusuma et al., 2016; Liao et al., 2010). These small-bodied fishes are a prominent component of Asian freshwater ecosystems (Brittan, 1972). They are morphologically distinguished by an elongated, laterally compressed body (5–20 cm SL), a symphyseal knob on the lower jaw, absence of barbels, and a lateral stripe running from the operculum to the caudal-fin base (Kusuma et al., 2016).

Received: Oct 1, 2025 Revised: Feb 12, 2026 Accepted: Apr 24, 2026

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Despite their ecological and taxonomic importance, species-level identification in *Rasbora* is notoriously difficult. Many species are morphologically similar, leading to frequent misidentifications (Aminan et al., 2020). Previous attempts to subdivide the genus into subgenera or species complexes (Brittan, 1972; Kottelat & Vidthayanon, 1993) have not resolved these ambiguities, highlighting the need for molecular tools to complement traditional taxonomy.

In the Philippines, the diversity of the genus *Rasbora* is greater than previously recognized. Early accounts documented only a limited number of species, including *Rasbora argyrotaenia* (Bleeker), *Rasbora taytayensis* (Herre), and *Rasbora philippina* (Günther) (Brittan & Brattstrom, 1952). However, recent syntheses and database updates indicate that at least six to seven *Rasbora* species occur in the country (Froese & Pauly, 2026; Jamandre, 2023). Based on the provisional checklist of Philippine freshwater fishes (Jamandre, 2023), the currently recognized *Rasbora* species in the Philippines include *R. argyrotaenia* (native), *Rasbora borapetensis* (introduced), *Rasbora everetti* (endemic to Palawan), *R. lateristriata* (native to southwestern Mindanao), *R. philippina* (endemic to Mindanao and Palawan), and *Rasbora taytayensis* (endemic to northern Palawan). FishBase additionally lists *Rasbora semilineata* as occurring in Philippine waters (Froese & Pauly, 2026). These species vary in distribution, conservation status, and morphological traits, underscoring the need for careful taxonomic assessment when identifying Philippine *Rasbora*. Within Mindanao, historical records recognize *R. philippina* and *Rasbora punctulata* (Seale & Bean, 1907). The latter was synonymized with *R. philippina* by Brittan & Brattstrom (1952), who placed the species within the *R. argyrotaenia* complex and suggested divergence during the Pleistocene or late Tertiary.

Recent investigations have highlighted persistent taxonomic uncertainty surrounding *Rasbora* populations in Mindanao. In Lake Wood, an ancient lake of ecological and cultural significance to the Subanen people (Baludo et al., 2021), a local fish known as “porang” has been variously identified as *R. philippina* (Genovia et al., 2023) or *Rasbora argyrotaenia everetti* (Ganzon & Demayo, 2022), while others have conservatively referred to it as *Rasbora* sp. (Gonzaga-Torino et al., 2025). Additional reports record *R. philippina* in Lake Mainit (Mercene, 1997), the Pasonanca River in Zamboanga (Kottelat & Vidthayanon, 1993), Titunod River in Lanao del Norte, Cagayan River in Misamis Oriental, and streams in Tawi-Tawi Island (Herre, 1924, 1953). These inconsistencies highlight the uncertainty surrounding the true

identity, distribution, and taxonomic status of *R. philippina*.

DNA barcoding using the mitochondrial cytochrome c oxidase subunit I (COI) gene has proven to be an effective method for resolving taxonomic uncertainty in freshwater fishes (Hebert et al., 2003, 2004; Ward et al., 2005) and has already been applied successfully to *Rasbora* species (Retnoaji et al., 2023) including those that are native in Malaysia (Aminan et al., 2020), Indonesia (Nazran et al., 2025), Sri Lanka (Sudasinghe et al., 2020), and India (Suryawanshi et al., 2024). However, no DNA barcode data currently exist for Philippine representatives of the genus.

Thus, the present study aims to clarify the taxonomic identity of *R. philippina* from Lake Wood, Mindanao, by integrating morphometric analysis with mitochondrial COI DNA barcoding. Specifically, this study seeks to (1) confirm species identity through comparison with established diagnostic morphological characters, (2) generate the first COI reference sequences for *R. philippina* and assess its genetic distinctiveness relative to congeners, and (3) provide baseline molecular data that will support future biodiversity assessments and conservation efforts involving Philippine freshwater fishes.

Materials and Methods

Specimen collection and processing

The putative *R. philippina* specimens analyzed in this study were collected from local fishermen in Lake Wood, Zamboanga del Sur, Mindanao, Philippines (Fig. 1). All specimens used for morphological and molecular analyses originated from this

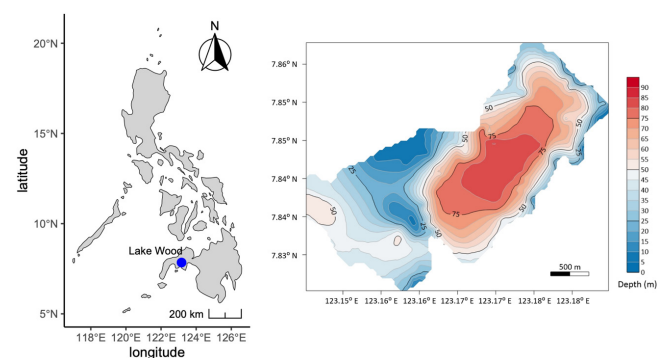


Fig. 1. Map of the sampling site. The left shows a map of the Philippines, highlighting the lakes' location in Zamboanga del Sur, Mindanao Island, and the right panel shows the bathymetric profile of Lake Wood. Map generated in R 4.3.1 (R Core Team, 2023).

single locality. Lake Wood, locally known as Danao, is situated at 320 m above sea level and covers an area of 7.38 km². The lake has a maximum depth of 85 m and a volume of 356 × 10⁶ m³ (Baludo et al., 2021). It is surrounded by mountainous terrain, with rugged northeast and southwest shores and relatively gentle plains along the western and eastern lakeshores.

The lake is fed by rainfall, small rivers, and groundwater, and drains into Dumanquilas Bay via the Biswangan River. The surrounding area is predominantly cultivated land, and the lake is currently classified as eutrophic (Baludo et al., 2021). Specimen identification followed the taxonomic keys of Brittan & Brattstrom (1952). Key diagnostic characters include a slender body with a blunt snout, a darkly pigmented dorsal surface gradually fading to yellowish on the ventral side, a body depth approximately 3.1–3.8 times the standard length, and the presence of 12 predorsal scales.

A tissue sample was excised from the epaxial muscle located posterior to the operculum on the right side of each specimen. Each muscle tissue was preserved in absolute ethanol and stored at –20°C prior to DNA extraction. Genomic DNA was extracted from 25 milligrams of the ethanol-preserved muscle tissue using the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA). On the other hand, to generate DNA barcodes for each specimen, the extracted crude DNA was amplified for the COI gene of the mitochondrial DNA (mtDNA) using the polymerase chain reaction (PCR) technique. The PCR amplification was carried out in a final reaction volume of 25 µL, consisting of 10.25 µL of ultra-pure water, 10.00 µL of 5X reaction buffer containing 5 mM of deoxynucleotide triphosphates (dNTPs), 0.50 µL magnesium chloride (MgCl₂), 1.00 µL each of forward and reverse primers at a concentration of 10 µM, 0.25 µL of Taq DNA polymerase, and 2.00 µL of purified genomic DNA template. The forward primer used was F1-5'TCAACCAACCA CAAAGACATTGGCAC-3', while the reverse primer used was R1-5'TAGACTTCTGGGTGGCCAAAGAATCA-3' (Aminan et al., 2020; Ward et al., 2005). The PCR condition was set at 95°C for 2.00 minutes, followed by 35 cycles of 94°C for 0.50 minutes, 59.7°C for 0.50 minutes, and 72°C for 0.50 minutes. Finally, samples were kept at 72°C for 1.00 minute and then held at 4°C (Aminan et al., 2020).

Visualization of the amplified PCR products was performed through electrophoresis on a 1% agarose gel stained with ethidium bromide. The DNA bands with an expected length of 650 bp for COI (Hubert et al., 2008) were excised and subjected to gel extraction using NucleoSpin Gel and PCR Clean-up. Finally,

the five (5) purified PCR products were sent to Macrogen, Inc. (Seoul, Korea) for bidirectional DNA sequencing using Applied Biosystems™ 3730xl DNA analyzer.

Data analyses

The COI sequences generated were processed using the Staden Package (Staden, 1996), wherein the forward and reverse reads were assembled using Pregap4 version 1.6 (The GAP Group, 2026). Subsequently, Gap4 version 4.11.2 (The GAP Group, 2026) was employed to generate the consensus sequences. The consensus sequence of each putative *R. philippina* specimen was then subjected to BLAST analysis (Altschul et al., 1997) to facilitate preliminary identification by comparing the sequences against publicly available sequences in the GenBank database.

A total of 123 COI sequences were analyzed, including five (5) sequences generated in this study and 118 additional sequences downloaded from the GenBank database (Appendix Table A1). The latter included COI sequences from other *Rasbora* species and outgroup taxa, with five sequences each from *Danio rerio*, *Trigonostigma somphongsi*, *Trigonostigma heteromorpha*, *Trigonostigma espei*, and *Trigonopoma gracile*. These 118 sequences were selected as the top 100 closest matches to the newly generated data, along with *Rasbora* species currently recorded in the Philippines. The outgroup taxa were selected based on their close phylogenetic affiliation (Tan & Armbruster, 2018) and their inclusion among the top 100 closest sequence matches to the newly generated data. The alignment of all the sequences was performed using MEGA version 7 (Kumar et al., 2016). A maximum-likelihood (ML) tree was constructed using the GTR + G + I model with 1,000 bootstrap replicates. The appropriate base substitution model was selected based on model testing in MEGA using the corrected Akaike Information Criterion (AIC). Lastly, the corrected genetic distances were calculated in PAUP*4.0 (Swofford & Bell, 2017) under the General Time Reversible model with gamma-distributed rate variation and a proportion of invariant sites (GTR + G + I).

Graphical representations of pairwise genetic distances among *R. philippina* and related taxa were generated in R 4.3.1 (R Core Team, 2023). A boxplot and a histogram were produced using the ggplot2 package (Wickham, 2016).

Results

The putative *R. philippina* specimens have an average total length of 8.36 cm, an average standard length of 6.52 cm, and

an average body depth of 1.72 cm. Likewise, the average body weight was recorded as 2.94 g, and each specimen exhibited 12 predorsal scales (Appendix Table A2). These are consistent with the key morphometric characteristics established by Brittan & Brattstrom (1952). Specifically, the body depth was approximately one-third (3.80 times) of the standard length, and each specimen exhibited 12 predorsal scales. Additionally, Lake Wood falls within the reported geographic range of *R. philippina*. A comparison of these morphological traits with other *Rasbora* species found in the Philippines (Appendix Table A3) further supports this identification. Additionally, the sampling site at Lake Wood falls within the known geographic range of *R. philippina*, further corroborating species identity.

A 643 bp fragment of the COI gene of the mtDNA was successfully amplified and sequenced from each of the five specimens. The COI sequences showed no deletions or insertions in the aligned sequences and no stop codons in the translated sequences. These sequences represent the first molecular data for *R. philippina* and will be deposited in GenBank as a species reference.

BLASTn results showed that each of the five specimens matched *Rasbora* sp. with sequence identity ranging from 92.32% to 92.99% (Appendix Table A4), whereas the closest match at the species level was *R. rheophila*, ranging from 92.06% to 92.72% (Appendix Table A5).

A total of 123 COI sequences, five (5) from this study and 118 from GenBank, representing 25 species, were aligned and analyzed. The calculated average genetic distance within *R. philippina* was 0.36%. In contrast, the average genetic distance between *Rasbora* species was 11.71%, while the mean genetic distance among genera within Rasborinae was slightly higher at 13.01%. The greatest divergence was observed between subfamilies, with *Danio rerio* (from subfamily Danioninae) and members of subfamily Rasborinae showing an average genetic distance of 21.04% (Table 1). Similarly, the distribution of the genetic distances is illustrated in a boxplot (Fig. 2), which shows an increase in divergence across taxonomic levels. Distances within *R. philippina* were clustered near zero, reflecting less intraspecific variation. Interspecific comparisons within the

genus *Rasbora* exhibited a broader spread with a median of 0.12, while comparisons between genera within the subfamily Rasborinae showed slightly higher divergence centered around 0.14. The highest divergences were observed between the two subfamilies, Rasborinae and Danioninae, where distances averaged above 0.20 and formed a distinct, non-overlapping cluster. In congruence with this, the histogram of pairwise genetic distances (Fig. 3) highlights the frequency distribution of genetic divergence across categories. Intraspecific distances were concentrated near zero, while interspecific and intergeneric comparisons peaked around 0.10 to 0.15.

Lastly, Fig. 4 shows the maximum likelihood (ML) tree generated from the members of the subfamilies Rasborinae and Danioninae. A total of 22 clades were observed in the ML tree, which do not strictly correspond to species designation, as some congeners clustered more closely with taxa from other groups rather than forming species-specific clusters. An exception to this pattern is *R. philippina*, whose sequences generated in this study

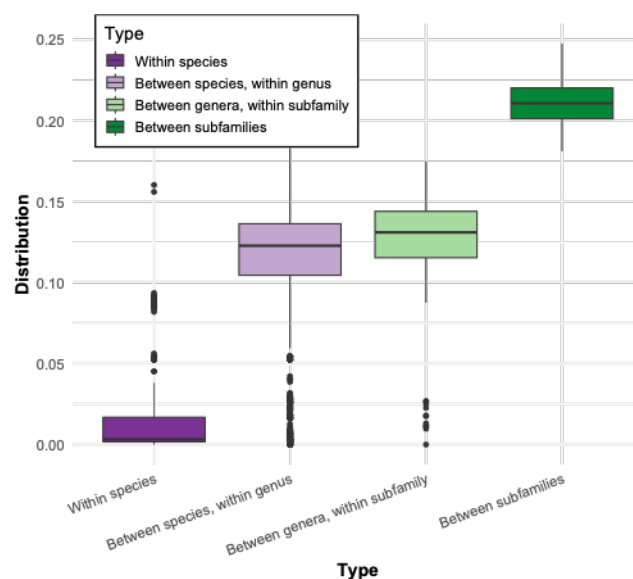


Fig. 2. Boxplot showing the distribution of pairwise genetic distances among *Rasbora philippina* and related taxa.

Table 1. Summary of percent genetic divergence across taxonomic groups

Comparison	No. of comparison	Minimum	Distance mean	Maximum	SE
Within <i>Rasbora philippina</i>	10	0.0000	0.0036	0.0048	0.0005
Between species (within <i>Rasbora</i>)	4330	0.0000	0.1171	0.1932	0.0005
Between genera (within Rasborinae)	2075	0.0000	0.1301	0.1749	0.0004
Between subfamilies (Danioninae vs Rasborinae)	590	0.1810	0.2104	0.2476	0.0006

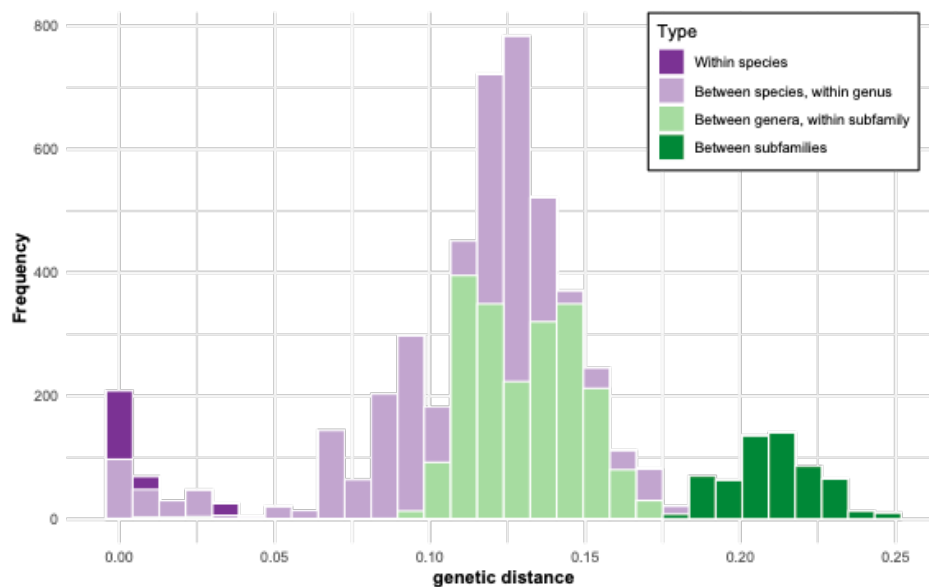


Fig. 3. Histogram showing the distribution of pairwise genetic distances among *Rasbora philippina* and related taxa.

formed an independent clade with 100% bootstrap support, clearly separated from *R. argyrotaenia* and other congeners (Fig. 4).

Discussion

This study successfully generated mitochondrial COI gene barcodes for five *R. philippina* specimens, representing the first published genetic sequences for this species to date. The amplified length of the COI gene sequence exceeded 600 bp without stop codons, indicating that the sequence represents a functional mitochondrial COI gene and the absence of nuclear DNA of mitochondrial origin (NUMTS) (Hubert et al., 2008). These DNA barcodes provide a valuable genetic resource that will support future taxonomic and conservation studies on the Philippine *Rasbora*.

Phylogenetic analysis using the maximum likelihood method revealed that the five *R. philippina* specimens clustered together with strong support (bootstrap = 100%). This clade was clearly separated from *R. argyrotaenia*, a species also reported from Mindanao (Ganzon & Demayo, 2022). This pattern may suggest that *R. philippina* represents a genetically distinct lineage and should not be regarded as part of the *R. argyrotaenia* species complex as previously described by Brittan & Brattstrom (1952). It is important to note, however, that the *R. argyrotaenia* sequences compared in this study were sourced from Indonesia (Hubert et al., 2019). At present, there are no available COI data on *R. argyrotaenia* from the Philippines (including the *R. a.*

everetti, native to the Palawan-Culion-Busuanga).

The calculated average intraspecific genetic distance for *R. philippina* was 0.36%, consistent with reported intraspecific distances in freshwater fishes (~0.60%; Aquilino et al., 2011). In contrast, the average interspecific genetic distances were substantially higher, measuring 11.71% between species within the *Rasbora* genus, 13.01% between genera within the subfamily Rasborinae, 21.04% between subfamilies Danioninae and Rasborinae. These values are approximately 32-, 36-, and 58-fold greater, respectively, than the intraspecific distance of *R. philippina*. The boxplot (Fig. 2) and histogram (Fig. 3) provide a visual summary of these patterns. On the other hand, the broader spread of interspecific comparisons within *Rasbora* and even among members of the subfamily Rasborinae is consistent with the ML tree (Fig. 4), which shows that not all *Rasbora* species form well-supported and clearly distinct clades. For instance, *R. myersi* was observed to cluster with the clade of *R. sumatrana*, suggesting that certain congeners remain genetically unresolved at the COI gene. This pattern may be attributed to various factors that could contribute to the observed taxonomic ambiguities within the genus and subfamily. Some of these factors may include biological processes (e.g., recent divergence, limited gene flow, or potential effects of selection acting on mitochondrial genes), as well as methodological limitations, including limited taxon sampling and the possible presence of misidentified reference sequences in public databases. These complexities

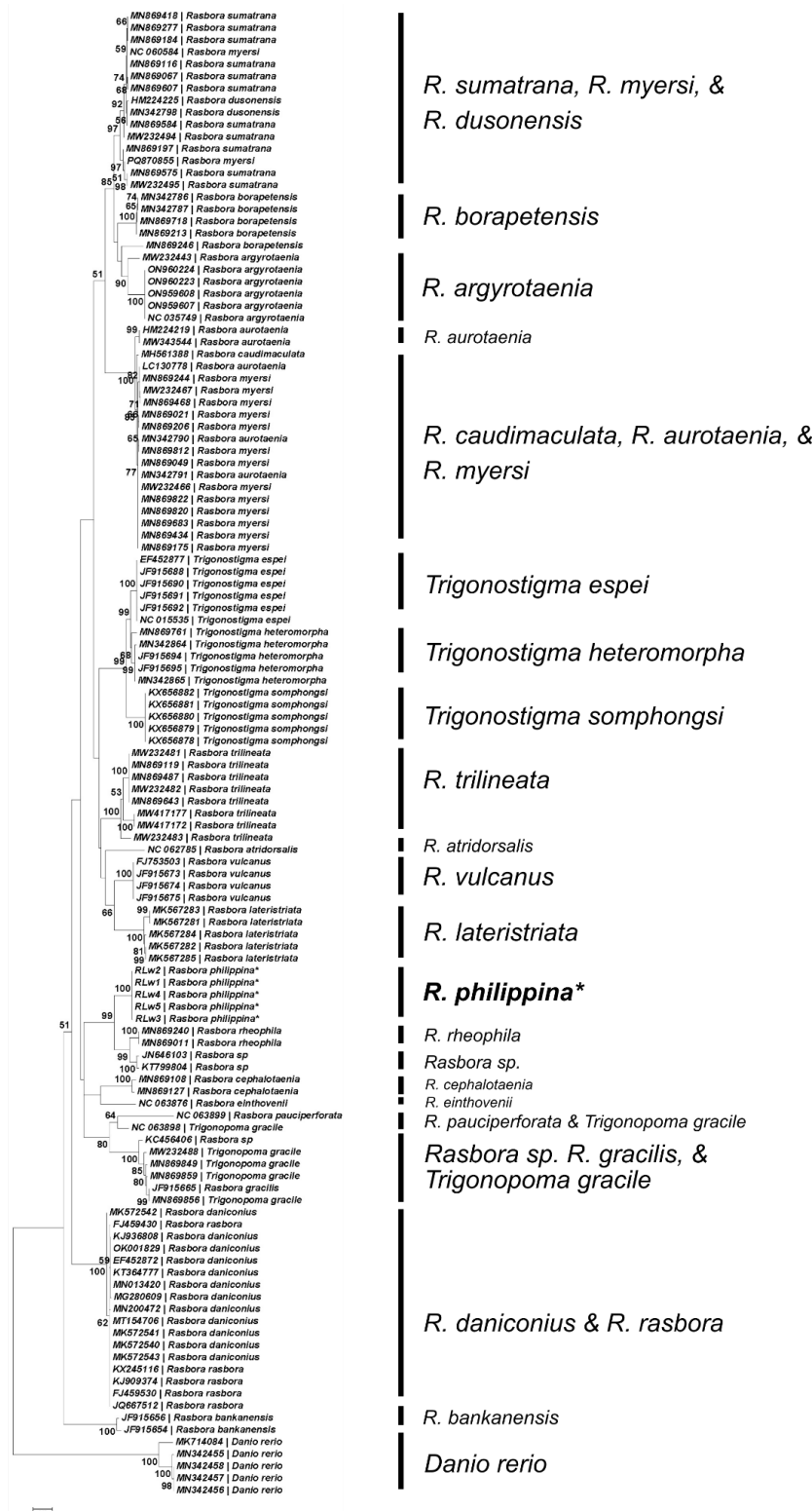


Fig. 4. Maximum-likelihood tree of 123 cytochrome c oxidase subunit I (COI) sequences from 20 *Rasbora* species, with 3 *Trigonostigma*, 1 *Trigonopoma*, and 1 *Danio* species as the outgroup, based on the GTR + G + I model. Sequence IDs with an asterisk (*) indicate specimens used in this study.

highlight the need for further research to resolve the taxonomic ambiguities in this group. Nevertheless, despite these challenges, the *R. philippina* sequences generated in this study consistently formed a distinct and well-supported clade, thereby showing its genetic distinctiveness relative to other congeners.

These results verify that fish locally referred to as “porang” in Lake Wood correspond to *R. philippina*. This resolves earlier ambiguity, as some populations had been variably identified as *R. argyrotaenia everetti* or simply identified as *Rasbora* sp. This study provides the first COI reference sequences for *Rasbora philippina*, which will serve as an important baseline for future DNA barcoding and genetic analyses.

Conclusion

The present study highlights the importance of integrating geographic distribution, morphological, and molecular data in elucidating the taxonomic identity of species, particularly within the *Rasbora* group. The COI sequences generated in this study represent the first DNA barcodes for *R. philippina*, providing a reference library for species identification and confirming *R. philippina* as a distinct species. Taxonomic re-examination of the *Rasbora* sp. in the country is needed, given that the existing descriptions were old and may require the incorporation of new diagnostic traits or methodologies, such as geometric morphometrics and genetic analysis. Implementing such approaches can enhance the accuracy of taxonomic resolution within the genus and can provide an extensive documentation of biodiversity in the country. This is especially important for *R. philippina* given the ecological and cultural importance of this endemic species.

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Not applicable.

Acknowledgements

We gratefully acknowledge the assistance of Marcelo Baludo for coordinating with the fishermen to obtain the samples. We also extend our thanks to the Municipality of Lakewood and the Department of Agriculture – Bureau of Fisheries and Aquatic Resources for granting permission to conduct this research.

Availability of data and materials

The DNA sequences were submitted to GenBank and were assigned Accession numbers PZ954598–PZ954602.

Ethics approval and consent to participate

An ethics permit (IRB/IACUC) is not applicable since the fish were obtained from local fishermen and were not subjected to laboratory trials.

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References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 1997;25:3389-402.
- Aminan AW, Lim LWK, Hui CH, Sulaiman B. Morphometric analysis and genetic relationship of *Rasbora* spp. in Sarawak, Malaysia. *Trop Life Sci Res.* 2020;31:33-49.
- Aquillino SVL, Tango JM, Fontanilla IKC, Pagulayan RC, Basiao ZU, et al. DNA barcoding of the ichthyofauna of Taal Lake, Philippines. *Mol Ecol Resour.* 2011;11:612–619.
- Baludo MY, Papa RDS, Magbanua FS. Limnology of Lake Wood: an ancestral lake of the Subanen Tribe. *Philipp J Sci.* 2021;150:1231-43.
- Brittan MR. The cyprinid fish genus *Rasbora* in Malaya. *Bull Raffles Mus.* 1954;25:129-56.
- Brittan MR. *Rasbora*: a revision of the Indo-Malayan freshwater fish genus *Rasbora*, with natural color photographs. Neptune City, NJ: T.F.H. Publications; 1972.
- Čandek K, Kuntner M. DNA barcoding gap: reliable species identification over morphological and geographical scales. *Mol Ecol Resour.* 2015;15:268-77.
- Froese R, Pauly D. FishBase [Internet]. World Wide Web electronic publication; 2026. <https://www.fishbase.org>
- Ganzon MAM, Demayo CG. Fish diversity in selected small lakes in Mindanao, Philippines. *Aquacult Aquarium Conserv Legis.* 2022;15:796-810.
- Genovia JA, Barquilla MB, Baludo MY. Reproductive biology of *Rasbora philippina* at Lake Wood for basis management. *J*

- Environ Sci Sustain Dev. 2023;6:149-66.
- Gonzaga-Torino FM, Gonzaga Jr. AM, Gonzaga FM, Tahiluddin AB, Pedroso FL. Gut content analysis of freshwater fishes in Lake Wood, Zamboanga del Sur, Philippines. *Philipp J Fish*. 2025;32:245.
- Günther A. Report on the shore fishes procured during the voyage of H.M.S. Challenger in the years 1873–1876. In: Thomson CW editor. Report on the scientific results of the voyage of H.M.S. Challenger during the years 1873–76: zoology vol 1. Edinburgh: Neill and Company; 1880.
- Hebert PDN, Ratnasingham S, de Waard JR. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc R Soc B*. 2003;270:S96-9.
- Hebert PDN, Stoeckle MY, Zemplak TS, Francis CM. Identification of birds through DNA barcodes. *PLOS Biol*. 2004;2:e312.
- Herre AW. Check list of Philippine fishes. Washington: U.S. Government Printing Office; 1953. Research Report No. 20.
- Herre AWCT. Distribution of the true freshwater fishes in the Philippines. I. The Philippine Cyprinidae. *Philipp J Sci*. 1924;24:249-307.
- Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burrige M, et al. Identifying Canadian freshwater fishes through DNA barcodes. *PLOS ONE*. 2008;3:e2490.
- Hubert N, Lumbantobing D, Sholihah A, Dahrudin H, Delrieu-Trottin E, Busson F, et al., *Rasbora argyrotaenia* voucher BIF3636 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial [Internet]. National Center for Biotechnology Information. 2019 [cited 2026 Jan 15]. <https://www.ncbi.nlm.nih.gov/nuccore/MK567225.1>
- Jamandre BW. Freshwater fishes of the Philippines: a provisional checklist. *Zootaxa*. 2023;5301:151-81.
- Kottelat M, Vidthayanon C. *Boraras micros*, a new genus and species of minute freshwater fish from Thailand (Teleostei: Cyprinidae). *Ichthyol Explor Freshw*. 1993;4:161-76.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33:1870-4.
- Kusuma WE, Ratmuangkhwang S, Kumazawa Y. Molecular phylogeny and historical biogeography of the Indonesian freshwater fish *Rasbora lateristriata* species complex (Actinopterygii: Cyprinidae): cryptic species and west-to-east divergences. *Mol Phylogenet Evol*. 2016;105:212-23.
- Liao TY, Kullander SO, Fang F. Phylogenetic analysis of the genus *Rasbora* (Teleostei: Cyprinidae). *Zool Scr*. 2010;39:155-76.
- Mercene EC. Freshwater fishes of the Philippines. Los Baños: Philippine Council for Aquatic and Marine Research and Development (PCAMRD); 1997.
- Nazran, Syofriani, Ulfauza, Valen FS, Oktaviandi D, Prayoga A, et al. DNA barcoding of *Rasbora* sp. from Bangka Island: a genetic identification basis and its potential application in aquaculture. *Egypt J Aquat Biol Fish*. 2025;29:213-26.
- Retnoaji B, Muslimin B, Wibowo A, Trismawanti I. Morphometric and genetic diversity of *Rasbora* several species from farmed and wild stocks. *Fish Aquat Sci*. 2023;26:569-81.
- Seale A, Bean BA. On a collection of fishes from the Philippine Islands, made by Maj. Edgar A. Mearns, surgeon, U.S. Army, with descriptions of seven new species. *Proc U S Natl Mus*. 1907;33:229-48.
- Staden R. The staden sequence analysis package. *Mol Biotechnol*. 1996;5:233-41.
- Sudasinghe H, Pethiyagoda R, Ranasinghe RHT, Raghavan R, Dahanukar N, Meegaskumbura M. A molecular phylogeny of the freshwater-fish genus *Rasbora* (Teleostei: Cyprinidae) in Sri Lanka reveals a remarkable diversification—and a cryptic species. *J Zool Syst Evol Res*. 2020;58:1076-110.
- Suryawanshi R, Khedkar GD, Kalyankar A, Khangale D, Shitale K. DNA barcoding for the assessment of the taxonomy of fish from Chilika Lagoon, India. *Genet Aquat Org*. 2024;8:GA812.
- Swofford DL. PAUP: phylogenetic analysis using parsimony (and other methods). Version 4.0a169 [Internet]. Sinauer Associates. 2017 [cited 2026 Jan 15]. <https://paup.phylosolutions.com/>
- Tan M, Armbruster JW. Phylogenetic classification of extant genera of fishes of the order *Cypriniformes* (Teleostei: Ostariophysi). *Zootaxa*. 2018;4476:6-39.
- The GAP Group. GAP: a system for computational discrete algebra. Version 4.16.0 [Internet]. The GAP Group. 2026. <https://www.gap-system.org>
- Ward RD, Zemplak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australia's fish species. *Philos Trans R Soc B*. 2005;360:1847-57.
- Wickham H. ggplot2. Cham: Springer International Publishing; 2016.

Appendix

Appendix Table A1. List of sequences downloaded from GenBank

Species	Number of sequences	GenBank accession number
<i>Rasbora argyrotaenia</i>	6	ON960223, ON960224, ON959607, ON959608 NC035749 MW232443
<i>Rasbora atridorsalis</i>	1	NC062785
<i>Rasbora aurotaenia</i>	5	MN342790, MN342791 LC130778 HM224219 MW343544
<i>Rasbora bankanensis</i>	2	JF915654, JF915656
<i>Rasbora borapetensis</i>	5	MN869213, MN869246, MN869718 MN342786, MN342787
<i>Rasbora caudimaculata</i>	1	MH561388
<i>Rasbora cephalotaenia</i>	2	MN869108, MN869127
<i>Rasbora daniconius</i>	12	MK572542, MK572541, MK572540, MK572543 MN200472, MN013420, KT364777 MT154706 EF452872 KJ936808, MK572543 MG280609 OK001829
<i>Rasbora dusonensis</i>	2	MN342798 HM224225
<i>Rasbora einthovenii</i>	1	NC063876
<i>Rasbora gracilis</i>	1	JF915665
<i>Rasbora lateristriata</i>	5	MK567281, MK567282, MK567283, MK567284, MK567285
<i>Rasbora myersi</i>	15	PQ870855 NC060584 MW232467, MW232466, MN869822, MN869820, MN869812, MN869468, MN869683, MN869244, MN869434, MN869175, MN869021, MN869049, MN869206
<i>Rasbora pauciperforata</i>	1	NC063899
<i>Rasbora rasbora</i>	5	KX245116 KJ909374 FJ459430, FJ459530 JQ667512
<i>Rasbora rheophila</i>	2	MN869240, MN869011
<i>Rasbora</i> sp.	3	JN646103 KT799804 KC456406
<i>Rasbora sumatrana</i>	11	MN869067, MN869607, MN869575, MW232494, MN869184, MW232495, N869116, MN869584, MN869418, MN869197, MN869277
<i>Rasbora trilineata</i>	8	MW417177, MW417172 MN869643, MW232483, MW232482, MN869487, MW232481, MN869119

Appendix Table A1. Continued

Species	Number of sequences	GenBank accession number
<i>Rasbora vulcanus</i>	4	JF915673, JF915674, JF915675 FJ753503
<i>Trigonopoma gracile</i>	5	MW232488, MN869859, MN869856, MN869849, NC063898
<i>Trigonostigma espei</i>	6	NC015535 JF915688, JF915690, JF915691, JF915692 EF452877
<i>Trigonostigma heteromorpha</i>	5	JF915694, JF915695 MN869761 MN342864, MN342865
<i>Trigonostigma somphongsi</i>	5	KX656878, KX656879, KX656880, KX656881, KX656882
<i>Danio rerio</i>	5	MN342455, MN342456, MN342457, MN342458 MK714084

Appendix Table A2. Raw data of the morphometric values of putative *Rasbora philippina*

Specimen code	Body weight (g)	Total length (cm)	Standard length (cm)	Body depth (cm)	Number of predorsal scale
RLw1	2.56	8.30	6.40	1.80	12
RLw2	2.98	8.50	6.60	1.70	12
RLw3	3.53	8.20	6.50	1.70	12
RLw4	2.91	8.50	6.60	1.70	12
RLw5	2.71	8.30	6.50	1.70	12
Average	2.94	8.36	6.52	1.72	12

Appendix Table A3. Description and morphological characters that significantly differentiate *Rasbora* species

	Geographic location	Number of predorsal scale	Body depth	Snout shape
<i>Rasbora philippina</i>	Mindanao	12	3.4 times standard length (3.1–3.8)	Blunt
<i>Rasbora argyrotaenia</i>	Palawan	14–15	4.0 times standard length (3.6–4.5)	Pointed
<i>Rasbora borapetensis</i>	River basins across the Philippines	12	3.5 times standard length (3.4–3.7)	Pointed
<i>Rasbora everetti</i>	Palawan	12–14	4.0 to 4.7 times standard length	Pointed
<i>Rasbora lateristriata</i>	Tawi-Tawi	12	4.4. times standard length (4.3–4.6)	Pointed
<i>Rasbora taytayensis</i>	Northern Palawan	11	3.3 to 3.6 standard length	Blunt

Appendix Table A4. BLASTn hits at the genus level for the five putative COI sequences of *Rasbora philippina*

Specimen code	Match species	Query cover (%)	Percent identity (%)	Accession number	Source
RLw1	<i>Rasbora</i> sp.	95	92.78	KT799804	Malaysia
RLw2	<i>Rasbora</i> sp.	85	92.99	KT799804	Malaysia
RLw3	<i>Rasbora</i> sp.	90	92.32	KT799804	Malaysia
RLw4	<i>Rasbora</i> sp.	92	92.67	KT799804	Malaysia
RLw5	<i>Rasbora</i> sp.	82	92.95	KT799804	Malaysia

BLASTn, basic local alignment search tool for nucleotides; COI, cytochrome c oxidase subunit I.

Appendix Table A5. BLASTn hits at the species level for the five putative COI sequences of *Rasbora philippina*

Specimen code	Match species	Query cover (%)	Percent identity (%)	GenBank accession number	Source
RLw1	<i>Rasbora rheopila</i>	94	92.50	MN869240	Indonesia
RLw2	<i>Rasbora rheopila</i>	84	92.72	MN869011	Indonesia
RLw3	<i>Rasbora rheopila</i>	89	92.06	MN869011	Indonesia
RLw4	<i>Rasbora rheopila</i>	91	92.40	MN869011	Indonesia
RLw5	<i>Rasbora rheopila</i>	81	92.68	MN869011	Indonesia

BLASTn, basic local alignment search tool for nucleotides; COI, cytochrome c oxidase subunit I.