

First report of morphological and molecular identification of greater scissortail *Rasbora caudimaculata* from Rokan Hulu District, Riau Province, Indonesia

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Abstract. The greater scissortail (*Rasbora caudimaculata*) is an appreciated food fish, widely distributed, and being subject of economical fishery in Indonesia. *R. caudimaculata* is commonly found in Indonesian rivers plenteously. In the villages of Riau Province many people use this species as daily food. *R. caudimaculata* in Indonesia is distributed in Java, Sumatra, Borneo, Natuna Besar, Lombok, Bali, Sumbawa and Nias. In Rokan Hulu district *R. caudimaculata* can be found in rivers around oil palm plantations and secondary forests. In addition to the morphological characters in fish there are genetic characters, which function to provide genetic information. Information on genetic diversity can be obtained by analyzing the gene encoding proteins from mitochondrial DNA. Among the protein coding genes that are often used to study genetic diversity are cytochrome genes b. Further analysis was made to access the utility of partial cytochrome c oxidase subunit 1 (CO1) gene to delineate the mullet specimens. PCR amplification was conducted using the universal primers and approximately 660-bp of partial CO1 gene was sequenced for all specimens. Molecular identification was performed by deploying the specimens' partial CO1 gene in the GenBank using the basic local alignment search tool (BLAST). The *R. caudimaculata* of Rokan Hulu was no confirmed as 100% similar with other *Rasbora* species. The morphological characters of the *R. caudimaculata* specimens in this study are congruent with those that have been described by previous studies. The identification of this species is important as it could be added into the vast and intricate taxonomic status of *R. caudimaculata*. Moreover, it serves as a basis of gen stock identification for fishery management of Indonesian stocks.

Key Words: *Rasbora* sp., genetic identification, cytochrome c oxidase subunit 1 (CO1), morphological measurement, specimen, blast analysis.

Introduction. Fish as part of an ecosystem, play a role in the nutrient cycling sustainability, in food chain and ecosystem resilience. Mobility of fish within nested set of temporal and spatial cycles of ecological systems enhances the functional importance of fish as ecological memory in the form of energy, nutrients and genetic reserves. Their population is used as environmental management tools in enhancing rice production, mitigating diseases in tropical zones, mitigating algal blooms, indicating ecosystem stress and mitigating growth of lake vegetation (Holmlund & Hammer 1999). Fish is usually provides high-value protein, carbohydrates, saturated fats, vitamins, minerals and polyunsaturated omega-3 fatty acids. Currently they represent about 16.6% of animal protein and 6.5% of all proteins for human consumption (The World Bank 2013).

The greater scissortail (*Rasbora caudimaculata*) is an appreciated food fish, widely distributed, and being subject of economical fishery in Indonesia. *R. caudimaculata* is commonly found in Indonesian rivers plenteously. These fish can easily be caught in rivers by using nets. Buy it on traditional markets can be purchased at cheap prices.

Because of these conditions these fish are a source of protein that is widely consumed by people in Indonesia. In Indonesia 44 species of *Rasbora* genus are found, with distribution in Java, Sumatra, Borneo, Natuna Besar, Lombok, Bali, Sumbawa and Nias. In other countries they can be found in Malaysia, Thailand, Cambodia, Singapore and Philippines (Kottelat et al 1993). *R. caudimaculata* is included in Phylum Chordata, Class Osteichthyes, Order Cypriniformes, Family Cyprinidae and Genus *Rasbora*. The morphological features of *R. caudimaculata* are easily recognized, its body shape is elongated and slightly flattened on its stomach. Mouth looking up with a not too long gap. At the end of the caudal fin is blackish. The body on the back is slightly shiny black, blackish scaly covering the upper half of the body. Half of that the lower part of the body is rather bright and in the water looks shiny silvery. *Rasbora* have anal fins five branched fingers, sometimes having a maxilla that extends well below or beyond the eye. The body size of *Rasbora* is small and is usually found in high quantities. These fish lives in freshwater on fast-flowing clear rivers, with sandy bottom and small rocks, rarely found in muddy waters as in parts downstream near estuaries. *Rasbora* lives on the surface of the river water, and many are found at depths less than one meter, but have also been found at the depth of six meters of the rivers.

In addition to the morphological characters in fish there are genetic characters that function to provide genetic information. Information on genetic diversity can be obtained by analyzing the gene encoding proteins from mitochondrial DNA. Among the protein coding genes that are often used to study genetic diversity are cytochrome genes b. The cytochrome b gene can be used as a genetic marker for studying species diversity and species relationships between the groups (intraspecies) and other groups (interspecies), because the code is positioned, has a more conserved region and more diverse regions (Farias et al 2001). Genetic diversity in an organism is controlled by genes contained in the chromosomes. The chromosome is obtained from both parents through inheritance. However, the expression of an organism's genes is strongly influenced by the conditions of the environment in which it lives. Increased gene diversity can occur through hybridization (cross-breeding) between organisms of one species with different characteristics, or through the domestication process.

The encoding genes in the DNA genome include genes cytochrome oxidase subunit I (CO1). CO1 has been chosen as one of the genes whose sequences are used in barcoding. This gene has properties that meet the requirements for use in determining species identity in almost all vertebrates. The CO1 gene has many advantages to studying genetic characteristics because it has very little deletion and insertion in its sequence, and many parts are conserved (sustainable) that can be used as DNA barcoding in most species. CO1 can also be used to reconstruct phylogeny at species-level evolutionary. In addition, the amino acid sequence of proteins encoded by the CO1 rarely undergo substitution so that the CO1 gene is stable and can be used as a marker of phylogeny analysis (Hebert et al 2003).

The existence of fish is strongly influenced by the condition of the waters, to be able to live and breed fish must adapt to the environment. *Rasbora* is an active fish in the aquatic environment with temperatures around 24-25°C. *Rasbora* feeds, especially small crustaceans and other larvae. Fertilized *rasbora* eggs will stick to aquatic plants and hatch after 24-30 hours. After hatching the fish can swim freely after 3-5 days. *Rasbora* lives in the river in Rokan Hulu district, Riau province with oil palm plantations dominated and small part of secondary forests around the river. This river is used by the local community as fishing ground and for households blackwater discard. *Rasbora* sp. is primary freshwater fish described from Sumatra Island of Indonesia but its taxonomy and molecular phylogeny have not been fully studied (Purnama & Yolanda 2016; Roza et al 2014; Kottelat et al 1993).

According to IUCN (International Union for Conservation of Nature and Natural Resources) most of this genus was included in the least concern status (IUCN 2018). Research on the study of morphological and genetic characters of *R. caudimaculata* in Rokan Hulu is still lacking. Some previous studies were only about ecological and taxonomic classification, while the morphology and genetic identification has never been done.

The present study was conducted to identify the *R. caudimaculata* using the morphological approach and analysis of the CO1 gene, performed in order to build a genetic database based on morphometric, meristic and phylogenetic status, required in knowing the pattern of distribution and conservation of fisheries in Indonesia to prevent a decline in population from one of biodiversity and maximize economic benefits from one of fisheries resources

Material and Method. The present research was conducted from December 2017 to June 2018 by using fishnet with random sampling methods. Twenty *R. caudimaculata* specimens from several rivers in Rokan Hulu District, Riau were collected and preserved in 96% alcohol. Fish samples that were obtained were identified using relevant identification books (Nelson 2006; Kottelat et al 1993). Identified fish were stored in proportional bottle containing and labeled with classification information, location of discovery and collector. Genetic analysis was conducted in the Genetics Laboratory, Biology Department Faculty of Mathematics and Science Riau University, Indonesia.

Morphological assessment. Morphometric measurements were done with digital vernier caliper with 0.01 mm accuracy. All morphological and meristic characters of fish were measured and counted to complete species identification. For accuracy the characters were measured and counted three times for each specimen. A total of 32 morphometric measurements (Table 1 & Figure 1) were taken for each specimen.

Table 1

Morphometric measurements of *Rasbora caudimaculata*

No	Character	Acronym	Mean±SD (mm)	Range (mm)
1	Total length	TL	106.88±12.5	87.50-132
2	Standard length	SL	81.17±9.34	64.7-99.90
3	Snout length	ML	4.33±0.58	2.60-5.40
4	Eye diameter	ED	3.71±0.65	2.40-4.60
5	Post orbital length	EOD	7.96±1.18	6.2-10.00
6	Head length	HL	17.87±1.87	14.80-22.40
7	Pre-pectoral length	PrPcD	19.49±1.56	17.8-22.20
8	Pre-pelvic length	PrPeD	41.40±2.43	38.00-46.00
9	Pre-dorsal length	PrDD	41.71±13.63	4.00-53.00
10	Pre-anal length	PrAD	62.09±4.66	55.60-72.00
11	First dorsal fin base	DL 1	6.13±2.71	3.80-17.00
12	Anal fin base	AL	6.90±1.40	5.30-10.00
13	Pectoral fin length	PcL	26.77±2.70	23.60-33.00
14	Caudal peduncle length	PeL	13.15±1.95	10.10-17.00
15	Caudal peduncle depth	CPDe	8.97±1.36	7.00-12.00
16	Body depth of origin at first dorsal fin	Bde	19.66±3.00	14.90-25.00
17	First dorsal spine length	DSL 1	16.00±2.35	13.40-20.00
18	Second dorsal spine length	DSL 2	14.29±2.41	9.80-17.00
19	Third dorsal spine length	DSL 3	12.76±2.31	8.00-16.00
20	Fourth dorsal spine length	DSL 4	11.43±1.91	7.30-14.00
21	Fifth dorsal spine length	DSL 5	9.68±1.72	6.20-12.50
22	Sixth dorsal spine length	DSL 6	8.32±1.47	5.10-10.20
23	Seventh dorsal spine length	DSL 7	6.67±1.30	4.3-8.70
24	Eighth dorsal spine length	DSL 8	5.03±1.16	3.00-6.60
25	Pelvic spine length	PeSL	13.29±1.94	10.80-17.40
26	First anal spine length	ASL 1	14.12±1.78	10.20-17.20
27	Pectoral fin insertion-dorsal fin origin	PCDD 1	30.25±2.74	24.68-7.41
28	Pectoral fin insertion-pelvic fin insertion	PcPeD	24.68±7.41	20.00-45.00

No	Character	Acronym	Mean±SD (mm)	Range (mm)
29	Pelvic fin insertion-anal fin origin	PeAD	23.00±6.86	19.00-42.20
30	Anal fin origin-dorsal fin origin	ADD 1	23.34±1.92	20.50-26.20
31	Pelvic fin insertion-dorsal fin origin	PeDD	20.84±3.08	16.00-25.00
32	Pectoral fin insertion-anal fin origin	PcAD	40.42±6.88	22.40-49.00



Figure 1. Morphometric measurement of *Rasbora caudimaculata*. Digits indicate the characters described in Table 1 (original).

Molecular analysis. The total genomic DNA was extracted using the Cell/Tissue DNA Extraction Kit (Geneaid). The polymerase chain reaction (PCR) was conducted to amplify the partial fragment of cytochrome c oxidase subunit 1 (*CO1*) gene. Amplification of the *CO1* gene fragments was carried out using the universal primer: Fish F1 ((5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FishR1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward et al 2005). PCR was carried out in 25 μ L reaction mixture containing 2.5 μ L of 10 \times PCR Buffer, 2 μ L of DNA template, 1 μ L of each primer (25 mM), 2 μ L of dNTPs (2.5 mM) and 0.3 μ L of Taq polymerase. The PCR cycling conditions consisted of an initial 5 minutes of denaturation at 95°C followed by 35 cycles for each of the reactions; 40 seconds at 94°C, 40 seconds at 48°C and 1 min at 72°C. Lastly, an elongation step for 7 minutes at 72°C completed the cycle (Bowen et al 2008). The amplifications were done in a Thermal Cycler (Eppendorf AG, Germany). Prior to sequencing, the PCR products were purified using the DNA Purification Kit according to the manufacturer's instructions. For measurement accuracy, all the PCR products sequenced bidirectionally, were purified using the same primers as those for the PCR. The reaction component that occurred in a single-stranded DNA print was printed by a primer or an oligonucleotide sequence that completes a predetermined DNA mold sequence.

Genetic analysis. The raw sequences of the specimens were edited using eBioX (<http://www.ebioinformatics.org/ebiox/>). The chromatograms were viewed using 4Peaks (Nucleobytes Inc.) and FinchTV (Geospiza Inc.) as references to identify any noises and gaps. Unwanted sequences were then removed. All of the sequences were identified using the nucleotide Basic Local Alignment Search Tool (BLAST) (Altschul et al 1990) at the National Center of Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>). As a general rule, a top match with a sequence similarity of at least 98% was used as a criterion to designate potential species identifications (Barbuto et al 2010). All sequences were then aligned together using CLUSTAL-W program in MEGA software (version 6.06). MEGA version 6.06 was also used to construct phylogenetic trees using Neighbour-Joining (NJ) algorithm, Maximum-Likelihood (ML) algorithm and Maximum-Parsimony (MP) algorithm. Kimura-2 parameter was chosen as best-fit model. The genetic distance was also calculated using MEGA version 6.06. The confidence level of the trees was tested with 1000 replications (Tamura et al 2011).

Results and Discussion. Morphologies of *R. caudimaculata* specimens from Rokan Hulu, Riau were examined to determine its species by referring to Kottelat et al (1993), Lumbantobing (2014) and Purnama & Yolanda (2016). The specimens possessed a phenotype of golden yellow body at the dorsal part, while ventral a blackish silver spine, except at the bottom of the silvery side line, thin black caudal fin lobe. The dorsal and pectoral fins are yellowish, while the pelvic and anal fins are transparent. The type of mouth is terminal, which is the type of mouth where the lower jaw and upper jaw are the same length, the type of scales is cycloid, namely the edges of the scales are smooth, type of caudal fin is forked, which is much shorter rather than the superior and inferior radius of the tail fin. Specimens in the present study exhibited TL 106.89 \pm 12.5, SL 81.17 \pm 9.34, ML 4.33 \pm 0.58, ED 3.71 \pm 0.65, EOD 7.96 \pm 1.18, HL 17.87 \pm 1.87, PrPCD 19.49 \pm 1.56, PrPeD 41.40 \pm 2.43, PrDD 41.71 \pm 13.63, PrAD 62.09 \pm 4.66, DL 16.13 \pm 2.71, AL 6.90 \pm 1.40, Pcl 26.77 \pm 2.70, Pel 13.15 \pm 1.95, CPDe 8.97 \pm 1.36, BDe 19.66 \pm 3.00, DSL1 16.00 \pm 2.35, DSL2 14.29 \pm 2.41, DSL3 12.76 \pm 2.31, DSL4 11.43 \pm 1.91, DSL5 9.68 \pm 1.72, DSL6 8.32 \pm 1.47, DSL7 6.67 \pm 1.30, DSL8 5.03 \pm 1.16, PeSL 13.29 \pm 1.94, ASL1 14.12 \pm 1.78, PCDD1 30.25 \pm 2.74, PcPeD 24.68 \pm 7.41, PeAD 23.00 \pm 6.86, ADD1 23.34 \pm 1.92, PeDD 20.84 \pm 3.08, and PcAD 40.42 \pm 6.88.

A sequence of 660-base-pair of partial *CO1* gene was amplified for all the *R. caudimaculata* specimens through PCR amplification. The *CO1* sequences were deposited in GenBank under accession number MH561388 (Figure 2).

AAAGATATCGGCACCCTTTATCTCGTATTCCGGTGCCTGAGCCGGACCGGATAGTTGGAACCGCCCTTAGTCTTC
 TTATTCGTGCTGAGCTAAGCCAGCCGGGATCACTCCTAGGAGATGACCAAATCTATAATGTAATTGTAACCTGCT
 CATGCTTTTGTAATAATTTTCTTTATAGTAATACCAATACTAATTGGAGGGTTTGGTAACCTGATTAGTCCCACTA
 ATAATTGGGGCACCAGACATGGCATTCCACGAATGAATAACATAAGTTTCTGACTTCTCCCCCATCATTCTT
 CTACTATTAGCCTCTTCTGGTGTGGAAGCCGGGGCCGGGACAGGATGAACAGTATATCCACCCTCGCAGGTA
 ATCTTGCTCACGCAGGGGCATCAGTAGACCTAACAATTTTTTTCACCTCATTAGCAGGTGTATCATCAATTCTAG
 GGGCTATTAACCTTATTACAACAATTATTAATATGAAGCCCCAGCTATTACCAATACCAAACCCCTTTATTG
 TTTGAGCAGTATTAGTCACAGCTGTACTATTGCTCCTATCATTACCAGTTTTAGCTGCCGGAATTACAATGCTCC
 TCACAGACCGAAACCTTAATACCACATTCTTGACCCAGCCGGGGGAGGGGATCCAATTCTATATCAACACTTA
 TTCTGATTCTTCGGT

Figure 2. The *matK* sequence of *Rasbora caudimaculata* from Rokan Hulu, Riau.

The single specimen (J1) was then further analyzed by deploying to BOLDSYSTEM (<http://www.boldsystems.org/>). The results showed that the specimen was 98% identical with *Rasbora aurotaenia*, whereas with species *Rasbora argyrotaenia*, *Rasbora dusonensis*, *Rasbora borapetensis*, *Rasbora cf. borapetensis* has 91% similarity, and with *Trigonostigma heteromorpha* and *Trigonostigma espei* 90% similarity (Figure 3 & Table 2).

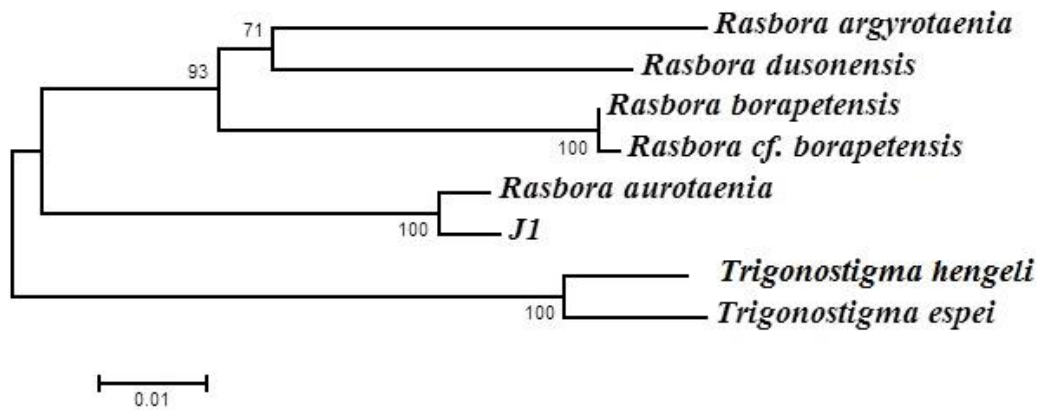


Figure 3. Dendrogram constructed (J1: *Rasbora* sp.) based on the *matK* sequences using Neighbor Joining method with 1000 bootstraps.

Table 2

The alignment analysis using BLAST on the *matK* sequence of *Rasbora caudimaculata* from Rokan Hulu Riau

Description	Max score	Total score	Query cover	E value	Identical	Accession
<i>Rasbora aurotaenia</i>	1142	1142	96%	0.0	98%	LC130778.1
<i>Rasbora argyrotaenia</i>	942	942	100%	0.0	91%	EF452880.1
<i>Rasbora dusonensis</i>	931	931	96%	0.0	91%	HM224225.1
<i>Rasbora borapetensis</i>	919	919	96%	0.0	91%	HM224221.1
<i>Trigonostigma heteromorpha</i>	917	917	99%	0.0	90%	JF915695.1
<i>Trigonostigma espei</i>	915	915	100%	0.0	90%	AP011449.1
<i>Rasbora cf. borapetensis</i>	913	913	96%	0.0	91%	HM224222.1

The combination of morphometric and molecular approaches is an analysis that can identify a species. The fish species found in Rokan Hulu were analyzed as *R. caudimaculata* species, but after phylogenetic analysis the results obtained showed no 100% similarity with other *Rasbora* species found in genbank. Incorporating genetic markers of mitochondrial and nuclear genes is a method that can be done next. To justify that this is a finding of a new species, identification of stock must be carried out

thoroughly by combining several methods from genetic analysis and phenotypic approaches (Coyle 1998).

Conclusions. The *R. caudimaculata* of Rokan Hulu was not confirmed as 100% similar with other Rasbora. The morphological characters of the *R. caudimaculata* specimens in this study are congruent with those that have been described by previous studies. The identification of this species is important as it could be added into the vast and intricate taxonomic status of *R. caudimaculata*. Moreover, it serves as a basis of gen stock identification for fishery management of Indonesian stocks.

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