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Genetic diversity and differentiation of cytochrome oxidase subunit I (COI) gene of *Rasbora lateristriata* bleeker in different habitat

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Abstract. *Rasbora lateristriata* is a freshwater fish that has small size and schooling fish in the shallow river. This fish is called Nyalian by the people in Bali. The population of *Rasbora lateristriata* has decreased at upstream in Sungai river that already lightly polluted. The genetic diversity of *Rasbora lateristriata* based on the Cytochrome Oxidase subunit I gene as a marker will be detected in the upstream, branch river, middle stream and downstream of the Sungai river. The method that used is the polymerase chain reaction (PCR) with two primers are Fish-BCL (forward) and Fish-BCH (reverse) were followed by sequencing using the method of Sanger sequencing to obtain its nucleotide sequences. Genetic diversity was analyzed using Mega 5.2 version. Nucleotide composition of *Rasbora lateristriata* are Thymine 29.8%, Cytosine 25.8 to 26.2%, Adenine 26.0% and Guanine 18.5-18.6% with total nucleotide are 633 to 682. The branch of the river, middle stream and downstream which the genetic diversity of locus COI experienced a change of nucleotide at the sixth base, Thymine (T) is replaced with Cytosine (C). Substitution Thymine to Cytosine is occurring on a polluted part of the river that may be caused by the parameters of the water quality.

1. Introduction

Rasbora is a freshwater fish that are easily found in rivers, lakes, ponds, ditches, and swamps that high oxygen content and a river where the current is not strong [1]. Rasbora fish used as an alternative source of protein because protein is high at 33.4g/100 g. The protein content Rasbora higher than the protein content of carp and milkfish, Rasbora also contains flour of high and contains omega 3 [2], while in other countries in Southeast Asia used as ornamental fish [3]. Kottelat showed that *Rasbora lateristriata* in Indonesia spread in Sumatra, Kalimantan, Java, Bali, and Lombok [4]. Nyalian or waderpari fish is an alternative source of protein which is important for the community around the river as an important food fish in Indonesia with a delicious meat flavor [5]. The delicious taste and high nutritional content make this fish attracted to many people [6]. Nyalian fish is also an economically important fish in the streams so that people make it a prime target of the catch.

Waderpari fish (*Rasbora lateristriata*, Bleeker) called yellow rasbora is a genus of freshwater fish from family *Cyprinidae* are often found living in groups based on small rivers rocky, medium flow water with a temperature range between 22°C - 24°C, pH waters 6.0 -6.5 [7, 8]. Rasbora found in the upstream



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and midstream areas but is extremely rare in the downstream areas and estuaries. These fish usually live at depths of less than 1 meter [9]. Rasbora fish is a fish that lives clustered or schooling fish [10].

The population structure of *Rasbora lateristriata* fish has been decreased especially in the upstream

The population structure of *Rasbora lateristriata* fish has been decreased especially in the upstream of the Sungai River. Sungai River is one of the ten rivers that deteriorated due to contamination by sewage [11]. Pollutant parameters that have exceeded the quality standard that is BOD, COD, total phosphate, total coliform and faecal coliform where conditions are important to note because Sungai River is a collection point for drinking water taps at Tabanan Regency [11]. Activities at Sungai River dominated by agricultural activities, settlements, farms in household and industrial-scale contained in the downstream areas. Waste from these activities led to the deterioration of water quality can be shown as an increase in the content of BOD, COD, total coliform and faecal coliform. Increased content of this occurs in the middle part of the river and decreases in the downstream areas. Sungai River quality status on the upstream same with water quality grade 1, while the middle and lower reaches where relatively lightly polluted water quality parameters exceeded the water quality standard grade 1 in Sungai River is Total Phosphate 0,55mg/l in the middle stream, 0,61mg/l downstream and faecal coliform with an average value of 280/100ml in the middle and downstream of 200/100ml [12].

This research can provide information and an overview of the Sungai River overall that genetic diversity and differentiation population of *Rasbora lateristriata* based gene COI on the upstream, branch, middle stream and downstream, as scientific information in the conservation of fish *Rasbora sp* and obtained marker genes to make improvements genetic quality and can support or strengthen the theory of DNA fragment polymorphism *Rasbora lateristriata*.

2. Methods

Sungai river area will be divided into four namely upstream, branch, middle and downstream. In the upstream point 8° 21'4"S - 115° 10'49"E, on the branch 8° 22'25"S - 115° 11'5"E. Midstream at the point 8° 33'695"S - 115° 09'538"E and downstream at the point of 8° 3'053"S - 115° 06'068" E. Fish samples collected from *Rasbora lateristriata* fishing on the upstream, branch, middle stream and downstream in Sungai River. Where in at each location were collected 15 fishes so that a total of 60 fishes (upstream, branch, midstream and downstream) to be analyzed morphological diversity with measurement morphometry and 20 fishes (total for upstream, branch, middle, and lower) *Rasbora lateristriata* to be analyzed genetically. *Rasbora lateristriata* COI gene detection by PCR using Primer Fish-BCL5'TCA-ACY-AAT-CAY-AAA-GAT-ATY-GGC-AC-3' (forward) and Fish-BCH5'-ACT-TCY-GGG-TGR-CCR-AAR-AAT-CA-3' (Reverse). Sequencing COI genes are amplified PCR to analyze the DNA fragment *Rasbora lateristriata* fish in the upstream, midstream and downstream Sungai River with the Sanger method.

2.1. Sample preparation

Equipment for sample preparation are gloves, cotton bud, labels, forms the collection of samples, cameras, icebox, micropipette, centrifuge tubes, a buffer solution, the autoclave to avoid contaminants. Organ muscle tissue of the fish *Rasbora lateristriata* collected from the Sungai River, 5 fishes each location and a total of 20 fishes with a distance of sampling is 15 km and the samples were stored in alcohol 95% were taken to laboratories for analysis of gene COI by PCR and continued sequencing.

2.2. Procedure research

2.2.1. *Extraction.* Sample extraction carried out by using a solution of 10% Chelex referring to Walsh et al., 1991. Tissue samples were taken of + 2 mm by using a pair of tweezers and put into a tube containing a solution of chiles. Before and after the tissue is taken, tweezers dipped in 95% ethanol and burned with a bunsen flame. Chelex solution that includes network already included samples, vortex, and in-centrifuge for + 20 seconds, and then heated in a heating block at a temperature of + 95°C for 45 minutes. Once heated, back in the vortex tube and in - centrifuge for ± 20 seconds. Extraction solution ready to be used for amplification.

R.lateristriata COI gene polymorphic site (figure 3). The branch Sungai river there is 5 polymorphic site (100%) that is on-site to 23 which is supposed to base Thymine but replaceable with a base Cytosin in all samples, in Middle Sungai River there is 3 polymorphic site (60%) at the site to 23 where Thymine is replaced by Cytosin and downstream there are three polymorphic sites (60%) at the site to 23 where Thymine is replaced by Cytosine. Thymine base changes into Cytosin show branch river, midstream and downstream substitution mutations occur in genes COI *Rasbora lateristriata* on a branch, midstream and downstream, while upstream has not undergone mutation in the gene COI. Comparison with *R.baliensis* shows the difference in a polymorphic site that is on the 398 site adenine become Thymine, 443 sites Adenine and Guanine be on site 620 Cytosin become Thymine. These results indicate the presence of the mutation site of 101 sites. In the upstream, no changes in nucleotide sequences due to the occurrence of inbreeding as indicated by the low population structure on the upstream [15]. At the

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