Organized by:

RUMAH PUBLIKASI INDONESIA

CERTIFICATE

No. 3425/UN40.R4/DT/2020

This certificate is awarded to

S A M P Suryani, I G P Wirawan, R Dwiyani and M Sritamin

as a **Presenter** of a paper entitled:

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

in the 5th Annual Applied Science and Engineering Conference (AASEC) 2020 Universitas Pendidikan Indonesia "Green Technologies for Environmental Sustainability", 20-21 April 2020.

Prof. Dr. Didi Sukyadi, MA.
Vice Rector for Research, Partnership, and Business
Universitas Pendidikan Indonesia

Prof. Dr. Ade Gafar Abdullah, M.Si.
Conference Chair

the 5th aasec 2020 "Green Technologies for Environmental Sustainability

Co-hosted by:

Green Conference

SURAT TUGAS
Nomor :333/UW-FP/PD-11/2020

Yang bertanda tangan dibawah ini :

Nama : Ir.Dewa Nyoman Sadguna,M.Agb

NIK : 230 500 084

Jabatan : Dekan Fakultas Pertanian Universitas Warmadewa

Dengan ini menugaskan,

No	Nama	NIP
1.	Dra.Sang Ayu Made Putri Suryani,M.Si	196704281992032001

Untuk melaksanakan tugas mengikuti seminar Internasional ***The 5th Annual Applied Science and Engineering Conference*** dengan tema ***"Green Technologies for Environmental Sustainability*** sebagai presenter, yang akan dilaksanakan secara daring pada :

Hari / Tanggal : Selasa , 21 April 2020

Waktu : 09.00-13.00 WIB

Tempat : Daring (Zoom Meeting)

Demikian surat ini kami sampaikan, untuk dilaksanakan sebagaimana mestinya.

Denpasar, 20 April 2020
Dekan,

Ir. Dewa Nyoman Sadguna, MAgb
NIK. 230 500 084

Tembusan : Arsip

PAPER • **OPEN ACCESS**

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

To cite this article: S A M P Suryani *et al* 2021 *IOP Conf. Ser.: Mater. Sci. Eng.* **1098** 052043

View the article online for updates and enhancements.

You may also like

- *Rasbora* Population in Bahorok rivers, Langkat regency, North Sumatra
Hesti Wahyuningsih, Erni Jumilawaty and Desrita
- Expression of heat shock protein 70 (hsp70) in liver and kidney organ of silver rasbora (*rasbora argyrotaenia*) exposed by sublethal organophosphate pesticides
A N Fadilah, L Sulmartiwi and L Lutfiyah
- Longitudinal distribution and population structure *Rasbora lateristriata* bleeker, 1854 (osteichthyes: cyprinidae) in Sungi River
S A M P Suryani, I W Arya and I M Kawan

Free the Science Week 2023 April 2–9

Accelerating discovery through open access!

ECS www.ecsdl.org

Discover more!

This content was downloaded from IP address 182.253.132.181 on 26/03/2023 at 10:30

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

S A M P Suryani[1,*], I G P Wirawan[2], R Dwiyani[2] and M Sritamin[2]

[1] Aquatic Resources Management department, Agriculture Faculty, Warmadewa university, Denpasar, Indonesia
[2] Faculty of Agriculture, Udayana University, Denpasar, Indonesia

*suryanip@rocketmail.com

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 Sungi 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 Sungi 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

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

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 of the Sungi River. Sungi 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 Sungi River is a collection point for drinking water taps at Tabanan Regency [11]. Activities at Sungi 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. Sungi 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 Sungi 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 Sungi 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

Sungi 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 Sungi 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 Sungi 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 Sungi 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.

2.2.2. Amplification of DNA. DNA amplification or multiplication performed by PCR (polymerase chain reaction). Sample extraction results were amplified at the locus COI (cytochrome oxidase I) with the Hotstart method. The parameters used in this method is as follows: denaturation at 94°C for 30 s, annealing at 50°C for 30 seconds, and extension at 72°C for 30 seconds, and the PCR process is repeated as many as 38 cycles [13]. In this method, used two primers, the forward primer is Fish-BCL5'TCA-ACY-AAT-CAY-AAA-GAT-ATY-GGC-AC-3 '(forward) and Fish-BCH5'-ACT-GGG-Tcy -TGR-CCR-AAR-AAT-CA-3' reverse primer [14].

2.2.3. Sequencing. Samples that have been amplified by PCR, and then sequenced on sequencing service facilities to obtain the nucleotide sequence, using the Sanger sequencing method.

2.2.4. Analytical method. To calculate sequence DNA with MEGA version 6.2

3. Results

3.1. Amplification Gen COI Rasbora lateristriata with PCR (Figure 1, 2)

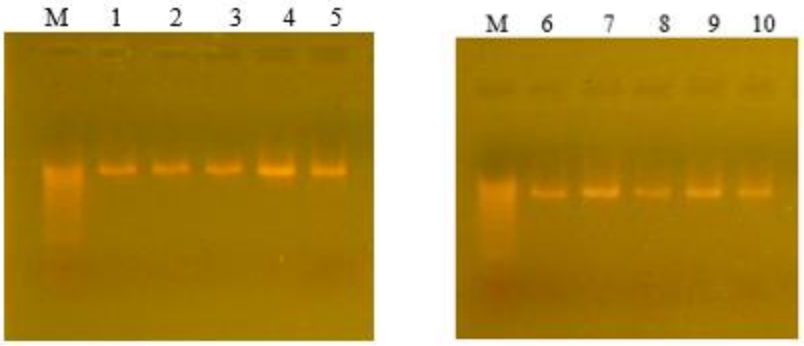

Figure 1. DNA amplification species *Rasbora lateristriata* (Upstream 1-5 and Branch river 6-10).

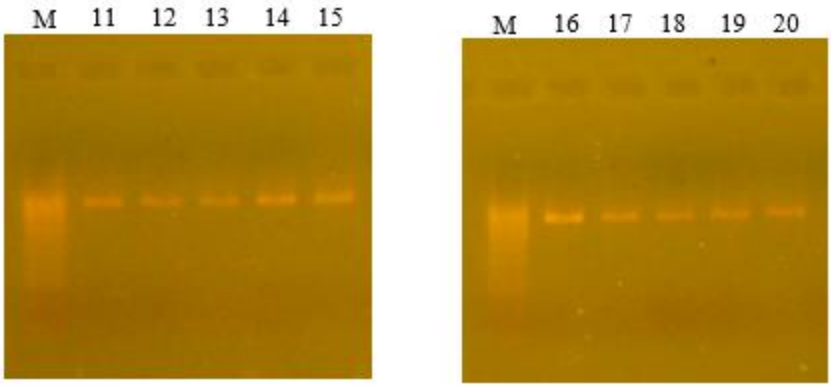

Figure 2. DNA amplification species *Rasbora lateristriata* (Midstream 11-15 and Downstream 16-20).

3.2. The results of DNA sequencing and alignment of the nucleotide sequences of COI gene Rasbora lateristriata

Nucleotides that is obtained is 633 bp to 682 bp, the sequence is then aligned to obtain a 679 bp nucleotides. Site-specific nucleotide *R. lateristriata* COI gene obtained after alignment with *R.lateristriata* from gene banks (LC130769), with species out-group, namely *R.sumatrana* (APO11221) and (EF452882*), R.aprotaenia* (LC021504), *R.baliensis* (KT960806) acquired 101 specific sites which are nucleotide bases identifier as a differentiator from other species.

R.lateristriata COI gene polymorphic site (fogure 3).The branch Sungi river there is 5 polymorphic site (100%) that is on-site to 23 which is supposed to base Thymine but replaceable with a base Citosin in all samples, in Middle Sungi 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 Citosin 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 branch area of 5 examples and samples, 100% where in the nucleotide sequence substitution of Thymine be Cytosin at 23 sites. This mutation caused due to exposure to the process of adaptation to changes in water quality parameters exceed the standards for *R. lateristriata*. In the Midstream of the nucleotide sequence of 60% had a substitution of Thymine be Cytosin at 23 sites for mutation is caused *R.lateristriata* exposed to polluted environment.

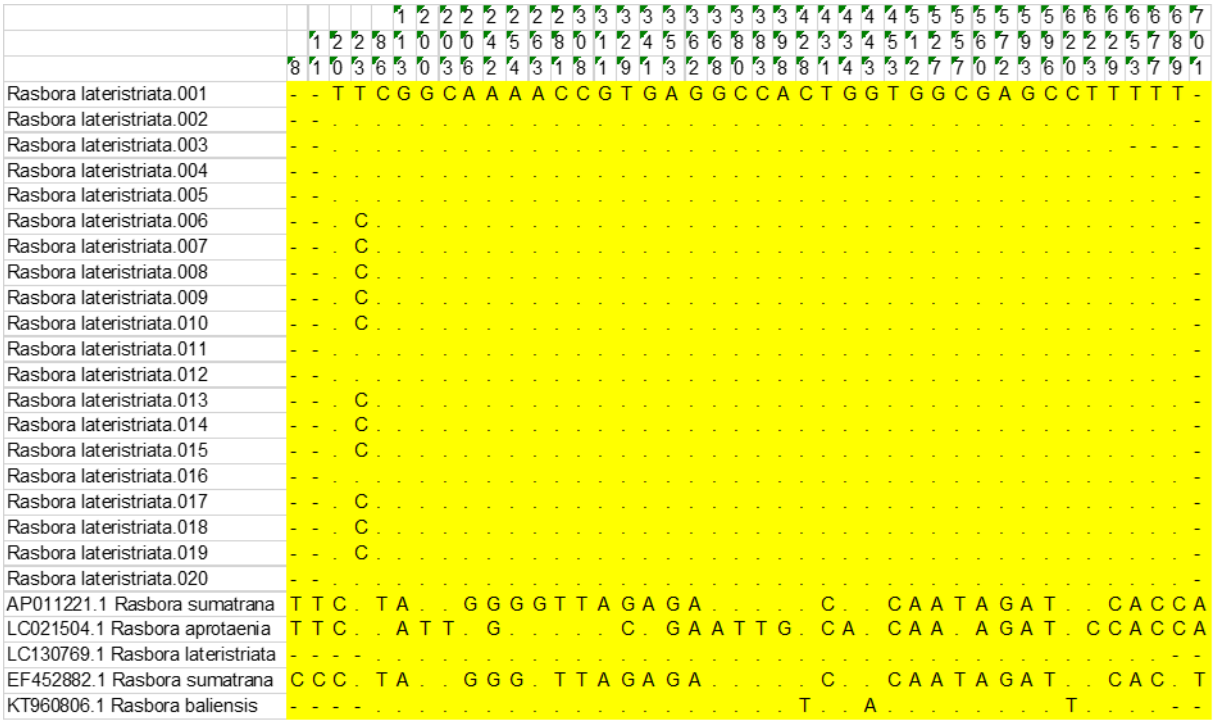

Figure 3. Polimorfic site gen COI R. lateristriata with outgroup.

4. Conclusion

Substitution mutations occur in genes COI *Rasabora lateristriata* on branch river, middle stream and downstream. The nucleotide sequences of the COI gene showed a mutation substitution on-site to 23 that Thymine are replaced with Cytosine. This mutation has not changed the COI gene as a marker of species on *R. lateristriata* in Sungi river. Comparison with outgroup species indicates that *Rasbora lateristriata* has a closeness with *Rasbora balinensis* that is species endemic Bali in the COI gene extinct. Mutation *Rasbora lateristriata* in Sungi river due to the process of adaptation to environmental changes that exceed the standards of water quality parameters for fish life that extremely increase temperatures

References

[1] Brittan M R 1998 *Rasboras: Keeping & Breeding Them in Captivity* (TFH Publications)
[2] Utami A T, Sumantadinata K and Palupi N S 2006 *Jurnal Manajemen Pengembangan Industri Kecil Menengah* **1**(1)
[3] Said D S and Mayasari N 2010 *Limnotek* **17**(2) 201-209
[4] Kottelat M, Whiten A J, Kartikasari S N and Wirjoatmodjo S 1993 *Freshwater Fishes of Western Indonesia and Sulawesi* (Periplus Editions (HK) Ltd. Collaboration with the Envinmental Management Development ini Indonesia (EMDI) Project Minstry of State for Population and Environment, Republic of Indonesia)
[5] Djumanto D, Setyobudi E, Sentosa A A and Nirwati N 2008 *Jurnal Perikanan Universitas Gadjah Mada* **10**(2) 261-275
[6] Budiharjo A 2002 *Biodiversitas Journal of Biological Diversity* **3**(2)
[7] Froese R and Pauly D 2014 *FishBase* (World Wide Web electronic publication)
[8] Kusuma W E, Ratmuangkhwang S and Kumazawa Y 2016 *Molecular phylogenetics and evolution* **105** 212-223
[9] Ahmad M dan Nofrizal 2011 *Jurnal Perikanan dan Kelautan* **16**(1) 71-78
[10] Dina R, Boer M and Butet N A 2011 *Oseanologi dan Limnologi di Indonesia* **37**(1) 105-118
[11] BLH Provinsi Bali 2009 *Status Lingkungan Hidup Daerah Provinsi Bali* (Denpasar: BLH)
[12] Setiari N M, Mahendra M S and Suyasa I W 2012 *ECOTROPHIC: Jurnal Ilmu Lingkungan (Journal of Environmental Science)* **7**(1) 40-46
[13] Barber P H, Erdmann M V and Palumbi S R 2006 *Evolution* **60**(9) 825-839
[14] Baldwin C C, Mounts J H, Smith D G and Weigt L A 2009 Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx *Zootaxa*
[15] Suryani S A M P, Arya I W and Kawan I M 2019 *Journal of Physics: Conference Series*, **1402** (3) 033064