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Morphological variations and molecular phylogeny of *Oryzias sarasinorum* Popta, 1905 (Ricefish) from Lake Lindu, Central Sulawesi, Indonesia

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Abstract. *Zainal S, Tellu AT, Kasim A. 2022. Morphological variations and molecular phylogeny of* Oryzias sarasinorum *Popta, 1905 (Ricefish) from Lake Lindu, Central Sulawesi, Indonesia. Biodiversitas 23: 3443-3451. Oryzias sarasinorum* Popta, 1905 is a well-studied species endemic to Lake Lindu, Central Sulawesi, Indonesia and identified as scarce. This study aims to describe the morphological variation and phylogeny of *Oryzias sarasinorum* Popta, 1905 from Lake Lindu, Central Sulawesi Province, Indonesia. This research was conducted in February 2022. 10 samples were collected and preserved on 95% ethanol for morphological analysis and molecular purposes. Our morphological analysis showed that the meristic counts, morphometric measurement, and phylogenetic analysis also resolved that the samples that were analyzed are indeed an endemic species of *O. sarasinorum* from Lake Lindu. Finally, we expect there are future studies that pay attention to well-resolved phylogenies and thereby remove additional noise from the analysis. The research data are expected to contribute to the preservation and utilization of one of Indonesia's important biodiversity resources.

Keywords: Diversity, endangered, endemic species, identification

INTRODUCTION

Sulawesi, an island situated in the biogeographical change zone among Indomalaya and Australasia, is famous for its elevated degrees of endemism in both earthly and freshwater fauna (Hall 2013). This endemism indicates that these taxa diversified within the island. Sulawesi comprises three primary structural developments, two of which begin from the edges of the Asian and Australian landmasses, and the others emerge by orogeny because of structural impacts between the two plates. These three structural developments have coincided with one another since the Pliocene (ca. 4 Mya), and a large portion of the land has been elevated over the last 2-3 Myr, bringing about the state of Sulawesi today (Hall 2013). It is shown that the complex land history of this island might have unequivocally impacted the expansion of a portion of Sulawesi's endemic taxa (e.g., fish).

Somewhat recently, fish systematics has grown quickly. A few examinations have attempted to gather the connection between each gathering of fish. One way is to utilize phylogenetic investigation (Peloso et al. 2015). The phylogenetic investigation is a review that inspects the connection between different life forms by genetic and morphological analysis. Molecular analysis using the Cytochrome Oxidase Subunit 1 (CO1) gene has been proven successful in phylogenetic studies of fish, and the output is in the form of phylogenetic tree data (Viswambharan et al. 2015; Bingpeng et al. 2018). For example, Serdiati et al. (2020) confirm the identity of *Oryzias nigrimas* (Ricefish) using partial CO1 mitochondrial gene. Fish phylogenetic connections were laid out with tests from Lake Poso and different groupings acquired from the National Center for Biotechnology Institute (NCBI) GenBank data set. In the meantime, Serdiati et al. (2020) succeeded in reconstructing the phylogenetic tree sequence data from the mitochondrial gene of species from *Oryzias* genera to investigate the phylogenetic relationships, namely *Oryzias nigrimas* Kottelat, 1990. Mokodongan (2019) *Oryzias sarasinorum* Popta, 1905 is critically endangered according to the International Union for Conservation of Nature and Natural Resources (IUCN). This is essential information as it affects how the animals are treated in conservation.

Refreshes on scientific fish categorization have likewise been done with changes from taxonomists (Panprommin et al. 2019). Prior to utilizing DNA sequencing procedures, morphometric depiction became one of the pillars in recognizing species. Morphometric investigation is one method for deciding the variety of animal categories by testing its morphological characters. Morphometric information can be utilized to make sense of contrasts and similitudes among populaces and portray the morphology of populace family relationships (Kim et al. 2010; Marzouk et al. 2016). Meng et al. (2018) expressed that the consequences of morphometric examination can give an outline of the level of fluctuation of taxa. For the most part, each noticed person is the consequence of quality collaboration whose articulation is impacted by the climate.

In any case, the utilization of morphometric information has limits, for example, the low consistency esteem in showing phylogenetic connections at the degree of subspecies variety (Klingenberg and Gidaszewski 2010). So it should be contrasted and morphological varieties and atomic information to come by the most extreme outcomes. A review utilizing these two methodologies can give greater and more accommodating data for efficient data and phylogenetic recreation. Subsequently, this study portrays the morphological and atomic phylogenetic varieties of *Oryzias sarasinorum* Popta, 1905 from Lake Lindu, Central Sulawesi Province, Indonesia.

MATERIALS AND METHODS

Study area

This research was conducted in February 2022 on Lake Lindu, Central Sulawesi, Indonesia (Figure 1).

Collection and preservation

Fish sampling is carried out using a net or gill net measuring 15x15 m^2 by involving residents who are fishermen and used to catch Ricefish. 10 sample adults were collected and preserved on 95% ethanol for morphological analysis and molecular purposes.

Morphological characteristics, morphometrics, and meristic analysis

As many as 10 samples in each population sites were measured using morphological analysis. The morphological analysis consists of 15 morphometric measurements and 5 meristic counts following Holden and Raitt (1974). Morphometric characters include characteristics as follows: Total Length (TL), Head Length (HL), Standard Length (SL), Pectoral Fin Length (PFL), Eye Diameter (ED), Anal Fin Length (AFL), Preorbital Distance (PRD), Predorsal Distance (PD), Post Orbital Distance (POD), Pre Pectoral Distance (PPD), Pre Pelvic Distance (PPED), Length of Jaw (PRg), Body Depth (BD), Dorsal Fin Length (DFL), and Tail Stem Height (TSH). Meristic characteristics included the number of scales along the lateral line, the number of dorsal, ventral, pectoral and anal fin fingers. Data were analyzed using Principal Component Analysis (PCA) on PAST4 software.

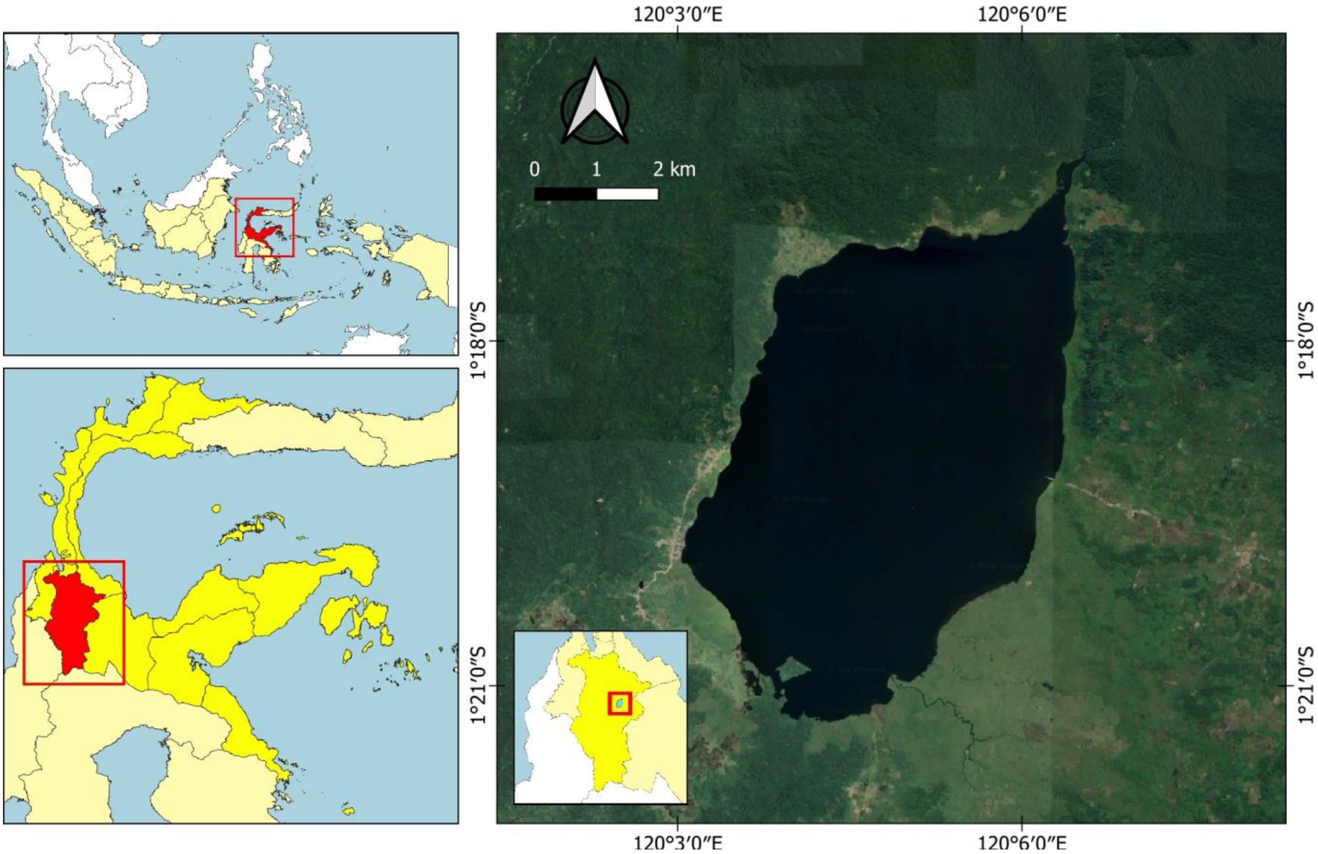

Figure 1. Study sites employed for the sampling of *Oryzias sarasinorum* Popta 1905 in Lake Lindu, Central Sulawesi, Indonesia

DNA extraction, amplification, electrophoresis, and sequencing

The total genomic DNA was extracted from part of the caudal fin from sample. DNA isolation was carried out using the gSYNC^{TM} DNA Extraction Kit (Genaid, GS300). The DNA Isolation steps follow its manufacturer protocol for species barcoding fish. DNA Amplification was performed with PCR Thermocycler using two CO1 primers. We amplified and sequenced the mitochondrial DNA specifically the CO1 gene using following primers: Primer FishR2_t1 5'-CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGAA-3' Primer FishF2_t1 5'-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCAC-3. PCR Master Mix, (2x) Dream Taq Green PCR Master Mix. The amplifications were carried out under the following condition: 1 cycle of initial denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 15 seconds, extension at 72°C for 45 seconds. The electrophoresis of PCR products was run on a 1% agarose gel stained with Florosafe (Bioline) and buffered with Tris-acetate EDTA (TAE) at 50 volts for 20 minutes. Visualization was conducted under UV light. All amplification samples were sent to First Base Sdn Bhd (Malaysia) through PT. Genetika Science (Jakarta) for purification and sequencing in both forward and reverse directions using the Big Dye Terminator (Applied Biosystems) and the ABI 3730xl Genetic Analyzer (Applied Biosystems).

Ethics approval and consent to participate

The protocol of this study had been approved by the Ethical Committee, Faculty of Animal Husbandry and Fisheries, Tadulako University (660A/UN28.1.31/PT/2022).

Data analysis

Principal Component Analysis (PCA) based on morphometric measurements

PCA was conducted using PAST software with meristic count and 15 morphometric measurements. The sum of the first and second Eigen values (>70%) was examined in the interpretation of the PCA output. Character loadings were generated for the first two principal components to determine the characters influenced in each component. Then, the first two principal component scores were plotted to visualize the result.

Sequence editing, alignment, nucleotide composition and genetic distance, and phylogenetic relationship

Information obtained from DNA sequencing results was altered in the GeneStudio program and approved with SeqMan and EditSeq on the DNASTAR program (DNASTAR Inc. Madison, USA). Sequencing responses were made on every individual utilizing both forward and switch groundworks. Chromatograms were assessed physically to actually look at vague bases and stop codons. The *Oryzias sarasinorum* CO1 arrangements were then adjusted utilizing Opal on Mesquite v.351 programs (Maddison and Maddison 2018) and ClustalW on the MEGAX program (Kumar et. 2018). The structure of the CO1 nucleotides was determined utilizing the MEGAX program. Genetic distance was dissected utilizing the MEGAX program with the Kimura-2 Parameter (K2P) model and summed up in a Neighbor-Joining (NJ) tree, which is the standard procedure utilized in barcoding studies with bootstrap 1.000 reproduces (Hebert et al. 2003). The reproduction of the phylogeny tree was examined utilizing the Neighbor Joining and Maximum Likelihood strategies with 1.000 bootstrap utilizing MEGAX program (Kumar et al. 2018) and Bayesian Inference utilizing the BEAST program (Suchard et al. 2018). The Bayesian Information Criterion (BIC) executed in jModelTest 2.1.10 (Darriba et al. 2012) was utilized to decide the best fit transformative model. This examination's most ideal grouping replacement model is HKY with invariant destinations (HKY + I) on the Bayesian Information Criterion (BIC). The Markov Chain Monte Carlo (MCMC) was run for 106 ages to appraise the back probabilities conveyance with an inspecting recurrence set to each 1.000 age. The agreement trees were envisioned in FigTree 1.4.4 (Rambaut 2019).

RESULTS AND DISCUSSION

Morphological characteristics, morphometrics and meristic analysis of *Oryzias sarasinorum* from Lake Lindu

Colour in life: Ground variety beige-yellowish with a 'focal "brilliant" stripe' that mirrors light unequivocally. Colour in alcohol: Ground variety pale yellow, midsection pale, whitish-yellow. An expansive, gleaming, parallel band stretches out from the back edge of the head to the caudal peduncle in certain examples, different examples blanched. A diffuse column of melanophores from the dorsal surface of the head to the dorsal-balance beginning, a sporadic, mid-lateral dark line from the back boundary of the head to the base of the caudal blade. Blades hyaline to gloomy (Figure 2).

Figure 2. Morphological characteristics of *Oryzias sarasinorum* Popta, 1905

The after-effects of the meristics count (Table 1) (Figure 3) and morphometrics estimation (Table 2) are displayed underneath. Our examination showed that meristics depends on the quantity of ventral balances beam, dorsal blade beam, butt-centric balance beam, and pectoral blade beam, are not altogether unique to make an accommodation that the species were morphologically unique. Nonetheless, the parallel line scale is fundamentally unique between the *O. sarasinorum* (this review) when contrasted and research results Sapitri (2020). Consequently, our investigations on morphometric estimation showed no huge contrasts in characters aside from body profundity, postorbital distance, and length of the jaw. The distinction on the parallel line-scale counts suggests that this character might be impacted by environmental conditions and furthermore both the physical and structure of these water (Quilang et al. 2007; Wark et al. 2010; Serdiati et al. 2020). Estimation of morphometric and meristic attributes can be utilized to decide the order and systematics of fish (Simon et al. 2010). Meristic counts depend on additional crude characters than morphometric estimations and would, thusly, give more grounded proof to speciation. Moreover, species status would possibly be acknowledged whether there was no cross-over in the scope of somewhere around one of the researched meristic characters or morphometric proportions (Fakunmoju et al. 2014). Ecological changes extraordinarily influence the freshwater fish variation that appeared on the progressions of morphological elements (Braich and Akhter 2015). No essentially divergent morphological qualities of *O. sarasinorum* other than those brought about by the closeness of living space or ecological elements in Lake Lindu may likewise be related to articulated contrasts in asset securing and hunter escape (Seebacher et al. 2016).

Furthermore, the morphometric information is dissected utilizing the important part examination to see the predominant person influence variety of the bunching example of people of *Oryzias* spp. PCA was led by the information gathered. The relationship examination between-bunch brings about eigenvalues and percent fluctuations displayed in Table 3, while the dissipate plot should be visible in Figure 4. The vital parts examination shows the example of test grouping in view of the job of each person in the bunching system. The stacking plot of part 1 should be visible in Figure 5, while the stacking plot of part 2 should be visible in Figure 6. In light of the consequences of PCA, there are varieties of individual *Oryzias* spp. estimations. The most dominant characteristics for the formation of seven clusters that determine to cluster of the species are the Total Length (TL), Length of Jaw (LOJ), and Tail Stem Height (TSH). This can be seen from the length of the resulted line (Figure 4), and also the picture of Loading Plot of Component 1 (Figure 5) and Loading Plot of Component 2 (Figure 6) produced. The longer the arrow and the higher the graph formed, hence the characters' role in group formation is also high.

Table 1. Meristic count of *O. sarasinorum* and *Oryzias* spp. from Lake Lindu, Central Sulawesi, Indonesia (latipes species group, celebensis species group, and javanicus species group) (Parenti 2008)

Species	Characters									
	Pc	**Ca**	**TV**	**DF**	**AF**	**PF**	**BR**	**PT**	**SLS**	**DFO**
Latipes species group										
O. mekongensis	10-11	17-20	27-31	5-7	13-18	5-6	4-5	6-8	29-32	19-20
O. latipes	11-13	17-20	27-32	5-7	17-22	5-7	5-6	9-11	28-32	22-23
O. luzonensis	11-12	18-19	29-31	5-7	15-19	6	5-6	11	30-35	20-21
O. curvinotus	11-12	17-18	28-30	5-6	17-20	6	4-5	10-11	27-28	22
Celebensis species group										
*O. sarasinorum**	15	19	34	11-13	21-23	7	5-6	11	71-75	20
O. sarasinorum	15	19	34	11-12	21-22	7	5-6	10-11	70-75	20-21
O. oophorus	15	21	36	8-10	20-22	6	5-6	12	58-65	22-23
O. celebensis	11-12	18-20	30-31	8-10	17-23	6	5-6	10-11	29-33	22-23
O. nigrimas	13-14	19	32-33	8-11	21-25	6	5	11-12	34-37	22-24
O. matanensis	12	18	30	8-9	20-25	6	5	11-12	41-47	21-22
O. marmoratus	12	18	30	8-12	20-26	6	5	10	31-32	20
O. profundicola	11	18	29	10-14	26-29	6	5	10-11	32-34	18-19
Javanicus species group										
O. minutillus	8-11	16-18	24-29	5-7	17-21	5	4-5	7-8	26-29	19-20
O. dancena	10-11	17-18	28-29	6-8	19-24	6	4-5	10-11	25-28	22-23
O. hubbsi	9-10	17-19	27-28	5-6	16-19	6	4-5	8-9	28-29	20
O. javanicus	10-13	17-18	27-31	6-8	18-25	5-6	5	10-13	27-30	22-23

Note: *sample in this study. Pc: precaudal or abdominal vertebrae, Ca: caudal vertebrae, TV: total vertebrae, DF: dorsal-fin rays, AF: anal-fin rays, PF: pelvic-fin rays, BR: branchiostegal rays, PT: pectoral-fin rays, SLS: scales in a lateral series, DFO: dorsal-fin origin

Table 2. Measurement of 15 morphometric characteristics of *O. sarasinorum* and *Oryzias* spp.from Lake Lindu, Central Sulawesi, Indonesia by Sapitri (2020) and Serdati et al. (2020)

Characteristics	**Species of *Oryzias* spp.**			
	***O. sarasinorum* (This study)**	***O. sarasinorum* (Sapitri 2020)**	***O. bonneorum* (Sapitri 2020)**	***O. nigrimas* (Serdiati et al. 2020)**
Total Length (TL)	47.52 – 48.66	47.13 – 47.94	49.12 – 49.50	51.03 – 52.51
Standard Length (SL)	40.94 – 41.25	40.15 – 40.65	41.40 – 41.60	40.96 – 44.30
Head Length (HL)	10.46 – 10.73	10.10 – 10.60	10. 88 – 10.90	11.80 – 12.31
Pectoral Fin Length (PFL)	3.19 – 3.26	3.14 – 3.23	3.20 – 3.32	3.22 – 3.35
Anal Fin Length (AFL)	6.18 – 6.19	6.01 – 6.13	6.10 – 6.16	5.46 – 6.17
Eye Diameter (ED)	3.96 – 4.00	3.94 – 3.97	4.00 – 4.01	3.83 – 4.15
Preorbital Distance (PRD)	2.50 – 2.75	2.45 – 2.52	2.78 – 2.80	2.77 – 2.96
Post Orbital Distance (POD)	3.00 – 3.05	2.99 – 3.02	3.08 – 3.10	2.79 – 3.37
Predorsal Distance (PD)	30.96 – 30.99	30.45 – 30.97	30.98 – 31.0	30.24 – 32.75
Pre Pelvic Distance (PPED)	19.01 – 19.15	18.98 – 19.05	19.00 – 19.10	17.24 – 19.27
Pre Pectoral Distance (PPD)	13.11 – 13.15	12.58 – 13.11	13.10 – 13.16	12.59 – 13.25
Body Depth (BD)	4.25 – 4.30	4.12 – 4.33	4.32 – 4.43	4.36 – 5.25
Dorsal Fin Length (DFL)	12.87 – 13.33	12.50 – 12.34	13.50 – 13.59	13.38 – 13.87
Length of Jaw (LOJ)	7.45 – 7.60	7.00 – 7.25	7.55 – 7.87	6.64 – 8.21
Tail Stem Height (TSH)	3.13 – 3.26	3.01 – 3.11	3.15 – 3.30	3.24 – 3.45

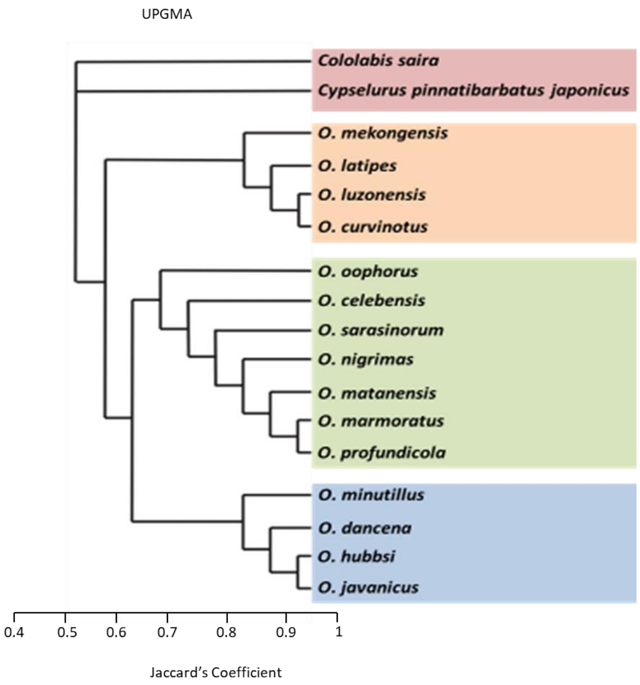

Figure 3. Meristic count of *O. sarasinorum* and *Oryzias* spp. from Lake Lindu, Central Sulawesi, Indonesia (red: outgroup, yellow: latipes species group, green: celebensis species group, and blue: javanicus species group) (Parenti 2008)

Table 3. Eigenvalue and % variance

PC	**Eigenvalue**	**% variance**
1	3.00689	96.064
2	0.07181	1.9288
3	0.05731	1.5710
4	0.02214	0.7761
5	0.02549	0.5662

PCR amplification and sequence identification of *Oryzias sarasinorum* from Lake Lindu

The result showed that the enhancement of the CO1 mitochondrial quality of five *O. sarasinorum* from Lake Lindu created a piece length of around 700 bp (Figure 7). The agreement arrangement results from the chromatogram altering process were between 661-701 bp, which can be converted into 221-235 amino acids. As indicated by the outcomes (Table 2), each of the five fish gathered from Lake Lindu were distinguished in one sort, specifically *Oryzias*, and comprised of one animal type *O. sarasinorum*. The similitude of the examples contrasted with the information in GenBank was 99.15-99.57% (Table 4).

Table 4. Species identification based on GenBank database using BLAST and BOLD identification

Sample code	**Identified species from GenBank / BOLD**	**Similarity (%)**	**Query cover (%)**	**Accession number**	**References**
OS-001	*O. sarasinorum*	99.15	99	LC154798.1	Direct submission
OS-002	*O. sarasinorum*	99.29	98	LC154798.1	Direct submission
OS-003	*O. sarasinorum*	99.16	99	LC154798.1	Direct submission
OS-004	*O. sarasinorum*	99.57	100	LC154798.1	Direct submission
OS-005	*O. sarasinorum*	99.30	98	LC154798.1	Direct submission

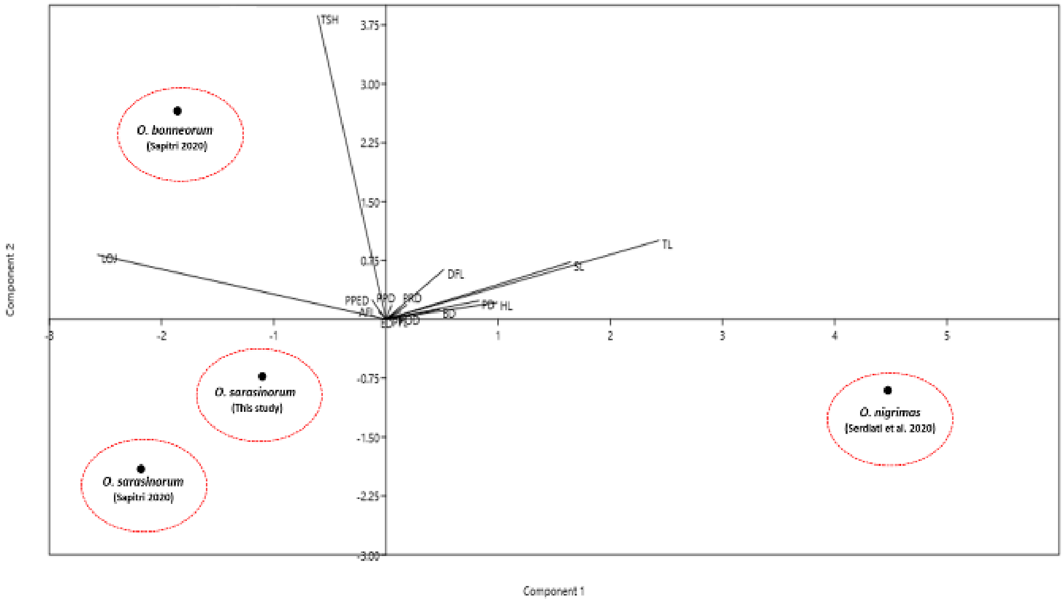

Figure 4. The PCA results of *Oryzias sarasinorum* from Lake Lindu, Central Sulawesi, Indonesia

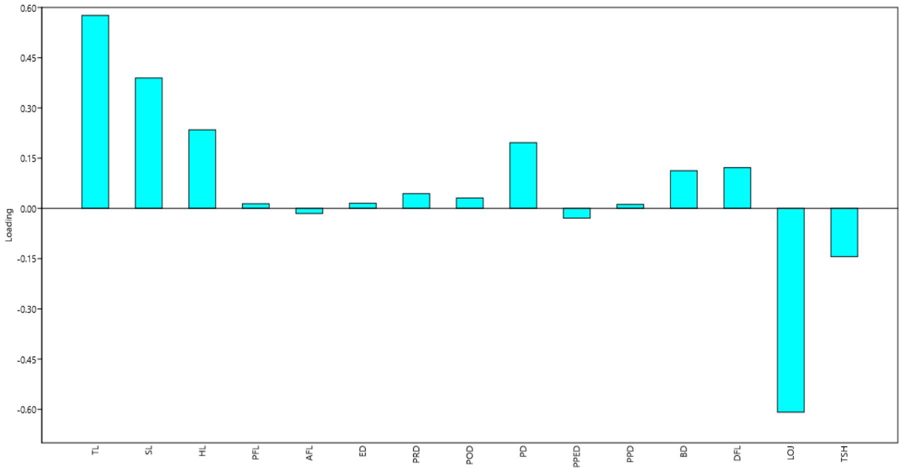

Figure 5. The loading plot of component 1

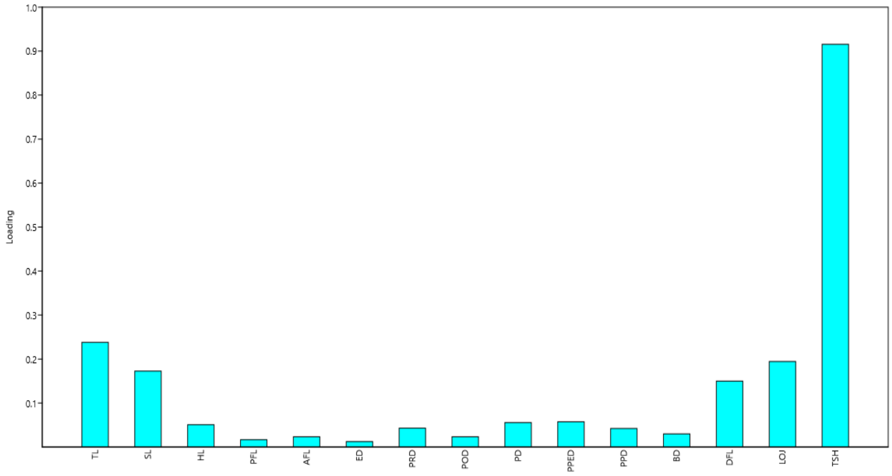

Figure 6. The loading plot of component 2

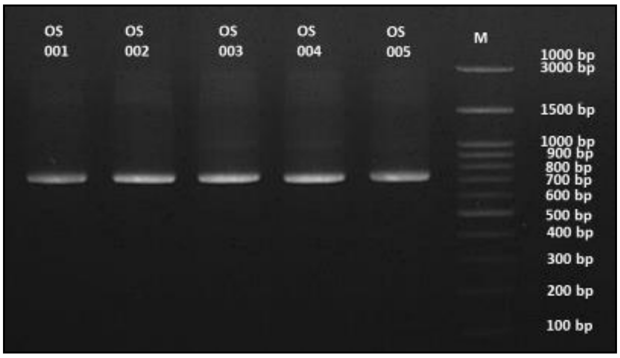

Figure 7. PCR amplification result of CO1 mitochondrial gene of *O. sarasinorum* from Lake Lindu, Central Sulawesi, Indonesia migrated in 1% agarose electrophoresis. OS 001 to OS 005 is the sample code. M is marker visualized from DNA ladder 1 kb (GENEAID)

Sequence alignment

The alignment of five *O. sarasinorum* arrangement tests from Lake Lindu yielded a spotless grouping (a succession that came about after the arrangement and cutting course) of 680 bp. These clean CO1 quality successions of every species were then utilized for intraspecies investigation. The CO1 groupings arrangement of *O. sarasinorum* from Lake Lindu and other Indonesian districts kept in GenBank and BOLD brought about a section length of 570 bp. This outcome was then exposed to the intraspecies investigation (nucleotide creation and hereditary distance). For phylogenetic tree examination, the clean CO1 groupings (565 bp) of 16 examples addressed 1 family and 8 species, in particular *O. sarasinorum, O. nigrimas, O. nebulosus, O. marmoratus, O. matanensis, O. celebensis, O. woworae,* and *O. asinua* from Lake Lindu and other Indonesian districts recorded on GenBank and BOLD were utilized. Two CO1 arrangement of *Gambusia affinis* (Accession number: KM220898.1 and MK628400.1) was utilized as an outgroup (Table 5).

Phylogenetic analysis and genetic distance

The tree reproduction results yielded indistinguishable tree geographies (Figure 8). The consequences of the jModelTes2 examination uncovered that the ideal arrangement replacement model is HKY with invariant locales (HKY + I) on the Bayesian Information Criterion (BIC). The phylogenetic tree remaking of *Oryzias* spp. from Lake Lindu and *Oryzias* spp. from different areas in Indonesia and Japan recorded on GenBank and BOLD framed 8 unique clades. The development of these 8 clades was upheld by a bootstrap worth of 80-100 percent in the Neighbor-Joining and Maximum Likelihood techniques. Also, the back likelihood esteem is 1.00 in Bayesian Inference. Bootstrap and back likelihood results showed that the development of these clades was strong and inflexible. The results of the grouping above were consistent with previous studies conducted by Serdiati et al. (2020), which showed that *O. sarasinorum* formed a group with *O. nigrimas, O. nebulosus, O. marmoratus, O. matanensis, O. celebensis, O. woworae,* and *O. asinua.*

Our phylogenetic investigation in view of effectively enhanced CO1 qualities likewise showed *O. sarasinorum* from Lake Lindu, Central Sulawesi are settled inside a similar clade of another *O. sarasinorum* from Aichi, Higashiyama Zoo, Japan (Accession number: LC154798.1) (Figure 8) with an all-around upheld bootstrap and on the hubs. The bootstrap and back likelihood esteem demonstrate the consistency of information appearing in rates. Low qualities imply that the arrangement gives alternate tree geography on each test. The considered all-around upheld bootstrap incentive for the greatest probability examination is at >75% (Yang 1994). In any case, in light of the perceptions of our arrangement, it was shown that there were varieties in a few locales, at this point, deficient in separating the examples. As per Kress and Erickson (2012), the CO1 quality has the ability to recognize the taxa up to species level on account of the exceptionally rationed varieties of the locale. These varieties of nucleotides can be utilized by a person that recognizes the species. The high variety of nucleotides among a grouping of tests is a successful device for distinguishing Rice fish species (Zhang and Hanner 2012).

Table 5. The CO1 sequences alignment of *O. sarasinorum* from Lake Lindu and other Indonesian regions recorded in GenBank and BOLD

Samples	Locality	Accession number	References
O. sarasinorum	Lake Lindu, Central Sulawesi	-	*This study*
O. sarasinorum	Lake Lindu, Central Sulawesi	-	*This study*
O. sarasinorum	Lake Lindu, Central Sulawesi	-	*This study*
O. sarasinorum	Lake Lindu, Central Sulawesi	-	*This study*
O. sarasinorum	Lake Lindu, Central Sulawesi	-	*This study*
O. sarasinorum	Aichi, Higashiyama Zoo, Japan	LC154798.1	Direct submission
O. nigrimas	Aichi, Higashiyama Zoo, Japan	LC153106.1	Direct submission
O. nebulosus	Aichi, Higashiyama Zoo, Japan	LC153098.1	Direct submission
O. marmoratus	Aichi, Higashiyama Zoo, Japan	LC154797.1	Direct submission
O. matanensis	Aichi, Higashiyama Zoo, Japan	LC153099.1	Direct submission
O. celebensis	Sulawesi and Timor, Japan	JX311942.1	Parenti et al. 2013
O. woworae	Muna Island, Sulawesi, Japan	JX311940.1	Parenti et al. 2013
Oryzias sp.	Asinua River, Sulawesi, Japan	JX311929.1	Parenti et al. 2013
G. affinis	USA	KM220898.1	Strecker and Oberdorster 2014
G. affinis	Thailand	MK628400.1	Panprommin et al. 2019

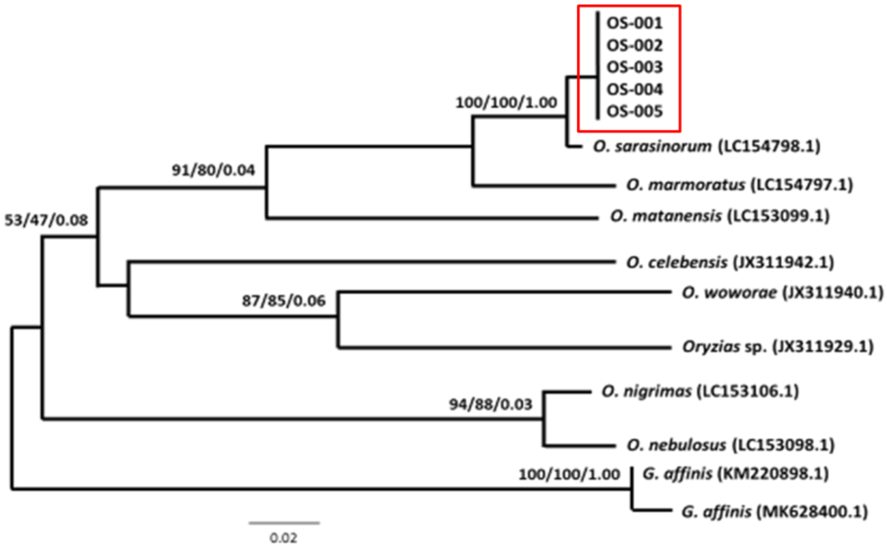

Figure 8. Phylogenetic tree reconstruction based on Neighbor-Joining (NJ), Maximum-Likelihood (ML), Bayesian Inference (BI) topology *Oryzias* spp. and outgroup based on CO1 gene sequence (565 bp). The node represented the number bootstrap (NJ and ML) and Bayesian Posterior Probability (Bayesian Inference). OS-001 to OS-005 is sequence data in this study

Table 6. Genetic distance of *Oryzias sarasinorum* from Lake Lindu, Central Sulawesi and additional sample *Oryzias* spp. from the GenBank and BOLD

	OS-001	**OS-002**	**OS-003**	**OS-004**	**OS-005**	***O. sarasinorum* (LC154798.1)**	***O. marmoratus* (LC154797.1)**	***O. matanensis* (LC153099.1)**	***O. celebensis* (JX311942.1)**	***O. woworae* (JX311940.1)**	***Oryzias* sp. (JX311929.1)**	***O. nigrimas* (LC153106.1)**	***O. nebulosus* (LC153098.1)**	***G. affinis* (KM220898.1)**	***G. affinis* (MK628400.1)**
OS-001	0.000														
OS-002	0.000	0.000													
OS-003	0.000	0.000	0.000												
OS-004	0.000	0.000	0.000	0.000											
OS-005	0.000	0.000	0.000	0.000	0.000										
***O. sarasinorum* (LC154798.1)**	0.002	0.002	0.002	0.002	0.002	0.000									
***O. marmoratus* (LC154797.1)**	0.037	0.037	0.037	0.037	0.037	0.035	0.000								
***O. matanensis* (LC153099.1)**	0.045	0.045	0.045	0.045	0.045	0.047	0.052	0.000							
***O. celebensis* (JX311942.1)**	0.106	0.106	0.106	0.106	0.106	0.104	0.109	0.114	0.000						
***O. woworae* (JX311940.1)**	0.115	0.115	0.115	0.115	0.115	0.109	0.116	0.121	0.120	0.000					
***Oryzias* sp. (JX311929.1)**	0.118	0.118	0.118	0.118	0.118	0.112	0.119	0.124	0.123	0.126	0.000				
***O. nigrimas* (LC153106.1)**	0.127	0.127	0.127	0.127	0.127	0.125	0.132	0.136	0.135	0.138	0.141	0.000			
***O. nebulosus* (LC153098.1)**	0.129	0.129	0.129	0.129	0.129	0.125	0.133	0.138	0.137	0.140	0.143	0.148	0.000		
***G. affinis* (KM220898.1)**	0.560	0.560	0.560	0.560	0.560	0.567	0.572	0.575	0.580	0.585	0.588	0.595	0.600	0.000	
***G. affinis* (MK628400.1)**	0.568	0.568	0.568	0.568	0.568	0.575	0.581	0.586	0.589	0.596	0.601	0.611	0.622	0.003	0.000

In view of our genetic distance investigations of 5 examples (OS-001 to OS-005) of CO1 mtDNA qualities of *O. sarasinorum* from Lake Lindu, Central Sulawesi, and 13 examples of *Oryzias* spp. Other Indonesian locales recorded on GenBank and BOLD, it was shown that our examples have a low hereditary distance (at worth of 0.002%) to the *O. sarasinorum* from Aichi, Higashiyama Zoo, Japan (Accession number: LC154798.1) (Table 6). It was considered that there was no genetic isolation occured and genetic structure is well maintained to form the single species (Braich and Akhter 2015) in Lake Lindu. As per Zemlak et al. (2009), the limit for intraspecies hereditary distance in fish species is 3.5%. Assuming that it surpasses 3.5%, it is viewed as an alternate animal group. In view of the rule from Zemlak above, *O. sarasinorum* from Lake Lindu and *O. sarasinorum* from the Indonesian locales kept in GenBank and BOLD were as yet delegated similar species (conspecific).

In conclusion, our morphological examination showed that the meristic counts, morphometric estimation, and our phylogenetic investigation additionally settled that the examples that were dissected are without a doubt endemic types of *O. sarasinorum* from Lake Lindu. Finally, we expect there are future studies that pay attention to well-resolved phylogenies and thereby remove additional noise from the analysis. The research data are expected to contribute to the preservation and utilization of one of Indonesia's important biodiversity resources.

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