Morphometric and genetic variations of the Poison Frog (Odorrana hosii Boulenger 1891) in Jatimulyo, Kulon Progo, Yogyakarta using microsatellite markers

Galuh Enggar Pramesti[1], and *Nurul* Suwartiningsih[2]

[1]Study Program of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia
[2]Laboratory of Ecology and Systematics, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta, Daerah Istimewa Yogyakarta, Indonesia

Abstract. One of the abundant amphibian species in Indonesia is *Odorrana hosii*. This species exhibits a wide distribution range and varies in morphometric and genetic characteristics. However, the morphometric and genetic variations of *Odorrana hosii* populations in Jatimulyo area of Kulon Progo, Yogyakarta, have not been previously studied. This research aims to investigate the morphometric and genetic variations of three populations: Sungai Mudal, Kembang Soka, and Kedung Pedut. Sampling was conducted in Jatimulyo ecotourism area, and morphometric measurements were taken from individual *Odorrana hosii* specimens. Genetic analysis was performed using *Polymerase Chain Reaction* (PCR) with microsatellite markers Rnh-9 and Rnh-13 at the Molecular Biology Laboratory, Ahmad Dahlan University. Morphometric analysis involved 27 characters observed in 9 male and 9 female individuals, while genetic analysis focused on amplified loci. Kruskall-Wallis analysis was used to compare interpopulation morphometric data, revealing four distinguishing characters with $P<0.05$. The results showed that head length (HL), nostril distance (IND), thigh length (TL), and total leg length (HLL) were longest in males from Kembang Soka population and shortest in males from Kedung Pedut population. Genetic variation analysis indicated the highest DNA polymorphism in Kembang Soka population (90.90%), followed by Kedung Pedut (60%) and Sungai Mudal (50%). Overall, Kembang Soka population exhibited the most significant morphometric and genetic variations.

1 Introduction

The Indonesian archipelago spans from Sabang to Merauke and is situated between two oceans and two continents [1]. This unique geographical position has endowed Indonesia with rich biodiversity in both flora and fauna. Evidently, numerous species with distinctive characteristics are found across various regions [2]. Among Indonesia's faunal treasures is the amphibian group [3]. Amphibians exhibit great diversity, and one abundantly distributed species in Indonesia is *Odorrana hosii*. *Odorrana hosii* can thrive in both rainy and dry

seasons, across elevations ranging from 300 meters above sea level (masl) to 1,430 masl [4]. The habitat distribution of *Odorrana hosii* across these elevation gradients suggests the potential for morphological and genetic variations [5].

Morphological characteristics are typically assessed through morphometrics, a technique used to gauge the general morphological diversity of a species [6]. The data derived from morphometric analyse are often utilised to elucidate differences and similarities among populations [7]. Each observed characteristic arises from gene interactions influenced by environmental factors [8]. Genetic variation, on the other hand, refers to differences in the genome of an organism, spanning nucleotide bases, genes, or chromosomes [9]. Such variation, typically observable among individuals within a population or among geographically separated populations, plays a pivotal role in conservation efforts and population resilience [10].

Research investigating the morphometric and genetic variations of *Odorrana hosii* has been undertaken in various regions, including West Sumatra and Yogyakarta [11]. Research on the genetic diversity of *Odorrana hosii* in West Sumatra using microsatellite markers showed the highest genetic diversity results in Padang population, this was due to the most suitable habitat and larger population size among the seven populations studied [12].

In Yogyakarta, morphometric studies on *Odorrana hosii* have been conducted, specifically in three locations within Jatimulyo, Kulon Progo, albeit limited to male frog samples [4]. The results of the study showed that there were morphometric variations in three populations of male frogs in Jatimulyo which were caused by differences in location with varying heights and abiotic factors, thus showing the potential for differences in morphometric variation in *Odorrana hosii* [4]. These differences may also influence genetic variations, although research on the genetic diversity of *Odorrana hosii* in Jatimulyo remains scarce.

Several studies conducted in Jatimulyo have explored the diversity of amphibians on Mount Kelir [2] and the herpetofaunal diversity in Jatimulyo [13]. This study aimed to analyze the morphometric and genetic variations of *Odorrana hosii* in Jatimulyo using microsatellite markers. Such research is crucial for understanding genetic diversity variations influenced by environmental conditions and location discrepancies within Jatimulyo ecotourism area, thereby informing decisions regarding the arrangement of these sites. The aim of the current study is in line with the Sustainable Development Goals (SDGs) in the research on *Odorrana hosii* not only enriches scientific knowledge about a particular species but also supports several broader SDGs goals, including biodiversity conservation, sustainable management of water resources, climate change mitigation and adaptation, and environmental education and awareness. Such studies are an integral part of global efforts to achieve sustainable development that harmonizes human needs with environmental sustainability.

2 Material and Methods

Sampling of *Odorrana hosii* frogs was conducted in Jatimulyo Ecotourism Object Area. Characterization was performed at the Molecular Biology Laboratory, Ahmad Dahlan University. Sampling and characterization were carried out from February to May 2023.

Sampling was conducted using the Visual Encounter Survey (VES) method and direct collection in the field [14]. Samples were taken from three populations, with three male and three female individuals sampled from each population. Morphometric character measurements were performed with 27 characters referring to body sizes according to [15], namely SVL, HL, HW, SL, SN, ED, EN, IND, IOD, UEW, TDV, TDH, ET, LAL, HAL, FE, TL, FL, IMTL, F1L, F2L, F3DW, T4DW, FLL, HLL, FPL, and TFL. Morphometric

character measurements were taken using a vernier calliper (Nankai) with an accuracy of 0.01 mm.

Genetic variation was assessed by calculating DNA polymorphism using microsatellite primers Rnh-9 (F: GCACAGTTAGCGAGATGGA; R: CTCACTAGAGCTGGGTGGTAT) and Rnh-13 (F: GATACGGGAGGCAAACG; R: TCCACAGCCCAGCACTC). DNA isolation was performed using the isolation kit protocol (PROMEGA). The isolated DNA was then amplified using a PCR kit (GoTaq Green PCR Master Mix (2X) Thermoscientific). PCR conditions included initial denaturation at 95°C (2 minutes), denaturation at 95°C (30 seconds), annealing at 58.3°C for Rnh-9 and 56.7°C for Rnh-13 (30 seconds), extension at 72°C (30 seconds), and final extension at 72°C (5 minutes). Denaturation, initial denaturation, annealing, extension, and final extension were performed for 33 cycles [16].

PCR products were then subjected to electrophoresis using a 1.75% agarose gel in 0.5x TAE buffer at 50 V for 60 minutes. Electrophoresis results were observed using a UV transilluminator (Bio-Helix).

Analysis of morphometric character data involved ratioing the measured data to the total length (TL) and logarithmic transformation. This was followed by analysis using the Kruskal-Wallis test to determine the comparative distinguishing morphometric characters among the three populations. Genetic variation analysis was conducted by calculating the percentage of allele polymorphism based on the percentage of polymorphic loci produced with the total number of loci amplified [17]. The formula used was total polymorphic loci/total loci observed x 100% [18]. Subsequently, the morphometric and genetic character data were converted into binary data using MS Excel 2019. Once binary data were obtained, they were transferred to PFE software to construct a dendrogram. The dendrogram was constructed using the MVSP 3.1 program to determine the similarity of characters, both morphometric and genetic.

3 Results and Discussion

3.1 Morphometric characteristics of three populations of *Odorrana hosii*

The research results using the Kruskal-Wallis test indicate that out of the 27 morphometric characters of *Odorrana hosii* studied across three populations, involving 18 individuals in Jatimulyo ecotourism area, there are four characters with a $P<0.05$. These distinguishing characters include HL (head length from snout tip to body boundary), IND (distance between the two nostrils), TL (thigh length, measured from tip to base), and HLL (total leg length), with their respective means, ranges, and significance levels presented in Table 1.

Based on the results of the Kruskal-Wallis test, it is evident that these four characteristics significantly differ among populations. The head length of the three populations of *Odorrana hosii* varies; Kedung Pedut population has a shorter head length compared to Sungai Mudal and Kembang Soka populations. *Odorrana hosii* populations inhabiting highland areas have longer hind limbs compared to other populations, influenced by their daily activities such as foraging and competing for mates with more competitors [19]. The differences in characteristics could be due to environmental factors, such as variations in elevation at the sampling site, leading to disparities in food availability, environmental conditions, and mating and foraging patterns [20].

Subsequently, the morphometric data results were utilised to construct a dendrogram, as depicted in Figure 1. The dendrogram was obtained from data processing using the UPGMA (*Unweighted Pair Group Method Arithmetic Average*) clustering method. There are two major clusters of populations with differences from other populations.

Table 1. The results of the Kruskal-Wallis analysis indicate the distinguishing characteristics of three populations of *Odorrana hosii* in Jatimulyo area of Kulon Progo

Character	Mean ± Stdev						Range	P
	Sungai Mudal Male	Sungai Mudal Female	Kembang Soka Male	Kembang Soka Female	Kedung Pedut Male	Kedung Pedut Female		
SVL/PT	0.45 ± 0.01	0.49 ± 0.03	0.45 ± 0.03	0.50 ± 0.03	0.51 ± 0.05	0.51 ± 0.05	0.45 – 0.51	0.150
HL/PT	0.17 ± 0.01^{a}	0.18 ± 0.00^{a}	0.22 ± 0.02^{b}	0.18 ± 0.01^{a}	0.23 ± 0.02^{b}	0.22 ± 0.01^{b}	0.17 – 0.23	0.020*
HW/PT	0.12 ± 0.01	0.10 ± 0.04	0.12 ± 0.00	0.12 ± 0.00	0.14 ± 0.01	0.12 ± 0.01	0.10 – 0.14	0.147
SL/PT	0.11 ± 0.03	0.09 ± 0.04	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 – 0.11	0.457
SN/PT	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.02 – 0.03	0.090
ED/PT	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.04 – 0.05	0.218
EN/PT	0.05 ± 0.01	0.06 ± 0.03	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.04 – 0.06	0.454
IND/PT	0.04 ± 0.00 ^{ab}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.06 ± 0.01^{c}	0.05 ± 0.00^{c}	0.04 – 0.06	0.016*
IoD/PT	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.04 – 0.06	0.083
UEW/PT	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.00	0.05 – 0.07	0.422
TDV/PT	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.03 – 0.04	0.215
TDH/PT	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.03 – 0.04	0.877
ET/PT	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.01 – 0.02	0.263
LAL/PT	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.12 ± 0.09	0.16 ± 0.04	0.12 – 0.16	0.815
HAL/PT	0.13 ± 0.03	0.13 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.07	0.14 ± 0.06	0.11 – 0.14	0.874
FE/PT	0.22 ± 0.01	0.23 ± 0.01	0.18 ± 0.00	0.19 ± 0.01	0.19 ± 0.07	0.22 ± 0.04	0.18 – 0.23	0.121
TL/PT	0.21 ± 0.01^{a}	0.25 ± 0.02^{a}	0.34 ± 0.03^{bc}	0.38 ± 0.02^{c}	0.35 ± 0.13^{bc}	0.35 ± 0.07^{bc}	0.21 – 0.38	0.045*
FL/PT	0.22 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.22 ± 0.02	0.30 ± 0.14	0.20 ± 0.00	0.19 – 0.30	0.245
IMTL/PT	0.18 ± 0.04	0.17 ± 0.04	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.15 ± 0.08	0.15 – 0.21	0.669
FIL/PT	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.12 ± 0.09	0.06 ± 0.01	0.06 – 0.12	0.864
F2L/PT	0.05 ± 0.02	0.06 ± 0.01	0.03 ± 0.00	0.07 ± 0.00	0.06 ± 0.02	0.05 ± 0.03	0.03 – 0.07	0.112
F3DW/PT	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.04 ± 0.04	0.02 ± 0.00	0.01 – 0.04	0.388
T4DW/PT	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 – 0.02	0.060
FLL/PT	0.12 ± 0.01	0.13 ± 0.03	0.12 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.12 – 0.14	0.864
HLL/PT	0.83 ± 0.01^{b}	0.82 ± 0.00^{b}	0.78 ± 0.02^{a}	0.82 ± 0.01^{b}	0.77 ± 0.02^{a}	0.78 ± 0.01^{a}	0.77 – 0.83	0.020*
FPL/PT	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.06 ± 0.06	0.10 ± 0.08	0.06 – 0.13	0.698
TFL/PT	0.19 ± 0.02	0.20 ± 0.03	0.17 ± 0.00	0.20 ± 0.01	0.07 ± 0.09	0.15 ± 0.12	0.07 – 0.20	0.346

Description: Morphometric characters that significantly differ, $p \leq 0.05$; *: significant from test results

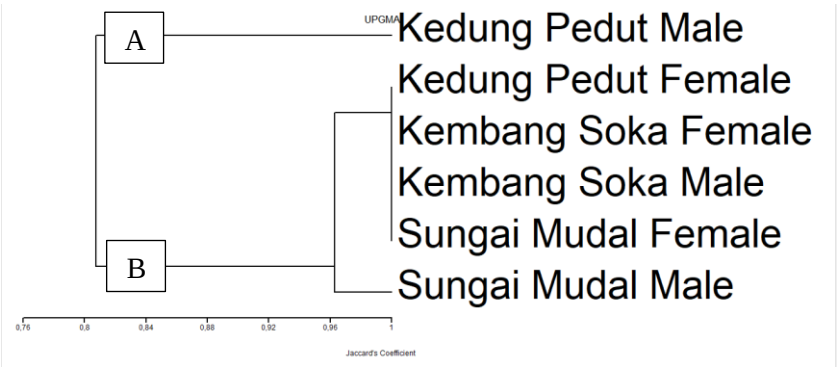

Fig. 1. Dendrogram of three populations *of Odorrana hosii* in Jatimulyo ecotourism area based on morphometric characters

Cluster A comprises the male population of Kedung Pedut, showing relatively low similarity with a similarity value of 0.778. This is evidenced by five differing characters from the 27 characters observed. These differing characters include IND (distance between nostrils), FL (total leg length), FIL (length of the first finger), FPL (length of the third toe), and TFL (length of the fourth toe). Kedung Pedut population also exhibits lower morphometric similarity with the female population of Sungai Mudal and the male and female populations of Kembang Soka, with a similarity value of 0.815 (Table 2). This difference may be influenced by the habitat of Kedung Pedut population being in a lower elevation compared to other populations.

Table 2. Morphometric similarity matrix of three populations of *Odorrana hosii* in Jatimulyo ecotourism area

	Sungai Mudal Male	**Sungai Mudal Female**	**Kembang Soka Male**	**Kembang Soka Female**	**Kedung Pedut Male**	**Kedung Pedut Female**
Sungai Mudal Male	1.000					
Sungai Mudal Female	0.963	1.000				
Kembang Soka Male	0.963	1.000	1.000			
Kembang Soka Female	0.963	1.000	1.000	1.000		
Kedung Pedut Male	0.778	0.815	0.815	0.815	1.000	
Kedung Pedut Female	0.963	1.000	1.000	1.000	0.815	1.000

Cluster B indicates that the male population of Sungai Mudal exhibits low similarity to other populations. This is evidenced by the presence of a distinct character difference among the 27 characters analysed. One notable differing character is thigh length (TL), which shows a low similarity value. The male and female populations of Kembang Soka, the female population of Sungai Mudal, and the female population of Kedung Pedut show moderate similarity among other populations, with a similarity value of 0.963. These populations also share similarities in humidity and air temperature conditions (Table 3) leading to similarities in morphometric characteristics.

Table 3. Abiotic factors in three populations in Jatimulyo ecotourism area

Factor	**Sungai Mudal**	**Kedung Pedut**	**Kembang Soka**
Height (mdpl)	646	563	435
Air humidity (%)	65-80	70-83	68-79
Air temperature (°C)	20-24	24-27	23-26

3.2 Genetic characteristics of three populations of *Odorrana hosii*

Polymorphism percentages varied among the three populations (Table 4). Kembang Soka population exhibited the highest polymorphism percentage at 90.90%. Sungai Mudal population showed the lowest polymorphism percentage at 50%, while Kedung Pedut population displayed higher polymorphism compared to Sungai Mudal at 60%. The findings suggest that Kedung Pedut population possesses a higher molecular variation compared to Sungai Mudal population. Genetic variation is high if the polymorphism percentage results are above 60% and is low if the polymorphism percentage results are below 40% [21]. Therefore, it can be inferred that Kembang Soka population demonstrates high genetic variation, while Sungai Mudal and Kedung Pedut populations show moderate genetic variation.

Table 4. DNA polymorphism of each population of the *Odorrana hosii* produced by primers Rnh-9 and Rnh-13

Population	Total Loci	Total Polymorphic Loci	Total Monomorphic Loci	Percentage of Polymorphism
Sungai Mudal	4	2	2	50.00%
Kembang Soka	11	10	1	90.90%
Kedung Pedut	5	3	2	60.00%

Genetic variation in *Odorrana hosii* based on Rnh-9 and Rnh-13 markers can be analysed using a dendrogram (Figure 2), which is constructed based on the average similarity coefficient using the MVSP 3.1 program. The dendrogram results obtained from the amplification of Rnh-9 and Rnh-13 (Figure 3) show that the number of amplified DNA bands is 67. The average percentage of polymorphic loci for both primers is 66.66%. The similarity coefficient calculation using the UPGMA clustering method and similarity using Jaccard's Coefficient. The similarity values of genetic characteristics of the *Odorrana hosii* can be seen in Table 5.

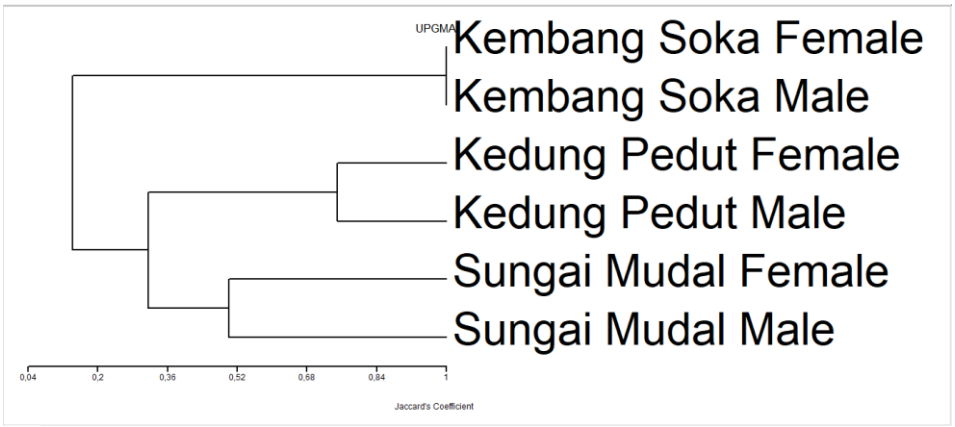

Fig. 2. Dendrogram of three populations of *Odorrana hosii* in Jatimulyo ecotourism area using primers Rnh-9 and Rnh-13

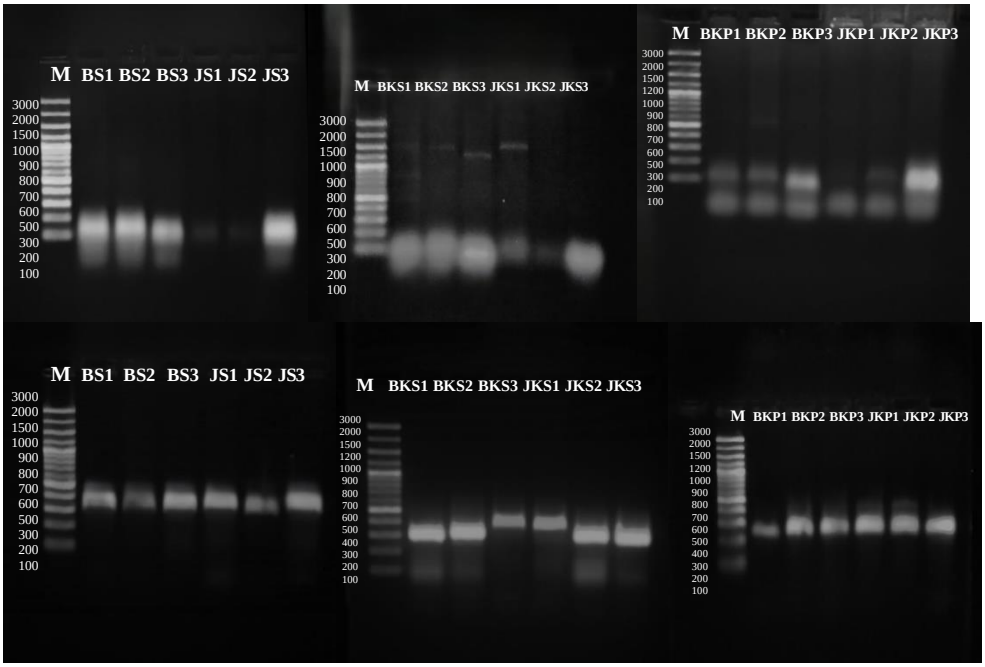

Fig. 3. Amplification results of Rnh-9 (above) and Rnh-13 (below). M=Marker, BS=Sungai Mudal Female, JS=Sungai Mudal Male, BKS=Kembang Soka Female, JKS=Kembang Soka Male, BKP=Kedung Pedut Female, JKP=Kedung Pedut Male.

Table 5. Similarity matrix of three *Odorrana hosii* populations in Jatimulyo ecotourism area using Rnh-9 and Rnh-13 primers

	Sungai Mudal Male	**Sungai Mudal Female**	**Kembang Soka Male**	**Kembang Soka Female**	**Kedung Pedut Male**	**Kedung Pedut Female**
Sungai Mudal Male	1.000					
Sungai Mudal Female	0.500	1.000				
Kembang Soka Male	0.000	0.200	1.000			
Kembang Soka Female	0.000	0.200	1.000	1.000		
Kedung Pedut Male	0.200	0.500	0.200	0.200	1.000	
Kedung Pedut Female	0.167	0.400	0.167	0.167	0.750	1.000

Kembang Soka population has the highest similarity value at 1.000. The lowest similarity value was found in Sungai Mudal population, which is 0.500, while Kedung Pedut population has a moderate similarity value of 0.750. The high similarity value in Kembang Soka population may be due to its geographical location, which is situated between highlands and lowlands. Behavioral patterns, environmental conditions, and reproductive patterns in Kembang Soka population may also contribute to the unique molecular characteristics [22].

The similarity results for *Odorrana hosii* in Table 5 also indicate that the three *Odorrana hosii* populations exhibit significant genetic differences between populations. This could be due to several factors such as geographic isolation in the form of physical barriers,

environmental differences, and reproduction. These factors influence the formation of genetic structure [23-25]. This study has successfully differentiated populations of *Odorrana hosii* based on morphometric characters. Genetic variations among populations have also been well characterized using microsatellite markers. However, only four morphometric traits were found to be significant in distinguishing *Odorrana hosii* populations, and these traits are still influenced by the sex of *Odorrana hosii*.

Research indicates that higher-altitude environments support the morphology and genetics of *Odorrana hosii*. Preserving these highland habitats not only protects this species but also contributes to the achievement of multiple SDGs focused on maintaining ecosystem balance and sustaining life on Earth.

4 Conclusion

The research results indicate that, based on morphometric and genetic analysis, Kembang Soka population has higher values compared to the other populations. Therefore, it can be concluded that Kembang Soka population exhibited the most significant morphometric and genetic variations.

This research was funded independently by the authors. We would like to express our deepest gratitude to everyone who supported us in the course of this study. Data will be made available on request. The authors disclose no conflict.

G.E. Pramesti and N. Suwartiningsih jointly conceived and designed the study. The computational analysis was performed by G.E. Pramesti. Both authors contributed to the writing of the manuscript. The research was funded by N. Suwartiningsih. The manuscript was revised with contributions from both authors. They have reviewed and approved the final manuscript, and both have made significant contributions to the study.

References

1. F. Allendorf, W., Luikart GH, Aitken SN. Konservasi dan genetika populasi. 2 daned. (New Jersey: Wiley-Blackwell, 2012).

2. D. Ardiyansyah. dan A. Priyono. Konservasi Amfibi dan Reptil di Indonesia. Prosiding. Bogor: Seminar hasil penelitian Departemen Konservasi Sumberdaya Hutan (2019).
3. T. Qurniawan, F., Trijoko, Species Composition of Amphibian in Gunungkelir Stream, Jatimulyo Village, Kulon Progo. J. Tekno Sains **2**, 15 (2012)
4. M. C. Pitaloka, Variasi Morfometrik *Odorrana hosii* Boulenger, 1891 (Anura: Ranidae) dari Tiga Populasi di Kawasan Jatimulyo Kulon Progo, Daerah Istimewa Yogyakarta. Skripsi. Yogyakarta: Universitas Ahmad Dahlan (2022).
5. F.R. Inger, D.T. Iskandar, A collection of amphibians from West Sumatra, with description of a new spesies of Megophrys (Amphibia: Anura). The Raffles Bulletin of Zoology **53**, 133 (2005)
6. A. Arista., D. Gunardi., W., Rudi Hilmanto. Keanekaragaman Jenis Amfibi untuk Mendukung Kegiatan Ekowisata di Desa Braja Harjosari Kabupaten Lampung Timur. Biosfera **34**, 103 (2017)
7. Fauzan. Variasi Morfomteri *Fejervarya limnocharis* (Grahenhorts, 1829). Menara ilmu **13**, 72 (2019)
8. D.S.J. Munshi, H.M. Dutta, Fish Morphologiy: Horizon of New Research. (Science Publisher, New York, 1996)

9. P. Bennet. Microsatellites. J. Clin. Pathol. Mol. Pathol, **53**, 177 (2000)
10. B.A. Kusuma, D.G. Bengen, H. Madduppa, B. Subhan, & D. Arafat, Close genetic connectivity of soft coral *Sarcophyton trocheliophorum* in Indonesia and its implication for marine protected area. Aceh J. of Animal Sci. **1**, 50 (2016)
11. D. Ibragimova, V., & S. Lyapkov, M. Demographic and morphometric characteristics of the moor frog *Rana arvalis* from a transformed habitatin the Khanty-Mansi Autonomous Region—Yugra. Biol. Bull. **45**, 831 (2018)
12. H.D. Tjong, V.A. Agesi, & D.I. Roesma, Genetic Variation at Microsatellite Loci in *Odorrana hosii* (Boulenger, 1891). Biogenesis: J. Ilmiah Biol. **8**, 62 (2020).
13. I. Musthofa, A.N. Raafi, T.P. Kuncoro, Panduan Lapangan Herpetofauna (Amfibi & Reptil) di Kawasan Ekowisata Desa Jatimulyo. (Masa Kini, Yogyakarta, 2021)
14. M. Kusrini, D. Pedoman Penelitian dan Survei Amfibi di Alam. Bogor: Pustaka Media Konservasi Institut Pertanian Bogor (2008)
15. L. J. Watters, S. T. Cummings, R. F. Flanagan, C. D. Siler, Review of Morphometric Measurements Used in Anuran Species Description and Recommendation Approach. Magnolia Press. Zootaxa **4072**, 477 (2016)
16. R. Nesty., D. Tjong, H. dan Herwina, H. Variasi Morfometrik Kodok *Duttaphrynus melanostictus* (Schneider, 1799) (Anura: Bufonidae) di Sumatera Barat yang Dipisahkan oleh Bukit Barisan. J. Biol. Univer. Andalas. **2**, 37 (2013)
17. R. Frankham, J.D. Ballou, & D.A. Briscoe, Introduction for genetic conservation, (Universitas Cambridge Tekan, Cambridge, 2002)
18. D. Nuryani. Analisis Keseragaman Genetik Tanaman Teh (*Camellia sinensis (L) O. Kuntze*) Asal Kultur Jaringan, Setek, dan Biji dengan Teknik RAPD. Skripsi. Bogor: Jurusan Kimia. Fakultas MIPA. Institut Pertanian Bogor (2003).
19. E. Chahyadi, Titrawani, & W. H. Rauf, 'Variasi Morfometrikk Bufo asper Gravenhorst (1829) di Kawasan Universitas Riau dan Desa Bencah Kelubi Tapung Kampar', AlKauniyah: J. Biol. **9**, 103 (2016)
20. K. Mistar, dkk., Buku Panduan Lapangan Amfibi dan Reptil Kawasan Hutan Batang Toru, (Herpetologer Mania Publishing, Medan, 2017)
21. K. Ramadhaniah, K. Jenis-Jenis Dan Morfometrik Amphibi (Ordo Anura) di Sekitar Kawasan Taman Wisata Alam Kawasan Danau Dendam Tak Sudah Kelurahan Dusun Besar Kecamatan Singaran Pati Kota Bengkulu. J. Pendidikan Biol. UMB **02**: 1 (2020)
22. Z. Q. Liu, YQ. Wang, & B. Su. The Mitochondrial Genome Organization of Tree Frog, *Fejervarya limnocharis* (Amphibia: Anura): a new gene order in the mtDNA. Gene. **346**, 145 (2010)
23. M. Arioli, C. Jakob, H. U. Reyer, Keanekaragaman genetik dalam Hibrida katak air (*Pelophylax esculentus*) bervariasi menurut struktur populasi dan lokasi geografis. Ekologi Molekuler. **19**, 1814 (2010)
24. A. Fouquet, J. B. Ledoux, V. Dubut, B. P. Noonan, I. Scotti, Interaksi keterbatasan penyebaran, sungai, dan peristiwa sejarah membentuk struktur genetik katak Amazon. Jurnal Biologi Masyarakat Linnean. **106,** 356 (2012)
25. E. Twomey, J. S. Vestergaard, P. J. Venegas, K. Summers, Divergensi mimetic dan kontinum spesiasi pada mimik racun katak *Ranitomeya imitator*. Naturalis Amerika. **187**, 205 (2016)