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Analysis Morphological Characters of *Chalcorana chalconota* (Schelgel, 1837) at Soraya and Ketambe Research Stations, Aceh

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Abstract: *Chalcorana* is a genus of amphibians that consists of species complexes which are difficult to distinguish morphologically. The difficulty in identifying species within this genus has prompted taxonomic research to utilize a more in-depth morphological approach in order to determine a more accurate taxonomic status and to examine the grouping of the *Chalcorana* genus originating from two different locations. This study used a principal component analysis (PCA) approach by measuring morphological characters. Based on the results of this study, it was found that different locations did not affect the variation in morphological characters, and therefore, no grouping was formed based on locality

Keywords: Anuran; *Chalcorana calconota*; Morphology; Principal component analysis

Introduction

Frogs of the genus Chalcorana, particularly Chalcorana chalconota, are one of the most widespread amphibian species in Southeast Asia, including Indonesia. The genus is known as the white-lipped frog with a green body on the dorsal side and white or cream on the ventral side (Arifin et al., 2018). One of its distinctive features is its wide webbed toes, which make it easy for them to adapt to aquatic ecosystems such as rivers, swamps, and tropical rainforests. In addition, Chalcorana chalconota has an important ecological role as an indicator of environmental quality and part of the food chain (Sabinhaliduna et al., 2025).

Despite its wide geographical distribution, morphological variation of Chalcorana chalconota in various locations has not been studied. Environmental factors such as topography, temperature, and ecological pressures can affect its morphological characters (Iskandar et al., 2015). Soraya and Ketambe Research Stations in Aceh are interesting locations for research because ecological differences in these two places have the potential to cause variations in local adaptations. Morphological analysis of these species is expected to help understand environmental influences on body structure and potential population differentiation (Adams et al., 2013).

Significant morphological differences in the genus Chalcorana can be an indication of cryptic species, which are species that appear morphologically similar but genetically different (Agustina et al., 2022). Interactions between genetic and environmental factors can also lead to population isolation, which in turn encourages species differentiation. The study by Hending (2025) highlighted that cryptic species are often difficult to recognize due to their very subtle morphological differences, despite considerable genetic differences. Therefore, modern morphometric and genetic analyses are essential to uncover hidden biodiversity within cryptic species groups such as Chalcorana. Morphometric research is particularly important in

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supporting taxonomic and systematic studies of Chalcorana chalconota, given the difficulty in distinguishing species in this genus (Agustina et al., 2022). Morphometry involves measuring morphological characters in general and can be used to describe variation (Wang et al., 2022).

Factors influenced by the environment can affect the formation of complex species through the mechanism of isolation between populations, limited migration, and differences in the pressure of environmental factors on species so that populations that are separated or have different ecotypes will show variation and differentiation of characters into different species (Priambodo et al., 2021). Therefore, this study aims to analyze the morphological variation of Chalcorana chalconota in Aceh, which is expected to provide scientific insights into biodiversity and support conservation efforts for this species.

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Method

Collecting Sampling Chalcorana chalconota at Soraya and Ketambe Research Stations

Chalcorana chalconota sampling was conducted in two different locations, namely, Soraya Research Station in Sultan Daulat District, Subulussalam City and Ketambe Research Station in Ketambe District, Southeast Aceh Regency. The method used in field sampling uses the Visual Ecounter Survey (VES) method which focuses on direct capture in the field by exploring areas suspected of being *C. chalconota* habitat. The total number of samples collected was 7 individuals from Soraya Research Station and 19 individuals from Ketambe Research Station.

Identification Sampling of Chalcorana chalconota

Morphometric identification of frog samples was carried out at the Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Syiah Kuala University, Banda Aceh. The measurements taken were 32 characters including snout to tail length (SVL), snout to tympanum distance (JMoT), internares distance (JIN), eye to tympanum distance (JMT), tympanum diameter (DT), manus to digiti length (PMD), antebrachium length (Pab), Length of first toe of forefoot (PJ1KD), length of third toe of forefoot (PJ3KD), length of femur/thigh (PF), Length of first toe of hindfoot (PJ1KB), Length of metatarsus to fourth toe of hindfoot (PMTJ4), Length of tarsus to fourth toe of hindfoot (PTJ4), Length of hindfoot (PKB), metatarsal tarsus length (PTM), third toe disc diameter (DJ3), tibia length (PT), hind toe length (PJ4KB), snout length (PM), branchium length (PBr), head width (LK), interorbital distance (JIO), eye length (PM), eye to nose distance (JMH), nostril to eye distance (JNM), head length (PK), nose to mandible distance (JHM), mandible to front eye distance (JMiMD), mandible to hind eye distance (JMiMB), eyelid length (PKM), nose to tympanum distance (JHT), and nose to snout distance (JHMo) (Djong et al., 2007; Nesty et al., 2013).

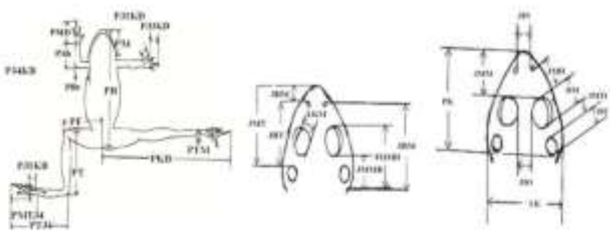

Figure 1. Amphibian morphometric measurements (Tjong et al., 2007)

Result and Discussion

Principal Component Analysis Based on Morphometric

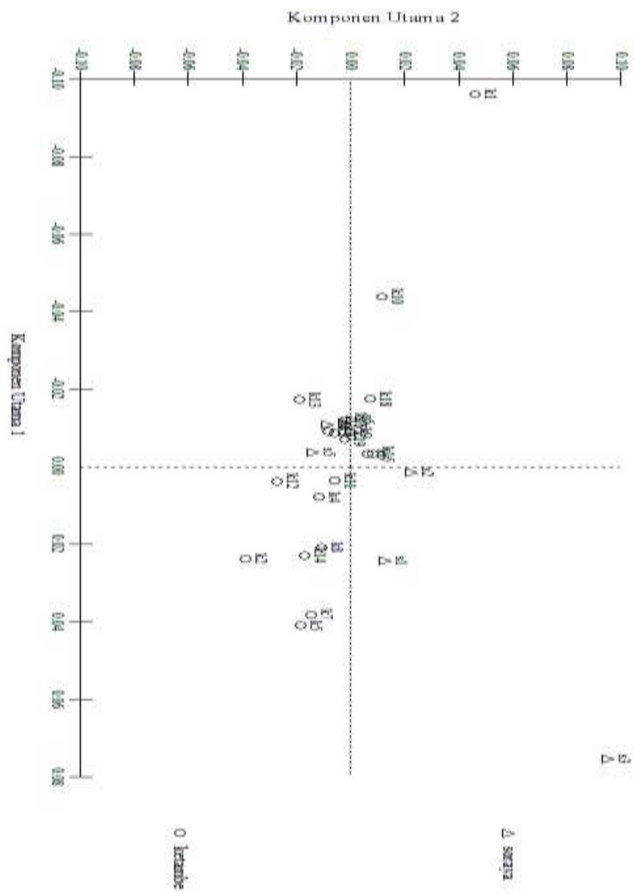

Figure 2. Graph of PCA sample

Samples of *Chalcorana chalconota* species were analyzed based on measurements of 32 morphological characters. Eigenvalues of principal component 1 (PC1) and principal component 2 (PC2) have a cumulative contribution of more than 50%, both of which are sufficient to represent factors affecting sample differences. The number of principal components is determined based on the percentage of cumulative contribution that at least dominates the total of 100% (>50%). In this calculation, two principal components with a cumulative contribution of 59.502% were selected, which is sufficient to represent all components.

Table 1. UPGMA Analysis, Bray-Curtis

Node	Objects			
	Group 1	Group 2	Dissimil.	in group
1	k9 (ketambe)	k19 (ketambe)	0.020	2
2	s7 (soraya)	Node 1	0.022	3
3	k4 (ketambe)	k11 (ketambe)	0.023	2
4	Node 2	k17 (ketambe)	0.023	4
5	k3 (ketambe)	k18 (ketambe)	0.024	2
6	Node 4	Node 3	0.027	6
7	s6 (soraya)	Node 5	0.027	3
8	k8 (ketambe)	k14 (ketambe)	0.028	2
9	Node 7	Node 6	0.029	9
10	k5 (ketambe)	k7 (ketambe)	0.032	2
11	Node 9	k16 (ketambe)	0.034	10
12	Node 11	k6 (ketambe)	0.037	11
13	Node 10	Node 8	0.039	4
14	Node 12	k15 (ketambe)	0.040	12
15	s5 (soraya)	Node 14	0.042	13
16	Node 15	k10 (ketambe)	0.047	14
17	s4 (soraya)	k13 (ketambe)	0.049	2
18	Node 16	Node 13	0.051	18
19	Node 18	k12 (ketambe)	0.054	19
20	Node 17	Node 19	0.058	21
21	s2 (soraya)	Node 20	0.061	22
22	Node 21	k2 (ketambe)	0.063	23
23	Node 22	k1 (ketambe)	0.067	24
24	s1 (soraya)	Node 23	0.072	25
25	Node 24	s3 (soraya)	0.090	26

The results of the clustering analysis showed that the cluster pattern did not follow a locality-specific pattern, indicating that the samples from Soraya Research Station and Ketambe Research Station did not form clear groups based on the sampling location. In other words, there were no significant differences between the two localities in terms of sample clustering patterns. This finding is in line with the results of the PCA visualization, where the distribution of samples showed that the influence of the principal component on each sample varied. For example, sample s3 has the highest value on the PC2 axis (0.091), which means that this sample is more influenced by PC2 with a positive correlation. In contrast, sample k1 has the highest value on PC1 (-0.093), so it is more influenced by PC1 with a negative correlation. The random cluster pattern reinforces that locality is not the main determinant in clustering the samples. These findings are consistent with the study by Seljestad et al. (2020), which showed that PCA and DAPC analyses can reveal population structures that do not always match geographic distributions, as well as the study by Struck et al. (2023), which emphasized the importance of modern morphometric and genetic analyses in uncovering hidden biodiversity in cryptic species complexes.

Based on UPGMA analysis with the Bray-Curtis similarity index, it was found that the sample with the highest similarity was sample k9 with sample k19 from SP Ketambe where both had the lowest dissimilarity index of 0.020, while the sample with the highest dissimilarity index was sample s3 from SP Soraya, which was 0.090. Apart from these samples, there are also samples from different localities but have the same dissimilarity index value, namely sample k13 from SP Ketambe with sample s4 from Soraya Research Station, which is 0.049. This reinforces that the locality samples from Ketambe and Soraya Research Station do not have significant differences. The visualization of the Bray-Curtis similarity index in the form of a Dendogram as shown in the figure below.

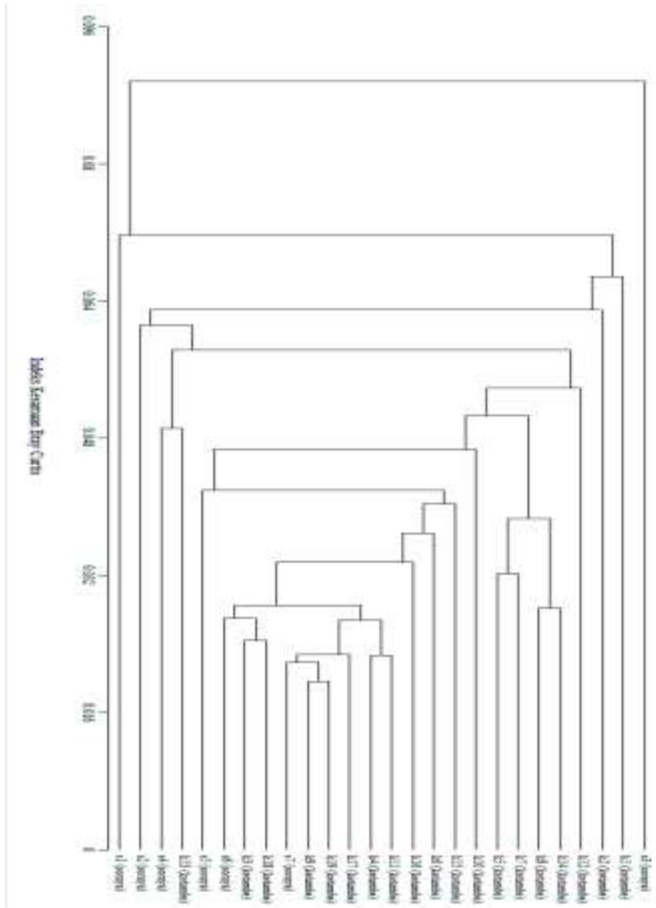

Figure 3. UPGMA phylogenetic dendrogram result

Environment Parameters

This study measured various environmental parameters such as water temperature, soil temperature, air temperature, humidity, soil pH, and water clarity at Soraya and Ketambe Research Stations to assess habitat suitability for the Anura order. The two locations have a significant difference in altitude, with Soraya at 110 meters above sea level (masl) and Ketambe at 1080 masl. However, measurements showed that water and soil temperatures at both sites were within the optimal range for Anura life, between 25-30°C (Mardinata et al., 2018). Temperatures at Soraya were slightly higher than Ketambe, but still within the tolerance range for this species.

High air humidity, which is 90% in Soraya and 100% in Ketambe, also benefits Anura because it is able to maintain water balance in the body and support the process of respiration and reproduction (Camacho et al., 2020). In addition, soil pH in Soraya (7.4) and Ketambe (7.0) is still in the neutral range (6.5-8.5), which is suitable to support the reproduction and development of Anura populations (Nasaruddin, 2008). Water clarity at both sites was also very good, at 0.080 ppm in Soraya and 0.157 ppm in Ketambe, in accordance with Hilsenhoff's (1988) standard classification, indicating low levels of water pollution. Thus, the measured environmental parameters indicate that the habitat conditions at Soraya and Ketambe Research Stations support the survival and development of Anura optimally.

Table 2. Parameter Measurements Result

Parameter Measurements	Research Stations	
	Soraya	Ketambe
Water pH	7.4	7.0
Soil temperature (°C)	26	25°C
Air humidity (%)	90	100
Water temperature (°C)	28.6	25.5
Clarity (ppm)	0.080	0.157
Air temperature (°C)	31	29

Description: Parameter measurements were taken at the same time (20.00-23.00 WIB)

Results should be clear and concise. The discussion should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

The results of this study indicate that morphological variation in *Chalcorana chalconota* across different ecological locations is strongly influenced by environmental factors, supporting the concept of phenotypic plasticity in amphibians. Amphibians are highly sensitive to environmental changes due to their permeable skin and biphasic life cycle (Duellman et al., 1994; Wells, 2010). This sensitivity allows them to respond rapidly to environmental pressures, resulting in observable morphological variation (Schmidt et al., 2006; Touchon et al., 2011).

Environmental heterogeneity plays a fundamental role in shaping morphological traits. Variations in habitat structure, water availability, and ecological pressures can significantly influence body size, limb proportions, and functional morphology (O'Brien et al., 2009; Stahlschmidt et al., 2011). Such variation is often associated with adaptive responses that enhance survival and locomotion efficiency, particularly in semi-aquatic amphibians (Emerson, 1978; Licht et al., 1972). In addition, climate-related factors such as temperature and precipitation also influence growth and development, leading to differences in morphological traits among populations (Sharma et al., 2007; Sheridan et al., 2011).

From an evolutionary perspective, morphological variation may indicate ecological divergence driven by natural selection. Populations occupying distinct ecological niches tend to develop different morphological characteristics as adaptive strategies (Harcombe, 2010; Schluter, 2000)(Losos, 2010; Schluter, 2000). This process is closely related to adaptive radiation, where species diversify morphologically in response to environmental opportunities (Grant et al., 2006). Therefore, differences observed between populations in this study may represent early stages of ecological differentiation.

Moreover, the possibility of cryptic species within Chalcorana chalconota cannot be ignored. Amphibians are known to harbor high levels of cryptic diversity, where genetically distinct lineages exhibit minimal morphological differences (Bickford et al., 2007; King et al., 2018). This highlights the importance of integrating molecular approaches such as DNA barcoding to complement morphological analyses and reveal hidden biodiversity (Lorenz et al., 2005; Sacks et al., 2005).

The use of morphometric approaches, particularly geometric morphometrics, provides a more precise method for analyzing subtle morphological differences. This method allows researchers to quantify shape variation and identify patterns that are not easily detected using conventional techniques (Adams et al., 2008; Zelditch et al., 2012). Additionally, multivariate statistical analysis enhances the ability to interpret complex morphological data and relate it to environmental gradients (Klingenberg, 2016).

Anthropogenic factors also contribute significantly to morphological variation. Habitat fragmentation, environmental degradation, and climate change can impose selective pressures that alter phenotypic traits (Cushman, 2006; Matenaar et al., 2015). These pressures may lead to long-term evolutionary changes or even population declines (Collins et al., 2003; Wake et al., 2008). Furthermore, global environmental changes have been shown to affect amphibian populations worldwide, making them one of the most threatened vertebrate groups (Alford et al., 1999; Stuart et al., 2004).

Ecologically, morphological traits are closely linked to fitness and survival. Body size, limb length, and structural adaptations influence locomotion, predator avoidance, and foraging efficiency (Emerson, 1978; Licht et al., 1972). Therefore, variations observed in this study may reflect adaptive strategies that optimize survival in specific environmental conditions.

In the broader context, this study contributes to understanding amphibian biodiversity, particularly in Southeast Asia, which is recognized as a global biodiversity hotspot (Griffiths et al., 2008). However, many amphibian species in this region remain understudied, especially regarding intraspecific variation. Documenting morphological diversity is crucial for conservation and biodiversity management (Parmesan, 2006).

Despite its contributions, this study has several limitations, particularly the absence of molecular data to validate morphological differences. Future research should adopt an integrative approach combining morphological, genetic, and ecological data to provide a more comprehensive understanding of species variation (Lorenz et al., 2005; Sacks et al., 2005). Long-term monitoring is also necessary to assess the stability of morphological traits in response to environmental changes.

Overall, this study confirms that morphological variation in amphibians is influenced by a complex interaction of environmental factors, ecological adaptation, and evolutionary processes. These findings emphasize the importance of integrative and multidisciplinary approaches in studying biodiversity, particularly for taxa with high potential for cryptic speciation such as *Chalcorana chalconota*.

Conclusion

The results of morphometric analysis showed that there were no significant morphological differences between Chalcorana chalconota populations from Soraya and Ketambe Research Stations. Principal component analysis (PCA) utilizing two principal components (PC1 and PC2) showed that samples from both locations were randomly distributed and did not form a clustering pattern based on locality. This was reinforced by the results of the clustering analysis, which did not show a cluster structure consistent with the sampling location. In addition, UPGMA analysis using the Bray-Curtis similarity index confirmed that samples from both areas had a high degree of morphological similarity. Overall, these results indicate that the geographical differences between the two sites are not sufficient to promote morphological isolation, which is one of the prerequisites for speciation, despite the significant elevation of the sites. This finding is in line with previous research in other locations. Priambodo et al. (2021) found that C. chalconota populations on Java Island also showed a clustering pattern that was not consistent with locality based on PCA analysis and clustering, and had a high Bray-Curtis similarity index. This indicates a strong level of morphological similarity between populations from different regions. Similarly, Hanifuddin et al. (2022) in a morphometric study of C. chalconota populations in Kulon Progo found that, despite some variations in body size and minor morphological characters, most populations were highly similar to each other. This study reinforces the assumption that local environmental factors have not been strong enough to shape significant morphological differentiation. This condition has also been reported in other amphibian groups, as discussed by Struck et al. (2023), that in the case of cryptic species, genetic differences are not always accompanied by clear morphological differences, so a multidisciplinary approach is needed to identify the overall population structure.

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Author Contributions

Fanny Lestari management and coordination responsibility, conducted a research, investigation process and making the journal draft; Essy Harnelly supervising, conducted literature review and provided critical feedback on the manuscript; Tjong Hon Djong conceptualized the research idea and designed of methodology.

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This research received no external funding.

Conflicts of Interest

The authors declare that this study on Chalcorana chalconota distributed in parts of Aceh was conducted without any conflict of interest, either financial or non-financial. All procedures of data collection and analysis related to the low genetic diversity and the uniformity of phylogenetic character distribution were carried out independently and objectively. The similarity of morphological characters observed across the two study areas was used solely to map species distribution patterns and to understand the relationship between environmental factors that contribute to the maintenance of distinct species traits, without influence from any external interests.

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