Morphological characters review on white-lipped frog (*Chalcorana chalconota*; Schelgel, 1837) based on morphometrical analysis, within the population of Java

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**Abstract.** *Chalcorana chalconota* or White-Lipped Frogs are widely distributed in Indonesia, Malaysia, and Thailand. Described as a species complex, *C. chalconota* from Java Island is very necessary to be studied its taxonomic status. Intra-population studies are important to determine the representation of a population in a particular place. Therefore, we have aimed to elaborate on the relationship of *C. chalconota* within the Java population using a morphometric approach. This research was conducted in the Laboratory of Herpetology, Research Center for Biology, Indonesian Institute of Sciences. Morphometric measurements were performed totally on 47 individuals of *C. chalconota* from Java Island with 29 morphological characters. Data analyses using Principal Component Analysis (PCA) and cluster analysis with Bray-Curtis Index. PCA on both sexes investigated a random grouping, both samples from West Java, Central Java, East Java, and Banten. Clustering analyses on male and female population are indicating high indexed of Bray Curtis with score up to 0.9, means very closely related among individual. Such as PCA, clustering analysis produces the same grouping patterns (random) from different localities. Based on PCA and clustering analysis, we interpreted that *C. chalconota* in the Javanese population was closely related with evidence of conserved morphological characters.

1. **Introduction**

Morphometry is one way to determine the diversity of a species by measuring the phenetic (morphological characters) in general. The analysis of morphometry can be used to describe the morphological relationship between populations by their presence or absence of variation and differentiation of species, it also can be collaborated with molecular data (genetic) so that it can reveal the presence or absence of plasticity, adaptive radiation or even genetic-based changes [1]. Each character observed is generally a result of the interaction of genes whose expression is influenced by the environment [2]. As a further application, the results of a morphometric analysis can also provide a general description of the level of variability or diversity of characters of taxa in various populations so that it becomes the initial basis for estimating genetic variability [3].
Differences in geographical position, ecological conditions, and the presence of physical barriers in an area are important factors that are suspected to strongly trigger the specification of the expression of genes that will cause variations in character differentiation between populations [1]. This condition can occur through the mechanism of isolation between populations, migration limitations, and differences in the pressure of environmental factors on species so that populations that are separate or have different ecotypes will show variations and differentiation of characters to become a species complex.

First described namely *Hyla chalconotus* (Schlegel, 1837) then this species underwent many revisions into nowadays called as *Chalcorana chalconota* [4,5]. Described originally from Java, *C. chalcononata* proposed as a species complex by Inger et al. (2009) [6]. *C. chalconota* species complex including *C. labialis*, *C. eschatia*, *C. parvaccola*, *C. megalonesa*, *C. raniceps*, and *C. rufipes* are suggested and applied to be a full species [6]. The characteristic of White – Lipped Frog or *C. chalconota* are small and medium-sized frog with brown tympanum. Long and slender legs with fully webbed toes to the tips of the fingers and lower thighs reddish and brownish. The skin is usually greenish-gray to yellowish-brown and for the males, dorsal are always covered by small nodules. It has a size ranging for males between 30-40 mm, and females 45-65 mm [7]. This species are widely-distributed in Southeast Asia such as Indonesia (Sumatra, Java, Bali, Borneo, and Sulawesi), Peninsular Malaysia, and Southern Thailand [8]. A species complex always interesting to study and taxonomically need to be solved. Because this species has the type locality from Java, we have aim to elaborate on the relationship of *C. chalconota* from Java, based on the morphological characters using morphometrical analysis.

2. **Material and Method**

This research was conducted in the Laboratory of Herpetology, Research Center for Biology, Indonesian Institute of Sciences. We used specimens stored in 70% alcohol from the collection of Museum Zoologicum Bogoriense (MZB) (Figure 1). Morphometric analysis was done using 25 individuals of female and 22 individuals of male (Table 1). We have modified the morphological observations from Islam et al. (2008) and using 29 morphological characters as follows: Inter-Narial Space (IN), Distance front of eye to nostril (EN), Eye Length (EL), Tympanum-eye Distance (TEL), Tympanum Diameter (TD), Distance from front of eye to tip of snout (SL, Head Length (HL), Inter-Orbital distance (IOD), Head Width (HW), Distance from back of mandible to nostril (MN), distance from back mandible to front of eye (MFE), distance from back of mandible to back of eye (MBE), maximum width of upper eyelids (UEW), distance from nostril to tip of snout (NS), Nostril tympanum length (NTL), Snout Tympanum Length (STL), Third Finger Length (3FL), First Finger Length (1FL), Mouth angle-snout Length (MSL), Forelimb Length (FLL), Lower Arm Length (LAL), Snout-Vent Length (SVL), Hindlimb Length (HLL), Thigh Length (THIGL), Tibia Length (TL), Length of Tarsus and Foot (TFOL), Inner Toe Length (ITL), and Fourth Toe Length (4TL). For standardizing the measurements, every character was divided by SVL (snout-vent length) and must be shown in percentage (%) [9,10]. Then data were transformed into log 10 values then analysed for Principal Component Analysis (PCA) and Clustering Analysis using open software PAST 4.02 [11].

![Figure 1. C. chalconota’s MZB specimen collection](image-url)
3. **Results and Discussion**

Males of *C. chalconota* are smaller than female, thus the measurements are separated by the sex. The length of the frog body is identified by the Snout-Vent Length (SVL), that measured along from snout to the vent. The morphological measurements of SVL in the female population showed that the largest individual was 7 cm and the smallest individual was 4.42 cm. Meanwhile, at the male population, the largest and the smallest individuals were 6.2 cm and 3.48 respectively. On the other hand, we also identified the webbing formula of *C. chalconota*’s toe. The results point out that mostly females and males got the webbing formula such as TOE: I 0 - 1/2 II 0 – 1 III 0 – 1 IV 1 – 0 V (Figure 2).

![Figure 2](image)

**Figure 2.** (a) Web of female *C. chalconota*, (b) Web of male *C. chalconota*

**Table 1.** Morphological characters measurement from Java population.

| Morphological Characters | Measurements in centimeters (cm)* |
|--------------------------|----------------------------------|
|                          | ♀, n=25                          | ♂, n=22                           |
| IN                       | 0.57 ± 0.08 (0.43-0.77)          | 0.43 ± 0.07 (0.34-0.61)           |
| EN                       | 0.69 ± 0.09 (0.48-0.91)          | 0.52 ± 0.09 (0.4-0.74)            |
| EL                       | 0.73 ± 0.12 (0.26-0.9)           | 0.6 ± 0.08 (0.42-0.8)             |
| TEL                      | 0.17 ± 0.07 (0.03-0.37)          | 0.12 ± 0.06 (0.05-0.34)           |
| TD                       | 0.48 ± 0.08 (0.3-0.68)           | 0.43 ± 0.07 (0.29-0.6)            |
| SL                       | 0.95 ± 0.12 (0.67-1.16)          | 0.71 ± 0.1 (0.55-0.9)             |
| HL                       | 2.13 ± 0.21 (1.63-2.42)          | 1.67 ± 0.23 (1.33-2.2)            |
| IOD                      | 0.59 ± 0.1 (0.4-0.89)            | 0.44 ± 0.08 (0.3-0.65)            |
| HW                       | 1.96 ± 0.29 (1.4-2.76)           | 1.35 ± 0.23 (0.96-1.96)           |
| MN                       | 2.04 ± 0.31 (0.84-2.47)          | 1.58 ± 0.24 (1.23-2.1)            |
| MFE                      | 1.43 ± 0.16 (1.14-1.78)          | 1.12 ± 0.16 (0.85-1.55)           |
| MBE                      | 0.87 ± 0.27 (0.6-1.96)           | 0.59 ± 0.09 (0.46-0.8)            |
| UEW                      | 0.48 ± 0.11 (0.32-0.8)           | 0.41 ± 0.13 (0.26-0.78)           |
| NS                       | 0.31 ± 0.05 (0.22-0.42)          | 0.24 ± 0.05 (0.15-0.34)           |
| NTL                      | 1.43 ± 0.21 (0.65-1.67)          | 1.1 ± 0.15 (0.87-1.46)            |
| STL                      | 1.65 ± 0.29 (0.51-1.97)          | 1.26 ± 0.19 (0.97-1.7)            |
| 3FL                      | 1.59 ± 0.22 (1.05-1.96)          | 1.14 ± 0.17 (0.85-1.59)           |
The data are shown in order by the average and standard deviation, and then followed by numbers in parentheses that indicate the minimum and maximum measurements.

Principal Component Analysis (PCA) on the female population showed no grouping cluster of samples (Figure 3). 25 individuals of C. chalconota were collected from different localities including Banten, West Java, Central Java, and East Java, then we suspect that it could be a separate cluster from different localities. However, the PCA results shows that the samples are indicating a random distribution. As well as the results from the females PCA, 22 male individuals of C. chalconota also show a random distribution from different localities (Figure 4). Seems different localities do not cause a significant difference in the C. chalconota morphology. Inger et al (2009) showed that the morphometric study of C. chalconota from Java had a normal body proportion (SVL) when compared to samples from Kalimantan or Sumatra (could be smaller or larger).

Several ecological factors such as climate, habitant, barriers, and species behaviour could be shaping a differentiation for the population structure [12,13]. Different localities certainly have the characteristics of each environment as complex topology and heterogeneous habitats. However, the presence of these factors does not make any significant difference in this species phenotype. As a species complex, unsurprisingly if this species can survive easily at several conditions of habitat. Hewitt (2000) proposed that a widely-spread species exist because of suitable habitats. This also indicates that this species able to do a long term persistent and could even cause a speciation [14].

![Figure 3. Principal Component Analysis (PCA) on female population. Different shape means sample localities (□ West Java, ○Central Java, ◇ East Java, + Banten).](image-url)
Figure 4. Principal Component Analysis (PCA) on male population. Different shape means sample localities (□ West Java, ○ Central Java, ◇ East Java, + Banten).

We are lacking samples from East Java. We only have two samples from Malang, East Java and it does not adequately represent the group representatives from East Java. We assumed that the results of the analysis of the PCA and clustering analysis still unresolved or random because of need more data. More complete data certainly needs to resolve a species complex.

Figure 5. Clustering analyses on female (left) and male (right) population by UPGMA with Bray-Curtis Similarity Index
Phenogram with Bray-Curtis Similarity Index reveals the high number of similarities, approximately ranging from 0.978 to 0.991. It means, either female or male population are close-related between samples, respectively. Bray-Curtis Similarity index is used to determine the level of similarity of several places where the biota or environment forms similarity. Bray- Curtis Index interval ranges from 0-1, the closer to 1, the higher the level of similarity was produced [15].

At the female phenogram, the most distantly-related sample was from LIPI Botanical Garden (West Java) with approximately 0.979 Bray-Curtis’s score (Figure 5 – left side). Meanwhile, at the male phenogram (Figure 5 – right side) showed two samples from Karumbi, West Java were farthest detected with the 0.9795 Bray-Curtis similarity. Even though the samples were detected as the most distant relatives, the similarity rate is up to 0.9. Thus, we classified it as a very high relationship is exist. That phenogram (Figure 5) were justified the PCA analyses (Figure 3 & 4) above that it still generates a close relationship among the Java population. According to Inger et al (2009), our results shows a similar results that *C. chalconota* is a species complex with a taxonomic confusion. We conclude that *C. chalconota* is morphologically conserved, even it came from different localities.

4. Conclusion
Based on the morphological approaches by PCA and clustering analysis, we observed that as a species complex which is widely distributed in the Java, *C. chalconota* showed a preserved and stable morph. The relationship of the Java population was close-related among samples, and there is no significant difference. For further research, genetic and molecular approaches are good tools to uncover this complexity of *C. chalconota*.

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References
[1] Hillis D M, and Wiens J J 2000 *Systematic Biology* **53**
[2] Munshi, J S D, and Dutta H M. 1996 *Fish Morphology: Horizon of New Research* (New York : Science Publishers, Inc.)
[3] Chernoff B 1982 *Amer.Zool.* **22** 425-439
[4] Schlegel H 1837 *Abbildungen neuer oder unvollständig bekannter Amphibien, nach der Natur oder dem Leben entworfen, herausgegeben und mit einem erläuternden Texte begleitet. Part 1.* (Düsseldorf: Arnd & Co)
[5] Oliver L E, Prendini, Kraus F, and Raxworthy C J 2015 *Molecular Phylogenetics and Evolution* **90** 176–192.
[6] Inger R F, Stuart B L, and Iskandar DT 2009 *Zoological Journal of the Linnean Society* **155** 123–147
[7] Iskandar D T 1998. *The amphibians of Java and Bali. Indonesia* (Bogor: Research and Development Centre for Biology-LIPI)
[8] Iskandar D T, and Colijn E 2000 *Truebia* 2009 1–133
[9] Islam M M, Kurose N, Khan M M R, Nishizawa T, Kuramoto M, Alam M S, Hasan M, Kurniawan N, Nishioka M, Sumida M 2008 *Zool Sci* **25** 1084–1105
[10] Kurniawan N, Djong T H, Islam M M, Nishizawa T, Belabut D M, Sen Y H, Wanichan R, Yasir I, and Sumida M 2011 *Zoolological Science* **28(1)** 12–24
[11] Hammer Ø, Harper D A T, and Ryan P D 2001. *Palaeontology Electronica* **4(1)** 9pp.
[12] Avise J T 2009 *Journal of Biogeography* **36** 3–15.
[13] Zhu F, Liu Q, Che J, Zhang L, Chen X, Yan F, Murphy R, Guo C, Guo, P 2016 *Zoologica Scripta* **45** 252–262.
[14] Hewitt, G 2000 *Nature* **405** 907–913.
[15] Bailey, R C, Richards H N, Trefor B R 2004 *Bioassessment of Freshwater Ecosystems* (USA : Kluwer academis Publishers)