

## Identification of Sexual Dimorphism in *Nyctixalus margaritifer* Based on Morphometric Analysis at Situ Gunung Sukabumi

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**Abstract.** The *Nyctixalus margaritifer* frog is a native species on Java island and nature's existence is increasingly threatened. This species has very limited distribution in West Java, and there is no information regarding sexual dimorphism. Therefore, this research aimed to explore the presence of *N. margarite* in the Situ Gunung area in the Gunung Gede Pangrango National Park (TNGGP) and analyze sexual dimorphism based on morphometrics. The research was conducted in September-December 2023, and data were collected using the Visual Encounter Survey (VES) method. The coordinates of individual *N. margaritifer* were recorded using GPSMAP, followed by analyzing 23 morphometric characters using the non-parametric Mann-Whitney. The Principal Component Analysis (PCA) was carried out to determine characters that form group structures. The data obtained were tested using the Bartlett homogeneity variance test and declared significant at  $p$ -value  $< 0.05$ . The statistical analysis was performed using R software. The results showed that there were 6 males and 10 females of *N. margaritifer* in 4.34-hectare home range found at 10-80 cm height from the ground, attached to the leaves or stems of fern *Shillaginella* sp., taro *Homalomena* sp., *Cyrtandra* sp., and *Laportea* sp. Regarding defensive behavior in the presence of humans, *N. margaritifer* were observed to stop moving and making sounds, pressed their limbs on the perches, remained still, and appeared dead. The results of the analysis obtained three characters that differentiate sex with significant  $p$ -value  $< 0.05$ , namely snout-vent length (SVL), third toe length (T3), and wrist width (Lt.M). PCA scatter plot showed that the three characters did not contribute maximally as separators. Meanwhile, the K mean cluster analysis produced clustering of SVL, T3, and Lt.M, which was not strong enough to be used as sex-distinguishing characters.

**Keywords:** frogs, morphometrics, *nyctixalus margaritife*, sexual dimorphism

### Citation

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## INTRODUCTION

The pearly tree frog *Nyctixalus margaritifer* is an endemic species on Java island, and its existence in nature is increasingly threatened. In Indonesia, the Anura diversity includes six families: *Bufonidae*, *Dicroglossidae*, *Microhylidae*, *Megophryidae*, *Ranidae*, and *Rhacophoridae*. *Rhacophoridae* is distributed in Africa, India, Sri Lanka, and Sundaland. The genus *Nyctixalus* belongs to the family *Rhacophoridae* and is distributed in the Philippines, South Vietnam, Malaysia, and Indonesia (Alhadi et al., 2021). The *N. margaritifer* is one of 28 Anura species that live in West Java (Alhadi et al., 2021; Frost, 2023)

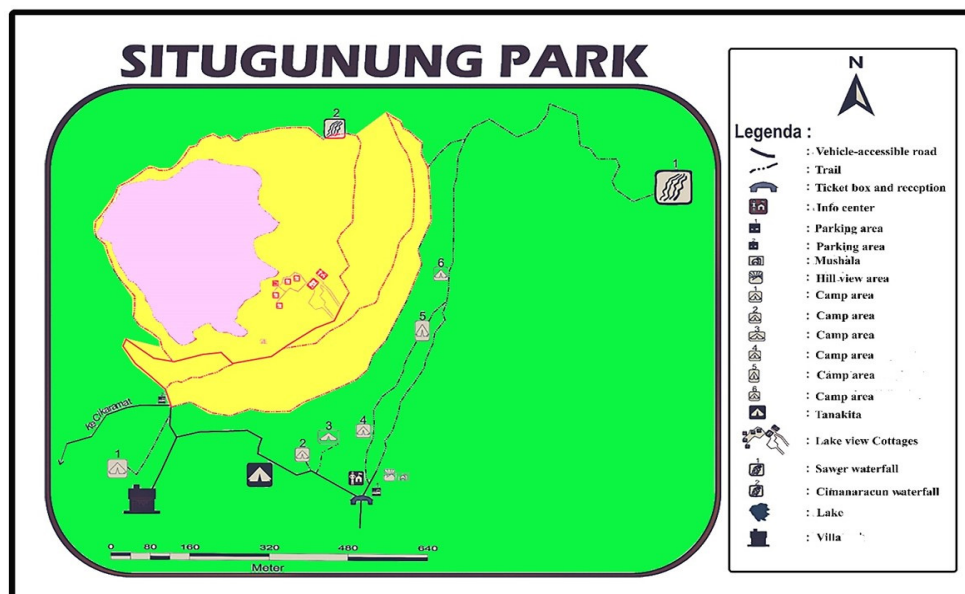
There are three species of *Nyctixalus*: *Nyctixalus margaritifer*, *Nyctixalus pictus*, and *Nyctixalus spinosus* (Pyrton & Wiens, 2011; Frost, 2023). The *N. margaritifer* is distributed in the Philippines, South Vietnam, Malaysia, and Indonesia, particularly on Java Island, mainly in Mount Gede Pangrango, Mount Halimun Salak, Situ Gunung, Mount Putri, and Mount Wilis (Frost, 2023). In the West Java region, *N. margaritifer* showed

very limited distribution and was rediscovered in 1997 at Mount Gede Pangrango and Situ Gunung Sukabumi (Hanifa et al., 2023), without information on sexual dimorphism.

Estimating the *N. margaritifer* population and its natural habitat is challenging due to its limited distribution, which is not recorded in West Java (Rumanta et al., 2019). Therefore, this research aimed to explore *N. margaritifer's* presence in Situ Gunung in the Mount Gede Pangrango National Park (TNGGP) and analyze the sexual dimorphism based on morphometrical characters.

## MATERIALS AND METHODS

The research *N. margaritifer* was carried out at Situ Gunung in the Mount Gede Pangrango National Park (TNGGP), using the Visual Encounter Survey (VES) method by walking through the observation location (Figure 1). Data collection was conducted in September-December 2023, at 19.00-23.00 WIB. The *N. margaritifers* were caught using a net, flashlight, and headlamp, followed by placement in the container, and brought to the ranger station for morphometric measurement.



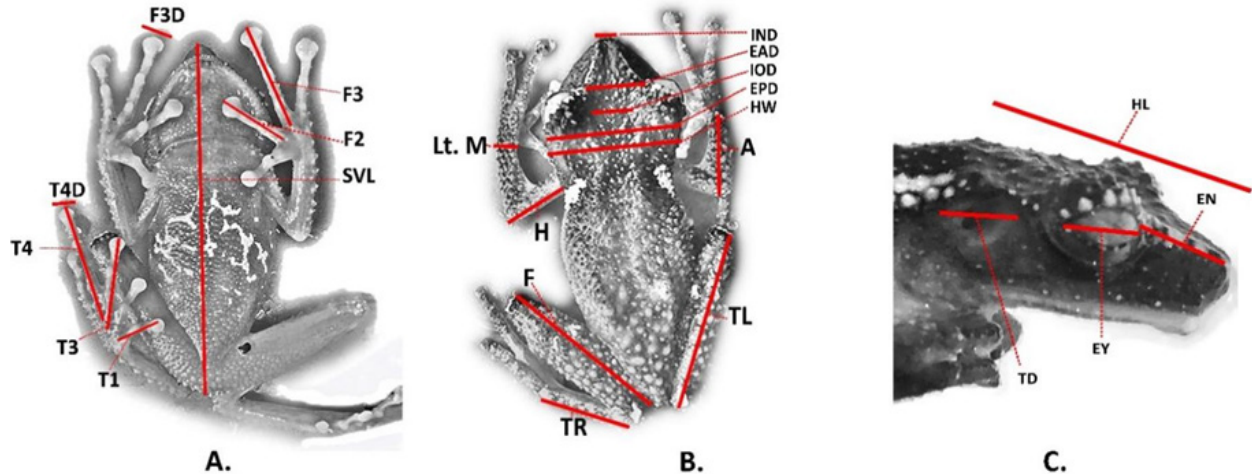
**Figure 1.** Map of the research area (yellow color, modified map from Nurjannah (2020) and conservation education Situ Gunung TNGGP)

The coordinates were recorded and 23 morphometric characters were measured using a digital caliper ( $\pm 0.1$  mm) for each individual, which was released back to their habitat (Table 1, Figure 2), according to Matsui

(1984) and Matsui et al. (2013). The data of characters measured was initially reduced by calculating the ratio of all morphometric data and divided with the snout-vent length (SVL) (Munir et al. 2018).

**Table 1.** The *Nyctixalus margaritifer* morphometric characters that measure

No.	Character	Description
1	Snout-vent length (SVL)	measured from the tip of the snout to the cloacal opening
2	Tibial length (TL)	measured from the outer margin of the knee joint to the external margin of the heel articulation
3	Tarsal length (TR)	measured from the external margin of the heel articulation to the proximal edge of the inner metatarsal tubercle
4	Femur Foot length (F)	measured from the middle of the cloacal gap to the outer border of the knee joint when the thigh and shin are in a position perpendicular to the body axis
5	Length of the first toe (T1)	measured from the distal edge of the inner metatarsal tubercle to the tip of the first toe
6	Length of the fourth toe (T4)	measured from the distal edge of the inner metatarsal tubercle to the tip of the fourth toe
7	Length of the third toe (T3)	measured from the distal edge of the inner metatarsal tubercle to the tip of the third toe
8	Fourth toe disc diameter (TD4)	measured as the diameter of the horizontal toe disc width
9	Upper arm length (H)	measured from the distal edge of the palmar tubercle to the tip of the third finger
10	Forearm length (A)	measured from the elbow to the wrist
11	Wrist width (Lt.M)	measured the leading edge of the upper ar
12	Length of the third finger (F3)	measure the distance from the tip of the finger to the proximal end of the outer palmar metacarpal tubercle
13	Diameter of the third finger disc (F3D)	measured by the diameter of the horizontal width of the third finger disc
14	Length of the second finger (F2)	measured from the distal edge of the palmar tubercle to the tip of the second finger
15	Head length (HL)	measured from the tip of the head to the posterior edge of the tympanum
16	Posterior eye distance (EPD)	measured between the posterior corners of the eyes
17	The inter-orbital distance (IOD)	measured as the shortest distance between the upper eyelids
18	Anterior eye distance (EAD)	measured as the distance between the front corners of the eyes
19	Distance between nostrils (IND)	measured by the distance between the centers of the nostrils
20	Eye nostril distance (EN)	measured as the distance between the anterior margin of the eye and the center of the nostril
21	Eye diameters (EY)	measure the eye diameter externally
22	Horizontal tympanic diameter (TD)	measure the distance across the circle of the tympanic membrane
23	Head width (HW)	measured at the widest distance from the head, measured at the commissure of the jaw



**Figure 2.** Physical location for the 23 morphometric measurements: (A) ventral view, (B) dorsal view, and (C) lateral view. See the main text for the definitions of these characters (Ibnudarda et al., 2024)

The coordinates of individual *N. margaritifer* were recorded using GPSMAP® 64s SEA. Subsequently, coordinate points were mapped with QGIS software version 3.34.1 (QGIS Development Team, 2023) using the Digital Elevation Model (DEM) map template, which was downloaded from the US Geological Survey's Earth Explorer (US Geological Survey, 2020). An administrative map of Java Island was obtained from ArcGIS Online. The data analysis was conducted using the non-parametric Mann-Whitney U-test to determine differences in morphometric characters between male and female *N. margaritifer*.

The results of 23 morphometric characters for each *N. margaritifer* are shown as mean values  $\pm$  SD. Values are considered significant and highly significant with a p-value  $<0.05$ . Data were analyzed using the Principal Component Analysis (PCA) and K Mean Cluster to determine the characters forming group structures that could be detected. The morphometric characters were determined using the Bartlett test of homogeneity of variance, with a significant p-value  $<0.05$ . The predictor variables from PCA analysis were based on Kaiser Meyer Olkin (KMO) values  $>0.5$  and Eigenvalues  $>1$ .

All statistical analyses used the R language application (Baptiste, 2015; Revelle, 2017; Kassambara & Mundt, 2020; R Studio Team, 2020; Gabriel, 1971).

## RESULTS AND DISCUSSION

In six males and ten females of *N. margaritifer* were discovered in an area of 4.34 hectares. The location of each individual frog is shown in Table 2. To determine how far the *N. margaritifer* mobilities from the nearest water source (Situ Gunung Lake), the distance of each frog was measured to the lake. The mean distance of individual males to the lake was  $155.1 \pm 124.7$  m (8.5-292.3 m), females were  $111.3 \pm 102.9$  m (3.7-393.3 m), while the distance between males and females was  $132 \pm 119.4$  m (10.8-383.1 m). Since tadpoles were not identified, there was no assumption that the species laid their eggs in the lake. The *N. margaritifer* was found in habitats filled with herbaceous plants and shrubs. Furthermore, the *N. margaritifer* were found perched on leaves or attached to the plant stems at a height of 10-80 cm from the ground, measured by using the roll meter. The plants at the location were the rane fern (*Shillaginella* sp.), forest taro (*Homalomena* sp.),



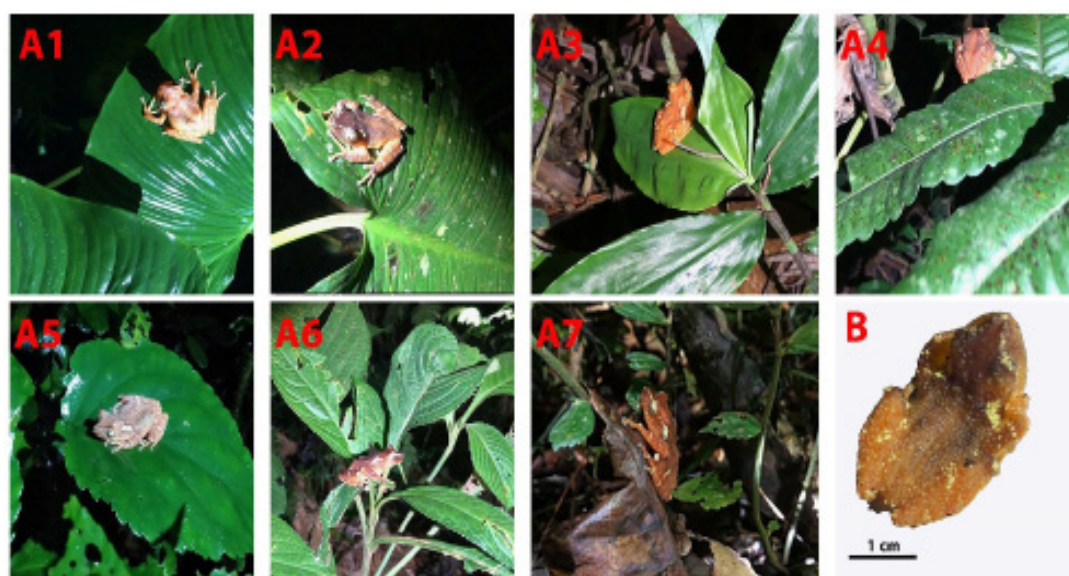
**Table 2.** The coordinate of *N. margaritifera* found at Situ Gunung TNGGP

No.	Male	Female
1	6°50'1.02"S, 106°55'29.45"E	6°50'1.53"S, 106°55'28.68"E
2	6°50'1.28"S, 106°55'29.32"E	6°50'1.56"S, 106°55'28.91"E
3	6°50'0.10"S, 106°55'29.94"E	6°50'0.30"S, 106°55'30.20"E
4	6°50'1.55"S, 106°55'29.55"E	6°50'1.67"S, 106°55'29.87"E
5	6°50'4.53"S, 106°55'21.75"E	6°50'1.90"S, 106°55'29.54"E
6	6°50'5.52"S, 106°55'22.11"E	6°50'1.52"S, 106°55'29.96"E
7		6°50'4.64"S, 106°55'21.30"E
8		6°50'2.38"S, 106°55'28.94"E
9		6°49'58.52"S, 106°55'32.40"E
10		6°50'0.06"S, 106°55'32.28"E

the plant stems at a height of 10-80 cm from the ground, measured by using the roll meter. The plants at the location were the rane fern (*Shillaginella* sp.), forest taro (*Homalomena* sp.), *Cyrtandra* sp., and pulus plant (*Laportea* sp) (Figure 3).

The defensive behavior of *N. margaritifera* was observed in the presence of humans. The observations showed cessation in moving and making sounds, pressing limbs together where perching occurred, and closing their eyes. The *N. margaritifera* flexed their body

when touched but remained motionless as when dead (Figure 3B). This behavior of pretending to be dead was seen by getting closer to the frog and observing it silently, causing it to fall from the perch to the ground. In several Anura species, the defensive behavior is a strategy for protection and escape from predators (Ferrante et al., 2014). This is in line with the reports by Priambodo et al. (2021) and Hanifa et al. (2023), where trees are the place to avoid predators by hanging on leaves.



**Figure 3.** The *Nyctixalus margaritifera* at the location: A1-2) Attached on leaf forest taro (*Homalomena* sp.), A3) Attached on leaf *Lophatherum* sp., A4) Attached on leaf *Helmithosachys* sp., A5) Attached on leaf *Cyrtandra* sp., A6) Attached on leaf pulus plant (*Laportea* sp.) and A7) Attached on stem of *Cyrtandra* sp B) Defensive behavior.

The results of morphometric analysis of males and females of *N. margaritifer* obtained three differentiating characters: SVL ( $P=0.04$ ), T3/SVL ( $P=0.02$ ), and Lt. M/SVL ( $P=0.02$ ) (Table 3). While the KMO analysis and Bartlett test results found that the sample

was adequate and all data could be used for PCA analysis. Moreover, PCA analysis based on the Eigenvalue  $>1$  and a cumulative value proportion  $>75\%$  determined five main components (Table 4) for subsequent analysis.

**Table 3.** Morphometric characters of adult males and females of *N. margaritifer*

Character	Males (mm)		Males (mm)		Mann-Whitney
	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD	Min-Max	U-Test
SVL	42,1 $\pm$ 2,1	38,5 – 43,9	44,4 $\pm$ 1,7	41,7 – 46,8	0,04*
TL	22,3 $\pm$ 1	20,8 – 23,1	23,4 $\pm$ 0,9	21,2 – 24,6	0,5
TR	13,2 $\pm$ 1,2	11,9 – 15,4	13,8 $\pm$ 1	11,7 – 15,6	0,96
F	20,4 $\pm$ 1,6	17,6 – 21,8	21,7 $\pm$ 4,7	18,1 – 33	0,48
F-L	28,9 $\pm$ 1,5	26,5 – 31,11	30,7 $\pm$ 2,1	27,4 – 33,7	0,79
T1	4,1 $\pm$ 0,2	3,8 – 4,4	4,7 $\pm$ 0,6	3,6 – 5,7	0,16
T4	11,9 $\pm$ 0,3	11,5 – 12,2	13,1 $\pm$ 0,9	12,1 – 14,5	0,07
T3	8,2 $\pm$ 0,6	7,8 – 9,3	9,5 $\pm$ 0,7	8,5 – 10,6	0,02*
TD4	1,7 $\pm$ 0,2	1,4 – 1,9	1,4 $\pm$ 0,4	0,9 – 2	0,05
H	9,4 $\pm$ 0,8	8,3 – 10,4	9,7 $\pm$ 1,4	7,7 – 11,4	0,54
A	10,2 $\pm$ 0,7	9,5 – 11,2	9,9 $\pm$ 3,2	0,97 – 12,1	0,96
Lt.M	2,5 $\pm$ 0,3	2,2 – 2,9	2,1 $\pm$ 0,4	1,6 – 2,7	0,02*
F3	11,5 $\pm$ 0,5	10,9 – 12,4	11,9 $\pm$ 1,2	9,2 – 13,4	0,7
F3D	2,3 $\pm$ 0,2	2 – 2,4	2,3 $\pm$ 0,8	1,5 – 4	0,31
F2	7 $\pm$ 0,6	6,2 – 7,9	7,1 $\pm$ 0,7	6,1 – 8,3	0,7
HL	14,4 $\pm$ 0,9	12,6 – 14,9	15,4 $\pm$ 1	14,3 – 17,7	0,43
EPD	13,8 $\pm$ 1,2	12 – 15,4	14 $\pm$ 0,9	12,7 – 15,4	0,21
IOD	6,4 $\pm$ 0,5	5,5 – 6,9	6,4 $\pm$ 0,5	5,5 – 7,1	0,18
EAD	7,3 $\pm$ 0,4	7 – 8	7,5 $\pm$ 0,6	6,3 – 8,3	0,91
IND	2,2 $\pm$ 0,3	1,6 – 2,6	2,4 $\pm$ 0,4	2,1 – 3,2	0,39
EN	5,5 $\pm$ 0,2	5,2 – 5,7	5,6 $\pm$ 0,7	4,7 – 6,6	0,3
EY	4,9 $\pm$ 0,2	4,6 – 5,2	4,8 $\pm$ 0,6	4,2 – 5,8	0,13
TD	3,5 $\pm$ 0,5	3 – 4,5	3,7 $\pm$ 0,4	3,2 – 4,3	0,9
HW	14,9 $\pm$ 1,3	13,1 – 16,1	14,3 $\pm$ 1,6	12,2 – 17,2	0,11

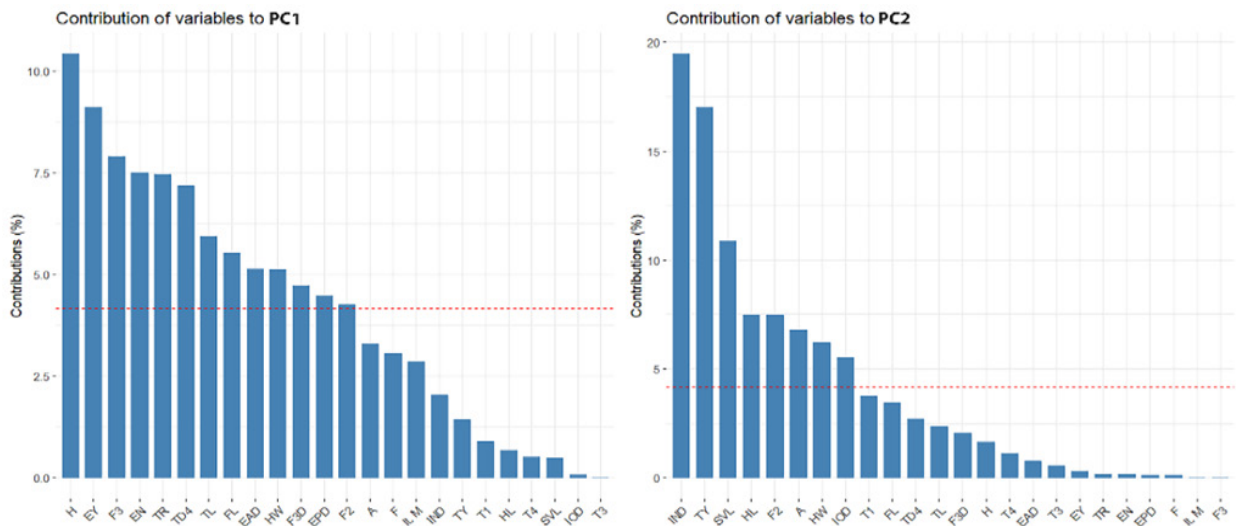
\*Significant at p-value  $<0.05$ . See text for a character name

**Table 4.** The character loads, eigenvalues, and percentage of variance explained

Character	PC1	PC2	PC3	PC4	PC5
SVL	0.07	-0.33	-0.15	-0.27	0.08
TL	-0.24	0.15	-0.19	0.18	-0.13
TR	-0.27	-0.04	-0.18	0.08	-0.13
F	-0.17	0.03	0.01	-0.30	-0.32
FL	-0.24	0.19	-0.21	-0.09	0.18
T1	-0.09	-0.19	-0.28	0.04	-0.24
T4	-0.07	0.11	-0.40	-0.25	0.07
T3	0.00	0.07	-0.40	0.00	0.40
TD4	-0.27	0.16	0.17	-0.27	0.03
H	-0.32	-0.13	0.06	-0.07	-0.06
A	0.18	0.26	0.13	-0.25	0.32
Lt.M	-0.17	0.01	0.38	-0.17	-0.14
F3	-0.28	0.01	-0.16	0.09	-0.22
F3D	-0.22	-0.14	0.27	-0.17	0.26
F2	-0.21	0.27	0.00	-0.06	0.14
HL	-0.08	-0.27	0.07	0.34	0.40
EPD	-0.21	0.03	0.22	-0.02	0.01
IOD	-0.03	0.24	0.17	0.34	-0.28
EAD	-0.23	-0.09	0.12	0.11	0.22
IND	-0.14	-0.44	0.04	0.20	0.07
EN	-0.27	0.04	-0.02	0.33	0.12
EY	-0.30	0.05	0.15	-0.10	0.18
TY	-0.12	0.41	-0.12	0.20	0.09
HW	0.23	0.25	0.20	0.27	0.04
Eigenvalue	2,8	1,8	1,7	1,6	1,5
Contribution of variables	<b>33%</b>	<b>14%</b>	12%	10%	9%
Cumulative portion	33%	47%	59%	69%	78%

Based on Table 4, a combination of PC1 and PC2 was used for the subsequent PCA analysis because the two components explained 47% of the total morphometric variation of males and females. The positive

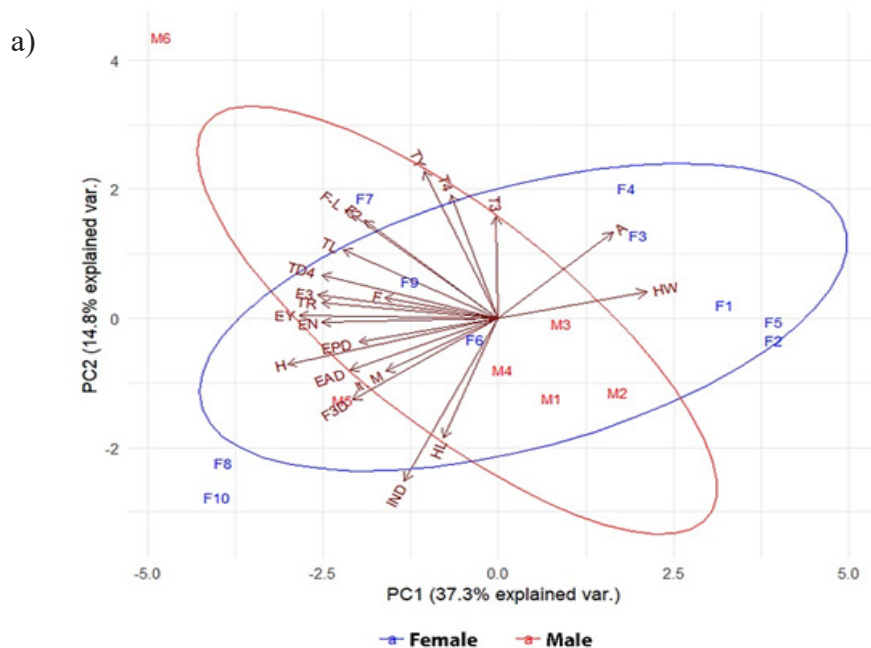
loading in PC1 was found in SVL and HW, while PC2 was in TL, F, FL, T4, TD4, A, Lt. M, F3, F2, EPD, IOD, EN, EY, TY, and HW, as shown in Figure 4.



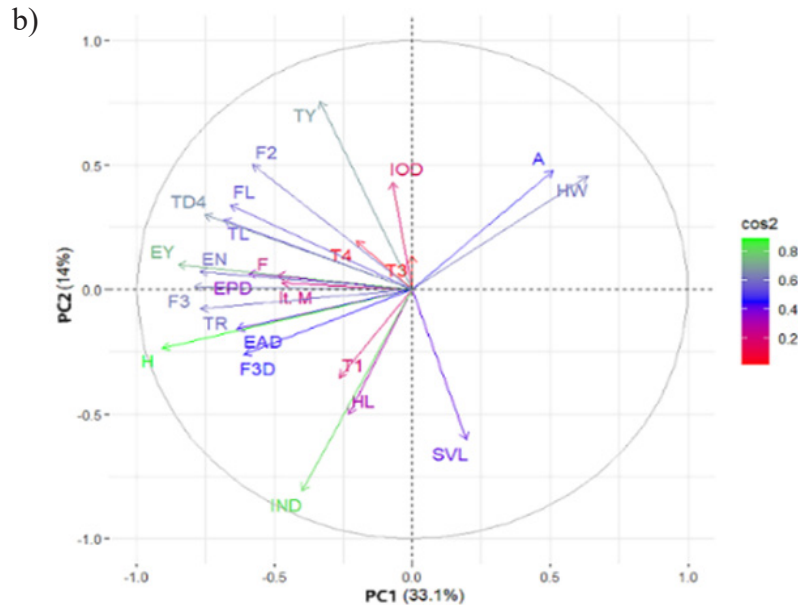
**Figure 4.** The percentage contribution (Y) of character variables (X) to PC1 and PC2 components

After comparing the highest values percentage of variance in PC1 and PC2, there was an overlap in morphometrics characters between the males and females of *N. margaritifer*, thereby limiting separation on the scatter plot (Figure 5a). The cosine

graphic representation showed that the SVL character contributed moderately to female no.6. The T3 character contributed lowly to the male no. 4. In contrast, the Lt.M character influenced lowly to the female no.7 (Figure 5b).



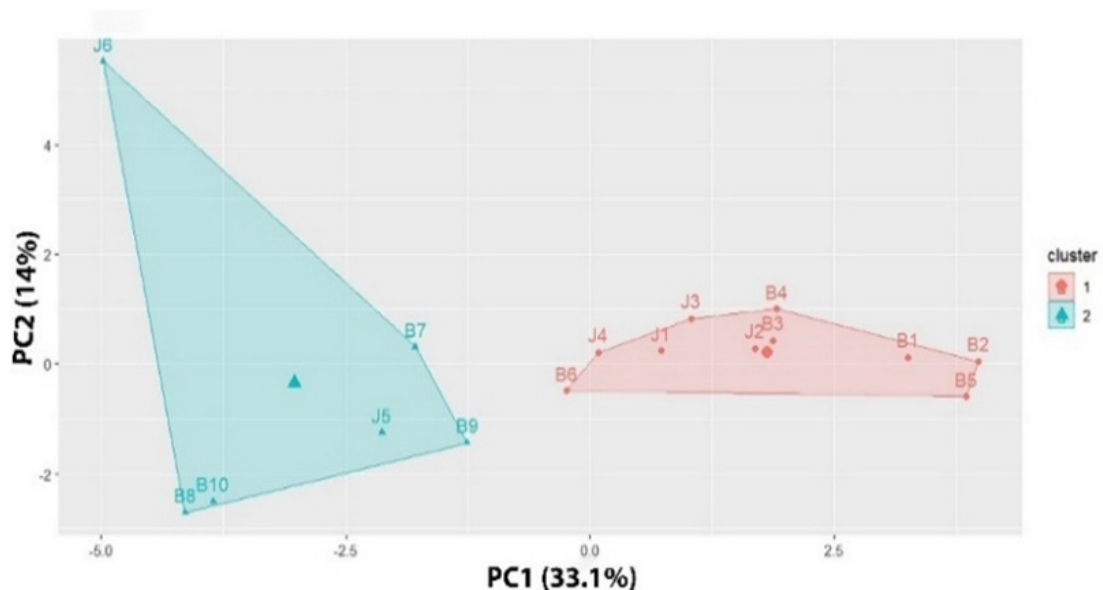




**Figure 5.** The morphometric PCA results of male and female of *N. margaritifer*: a) distribution of characters on PC1 and PC2; b) cosinus graph of PC1 and PC2. (See text for character names)

The K mean cluster test results showed that the optimum clusters for analysis were PC1 and PC2. The graphic representation formed two clusters based on SVL, T3/SVL, and Lt.M/SVL. The first cluster consisted of males no.1-4, and females no. 1-6, with

mean value of SVL 45.02 mm, T3/SVL 0.21 mm, Lt.M/SVL 0.05 mm. The second cluster consisted of males no. 5 and 6 and females no. 7, 8, 9, and 10, with mean values of SVL 41.57 mm, T3/SVL 0.2 mm, and Lt.M/SVL 0.05 mm (Figure 6).



**Figure 6.** The distribution of male and female individuals on the K mean cluster plot. Note: J = male; B = female. (See text for character names).

The K mean cluster analysis results showed an overlap, but there were clusters of SVL, T3, and Lt.M. The ratio of males to females in the first cluster was 4:6, while in the second cluster, it was 2:4 (Figure 6). The three characters were determinants of clustering, which was in line with previous non-parametric analysis (Table 3).

The results of PCA analysis showed that the SVL, T3, and Lt.M characters could distinguish males and females of *N. margaritifer*, but there was still overlap between the two populations. However, further research should be carried out using a larger number of samples and high comparison characters to confirm the sexual dimorphism of *N. margaritifer* in Situ Gunung's natural habitat at TNGGP.

### CONCLUSION

In conclusion, this research showed that *Nyctixalus margaritifer* was found in the TNGGP as their natural habitat in the West Jawa region. The three morphometric characters that could differentiate the sex were SVL, T3, and Lt.M. The discovery of 10 females and six males of *Nyctixalus margaritifer* provided significant opportunity, ensuring their existence in TNGGP. However, the TNGGP managers were expected to maintain the ecosystem's quality sustainably.

### AUTHOR CONTRIBUTION

Wahyu Prihatini designed the research and wrote the manuscript. Bobby Darmawan supervised the process. Rouland Ibnudarda collected and analyzed the data. Mutia Anggraini managed the administration and documentation and wrote the manuscript.

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### CONFLICT OF INTEREST

The Authors declares no conflict of interest regarding the research or the funding.

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