SAINTIFIK@: JURNAL PENDIDIKAN MIPA

E-ISSN: 2598-3822 | P-ISSN: 2087-3816

Available online at https://ejournal.unkhair.ac.id/Saintifik

Keanekaragaman Genetik Kupu-Kupu *Ornithoptera* spp di Pulau Bacan Berdasarkan Analisis Faktor Morfologi dan Lingkungan

(Diversity Genetics of Butterfly Ornithoptera spp in Bacan Island Based on Morphological and Environmental Factor Analysis)

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Received 02-10-2025, Revised 10-10-2025, Accepted 25-10-2025, Published 30-10-2025

Keywords:

Diversity; Genetic; Ornithoptera croesus; Morphological; Environmental.

ABSTRACT. *Ornithoptera* spp. on Bacan Island are endemic butterflies. *Ornithoptera croesus* in Bacan Island empirically has a variety of morphological characters. The purpose of this study was to determine the dissimilarity of specific characteristics of *O. croesus* based on morphometric analysis of environmental factors, especially elevation gradients. Data from morphometric character measurements were analyzed using UPGMA cluster analysis. The results showed that the intraspecific dissimilarity of *O. croesus* across elevation gradients in the Sibela Mountains Nature Reserve, Bacan Island, was low. Elevation gradients were not the main factor in determining intraspecific dissimilarity. Migration factors and the availability of food plants that determine the pattern of *O. croesus* can be seen from the highest similarity value in the dendrogram, found between O. croesus ♀ 800 masl and *O. croesus* ♀ 20 masl. Migration factors and the availability of food crops cause the grouping pattern to be inconsistent with the biplot analysis of environmental factor data, which groups the lowlands and midlands into a single cluster.

INTRODUCTION

The Ornithoptera croesus butterfly was discovered by Wallace in 1859 and is considered an important genetic resource and an endemic species of Bacan Island, South Halmahera District [1]. The natural hotspots of *O. croesus* are characterized by the presence of Mussaenda and Ashoka plants, which serve as food sources for *Ornithoptera* spp. [2]. *O. croesus*, in fact, has a variety of morphological characters that vary; the quantity of body length, wing length, leg length, antenna length, and length of proboscis vary greatly. The quality of body color and wing color pattern also varies. [3] described the size of the male *O. croesus* wing as more than 17 cm, the size of the female O. croesus wing as about 17-20 cm, and the female wing is larger than the male wing [1]. Furthermore, [4] states that male *O. croesus* has a wing span of 13.0-15.5 cm, and a front wing length of 7.5-9.7 cm. The female *O. croesus* has a wingspan of 14.5-19.0 cm and a front wing length of 9.6-11.3 cm. Current information about the morphological character of *O. croesus* at various altitudes has not been reported to date.

Although several studies have described the general morphological dimensions of *O. croesus*, none have examined how these characters vary across different altitudinal gradients. Altitude influences environmental factors such as temperature, humidity, vegetation composition, and foodplant availability, all of which may affect butterfly morphology. However, altitude-related morphological variation in *O. croesus* has not been addressed in previous research, creating a clear knowledge gap.

Intraspecific variability in butterflies can be observed in morphological traits such as body size and color [2]. Morphological character variations within an individual are generally difficult to use as a specific differentiating standard, so techniques or methods that support the verification of intraspecific variation are needed. [5][6] suggested that morphometry is a method that can describe morphological characters through measurement techniques and scoring, including quantitative and qualitative characters.

Therefore, this study assesses the morphological (phenotypic) diversity of *O. croesus* across different altitudes. This study aims to determine whether altitude influences morphological variation in *O. croesus* populations on Bacan Island. The results are expected to contribute to the field of zoology and entomology by providing information on phenotypic variation within an endemic species that may support future conservation management. This study provides the first altitude-based morphometric assessment of *O. croesus*, offering essential baseline data that fills a previously unaddressed gap in the literature and strengthens the scientific foundation for future biodiversity and conservation strategies.

MATERIALS AND METHODS

Study Area

Mount Sibela is located on the island of Bacan, which is geographically situated between 126°45'-129°30' East Longitude and 0°30' North Latitude and 2°00' South Latitude, having an altitude of 2,118 meters above sea level (masl). *The O. croesus butterfly collection was carried out at four altitude locations (altitude 20 masl, 200 masl, 400 masl, and 800 masl) in the Sibela mountain nature reserve area of Bacan Island, South Halmahera Regency,* by using insect nets [7]. The map of *O. croesus* distribution can be seen in Figure 1

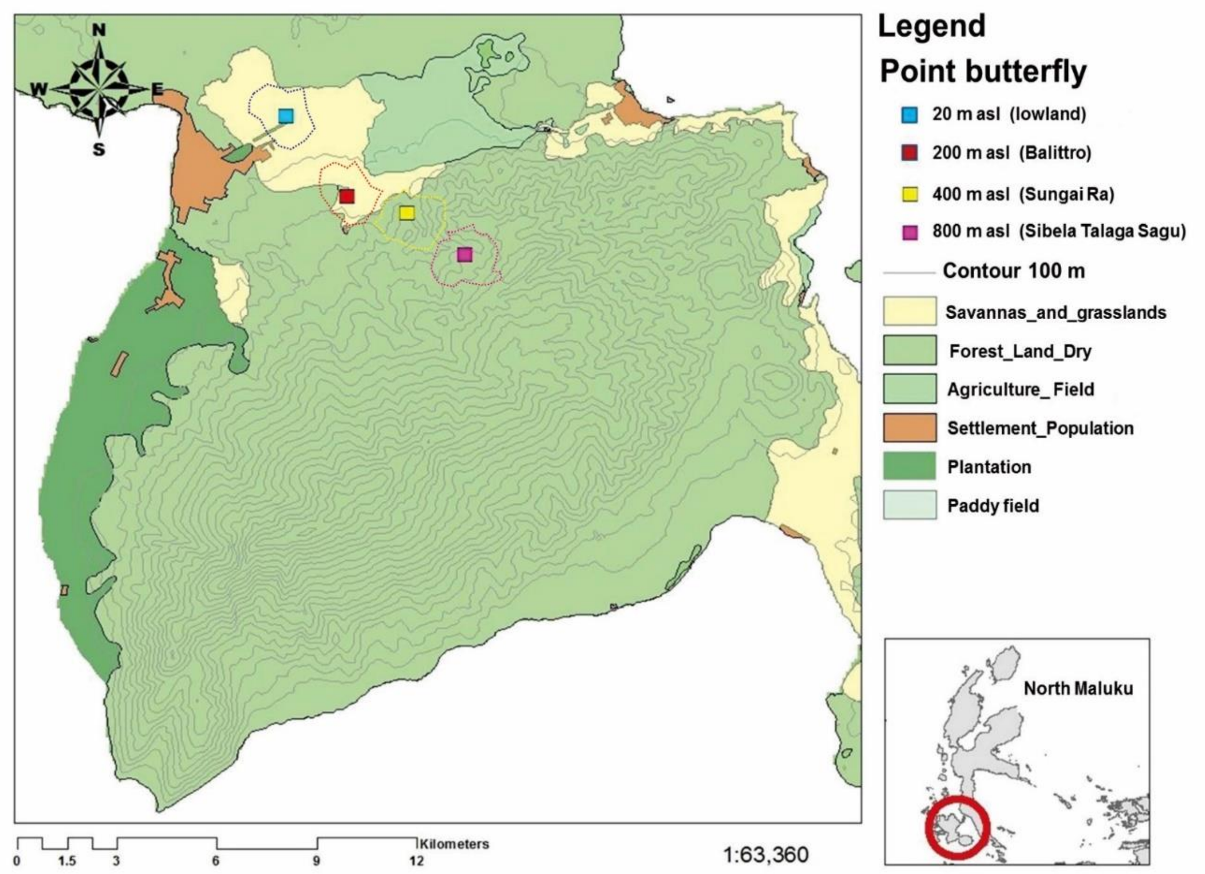

Figure 1. Location of the Habitat/Hotspot *O. croesus* Research Site in the Sibela Mountain Nature Reserve, Bacan Island, South Halmahera Regency, North Maluku.

Specimen Collection

O. croesus is preserved by using dry camphor to identify and measure the morphometric characteristics of *O. croesus* specimens, including as many as 32 individual *O. croesus* butterflies (16 male individuals and 16 female individuals). Furthermore, morphometric character measurement data obtained from the specimens were analyzed descriptively and quantitatively using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis in the Multivariate Statistical Package (MVSP) 3.22, as proposed by [8].

Measurement of morphometric Data

Standard measurements commonly used for butterflies include head length, thoracic length, abdominal length, antenna length, wing length, and wingspan [9]; [6]; [10]; [11]. In addition to standard characters in the form of body measurements, wing venation was also measured. Standard measurement of *O. croesus* can be seen in Table 1

Table 1. Measurements on butterfly body size

(1) WB = Whole Body (measured from the tip of the head to the tip of the abdomen); (2) LC = Length of Caput (measured from the edge of the thorax to the tip of the head);	(10) LBW = Length of Back Wings (measured from the base of the back wings to the tip of the back wings); (11) WFW = Width of the Front Wings (measured from the mid of the upper wing or Radius 2 until the mid of the lower wing or Cubitus anterior);

(3) LTh = Length of Thorax (measured from the base of the thorax to the border of the abdomen);
(4) LA = Length of Abdomen (measured from the base of the thorax to the tip of the abdomen);
(5) LPbs = Length of Proboscis (measured from the base of the mouth or proboscis until the tip part of the proximal proboscis);
(6) LAtn = Length of Antenna (measured from the base of the antenna to the tip of the proximal antenna);
(7) LRW = Long Range of Wings; measured from the front left wing tip to the right front wing tip;
(8) LW = Length of Wingspan (measured from the left wing tip to the right wing tip);
(9) LFW = Length of Front Wings (measured from the base of the wing to the tip of the front wing);
(12) WBW = Width of the Back Wing (measured from the anal vein tip of the back wing until the subcostal + radius 1 of the back wing);
(13) LFL = Length of the Front Legs (measured from the base of the femur, tibia, tarsus to the nail end of the front leg);
(14) LML = Length of the Middle Leg (measured from the base of the femur, tibia, tarsus to the tip of the middle toe nail);
(15) LBL = Length of the Back Leg (measured from the base of the femur, tibia, tarsus to the tip of the back toenail).

[9]; [6]; [10]; [11].

How to measure the morphological characters of Ornithoptera spp. can be seen in Figure 2.

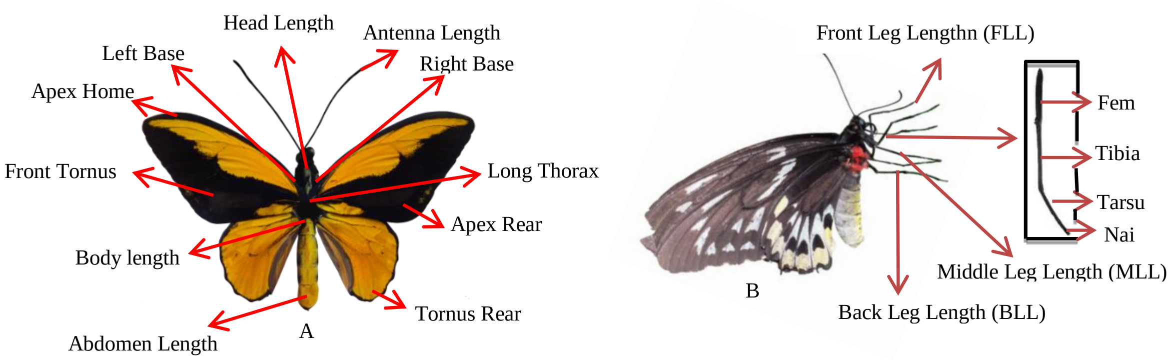

Figure 2. Morphological characters of the body and wing venation measured; (A = body character, and antenna B = foot character)

RESULT AND DISCUSSION

Morphometric Data

The data of this study are the mean morphological (quantitative) measurements of endemic butterflies on Bacan Island, with a total sample of O. croesus comprising 32 individuals (16 male and 16 female). Morphological description of *the O. croesus species* seen in Figure 3.

Figure 3. Species Description of *O. croesus*, Endemic Butterfly of Bacan Island

The data in this study were measurements of morphological characters of O. croesus, with 14 characters from 32 individuals (16 male and 16 female). Furthermore, the 14 characters obtained are converted into ratio data. Measurement of *O. croesus* morphometric ratio data is shown in Table 2.

Table 2. Morphometric measurement data of *the O. croesus endemic butterfly in Bacan Island*.

No	Karakter	*O. croesus*		*O. croesus*		*O. croesus*		*O. croesus*	
		20 masl		200 masl		400 masl		800 masl	
		♂	♀	♂	♀	♂	♀	♂	♀
1	CL: WBL	0,10	0,08	0,12	0,10	0,10	0,09	0,14	0,13
2	TL: WBL	0,29	0,28	0,24	0,25	0,27	0,27	0,25	0,25
3	AL:WBL	0,59	0,64	0,63	0,67	0,59	0,59	0,61	0,66
4	LPbs: WBL	0,71	0,7	0,76	0,75	0,73	0,71	0,71	0,77
5	AtnL: WBL	0,57	0,6	0,59	0,59	0,58	0,55	0,58	0,6
6	UWS: WBL	2,73	3,17	2,8	3,28	2,68	2,93	2,62	3,12
7	FWL: WBL	1,4	1,66	1,44	1,67	1,39	1,51	1,37	1,61
8	RWL: WBL	0,71	0,99	0,73	0,99	0,75	0,96	0,75	1,10
9	FWW: WBL	0,68	0,94	0,68	0,89	0,68	0,84	0,68	0,91
10	RWW: WBL	0,57	0,79	0,57	0,76	0,55	0,71	0,57	0,77
11	FL:WBL	0,59	0,56	0,63	0,6	0,59	0,51	0,57	0,59
12	MFL: WBL	0,74	0,74	0,74	0,75	0,7	0,68	0,7	0,71
13	BFL: WBL	0,76	0,76	0,77	0,77	0,73	0,7	0,71	0,77
14	CL: TL	0,36	0,32	0,48	0,42	0,37	0,35	0,57	0,55
15	CL:AL	0,17	0,13	0,18	0,15	0,16	0,16	0,23	0,20
16	CL: LPbs	0,14	0,12	0,15	0,13	0,13	0,13	0,2	0,17
17	CL: AtnL	0,18	0,14	0,2	0,17	0,17	0,17	0,24	0,22
18	CL: UWS	0,03	0,02	0,04	0,03	0,03	0,02	0,05	0,03
19	CL:FWL	0,07	0,05	0,08	0,06	0,07	0,06	0,10	0,08
20	CL:RWL	0,14	0,08	0,16	0,10	0,14	0,09	0,18	0,12
21	CL: FWW	0,15	0,09	0,17	0,11	0,14	0,11	0,20	0,14
22	CL: RWW	0,18	0,11	0,20	0,13	0,18	0,13	0,25	0,17
23	CL:FL	0,17	0,16	0,18	0,17	0,16	0,18	0,24	0,22

No	Karakter	*O. croesus*		*O. croesus*		*O. croesus*		*O. croesus*	
		20 masl		200 masl		400 masl		800 masl	
		♂	♀	♂	♀	♂	♀	♂	♀
24	CL: MFL	0,13	0,12	0,15	0,13	0,14	0,13	0,20	0,19
25	CL: BFL	0,13	0,11	0,14	0,13	0,13	0,13	0,20	0,17
26	TL:AL	0,48	0,43	0,38	0,37	0,45	0,45	0,41	0,38
27	TL: LPbs	0,39	0,39	0,31	0,33	0,36	0,38	0,35	0,32
28	TL: AtnL	0,50	0,46	0,41	0,41	0,46	0,48	0,43	0,41
29	TL: UWS	0,10	0,08	0,08	0,07	0,09	0,08	0,10	0,07
30	TL: FWL	0,20	0,16	0,16	0,14	0,19	0,17	0,18	0,15
31	TL: RWL	0,40	0,28	0,32	0,25	0,35	0,28	0,33	0,22
32	TL: FWW	0,42	0,29	0,35	0,28	0,39	0,31	0,37	0,27
33	TL: RWW	0,51	0,35	0,41	0,32	0,49	0,37	0,44	0,32
34	TL: FL	0,49	0,5	0,38	0,41	0,45	0,52	0,44	0,42
35	TL: MFL	0,38	0,37	0,32	0,33	0,38	0,39	0,36	0,35
36	TL: BFL	0,37	0,37	0,31	0,32	0,36	0,38	0,35	0,32
37	AL:LPbs	0,82	0,91	0,81	0,89	0,8	0,84	0,86	0,85
38	AL:AtnL	1,04	1,06	1,05	1,11	1,01	1,07	1,05	1,09
39	AL: UWS	0,21	0,2	0,22	0,2	0,21	0,2	0,23	0,20
40	AL:FWL	0,42	0,38	0,43	0,39	0,42	0,39	0,45	0,40
41	AL:RWL	0,83	0,65	0,85	0,68	0,78	0,61	0,81	0,59
42	AL:FWW	0,86	0,68	0,91	0,74	0,86	0,71	0,90	0,72
43	AL:RWW	1,04	0,82	1,09	0,88	1,05	0,83	1,08	0,85
44	AL:FL	1,01	1,15	0,98	1,09	0,99	1,16	1,06	1,11
45	AL:MFL	0,79	0,87	0,84	0,88	0,83	0,86	0,86	0,93
46	AL:BFL	0,78	0,84	0,80	0,86	0,80	0,85	0,86	0,85

Primary Data Sources processed (2025)

Note:

1. WBL = Whole body length
2. CL = Caput length
3. TL = Torax length
4. AL = Abdomen Length
5. LPbs = Length of Probosis
6. AtnL = Antenna Length
7. UWS = Upper wing stretch
8. FWL = Front wing length
9. RWL = Rear wing length
10. FWW = Front wing width
11. RWW = Rear wing width
12. FL = Foreleg length
13. MFL = Middle foot length
14. BFL = Back foot length

In the data (Table 2), there are no striking variations in the morphological measurements of each O. croesus, male or female, in terms of height. The existence of this phenomenon shows that there is no variation in body size (ratio scale) on the morphological character of *O. croesus*, both male and female. Data on the genetic distance analysis results of male and female *O. croesus* morphological characters are presented in Table 3.

Table 3. Data on the genetic distance of intraspecific morphological characters of the O. croesus endemic butterfly in the Bacan Islands based on morphological characters were analyzed using UPGMA clusters.

	O. croesus		*O. croesus*		*O. croesus*		*O. croesus*	
	♂	♀	♂	♀	♂	♀	♂	♀
	20 masl		200 masl		400 masl		800 masl	
O. croesus ♂ 20 masl	1							
O. croesus ♀ 20 masl	0,609	1						
O. croesus ♂ 200 masl	0,826	0,609	1					
O. croesus ♀ 200 masl	0,717	0,674	0,674	1				
O. croesus ♂400 masl	0,63	0,63	0,587	0,652	1			
O. croesus ♀ 400 masl	0,674	0,63	0,63	0,696	0,739	1		
O. croesus ♂ 800 masl	0,696	0,826	0,739	0,674	0,543	0,543	1	
O. croesus ♀ 800 masl	0,522	**0,870**	**0,522**	0,717	0,543	0,543	0,696	1

The data in Table 3 show that the two locations with the highest altitude have the highest similarity value (0.870 between O. croesus ♀ 800 masl and O. croesus ♀ 20 masl). This implies the highest similarity based on morphological characters. The lowest similarity value (coefficient of similarity) is 0.522 at *O. croesus* ♀ 800 masl with *O. croesus* ♂ 20 masl and *O. croesus* ♂ 200 masl. This implies that the morphological characters are least similar to one another, or at least that they are similar to one another. Based on the morphological similarities of O. croesus described above, a dendrogram can be constructed to show the grouping of individuals into clusters, as in Figure 3.

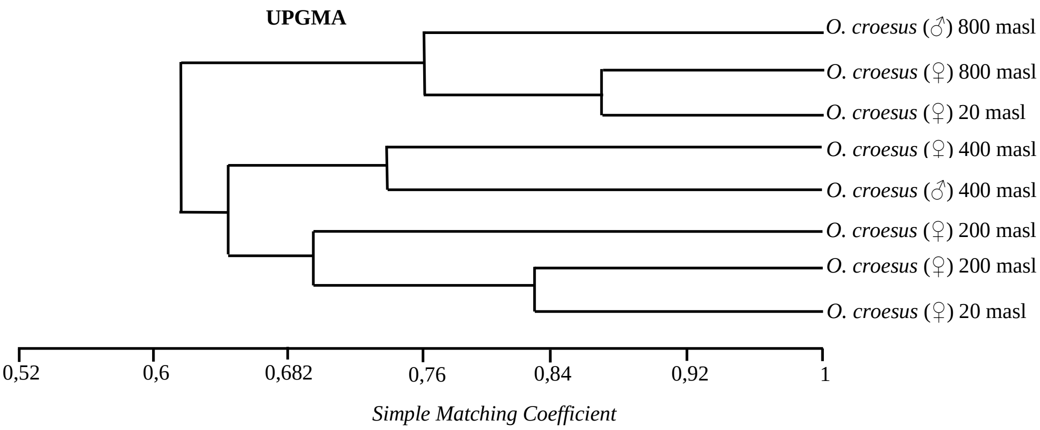

Figure 3. Analysis of the dendrogram *of the O. croesus endemic butterfly on Bacan Island* based on morphological characters analyzed using UPGMA clusters.

The dendrogram results above show that the similarity value of 0.62 formed two main clusters. Furthermore, in the main cluster, I can be divided into two subclusters, namely subcluster one consisting of *O. croesus* ♂ 800 masl and subcluster two consisting of *O. croesus* ♀ 800 masl with *O. croesus* ♀ 20 masl. Based on the position of this subcluster, O. croesus ♀ 800 masl may be very similar to O. croesus ♀ 20 masl, with a similarity value of 0.870. In the main cluster II can be divided into three subclusters, namely subcluster one consisting of *O. croesus* ♀ 400 masl, with *O. croesus* ♂ 400 masl, subcluster 2 consists of *O. croesus* ♀ 200 masl, and subcluster consists of *O. croesus* ♂ 200 masl with *O. croesus* ♂ 20 masl with similarity values (0.820).

Based on the position of the main cluster and subcluster (Figure 4), it can be explained that the height of the place in the Sibela mountain does not contribute to the dissimilarity of intraspecies *O. croesus*; in other words, *O. croesus* from the highlands (800 masl) has similarities with *O. croesus* from the lowlands (20 masl), while *O. croesus* from the plain (200 and 400 masl) are distributed to all clusters on the dendogram.

These clustering patterns suggest that morphological similarity in *O. croesus* is not structured by altitude but is likely shaped by ecological factors that operate across elevations, such as food-plant availability, microhabitat characteristics, or movement behavior among populations. The close clustering of individuals from different altitudes suggests that gene flow or phenotypic plasticity may be maintaining morphological uniformity despite environmental differences. This interpretation is consistent with the view that morphological traits in particular butterfly species can remain stable across environmental gradients when selective pressures are similar or when individuals move frequently between habitats. Therefore, the dendrogram supports the conclusion that altitude alone does not drive phenotypic divergence in *O. croesus* on Bacan Island.

Environmental Data

The result of the similarity between the matrix and dendrogram morphological characters of O. croesus shown above can then be analyzed to determine the contribution of environmental factors to the intraspecific disimilarity of O. croesus using morphological characteristics at different elevations along the Sibela Mountain gradient. The results of an analysis of the contribution of environmental factors to the genetic diversity of *O. croesus* butterflies can be shown in Figure 4.

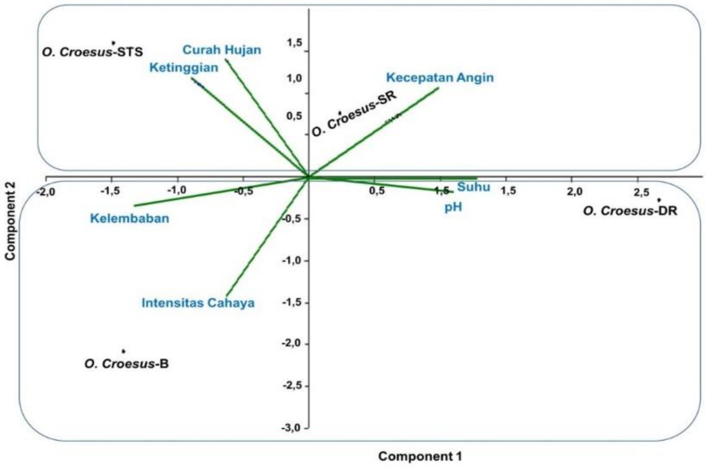

Figure 4. Analysis of environmental factors based on Principal Component Analysis (PCA) at hotspot O. croesus endemic butterfly, Bacan island, with a height (L) of 20 masl, (B) 200 masl, (SR) 400 masl, and (STS) 800 masl.

Note: L= Lowland B= Balitro SR= Sungai Ra STS= Sibela Talaga Sagu

The results of the analysis of the contribution of environmental factors to the disimilarity of *O. croesus* indicate that clustering, or lowland hotspot clustering, is influenced by temperature and pH. The Balitro hotspots are influenced by humidity and light intensity factors. Furthermore, River Ra hotspot clustering is influenced by wind speed, while Sibela telaga sagu hotspot is influenced by light factor and altitude. The clustering pattern of biplot analysis is shown in Figure 5.

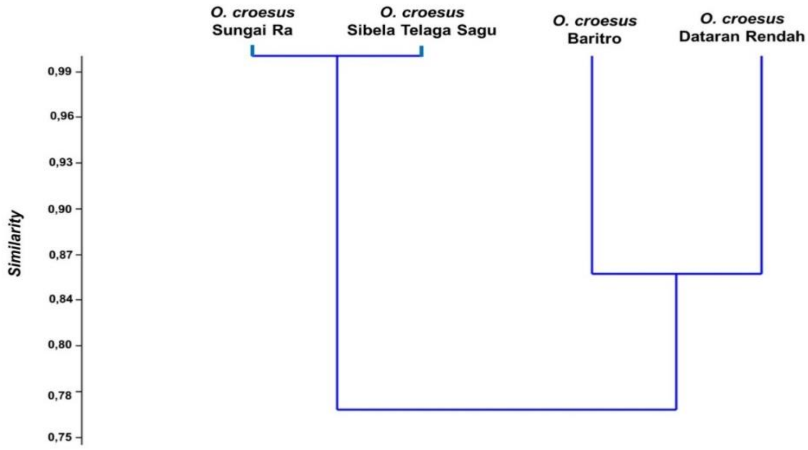

Figure 5. BIPLOT analysis of environmental factors based on Principal Component Analysis (PCA) at the hotspot O. croesus endemic butterfly island of Bacan, with a height of elevation (DR) 20 masl, (B) 200 masl, (SR) 400 masl, and (STS) 800 masl.

The results of the Biplot Analysis above indicate that, based on the contributions of several environmental factors, two main clusters were formed: cluster I with a similarity value of 0.87 and cluster II with a similarity value of 0.98. Cluster I consists of hotspots at altitudes of 20 masl and 200 masl. The most influential environmental factors of the height of 20 masl are pH, soil, temperature, and air. These factors are positively correlated with the morphological characters of *O. croesus* intraspecific. Environmental factors affecting height at 200 masl include light intensity and humidity. These factors are positively correlated and contribute to the similarity of *O. croesus* intraspecific morphological characters. Furthermore, cluster II consists of an altitude of 400 masl and an altitude of 800 masl. Environmental factors that most contribute to the morphological similarity of *O. croesus* at an altitude of 800 masl are rainfall. In contrast, the altitude has a negative correlation and contributes to the similarity of *O. croesus* intraspecific morphological characters. The most influential environmental factor at an altitude of 400 masl is wind speed, which is positively correlated to the morphological similarities of *O. croesus* intraspecifics.

The altitude factor in the Sibela Mountain Range, as assessed in the cluster analysis based on morphological characters, does not affect the intraspecific diversity of O. croesus. It can be proven that female O. croesus at an altitude of 800 masl (plateau) has morphological characteristics that are very similar to O. croesus females at an altitude of 20 masl (low altitude) (Figures 4 and 5). Thus, it can be explained that the morphological characteristics of *O. croesus* in various altitudes in the Sibela Mountain Range are most similar to those of *O. croesus* females (Table 3). The results of this study also showed that *O. croesus* of different sexes differed in body color and wing morphology.

The similarity of *O. croesus* females at an altitude of 800 masl with *O. croesus* females at an altitude of 20 masl is likely caused by female *O. croesus* migration factors at several altitudes in the Sibela Mountain Range. This is related to the availability of food (foodplant). *O. croesus* butterflies are mussaenda and asoka plants. At the observation site 20 masl, there are abundant mussaenda and asoka plants. At an altitude of 200 masl and 400 masl, mussaenda and asoka plants are not dominantly seen, while at an altitude of 800 masl, there are no mussaenda and asoka plants, but are dominated by Gusale (*Octomyrtus lanceolante*) plants, utilizing flowers as a food source. This phenomenon is consistent with [12], which finds that, across butterfly habitat types, the highest species abundance, wealth, diversity, and participation occur in shrub and plantation habitats, which offer many host-plant food sources for butterflies. In contrast, species diversity and evenness are lowest in the primary forest.

Furthermore, [13] states that the structural complexity of the habitat and the diversity of vegetation forms correlated with the diversity of butterfly species. High vegetation diversity will lead to high butterfly diversity [14]. Mussaenda plants can grow along the Sibela mountain range. *O. croesus* butterflies generally like to eat plants that live along the river as food to carry out their survival. This condition is used by *O. croesus* female butterflies, from an altitude of 800 masl, to look for mussaenda and asoka plants as a source of food and copulation at an altitude of 20 masl. The results of this study are consistent with [15], which reports that at lower altitudes, species richness, species abundance, and species diversity tend to be higher than at higher altitudes. Food factors influence this. Furthermore, [16] states that butterflies are nectarivorous animals whose lives suck the flower essence (honey). Nectar-producing plant species that are the source of adult *O. croesus* feed are generally of attractive flowering. Ashoka has red flowers, and Musaenda has yellow flowers. Adult butterflies are attracted to contrasting flower colors because this color spectrum can be accepted by adult butterfly eyes [17].

Habitat conditions in the Sibela mountain range include three types of habitats, namely at an altitude of 20 and 200 masl (settlements and plantations), an altitude of 400 masl (limited production forest habitat), and at an altitude of 800 masl (protected forest), surrounded by river areas along the area of the Mount Sibela nature reserve. *O. croesus* butterflies are often found and look for food in the river area, because in that area, there is available water and minerals attached to the rocks around the habitat. According to [18], many butterflies are found in the river area, which sometimes suck mineral water from the sand and rocks; one of them is from the Pieridae group. Furthermore, [3] suggested that the *O. croesus* butterfly lived in the lowlands found in swamps and wet places.

In general, altitude in Sibela Mountain is not a major factor in determining *intraspecific diversity of O. croesus*; other environmental factors, such as pH, rainfall, and humidity, also play a role. The main factor that determines the pattern of *O croesus clustering is the availability of food, which can be seen from the highest similarity value in the dendrogram* found in *O. croesus* ♀ 800 masl with *O. croesus* ♀ 20 masl. Migration and food availability lead to clustering patterns that are inconsistent with the biplot analysis based on environmental factor contributions, which classify low- and medium-land areas into one cluster and the plateau into another. This research needs molecular analysis to confirm the clustering pattern at each altitude in Mount Sibela.

CONCLUSION

The intraspecific diversity of O. croesus across several altitudes in the Sibela Mountain Nature Reserve area of Bacan Island is low; in other words, there is high intraspecific similarity. Based on the morphological characteristics of *O. croesus* in the Sibela Mountain Range, the most similar is found in O. croesus females, followed by O. croesus with different sexes, which have variations in body color and wings. Biplot analysis indicates that site elevation is not the primary determinant of intraspecific diversity in O. croesus. This is because food availability determines female butterfly migration more.

ACKNOWLEDGEMENTS

The researcher would like to thank the Faculty of Teacher Training and Education, Khairun University, for providing research funding for the assignment with contract number 061/PEN PKUPT/PG.12/2025.

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