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Full Length Research Paper

# Elevational diversity of butterflies in the Bioko Island

# Mercy Murkwe<sup>1, 5</sup>, Geraud C. Tasse Taboue<sup>2, 5</sup>, Maximiliano S. Fero-Meñé<sup>3</sup>, Mary K. Gonder<sup>4</sup> and Eric B. Fokam<sup>5,6</sup>

<sup>1</sup>Department of Biology, Higher Teachers Training College, University of Bamenda, P.O.Box 39 Bambili, Cameroon <sup>2</sup>Multipurpose Agricultural Research Station Bangangte, Institute of Agricultural Research for Development, P.O.Box 222 Bangangte, Cameroon

<sup>3</sup>Environmental Science Programme, Universidad Nacional de Guinea Ecuatorial, Malabo, Bioko Notre, Equatorial Guinea

<sup>4</sup>Department of Ecology and Conservation Biology, Texas A&M University, College Station, Texas, United States of America

<sup>5</sup>Laboratory for Biodiversity and Conservation Biology, Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O.Box 63 Buea, Cameroon

<sup>6</sup>Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O.Box 63 Buea, Cameroon

# Abstract

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Understanding the diversity and distribution of species composition is crucial for informing conservation strategies. This study documented the diversity of butterflies at various elevations on Bioko Island, specifically between 618 m and 1075 m above sea level. Butterfly sampling was carried out in November 2015 using 15 baited traps and nets across 15 locations. Generally, 1,029 individuals were collected, representing 92 species from 5 families: Nymphalidae, Papilionidae, Heliconiinae, Pseudopontiinae, and Lycaenidae. The species collected varied by elevation with the mid elevations (540-618 m) harbouring the most (76 species), followed by high elevations (655-1075 m) with 28 species and low elevations (4-116 m) with the fewest (19 species). Overall diversity patterns, as indicated by the Shannon-Wiener and Simpson's Index, revealed that butterfly species richness and abundance were significantly higher within the mid and high elevations. Diversity indices were relatively similar between high and low elevation. Importantly, this study identified three endemic species: *Bicyclus feae, Cymothoe fumana, and Ceratrichia flava Fernanda*, most of which were found at elevations exceeding 500 m a.s.l. The findings improve understanding of elevational diversity patterns in tropical ecosystems, particularly Bioko Island, aiding conservation management in predicting species shifts due to elevation, population growth, deforestation and climate change.

Keywords: Elevation, Butterfly diversity, Bioko Island, conservation management.

### Introduction

West African tropical rainforests of the Congo Basin and Gulf of Guinea including the Bioko Island, Equatorial Guinea and the Cameroon highlands have long been

Corresponding authors email: mmurkwe@yahoo.com

known to harbour unique ecological and biological diversity (Myers *et al.*, 2000; Cronin *et al.*, 2014). The Bioko Island in particular, located off the western coast of Africa in Equatorial Guinea serves as a unique ecological laboratory showcasing remarkable elevational diversity in its butterfly populations. The varied topography of the Island, characterised by its steep mountains and associated



Figure 1: Map of Bioko Island, with sampling sites and associated elevation levels.

microclimates offers a multitude of habitats such as rainforest, savannahs, and coastal areas that can support diverse species of butterflies. This variability in elevation creates distinct ecological niches that influence species distribution, life cycle and interactions with other organisms (Mahata *et al.*, 2023). However, these unique ecological niches face numerous conservation challenges, such as deforestation, illegal hunting, and the effects of climate change (Perella *et al.*, 2021; Cronin *et al.*, 2016).

Studies conducted so far on biodiversity in this region focus mostly on mammals, birds, fishes, reptiles and plants (Oates *et al.*, 2004; Miller *et al.*, 2021; Fonteyn *et al.*, 2023; Perella *et al.*, 2021) with little publications on invertebrate taxa such as butterflies. Besides their wellknown taxonomy (Comay *et al.*, 2021) butterflies render several ecological services including pollination, food sources to predators (Devries *et al.*, 1997); ecological indicators of the presence of other invertebrate group of organisms (Kumar *et al.*, 2009) and habitat disturbances on ecosystems (Brown & Frieta, 2000).

Monitoring the patterns of butterfly diversity across different altitudes on Bioko Island is crucial for

comprehending broader ecological dynamics and the impacts of environmental changes. Furthermore, studying these butterflies can shed light on evolutionary processes as variations in altitude often lead to adaptations that enhance survival (Montejo *et al.*, 2022). Lastly, investigating the elevational diversity of butterflies on Bioko Island will aid in guiding conservation efforts by providing crucial insights into the composition of this Island's unique ecosystems.

Here, we bring a study of nine families of adult butterflies including both fruit feeding and nectar feeding butterflies taking place in November 2015 at different Eco regions of the Bioko Island. We combined sampling by fruit-baited traps and sweep net catching. We hypothesized that changes in elevation will affect the diversity of butterflies in Bioko Island. This hypothesis was supported by the observation of differences in species richness, abundance and distribution along elevational gradient on the Island. Our study represents the first multi-taxa survey of butterflies sampling by fruit baited traps and sweep nets in the Bioko Island region. Because Bioko Island is known to have a high degree of isolation (Jones, 1994) we used butterflies as models to predict the suitabi-

Sampling sites	Elevation	Gradient	Longitude	Latitude
Northcamp	1075	High	3.36524	8.50036
Hormingas	540	Middle	3.32992	8.48013
Moka Middle Point (MMP)	655	Middle	3.28454	8.63835
Ureca Mid	618	Middle	3.301017	8.580283
Ureca	618	Middle	3.25476	8.585533
Moraka	4	Low	3.26014	8.4861
Moaba	116	Low	3.23931	8.62831

Table 1: Sampling sites used in this study, with elevation, elevational gradient and geographic coordinates.

lity of diversity for which distributional data are scarce.

### Materials and Methods

### Study site

Bioko Island, formerly known as Fernando Po, is the largest island in the Gulf of Guinea, located on the continental shelf at latitudes 3°48'N to 3°12'N and longitudes 8°25'E to 8°57'E. It spans a total area of 2,017 km<sup>2</sup> (Juste and Fa, 1994). The island has a distinctive boot-like shape and features two prominent volcanic massifs linked by a central depression, with the highest point reaching 3,011 meters. Bioko boasts a 195 km coastline that is steep and rugged in the south, while the northern coast is lower and more accessible (Figure 1) (Fa et al., 2006). The climate is typically equatorial, with sealevel temperatures exceeding 25°C, though they drop to around 5°C at higher elevations of 3,011 meters (Juste and Fa, 1994; Atlas 2013). Rainfall varies across the island, decreasing from southwest to northeast, particularly on the north-facing slopes, with precipitation levels exceeding 2,500 mm and reaching over 10,000 mm in the southern regions of Bioko (Fa et al., 2006).

Bioko Island is home to six primary types of natural vegetation (Island Africa, 1989). The lowland rainforest extends from sea level up to 800 meters, featuring a composition similar to that of mainland rainforests with a lower diversity of species. This reduced diversity is mainly due to the absence of okoume trees (Aucomae spp) and creeper palms, while a variety of endemic Ficus species can be found. Montane forests, located between 600 and 1,400 meters, host numerous endemic plant species, with tree ferns (Cyathea spp) being a notable This was in order to maximise the chances of trapping species attracted by different understory habitats along the different elevations. The traps were checked daily between 10 AM and 3 PM over a five-day sampling period. The collected butterflies were sorted according to the day of capture and sampling site.

characteristic. Mossy forests thrive at elevations between 1,500 and 2,500 meters, showcasing a rich array of endemics, most of which are significantly stunted in height. Above 2,500 meters, two types of vegetation can be found: shrub formations and subalpine meadows which are primarily dominated by temperate species such as *Hypericum lanceolatum* and Agauria salicifolia, along with various grasses like *Festuca scimpeana* and *Eragrostis mokensis*. Mangrove forest is located at the mouths of rivers on the island. These habitats support over 268 butterfly species, 10 of which are endemic (African butterfly database, 2019).

During the annual sampling of biodiversity at the Bioko Biodiversity Protection Programme in the Caldera Mountains in November 2015, fruit and nectar feeding butterflies were sampled across 5 sites in lowland and mountain forest (Figure 1) by means of fruit baited traps and sweep nets respectively.

### **Data collection**

**Sampling of fruit feeding butterflies**: Our survey of fruitfeeding butterflies was conducted in November 2015, during the transition from the wet to the dry season, at various elevations: Moraka (4), Moaba (116 m), Hormigas (540 m), Ureca (618), Ureca Mid (618 m), Moka Middle point (655 m), and Bioko Northcamp (1075 m). In each focal area, we set up 15 traps, which were deployed for five days to capture fruit-eating butterflies resulting in a total of 35 trap days across all sites. All traps were baited with overripe bananas and were placed irregularly in the understory, approximately 1m above ground level, with each trap located 50 m to 100 m apart.

**Sampling of nectar feeding butterflies**: Non-fruit-eating butterflies, along with certain fruit-eating species that did not respond to the baited traps, were collected using the transect walk and catch method across the five elevations. This sampling occurred concurrently with the collection of fruit-feeding butterflies from 10:00 AM to 3:00

**Table 2:** Number of individuals per species captured at each of the three elevations. Numbers in bold indicate a potential elevational preference.

Species	High	Middle	Low	Total
Acrea egina	0	1	1	2
Acrea rogersi	7	0	0	7
Amauris niavius	0	1	0	1
Appias epaphia	0	2	11	13
Aterica galena	0	1	0	1
Beaberia barce maculate	0	1	0	1
Bicyclus analis	0	1	0	1
Bicyclus buea	4	0	0	4
Bicyclus dorothea	0	17	0	17
Bicyclus golo	8	0	0	8
Bicyclus hewitsoni	13	2	0	15
Bicyclus italus	6	72	0	78
Bicyclus martius	0	1	0	1
Bicyclus feae	2	0	0	2
Bicyclus sophrosyne	36	5	0	41
Biclyclus sandace	0	49	2	51
Bicyclus sciathis	0	11	0	11
Bicyclus safitza	17	0	0	17
Catuna crithea	0	13	0	13
Ceratrichia flava	1	0	0	1
Ceratrichia nothus	0	0	1	1
Charaxes bipunctatus	0	3	0	3
Charaxes brutus	32	28	1	61
Charaxes candiope	1	0	0	1
Charaxes castor	0	5	0	5
Charaxes cynthia	0	4	0	4
Charaxes eudoxus	0	2	0	2
Charaxes eupale	0	1	0	1
Charaxes fulvescens	7	25	1	33
Charaxes herminia	0	4	0	4
Charaxes lucrecius	0	70	2	72
Charaxes numenes	44	5	0	49
Charaxes pollux	1	6	0	7
Charaxes protoclea	0	27	0	27

Table 2 Continues				
Charaxes tiridates	0	20	1	21
Charaxes uniformis	0	4	0	4
Charaxes viola	1	3	0	4
Charaxes zingha	0	8	0	8
Cymothoe beckeri	0	14	0	14
Cymothoe ceanis	3	26	4	33
Cymothoe coccinata	0	0	8	8
Cymothoe fumana	1	0	0	1
Cymothoe oemillius	0	4	0	4
Cymothoe ogova	0	0	3	3
Cymothoe sangaris	0	6	0	6
Eupheadrta auroela	0	4	2	6
Eupheadra controversa	0	1	0	1
Eupheadra duseni	0	59	0	59
Eupheadra eleus	4	2	0	6
Eupheadra hewitsoni	0	2	0	2
Euphaedra losinga	0	6	0	6
Eupheadra permixtum	0	1	0	1
Eupheadra preusiana	0	1	0	1
Eupheadra ravola	0	1	0	1
Euriphene barombi	0	3	0	3
Euriphene dargeana	0	3	0	3
Euriphene insecta	1	13	0	14
Euriphene duseni	0	33	0	33
Euriphene bernaudi	0	4	0	4
Euriphene gambiae	2	58	0	60
Euriphene incerta	0	2	0	2
Euriphene schultzi	0	17	0	17
Euriphene tadema	0	1	0	1
Gnophodes betsimena	1	10	0	11
Gnophodes chelys	0	13	0	13
Graphium illyris	0	1	1	2
Graphium policenes	0	7	2	9
Graphium ucalegon	0	6	0	6
Hypolynas anthedon	0	1	2	3
Hypolimnas salmacis	0	3	2	5

Table 2 Continues						
Kallimoides rumia	7	25	0	32		
Melanitis Libya	0	0	1	1		
Melanitis leda	0	1	2	3		
Metisella medea	2	0	0	2		
Mylothris dimidiate	0	2	0	2		
Neptis nemetis	0	4	0	4		
Nephronia argia	1	0	0	1		
Palla decius	0	1	0	1		
Palla usheri	0	2	0	2		
Papilio charopus	1	0	0	1		
Papillio dardanus	1	0	1	2		
Papilio menestheus	0	3	0	3		
Papilio phorias	7	0	0	7		
Papillio chrapkowskoides	1	1	0	2		
Paraderos placidus	1	0	0	1		
Protogonomorpha parhassus	0	4	0	4		
Pseudopontia paradoxa	3	5	0	8		
Pseudacrea eurytus	0	9	0	9		
Pseudacreae Lucretia	0	9	0	9		
Pseudacreae semire	0	1	0	1		
Pseudacrea warburgi	0	1	0	1		
Sexenia boisduvalii	0	3	0	3		
Total	216	765	48	1029		

PM under sunny conditions. Butterflies observed in various habitat types within the established plots, as well as in adjacent forest patches, hilltops, and areas between the forest and cleared sections, were captured using aerial sweep nets, identified, and documented. They were also sorted according to the day of capture and sampling site. Butterflies identification was conducted in situ using the Butterflies of West Africa field guides of Larsen (2005), and consistently down to the species level.

### Data analysis

Butterfly sampling locations were combined to reflect elevational gradients (i.e., low, mid and high). To facilitate comparative analysis, specific indices were calculated to depict the species abundance, richness and diversity across various gradients. Butterfly samples were aggregated at each elevational gradient (low, mid, and high). Species richness at each elevation was assessed using the ACE (Abundancebased Coverage Estimator) and the Chao1 richness estimator (Mangurran, 2013). The ACE method focuses on species with one to ten individuals, estimating richness by adding the count of more abundant species (those with over ten individuals). The Chao1 estimator operates on the idea that if rare species (singletons) are still being found during sampling, it indicates that there are likely more rare species yet to be discovered. Once a species has been recorded at least twice (doubletons), it is assumed that no additional species remain to be identified (Colwell, 2013). The Shannon-Wiener Index and the Simpson Diversity Index were calculated based on the observed species abundance data. The Shannon-Wiener Index assumes that all species in a community are represented in the

 Table 3: Pairwise comparisons of species similarities between the three elevational gradients using four similarity indices.

Pairwise elevation	Number of	Jaccard	Morisita–Horn Index	Sørensen Index	Bray–Curtis
comparison	shared	Index			Index
	species				
High-Middle	16	0.057	0.81	0.823	0.95
High-Low	4	0.034	0.80	0.692	0.847
Middle-Low	14	0.04	0.94	0.705	0.94



Figure 2: Ranked species abundance of butterflies recorded across all sampling sites. Fruit feeding butterfly *Bicyclus italus* had the highest catch while nectar feeders like *Ceratrichiia nothus, Papillio charopus* recorded the least number.

sample and that they are randomly selected, while also considering their abundance. The values of the Shannon– Wiener Index for each elevation were converted into true diversities, or effective species numbers, as described by Jost (2010) and Jost *et al.* (2010). In contrast, the Simpson Diversity Index measures dominance, giving more weight to the more common or dominant species within a community. Based on recommendations by Manguran (1988, 2004) on diversity indices, we utilized four different similarity coefficients to compare various elevational communities: the Morisita–Horn, Jaccard, Sørensen, and Bray–Curtis coefficients, all calculated using EstimateS (Colwell, 2013). The Morisita–Horn Index is particularly influenced by the most abundant species and is based on estimates of their proportions. According to Jost (2007), this index is

= abundance-based coverage estimator. Per cent completeness indicates the proportion of the number of species expected.									
Elevation	Number of species	Estimated number of species	Number of individuals	ACE mean ± SE	Chao-1 <b>m</b> ean ± S <b>E</b>	Percentage completeness	Shannon- Wiener Index	Simpson Inverse Index	Evenness among species abundances (Pielou J)
High	28	58.1	213	40.2±8.2	58.1±28.5	47.45	2.59	8.93	0.78
Middle	76	98.1	768	92.279±7.7	98.19±13.2	79.16	1.66	22.24	0.82
Low	19	23.48	48	29.24±7.6	23.48±4.2	76.76	2.01	9.37	0.88

**Table 4:** Comparison of butterfly diversity, species richness estimates, and community evenness across elevation zones. ACE = abundance-based coverage estimator. Per cent completeness indicates the proportion of the number of species expected.





Figure 3: Species accumulation curves for each elevational gradient across study sites.

more reliable, informative, and discriminating than the Jaccard and Sørensen indices. The latter two only consider species presence and do not take into account variations in species frequency, which means that rare species are treated the same as dominant species in the similarity assessments.

To assess the sampling level, we plotted species accumulation curves and ranked species abundance. The analyses were performed using the abdiv package (Bittinger, 2020) and the iNext package (Hsieh et al., 2016) in the R environment (R Core Team, 2021). Furthermore, we employed Estimates V9.1.0 software



Figure 4: Cumulative site-level species richness as a function of elevation, showing a positive trend.

(Colwell, 2013) to calculate various diversity metrics, including species diversity indices, richness estimators, pairwise similarity indices, and species accumulation curves based on 1,000 randomizations.

### Results

# Diversity of butterflies along elevation gradients on the Bioko Island

In total, 1029 specimens were collected belonging to 92 species (Table 2). The lowest elevation recorded the least number of individuals (48), whereas mid elevation scored the highest number of individuals (765) followed by the high elevation (216). These butterflies were classified into five subfamilies (Nymphalinae, Danainae, Satyrinae, Charaxinae, and Limenitidae) within the fruit-feeding butterfly family Nymphalidae, and four subfamilies (Papilionidae, Heliconiinae, Pseudopontiinae, and Lycaenidae) among the nectar feeders.

### **Species Abundance**

Species abundance in the Bioko Island varied with elevation, with some species being more prevalent than others (Figure 2).

The four similarity indices used (Table 3) generally indicated that the communities in the low and midelevations and the mid- and high-elevations are the most similar. In contrast, the communities at low and high elevations show the least similarity.

### **Species richness**

The number of species collected varied by elevation with a higher number of species captured at mid elevations (76),

followed by the high elevation with 28 species captured then 19 species at low elevation (Table 4). Although the species richness differed in each elevation, diversity indices were relatively similar between high and low elevation with a value of 2.59 and 2.01 and the mid-elevation scoring 1.66 for the Shannon–Wiener Index and a range of 8.9 to 22.2 for the Inverse Simpson Index.

The species accumulation curves indicate different mean rates of species capture across the three sampled elevational gradients (Figure 3). The >75% completeness (Table 4) of the species sampling at low and mid elevation suggests the majority of butterflies present at these sites and attracted to ripe banana were captured. This is similar to the information displayed in the species accumulation curves as they appear close to asymptote (Figure 3). Additionally, species richness estimators (ACE and Chao1) gave close results (Table 4), with 40 species for ACE and 58 species for Chao1 estimated at high elevations (28 captured), 92 species for ACE and 98 species for Chao1 for the mid- elevation (76 captured), 29 species for ACE and 23 species for Chao1 for the midelevation (19 captured).

Finally, highlands and mid elevations were observed to harbour the greatest species concentrations (Figure 4).

#### **Discussions and conclusions**

This study aimed to document the diversity of butterflies as well as to uncover their distribution model on the Bioko Island, focusing specifically on elevation gradients. During a field survey conducted in November 2015, we recorded a total of 1,029 butterflies across 92 genera from five primary butterfly families: Papilionidae, Pieridae, Lycaenidae, Nymphalidae, and Hesperiidae. The survey utilized both baited traps and sweep net techniques. A substantial sample size was collected, with lowest elevation recording the least number of individuals (48), whereas mid elevations scored the highest number of individuals (765) followed by the high elevations (216). The findings indicated that the abundance and diversity of butterfly species on Bioko Island varied significantly with elevation gradients, mirroring broader ecological trends identified in various biogeographical studies (Beck *et al.*, 2017; Dani *et al.*, 2023; Popovic *et al.*, 2021; Jaiswal & Jayakumar S, 2024). These trends were evident across all diversity indices measured, including both Shannon-Wiener and Inverse Simpson's indices.

The elevational diversity of butterflies on Bioko Island is likely shaped by several interrelated factors, including climate, precipitation, types of vegetation, and climatic stability. As elevation rises, temperature and humidity generally decrease, resulting in unique microclimates that can host different butterfly communities (Mtui et al., 2022). These environmental variations often affect the availability of essential resources, such as nectar and host plants, which are vital for the survival and reproduction of butterflies. Many plant species show variation in chemical composition along altitudinal gradients with plant populations at mid and higher elevation often having higher nitrogen concentrations than those at low elevations. The high levels of nitrogen concerntrations in the host plants serve as an attractive element for butteflies at these elevations. Moisture levels, influenced by rainfall and humidity, also affect butterfly distribution. gambiae, Eupheadra duseni, Bicyclus sandace, Cymothoe ceanis, Euriphene duseni, Charaxes protoclea, Euriphene insecta, Cymothoe beckeri, Gnophodes chelys, Catuna crithea which were more abundant at mid elevation due to optimal productivity of their host plants Hypericum lanceolatum, Festuca scimpeana as well as favorable temperature and rainfall conditions (Pires et al., 2020).

Other indicator species like Acrea rogersi, Papilio phorias, Bicyclus golo, Bicyclus safitza, Bicyclus sophrosyne, Charaxes numenes and endemic species like Bicyclus feae, Cymothoe fumana, and Ceratrichia flava fernanda, from the highland regions seem to prefer highland regions. These species are among the deep forest indicators and ten endemic butterflies identified in the country, each confined to mountainous environments, either within a specific highland area or among a small group of highlands with endemic plant species. This situation underscores the urgent need for their conservation, especially in light of the increasing human population (Dinersten et al., 2017). Unfortunately, these highland regions remain insufficiently protected (Bergl et al., 2007), likely due to the limited number of studies highlighting their unique biodiversitysuch as the one presented here.

The mountain forests of Bioko Island are acknowledged as a hotspot for biodiversity and endemism across various taxonomic categories (Myers *et al.*, 2000). Findings demonstrate significant variations in species diversity at different altitudes, indicating that certain Some species are adapted to drier environments, while others thrive in more humid conditions.

Research on mountain ecosystems suggests that this ecological diversity is key to influencing butterfly populations, as distinct climatic conditions can lead to variations in species richness and composition across different altitudes (Maicher et al., 2018). Additionally, the types of vegetation found at various elevations play a significant role in determining species diversity, with diverse native flora typically supporting higher butterfly populations compared to less varied plant life which can lead to a decline in species richness, as demonstrated for example by studies conducted by Pires et al., (2020). As altitude increases, habitats shift from tropical lowlands to montane forests, thereby changing the availability of essential resources for various butterfly species (Dar et al., 2022; Srivastava & Lawton, 1998). Furthermore, habitat stability, affected by human activities such as agriculture and deforestation, poses ongoing threats to butterfly diversity in these elevational zones (Habel et al., 2021). Natural disturbances can either enhance or impede butterfly populations, highlighting the complex interactions that shape the elevational diversity of butterflies on Bioko Island.

During the expedition, we collected samples of butterflies, including *Bicyclus italus, Charaxes brutus, Euriphene* 

endemic butterfly families, such as Hesperidae, Papilionidae, and the subfamily Satyrinae, show a higher degree of specialization and are more confined to specific elevational zones compared to the more adaptable Pieridae. The potential destruction of these habitats could trigger regional species migration and a significant decline in biodiversity, ultimately leading to the extinction of numerous species that are not present in lowland areas or other tropical environments.

Our study however had limitations as it concentrated on small spatial scales and short timeframes, which restricted our understanding of long-term ecological changes and the effectiveness of restoration efforts at larger scales. To address this, we recommend expanding the scope to include larger areas across the Island and implementing longer monitoring periods and protocols. This approach will enhance mountain restoration efforts on the Island and globally, thus improving success through adaptive management strategies.

In conclusion, the research notably revealed distinct species assemblages at different altitudes, indicating that elevational gradients play a significant role in shaping butterfly biodiversity. This provides valuable insights into how environmental factors, especially temperature and vegetation changes linked to elevational changes, shape species distributions and community composition.

This distinction emphasizes the urgent need for focused conservation initiatives tailored to these diverse habitats, which are increasingly at risk due to environmental changes. The conservation implications are significant; focused efforts are necessary to safeguard these specific habitats, especially in regions where human activities threaten to reduce butterfly populations. It is essential to develop and implement a comprehensive land use zoning plan that prioritizes and protects areas with distinct butterfly diversity of considerable conservation importance.

This plan should actively engage local communities to cultivate a sense of ownership and responsibility for conservation initiatives. It should allocate critical habitats and ecological corridors to promote biodiversity, while also addressing population growth. Furthermore, implementing conservation strategies that account for the effects of climate change on elevational ranges is vital for preserving ecological balance. A robust monitoring system should be developed to track climate variations, assess ecosystem health, and evaluate the effectiveness of conservation initiatives. Ultimately, protecting the unique biodiversity of Bioko Island will not only support butterfly species but also enhance overall ecological health, emphasizing the interconnections of species and their environments in conservation efforts.

### Data Availability Statement

All data generated or analyzed during this study are included in this published article in the form of figures and tables. Additional information about the dataset or accessing the dataset in a different format than what is presented in this article can be obtained from the corresponding authors upon request.

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# **Conflicts of Interest**

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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