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Abundance, Distribution and Association of *Paphiopedilum fairrieanum* (Lindl.) Stein with Site Factors in Brongshing, Samdrup Jongkhar District

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Abstract: The long-lived and attractive flower of *Paphiopedilum fairrieanum* (Lindl.) Stein has made it one of the most commercialized flowers in the world, and its distribution is restricted to small areas. Thus, it was listed as a critically endangered orchid species by the IUCN in 2015. Therefore, the aim of this study was to generate information on the abundance and distribution of *Paphiopedilum fairrieanum* and assess the relationship between the abundance and distribution of *Paphiopedilum fairrieanum* with site factors in the study area. A systematic sampling method was followed for the data collection, with a 200 m distance between the plots. A total of 34 sample plots with 5.64 m radii were established. In total, 1004 individuals of orchid species were recorded. The abundance and distribution of the *Paphiopedilum fairrieanum* showed statistically significant associations with the elevation ($r = -0.350$, $p = 0.042$), slope ($r = 0.666$, $p = 0.000$), precipitation ($r = -0.630$, $p = 0.000$) and temperature ($r = 0.371$, $p = 0.031$). A southeast aspect was found to have the highest number of *Paphiopedilum fairrieanum*, and a northwest aspect had the least. The findings of this study would help researchers to find suitable sites and the distribution of *Paphiopedilum fairrieanum* in unexplored areas.

Keywords: aspect; elevation; precipitation; slope; temperature

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1. Introduction

Orchidaceae is one of the major families of flowering plants, and it constitutes five subfamilies with approximately 870 genera and 30,000 species worldwide [1,2]. They are known for their variety in habits and habitats. They are either saprophytes, terrestrials, lithophytes or epiphytes. Orchidaceous plants are considered a gem because of their beautiful flower and medicinal and economic values [3].

Bhutan has 469 species of orchids recorded to date [4]. According to [5], Bhutan has 16 species of orchids listed as threatened and 14 species as endemic to the country, and from these 14 endemic species, 6 are epiphytic and 8 terrestrials. *Paphiopedilum fairrieanum* (Lindl.) Stein is one such species terrestrial in nature found in Bhutan, which is listed as CR by the IUCN. Globally, the orchid is found in Bhutan, India and Nepal [6]. It was found that the population of *Paphiopedilum fairrieanum* (Lindl.) Stein has dropped globally over the years due to over-collection and habitat degradation [7]. It was also stated that *Paphiopedilum* sp. are one of the most commercialized as a potted plants and cut flowers owing to their attractive appearance [8].

According to [7], the population of the *Paphiopedilum fairrieanum* (Lindl.) Stein was estimated to be less than 50 mature individuals, and the population is severely fragmented globally. The orchid is also known as the lost orchid due to its rarity [9]. According to [6], there are more than 6700 individuals distributed in 10 districts in Bhutan. *Paphiopedilum fairrieanum* particularly grows on arid, grassy limestone hills at high altitudes [5]. The species was also found growing in limestone, gneiss and dolomitic gravels

and rocks under oak forests with a very good drainage system [10]. In Bhutan, the species is found growing in limestone hills and ridges of subtropical forests [6]. According to [11], these orchid species were found on limestone substrates with soil pH values ranging from 7.3 to 8.0 and moisture content ranging from 1.4 to 2.5%.

October until January is *Paphiopedilum fairrieanum*'s blossoming period. The orchid has the most distinguishing flower. The flower has a large white and purple-striped dorsal sepal with an undulate margin and rounded at the apex. It has S-shaped petals with undulate margins and the staminate, which is three-toothed at the apex, with the central tooth protruding [10]. The length of the flower measures between 5 to 10 cm, and it has a width of more than 8 cm [12]. Mature individuals produce one or more peduncles, and each peduncle ends in a solitary flower [13]. According to [12], an individual *Paphiopedilum fairrieanum* has three to six numbers of leaves being more than 10 cm in length and more than 2 cm in breadth. The shape of the leaf is oblong with an obtuse apex. It has an undulated margin with the absence of variegation on the leaves. *Paphiopedilum* has short rhizomatous roots. Their roots are hairy, and they are hardly stoloniferous [5,14]. *Paphiopedilum* were found to be drought tolerant due to its leaf's anatomical structures. The lifespan of leaves was found to be more than three years [15].

In Bhutan, the species has been assessed in Ngala under Zhemgang district, Kheng-Gongdu under Mongar district, Martshalla Sarjung under Samdrup Jongkhar district [6] and Gomdar and Wangphu geog under Samdrup Jongkhar [16]. However, there were no studies regarding the association of *Paphiopedilum fairrieanum* with site factors globally. Its abundance and distribution have not been studied so far in the Brongshing locality in Gomdar in the Samdrup Jongkhar district. The Bhutan Trust Fund for Environmental Conservation reported that the number of mature individuals is declining as per villagers of Gomdar [17].

Therefore, this study tries to fill up this gap by determining its distribution, abundance and relationships with geophysical factors (slope, and elevation) and climatic factors (annual temperature and annual precipitation) in the study site; this information would be useful in finding distribution in other unexplored parts as well as finding favorable locations for growing this species. Though there are few works of literature regarding the habitat assessment of the *Paphiopedilum fairrieanum*, there is no scientific record stating how the geophysical and climatic factors affect the abundance and the distribution of the species.

2. Materials and Methods

Study Site

Bhutan is located at the latitude of 26.5° to 28° N and longitude ranging from 88.5° to 92° E in Himalayas [18]. The study was conducted in the southeastern part of the country and the area is known as Brongshing under Samdrup Jongkhar district (Figure 1). The study area covers approximately 170 hectares with an altitude ranging from 980 m.a.s.l to 2000 m.a.s.l. The study falls under the subtropical climatic zone. There are 26 households in this locality, and approximately 100 people living there. On average, the study area receives 1458.66 mm of precipitation annually and an annual average temperature of 17.1 °C [19]. The main food grain grown is maize, and orange is their main cash crop. It has a southern aspect.

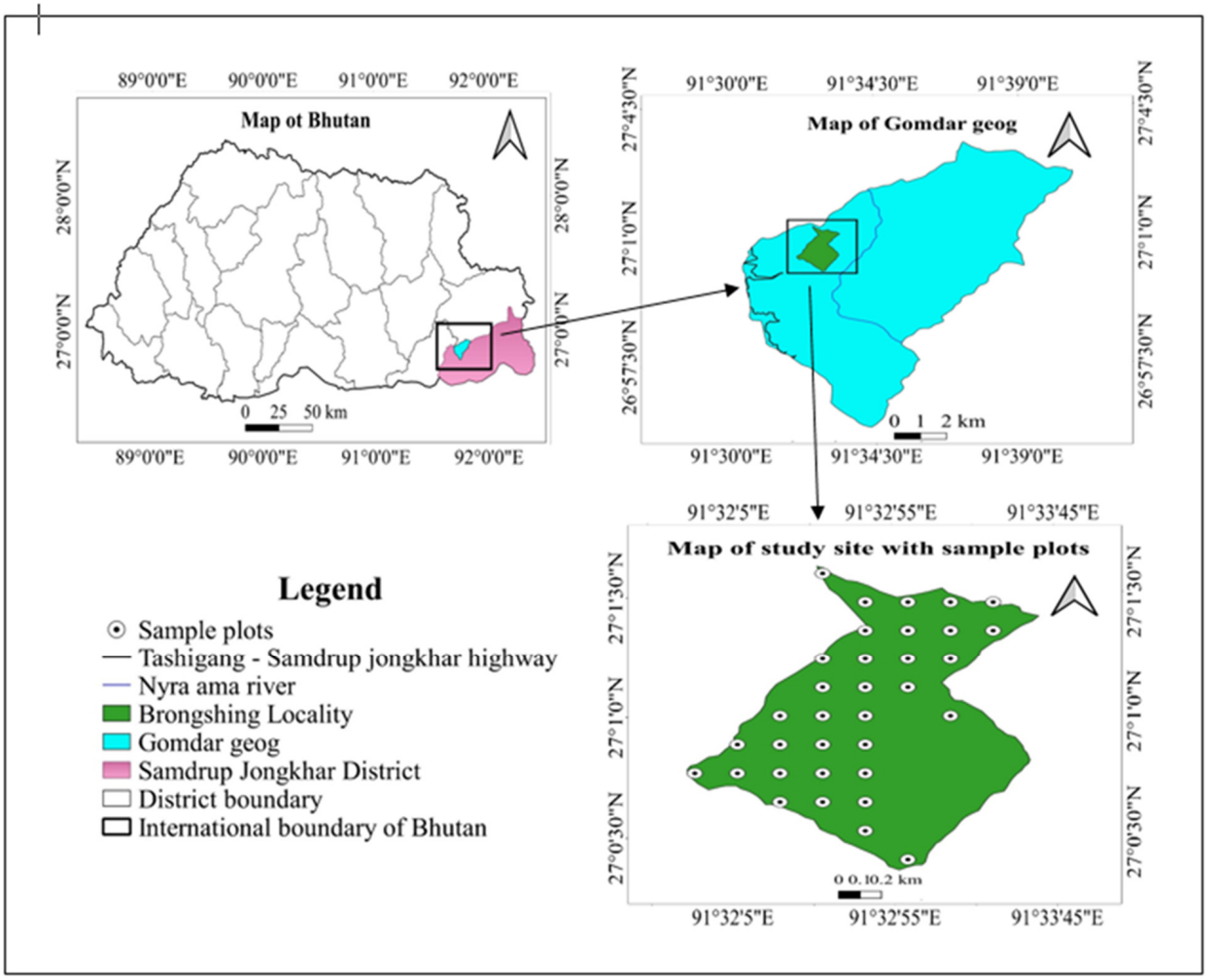

Figure 1. Map of the study site.

3. Data Collection, Method and Data Analysis

Systematic sampling was applied to collect the data with circular plots of a radius of 5.64 m. The plots had an area of 100 m^2. The circular plots were followed because the ratio of the perimeter to the area was minimum in circular plots. Laying out plots in the field avoids the rhombus laying of plots. In the case of other shaped plots such as squares and rectangles, there is a chance of laying out the plots in a rhombus shape, especially in hilly areas, which results in biased data. Circular plots are easier to lay out, and also, the data will be not biased. The sample points were laid in a GIS Arc Map with a distance of 200 m between the plots. The plots, which fell in human settlements and inaccessible parts of the study area, were discarded. The plots in the field were located by using GPS. In the field, the plots were laid using meter tape with a 5.64 m radius. There were 34 plots in total (see Appendix A), excluding those in the settlement areas and inaccessible parts. The field data collection was carried out from October 2019 to February 2020.

Information regarding *Paphiopedilum fairrieanum* from the plots was collected on different levels—individual, clump (having more than two individuals), matured individuals (individuals with peduncles) and immature individuals (individuals without peduncles) [13] were counted. The slope and aspect were measured from the center of the plots. The slopes were corrected using the formula $r' = r/\cos^2(a/2.1)$ as per [20], where r' is the corrected radius, r is a radius on the horizontal plane (5.64 m) and a is the slope angle. It was carried out so that the areas of the plots were not underestimated in slopy areas.

For the determination of the spatial distribution of this species, GIS, ArcMap 10.4 was used to produce a distribution map of *Paphiopedilum fairrieanum* in the study area. The abundance of *Paphiopedilum fairrieanum* was determined by counting the number of *Paphiopedilum fairrieanum* individuals in the plots as per [21].

The relationship between the abundance and distribution of *Paphiopedilum fairrieanum* with its site factors such as geophysical factors (slope and elevation) and climate factors (annual temperature and annual precipitation) of the plots were determined by running the Spearman's rho correlation test in statistical software SPSS version 23.0, as the data were not normal. The annual temperature and annual precipitation of the plots were calculated using the climatic model developed by [19]. To organize the collected data from the field, Microsoft Excel sheets (.xlsx) and Microsoft Word documents 2016 were used.

4. Results

4.1. The Abundance of Paphiopedilum fairrieanum (Lindl.) Stein

A total of 1,004 individuals of *Paphiopedilum fairrieanum* were encountered, with 927 matured individuals, 77 immature individuals and 97 clumps (Figure 2) within the sampled area of 1000 m^2 (0.1 hectares). In a clump, the maximum numbers of *Paphiopedilum fairrieanum* were 23 individuals, and the lowest was 2. On average, there were 12 to 13 individuals in a clump. The highest count of 271 individuals was recorded at an elevation of 1070 m.a.s.l, and the lowest count of nine individuals was at 1509 and 1701 m.a.s.l. On average, the abundance of *Paphiopedilum fairrieanum* per 100 m^2 in the study area was around 100 individuals (Figure 3).

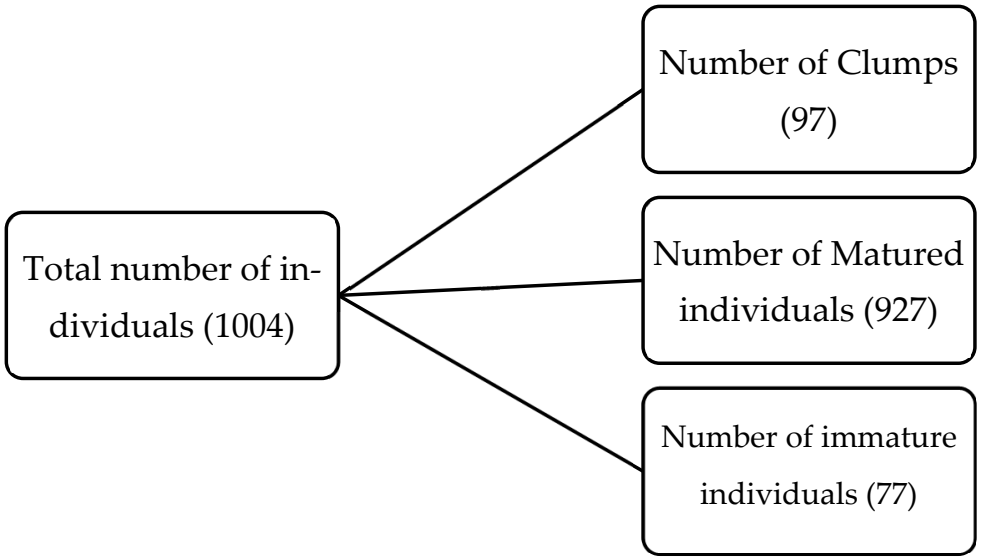

Figure 2. Total *Paphiopedilum fairrieanum* individuals, number of clumps, matured individuals and immature individuals.

In the study area, *Paphiopedilum fairrieanum* was found growing on the woody shrub *Spermadictyon suaveolens*, which were slanting and covered by mosses. The Orchid species was found to be grazed by wild ungulates such as Deer (*Muntiacus Muntjac*), Goral (*Neomorhadus goral*) and Serow (*Capricornis sumatraensis*) at the study site.

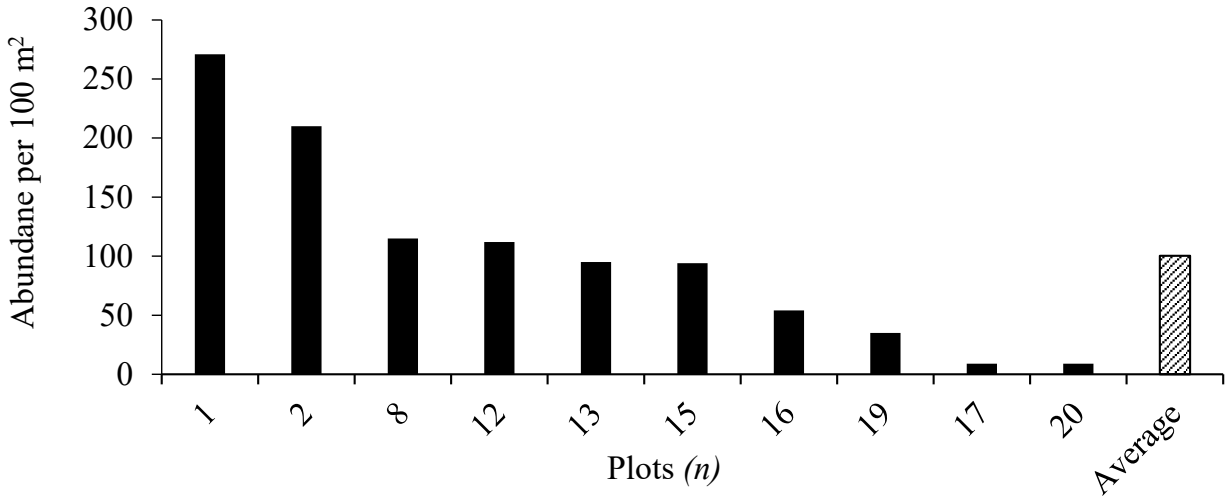

Figure 3. The abundance of *P. fairrieanum* per 100 m^2 and its average.

4.2. Spatial Distribution of Paphiopedilum fairrieanum (Lindl.) Stein in the Study Site

In the study area, *Paphiopedilum fairrieanum* (Lindl.) Stein was found in 10 plots (Figure 4), in which the slope gradient ranges from 65° to 90°, with a slope aspect from southeast to southwest and elevation ranging from 1070 to 1709 m.a.s.l. The annual precipitation ranges from 1150.56 mm to 1164.54 mm, and the annual temperature ranges from 16.20 °C to 19.68 °C. The areas with *Paphiopedilum fairrieanum* in the study area were mainly covered by herbs, grasses, shrubs and trees with open-canopy vegetation.

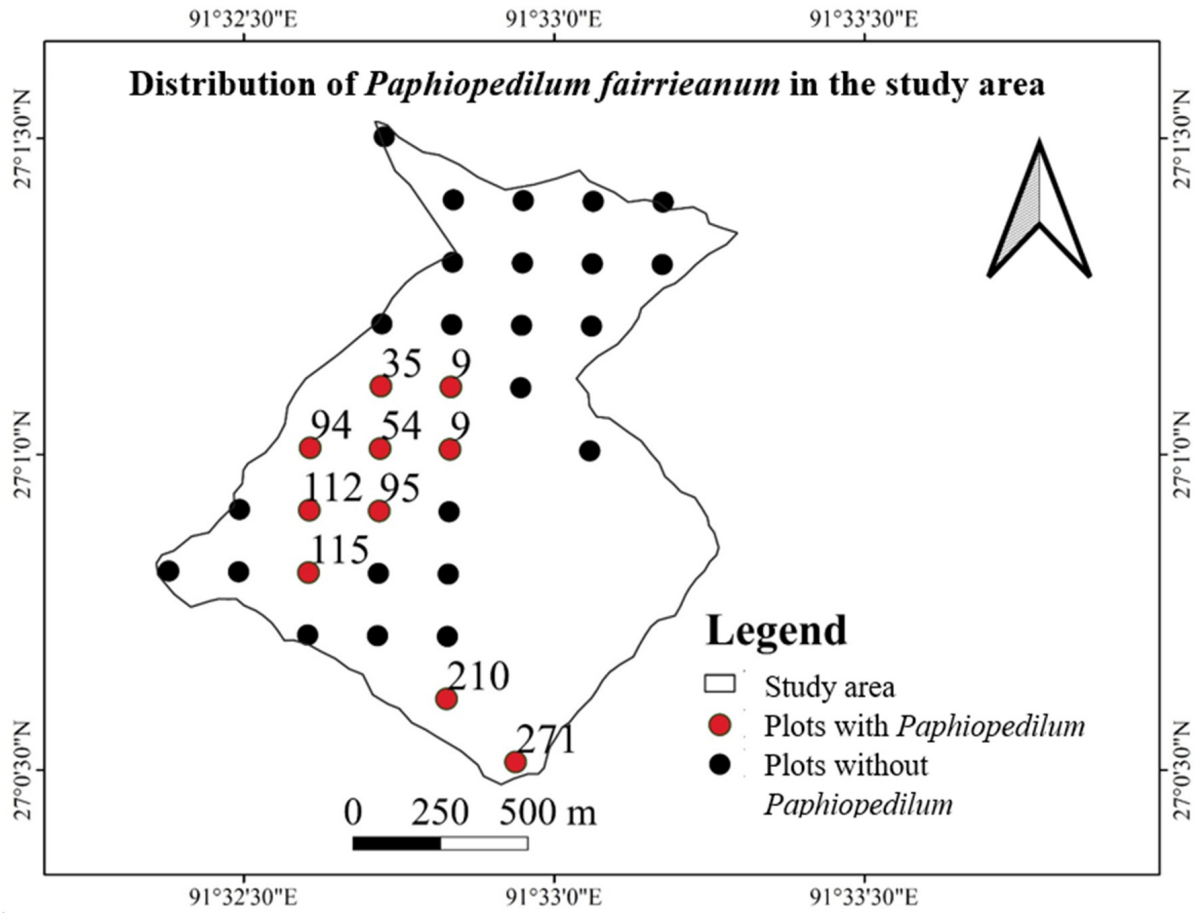

Figure 4. Spatial distribution of *Paphiopedilum fairrieanum* in the study area.

4.3. Relationship between Abundance and Distribution of Paphiopedilum fairrieanum (Lindl.) Stein with Site Factors (Climate and Geophysical Factors)

4.3.1. Relationship between Abundance and Distribution of *Paphiopedilum fairrieanum* with Annual Precipitation

As per Spearman's rho correlation, the abundance and distribution of the *Paphiopedilum fairrieanum* in the study area were significantly and negatively associated with annual precipitation ($r = -0.630$, $p = 0.000$) (Table 1). This showed that with the increase in annual precipitation in the study area, the abundance and distribution of the *Paphiopedilum fairrieanum* decreases. We found out that the orchid species is adapted to dry, grassy and steep slope limestone areas.

Table 1. Spearman's rho correlation between annual temperature, annual precipitation, slope, elevation and aspect with distribution and abundance of *Paphiopedilum fairrieanum*.

	Total Number of Orchids	Annual Precipitation (mm)	Annual Temperature (°C)	Slope (°)	Elevation (m.a.s.l.)
Total number of orchids	1	−0.630 **	0.371 *	0.666 **	−0.350 *
Annual precipitation (mm)		1	−0.129	−0.986 **	0.133
Annual temperature (°C)			1	0.266	−0.987 **
Slope (°)				1	−0.27
Elevation (m.a.s.l.)					1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

4.3.2. Relationship between Abundance and Distribution of *Paphiopedilum fairrieanum* with Annual Temperature

The result of Spearman's rho correlation demonstrated that in the study area, there was a significant positive association between the distribution and abundance of *Paphiopedilum fairrieanum* with the annual temperature ($r = 0.371$, $p = 0.031$) (Table 1), which indicates that with the increase in annual temperature, the distribution and abundance of the species increases. In the study area, *Paphiopedilum fairrieanum* was found in open-canopy areas, which indicates that they receive direct sunlight. Thus, these sites would be warmer. *Paphiopedilum fairrieanum* individuals were found growing in study areas with maximum, minimum and average temperatures of annual and for different seasons as given in Table 2.

Table 2. Maximum, minimum and average temperature [19] where *Paphiopedilum fairrieanum* was found growing in the study site.

	Maximum (°C)	Minimum (°C)	Average (°C)
Temperature (Annual)	19.68	16.27	17.98
Temperature (Dec–Feb)	14.02	10.50	12.26
Temperature (Mar–May)	20.02	16.41	18.21
Temperature (Jun–Aug)	23.94	20.80	22.37
Temperature (Sep–Nov)	20.66	17.29	18.98

4.3.3. Relationship between Abundance and Distribution of *Paphiopedilum fairrieanum* with Slope Gradient

The distribution and abundance of the *Paphiopedilum fairrieanum* in the study site was affected by the slope gradient on which it grows. *Paphiopedilum fairrieanum* individuals were found growing on slopes ranging from 65° to 90° (A1). Based on Spearman's rho correlation result, this showed that the slope significantly and positively affects the distribution and abundance of *Paphiopedilum fairrieanum* in the study area ($r = 0.666$, $p = 0.000$) (Table 1). It shows that with the increase in the degree of the slope, the abundance and distribution of the species increases.

Generally, in the study area, the slope gradient ranges from the lowest of 45° to the highest of 90° (A1). This demonstrates that the study area was a hilly or sloppy area.

However, no individuals of *Paphiopedilum fairrieanum* were encountered below 65° of slope gradient in the study area.

4.3.4. Relationship between Abundance and Distribution of *Paphiopedilum fairrieanum* with Elevation

The Spearman's rho correlation results showed a negative association between the abundance and distribution of *Paphiopedilum fairrieanum* with the elevation ($r = -0.350$, $p = 0.042$) (Table 1). This shows that with the increase in elevation, the distribution and abundance of the *Paphiopedilum fairrieanum* decreases in the study area. The lowest elevation where it occurred in the study area was 1070 m.a.s.l., with a maximum orchid count of 271 individuals in a plot and the highest elevation of 1709 m.a.s.l. with an orchid count of 35 individuals in a plot being recorded (A1).

4.4. Distribution and Abundance of Paphiopedilum fairrieanum with Respect to the Aspect

This orchid species was only recorded from three slope aspects (southeast, south, and southwest) out of 10 plots (plots with *Paphiopedilum fairrieanum* individuals) in the study area. From three slope aspects, more individuals of *Paphiopedilum fairrieanum* were found facing southeast, followed by south- and then southwest-facing directions (Figure 5).

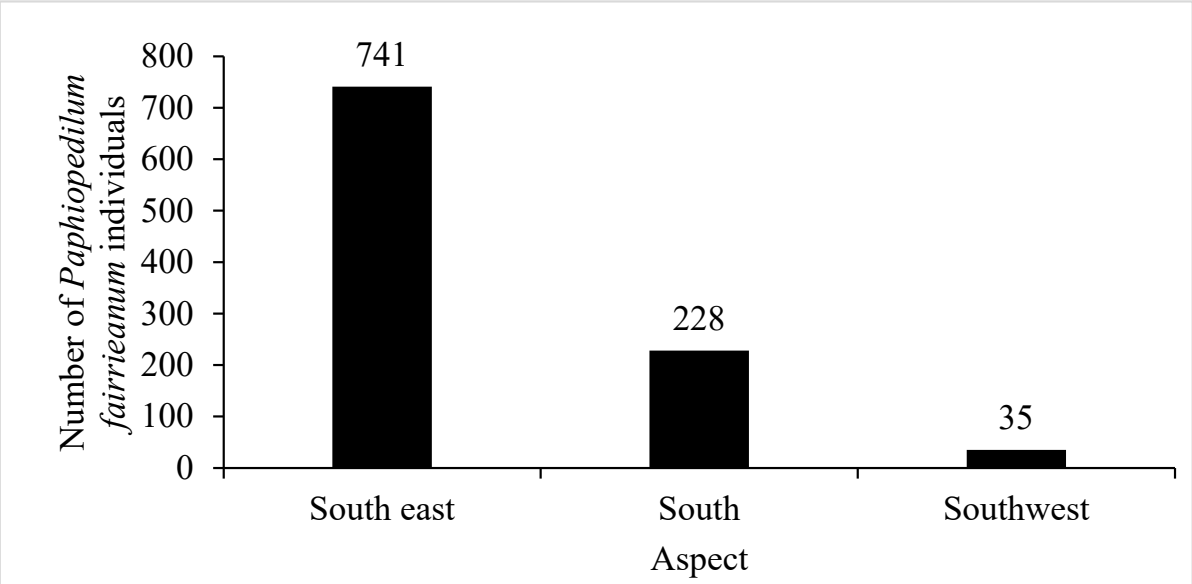

Figure 5. Abundance and distribution of *Paphiopedilum fairrieanum* with respect to slope aspect.

5. Discussion

5.1. The Abundance and Spatial Distribution

The study recorded 1004 individuals of *Paphiopedilum fairrieanum* with 927 matured individuals, 77 immature individuals and 97 clumps. However, [22] reported that 900 mature individuals were recorded from Gomdar gewog under Samdrup Jongkhar district. Similarly, [6] reported that in Gomdar, around 800 individuals of *Paphiopedilum fairrieanum.* Most of the *Paphiopedilum fairrieanum* individuals in the study area were found growing in open areas with herby, grassy and shrubby vegetation with an open canopy. The authors of [6,16] also reported that the habitat of this orchid species was deciduous shrubs and tree vegetation.

5.2. Abundance and Distribution of Paphiopedilum fairrieanum with Respect to Climatic and Geophysical Factors of the Study Site

We found that this orchid species was negatively affected by the annual precipitation of the study site. The finding of the study was supported by [15,23], in which they found

out that the natural habitat of *Paphiopedilum* has low soil water. They also found out that the species was found adapted to dry and nutrient-poor habitats. Moreover, [13] found out that the plants were growing on limestone substrates, and the soil moisture content ranged from 1.4 to 2.5%, which was very low. Furthermore, [6] stated that *Paphiopedilum fairrieanum* was found in dry, slopy areas, where the risk of forest fires was higher. *Paphiopedilum* was found to be drought tolerant due to its leaf structure having sunken stomata and thick cuticles on both sides of the leaves, which aids in reducing transpiration [15]. It was mentioned that the photosynthetic rate was very low in the case of *Paphiopedilum* [23]. It was also found that their leaf lifespan was very long, and it conserves nutrients, compensating for its low photosynthesis, indicating a lower requirement for water [15]. Therefore, all those traits correspond to the lower need for water by *Paphiopedilum fairrieanum*. It was found that its underground parts were rhizomatous in nature, which conserves water, and this indicates that its environment has less water. It can also be understood that limestone areas are all dry, and the survival of this orchid species in such harsh environments also shows its adaptation to areas with less water.

The annual temperature was found to positively affect the distribution and abundance of this orchid species in the study area. Similarly, [8] found that in China, *Paphiopedilum* sp. were found growing in warmer environments. It was also stated that many of the studies found that *Paphiopedilum* seed germination takes place between a temperature range of 22 °C to 28 °C [24]. Furthermore, for the in vitro shoot induction and plant regeneration from the flower buds, they have grown *Paphiopedilum*, where a medium was maintained at 25 °C to 27 °C [22]. Moreover, [25] found that *Paphiopedilum* seed germinated well on Norstog medium when the temperature was maintained at 25 °C, because almost all seeds sprout from the medium. All the above-mentioned temperatures were between 20 °C and 30 °C, which was moderately high. The authors of [13] also stated that *Paphiopedilum* could tolerate coldness but only for short period, which shows that it is found in warmer areas. In the study area, more populations of *Paphiopedilum fairrieanum* were found in lower elevations. There was also a significant and negative association between elevation and annual temperature ($r = -0.987$, $p = 0.000$) (Table 1), which showed that at lower elevations, the temperature was higher, and there was a greater number of *Paphiopedilum fairrieanum* individuals. However, presently, there is a lack of literature regarding the impact of annual temperature on the distribution and abundance of this orchid species. Therefore, there is a need for future studies to determine the impact of the abundance and distribution of the orchid species with geophysical and climatic factors.

Moreover, in the study area, the distribution and abundance of this orchid species was found to be affected positively by the slope gradient in the study area. There were more individuals of *Paphiopedilum fairrieanum* distributed in steep slope areas of the study area. The possible reasons could be to avoid disturbances from wild and domestic animals and also from human disturbances. Similarly, such a slope range and reasons were reported by [6]. They recorded 45° as the lowest slope in Samtse district, but commonly, the species occurs between slope ranges of 65° to 95°. The authors of [16] also reported the same range of slopes in Gomdar and Wangphu geogs. However, they did not evaluate its association with the slope in their study area. Another reason could be as this orchid species was negatively associated with annual precipitation, so to drain out rain easily, it may prefer steep slopes; [10] found out that the orchid species was growing in areas with very good drainage systems in limestones, gneiss and dolomitic gravels and rocks under oak forest.

In the study area, the number of *Paphiopedilum fairrieanum* decreases as the elevation of the study site increases and vice versa. There was a significant negative association between elevation and the number of this orchid's distribution and abundance. As mentioned above, it was due to a negative association between elevation and annual temperature. In the study area, despite having favorable conditions such as limestone substrates and a grassy, shrubby, and sloppy environment, the abundance and distribution of this orchid species decreases as elevation increases and was found until the elevation of 1790 m.a.s.l. and not above that elevation. However, in Bhutan, to date, its range was reported to be 980–1550 m.a.s.l. by [6],

but [16] found that the highest altitude was 1690 m.a.s.l. Therefore, there should be more studies to confirm its highest and lowest elevation ranges from Bhutan. However, [26] mentioned that at higher altitudes, due to less visitation by the pollinators, the abundance of orchid species is lower. This may be also the possible reason that the abundance and distribution of *Paphiopedilum fairrieanum* in the study area decreases as elevation increases, because it was mentioned that the effectiveness of the pollination in terrestrial orchids is a determining factor for the abundance and distribution of the terrestrial orchids [26].

Meanwhile, the maximum number of this orchid species was found facing southeast followed by the southern aspect and then by the southwestern aspect. The orchid species was not found in other aspects of the study area. Similar findings were reported by [16]. This may be due to its need for high temperature because the study area receives direct sunlight in those three aspects. This may also be because *Paphiopedilum* sp. were found to be drought tolerant [15]. However, according to [6], the orchid species mostly occurs in the northwest to southwest facing slopes. Furthermore, [11] found that this orchid species was distributed on north-, northwest- and northeast-facing slopes in Jigme Singye Wangchuk National Park in Bhutan and stated that the slope is not an important parameter for its distribution and abundance. Therefore, the aspect does not play an important role in the abundance and distribution of this orchid species.

6. Conclusions

In total, it was found that there are 1004 *Paphiopedilum fairrieanum* individuals with an average of 100 individuals per plot in the study area. The species was found distributed in 10 plots out of 34 plots in total, which has a slope range between 65° to 90° and elevation between 1070 to 1709 m.a.s.l. The species was found to be statistically significant in terms of elevation, slope, annual temperature and annual precipitation. The elevation and the precipitation were negatively associated, and slope gradient and temperature were positively associated. The orchid species was found in three aspects of the study area.

The people of the locality should be made aware that this species is critically endangered, and they should protect it. The concerned authority should balance the needs of the people and the conservation of this orchid species in the study area. Finally, the study was conducted in small areas, and therefore, the findings may or may not be generalized to other areas. Therefore, further study is still needed.

Author Contributions: Conceptualization, U.D.; Data curation, K.W.; Formal analysis, K.W.; Methodology, K.W.; Resources, K.W.; Software, U.D.; Supervision, U.D.; Writing—original draft, K.W.; Writing—review & editing, U.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: This research was conducted only after the approval of research clearance from the Ugyen Wangchuk Institute for Conservation and Environmental Research of Bhutan in 2020 and as per their research guidelines.

Abbreviations

Aug	August
BTFEC	Bhutan Trust Fund for Environmental Conservation
cm	Centimeter
CNR	College of Natural Resources
CR	Critically endangered
Dec	December
DoFPS	Department of Forest and Park Services
Feb	February
GIS	Geographic Information System
GPS	Global Positioning System
IUCN	International Union for Conservation of Nature
Jun	June
km	Kilometer
m	Meter
m.a.s.l	Meters above sea level
Mar	March
mm	Millimeter
n	Number
NEC	National Environment Commission
Nov	November
p	Significant value
Preci.	Precipitation
r	Correlation coefficient
Sep	September
Sp.	Species
SPSS	Statistical Package for the Social Sciences
Tempt.	Temperature

Appendix A. Elevation, Slope Gradient and Individual Count in Each Plot

Table A1. Table showing plot-wise elevation, slope gradient and individuals count of *Paphiopedilum fairrieanum* in the study area.

Plot Number	Total Number of Orchids	Elevation (m)	Slope Gradient (°)
1	271	1070	90
2	210	1140	85
3	0	1190	65
4	0	1195	55
5	0	1220	65
6	0	1249	50
7	0	1232	55
8	115	1233	90
9	0	1343	70
10	0	1358	60
11	0	1388	60
12	112	1497	75
13	95	1498	70
14	0	1473	65
15	94	1563	80
16	54	1530	65
17	9	1558	65
18	0	1590	50
19	35	1709	85
20	9	1697	70
21	0	1609	60
22	0	1682	55
23	0	1739	50

Table A1. *Cont.*

Plot Number	Total Number of Orchids	Elevation (m)	Slope Gradient (°)
24	0	1819	65
25	0	1714	70
26	0	1816	65
27	0	1793	85
28	0	1748	65
29	0	1755	75
30	0	1896	55
31	0	1973	50
32	0	1890	45
33	0	1892	50
34	0	1998	65

References

1. Dressler, R.L. *Phylogeny and Classification of the Orchid Family*; Dioscorides Press: Portland, OR, USA, 1993; 314p.
2. Mathew, J.; George, V.K. Checklist of Orchids of Kottavasal Hills in Achancoil Forests, Southern Western Ghats, (Kollam, Kerala), India. *J. Threat. Taxa* **2015**, *7*, 7691–7696. [CrossRef]
3. Hegde, S.N. *Orchid Wealth of India*; State Forest Research Institute, Arunachal Pradesh: Itanager, India, 1997; pp. 229–244.
4. DoFPS. Forest Facts and Figures 2019. Available online: https://www.dofps.gov.bt/reports/ (accessed on 12 September 2022).
5. Pearce, N.R.; Cribb, P.J. *The Orchids of Bhutan*; Royal Botanic Gardens Edinburg: Edinburgh, Scotland, 2002.
6. Gurung, D.B.; Gyeltshen, N.; Tobgay, K.; Dalstrom, S.; Wangdi, J.; Ghalley, B.B.; Chaida, L.; Phuntsho, N.G.; Dawa, K.; Wangchuk, T.; et al. Distribution and habitats of *Paphiopedilum* Pfitzer (Orchidaceae) known to occur in Bhutan. *J. Threat. Taxa* **2019**, *11*, 14101–14111. [CrossRef]
7. Rankou, H.; Kumar, P. *Paphiopedilum fairrieanum*. The IUCN Red List of Threatened Species 2015: E.T43320321A43327829. Available online: http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T43320321A43327829.en (accessed on 29 August 2022).
8. Zhang, F.P.; Huang, J.L.; Zhang, S.B. Trait Evolution in the Slipper Orchid Paphiopedilum (Orchidaceae) in China. *Plant Signal. Behav.* **2016**, *11*, e1149668. [CrossRef] [PubMed]
9. Chowdhery, H.J. Lady's Slipper Orchids in India. In *Orchid Memories: A Tribute to Seidenfaden*; Kumar, C.S., Ed.; Mentor Books: Calicut, India, 2004; pp. 35–48.
10. Cribb, P. *Paphiopedilum fairrieanum*. *Kew Mag.* **1985**, *2*, 351–354. Available online: https://www.jstor.org/stable/45066428 (accessed on 20 August 2022). [CrossRef]
11. Tshewang, S.; Gyeltshen, P.; Chetenla; Nepal, A.; Dendup, K.C.; Letro, L. Population status, habitat composition and threats of *Paphiopedilum fairrieanum* (Lindl.) Stein (Orchidaceae: Cypripedioideae) in Jigme Singye Wangchuck National Park. Bhutan. *J. Nat. Resour. Dev.* **2022**, *9*, 11–21. [CrossRef]
12. Dhiman, S.; De, L.C.; Singh, D.R.; Agrawala, D.K. DUS Test Guidelines in Paphiopedilum orchid ICAR-NRC for orchids. *Plant Variety Journal of India* **2015**, *9*, 219–238. [CrossRef]
13. Shi, J.; Luo, Y.B.; Bernhardt, P.; Ran, J.C.; Liu, Z.J.; Zhou, Q. Pollination by deceit in *Paphiopedilum barbigerum* (Orchidaceae): A staminode exploits the innate colour preferences of hoverflies (Syrphidae). *Plant Biol.* **2008**, *11*, 17–28. [CrossRef] [PubMed]
14. Zhongjian, L.; Xinqi, C.; Cribb, J.P. *Paphiopedilum* Pfitzer, Morphological Studies of Orchid. *Flora China* **2009**, *25*, 33–44.
15. Guan, Z.J.; Zhang, S.B.; Guan, K.Y.; Li, S.Y.; Hu, H. Leaf anatomical structures of *Paphiopedilum, Cypripedium*, and their adaptive significance. *J. Plant Res.* **2010**, *124*, 289–298. [CrossRef] [PubMed]
16. Samdrup, T.; Dorjee, S.; Phurpa, K.W.; Dorjee, S.; Cheda, K.; Rinzin, C.; Subba, K. Habitat assessment of *Paphiopedilum fairrieanum* (Lindl.) Stein (Orchidaceae: Cypripedioideae) in Samdrup Jongkhar, Bhutan. *NeBIO* **2020**, *11*, 40–41.
17. BTFEC. Reviving *Paphiopedilum fairrieanum*. 2017. Available online: www.bhutantrustfund.bt/?p=2114 (accessed on 12 August 2019).
18. NEC. *Second National Communication to the UNFCCC*; Phama Printers and Publishers: Thimphu, Bhutan, 2011.
19. Dorji, U.; Jørgen, E.; Olesen, J.E.; Bøcher, K.P.; Seidenkrantz, S.M. Spatial Variation of Temperature and Precipitation in Bhutan and Links to Vegetation and Land Cover. *Mt. Res. Dev.* **2016**, *36*, 66–79. [CrossRef]
20. Kleinn, C.; Traub, B.; Hoffmann, C. A note on the slope correction and the estimation of the length of line features. *Can. J. For. Res.* **2002**, *32*, 751–756. [CrossRef]
21. Djordjević, V.; Tsiftsis, S.; Lakušić, D.; Jovanović, S.; Stevanović, V. Factors affecting the distribution and abundance of orchids in grasslands and herbaceous wetlands. *Syst. Biodivers.* **2016**, *14*, 355–370. [CrossRef]
22. Liao, J.Y.; Tsai, C.Y.; Sun, W.Y.; Lin, S.R.; Wu, S.F. In vitro shoot induction and plant regeneration from flower buds in *Paphiopedilum* orchids. *In Vitro Cell. Dev. Biol.-Plant* **2011**, *47*, 702–709. [CrossRef]
23. Yang, H.Z.; Huang, W.; Yang, Y.Q.; Chang, W.; Zhang, B.S. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between *Cypripedium* and *Paphiopedilum*, Orchidaceae. *Photosynth. Res.* **2017**, *3*, 81–90. [CrossRef] [PubMed]

24. Zeng, S.; Huang, W.; Wu, K.; Zhang, J.; Teixeira, S.D.; Jaime, A.; Duan, J. In vitro propagation of *Paphiopedilum* orchids. *Crit. Rev. Biotechnol.* **2015**, *1*, 55–61. [CrossRef]
25. Tay, J.L.; Takeno, K.; Hori, Y. Culture conditions suitable for in vitro seed germination and development of seedlings in *Paphiopedilum*. *J.-Jpn. Soc. Hortic. Sci.* **1986**, *57*, 243–249. [CrossRef]
26. Djordjevic, V.; Tsiftsis, S. The Role of Ecological Factors in Distribution and Abundance of Terrestrial orchids. *Orchid. Phytochem. Biol. Hortic.* **2020**, *9*, 12–19. [CrossRef]