



Article Associations between Epiphytic Orchids and Their Hosts and Future Perspectives of These in the Context of Global Warming

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Abstract: Epiphytic species are ecologically important and a significant component of biodiversity. To ensure their efficient conservation, we need to understand their ecology and host plant associations. It is also important to investigate how the predicted temperature change will affect their future distribution. Here, we use data collected in Nepal to investigate how epiphytic orchids are associated with host species, their distribution patterns, and how they may be threatened by the predicted increase in temperature towards the end of the 21st century. We used the phi coefficient (Φ) of association to calculate the associations of epiphytic orchid species with plants and rarefaction to describe the diversity of orchids associated with a particular host species. We used interpolation to estimate the distribution of epiphytic orchids and their host species along altitudinal gradients. The phi (Φ) coefficient of association revealed that 30 species of host plants showed more association with different orchid species than expected. The number of epiphytic orchids increased with the number of host individuals. We predict that an increase in temperature by \sim 3 °C, which is a more moderate value of temperature increase by the end of the 21st century, will affect at least 52 narrow-ranged species of orchids and 58 narrow-ranged species of host plants. Therefore, we should make efforts to prevent many plant species from becoming extinct, as an increase in temperature is likely to affect their existence.

Keywords: climate change; epiphyte; Himalayas; host; phi coefficient; rarefaction

1. Introduction

Epiphytes are important components of plant diversity and typically benefit from their host plants as they grow on them and receive facilitative support for growth [1]. Epiphytic orchids, which constitute 70% of all the epiphytes in the world [2], are of topical interest to ecologists [3–5]. The patterns of association between epiphytic orchids and host species are not uniform [6]. Studies have shown that epiphytes are associated with different species and various characteristics of their host plants [1,3,7]. In addition, some of the factors that influence the occurrence and diversity of epiphytic orchids are (i) host type (evergreen or deciduous); (ii) abundance or rarity of the host species; (iii) microclimate; (iv) height of the host species; (v) altitude; and (vii) abiotic environmental factors [8–11]. The existence of



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). these different factors in nature leads to the situation of some host species hosting more epiphytic orchids than other host species. So, it is necessary to find which host species are associated with more orchid species than other host species so that epiphytic orchid conservation can be achieved by protecting host species with more epiphytic orchids.

Altitudinal gradients, which differ mainly in temperature and precipitation, are major determinants of the distributions of species [12,13]. In general, species diversity decreases with increasing altitude, and this pattern is well demonstrated by different studies and also for Nepal [14–16]. Li and Feng (2015) found a significant decrease in species richness in the biodiversity of tropical and temperate genera in Nepal. Likewise, altitudinal patterns of orchid species in Nepal are also available [14], but they do not mention the distribution patterns of epiphytic orchid species and associated host species in Nepal.

In Nepal, there are 251 species of epiphytic orchids [17]. They have been studied in terms of the effect of human activity on their ecological niches [18,19], diversity, composition, distribution, phenology, and host-species relationships at the species level [3,20,21] and larger spatial scales [11]. Yet, there are very few studies on epiphytic orchids that deal with their relationships with their host species, their spatial distribution, and their future survival, bearing in mind that many of them are threatened by human-induced disturbances, including climate change. In the present study, we investigate the relationships between epiphytic orchids and their hosts, their distribution patterns, and how these will be threatened by global warming. Specifically, we addressed the following questions: (i) Which host species are more likely to host multiple epiphytic orchids along altitudinal gradients? (iii) How many species of host and epiphytic orchids are potentially threatened by global warming, based on the projected increase in temperature? To answer these questions, we collected data on different host species that have epiphyte orchid species on them in five different regions of Nepal.

2. Materials and Methods

2.1. Data Collection

The data were collected between 2006 and 2011 in five different localities in Nepal: Annapurna region, Chitwan district, Palpa district, Shivapuri area, and Upper Tamakoshi valley. The details of these regions are given in Timsina et al. [11]. During the data collection, each tree was observed from the ground. The tree was inspected from different directions to see all the host branches. First, orchids were observed with the naked eye and then by using 10×42 mm binoculars. We did not climb the very large trees, but we used binoculars and Nikon cameras (D3200, D5300, Nikon Corporation, Amphur U-Thai, Ayutthaya, Thailand) with 55–300 zoom lenses to take photographs of orchids for correct identification. We documented orchid species for the 50 more common host species (mainly trees, but we also considered shrubs with orchids) in one transect and determined the identities of the host species and orchids growing on them in each transect. The numbers of transects used in each sampling region were variable, and there were 50 transects in Annapurna, 200 in Chitwan, 21 in Palpa, 100 in Shivapuri, and 101 in Tamakoshi. The forest areas in each region varied, which was the primary reason for the lack of a consistent number of transects. Each transect consists of the first 50 host plant individuals, comprising orchid species. We set up transects at random places with abundant epiphytic orchids, primarily in a horizontal direction. The length of each transect varied depending on the density of host individuals. When the density was high, the transects were shorter, and vice versa. There was at least a 10 m gap between the two transects, although this expanded to 20–30 m in larger areas such as Annapurna and Shivapuri. During data collection, each orchid species was photographed, tallied with available photographs in various literatures [22,23], and confirmed by B. B. Raskoti, an orchid expert.

In total, 141 different species of epiphytic orchids and 192 different host plant species were recorded in these localities. Finally, 16 epiphytic orchids (11.35% of the total) and 22 host plants (11.46% of the total) were not included in the final analysis because there was

no data on their altitudinal ranges. For the distributional ranges of the epiphytic orchids, we followed Rokaya et al. [17], and for host species, Press et al. [24]. For nomenclature, we used the 'Plants of the World Online (POWO) database' [25].

2.2. Epiphytic Orchid–Host Relationships

To determine the associations of epiphytic orchids with host individuals, we calculated the phi coefficient (Φ) of association [26,27] using 'optpart' and 'indispecies' packages in R 4.2.0 [28]. The phi coefficient (Φ) is independent of the size of the data set and gives values from -1 to +1. High positive values indicate the epiphytic species is occurring on a particular host more frequently than expected by chance [27]. According to Fisher's exact test, values greater than 0.2 indicate the numerical threshold, which yielded this required number of diagnostic species [29]. We determined the number of orchid species associated with each host species (having $\Phi > 0.2$) by summing the orchid species that showed a higher frequency of occurrence on a single host species than expected by chance, with a value greater than 0.2.

To describe the diversity of orchids on the hosts, we used a rarefaction function for each host plant. Rarefaction can be used to compare the species richness of samples of different sizes by calculating species richness for a given number of individuals based on rarefaction curves, which are plots of the number of species as a function of the number of samples—the number of host individuals in our case [30]. For calculating the rarefaction function, the 'vegan' package was used in R 4.2.0 [28].

We selected the value of 20 orchid individuals from the rarefaction as there was a sufficient number of orchid individuals for sample analysis found in all the host species. To depict the patterns in the species area, we determined the relationship between the number of individual epiphytic orchids recorded on a particular host plant and the number of individuals of that host plant. The numbers of host individuals were taken as a proxy for area. We used Pearson's correlation test because the data were normally distributed. The number of individual epiphytic orchids was $log_{10} + 1$ transformed, and the number of individual host plants was log_{10} transformed to obtain normal distributions.

2.3. Data Interpolation and Analysis

We divided the altitudinal gradient into 100 m intervals between the upper and lower altitudinal limits of a species [31]. In total, there were 44 altitudinal bands. This is widely used to determine the distribution of a particular plant along altitudinal gradients [15,32]. If a species is restricted to a certain altitudinal range, it is considered to be present in all the 100 m intervals within that range [32,33]. For the distribution of orchid and host plant species, we took total distributional ranges to interpolate and obtain the species richness per elevational band between the lower and upper elevational limits [34]. In our analysis, we divided the elevational gradient between 100 and 4400 m into 44,100 m elevational intervals for host species, and we divided the elevational gradient between 100 and 4200 m into 42,100 m elevational intervals for orchid species [15,31]. We used a generalized linear model (GLM) to relate orchid species and host species richness with elevation [31].

2.4. Species Threatened by Climate Change

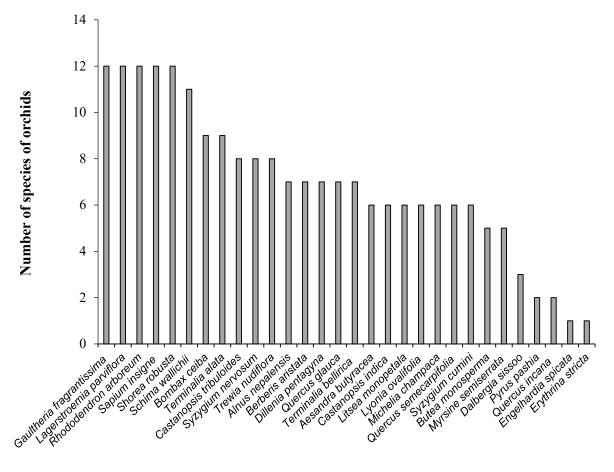
The method used to assess the effect of climate change on plant species was previously used by Subedi et al. [35] for different vascular plant species in central Nepal. Due to global warming, it is predicted that the global temperature will increase by 1.5–4.8 °C by the end of this century [36]. Following Bhattarai and Vetaas [31], we assumed that temperature declines by about 0.53 °C/100 m of altitude in Nepal. It is reported that the temperature tolerance range of a species can be determined based on the altitudinal distribution of any species, as the difference in temperature between the lower and upper limits of their altitudinal distributions. Thus, if the altitudinal range of a species is 100 or 200 m, then we expect that the difference between its upper and lower limits of occurrence is 0.6 °C and 1.2 °C. We used a moderate value of 3.15 °C for the global warming scenario

(i.e., a mid-value between 1.5 and 4.8 $^{\circ}$ C) in our analysis. Thus, orchids or host species with distribution ranges below 600 m are included as per the lapse rate of 0.53/100 m. In other words, the number of host and orchid species affected by the predicted increase in temperature is based on the distributional range of the respective plant species.

3. Results

3.1. Associations of Epiphytic Orchids and Their Host Species

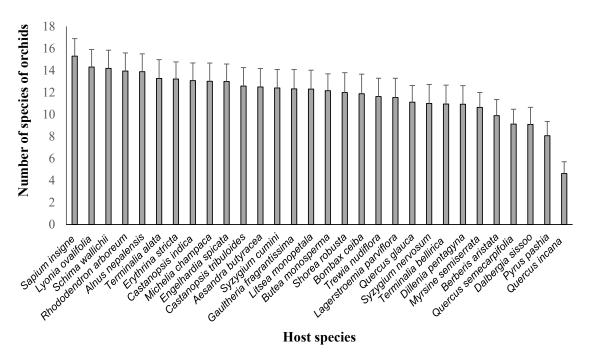
The phi (Φ) coefficient of association is greater than 0.2, indicating that epiphytic species occur on a particular host more frequently than expected by chance in 30 host species (17.64% of the total number included in the analysis), as depicted in Figure 1. Our result showed that the largest number of orchid species were associated mainly with four host species (*Gaultheria fragrantissima, Lagerstroemia parviflora, Rhododendron arboreum,* and *Sapium insigne*), each of which supported the growth of 12 species of orchids (9.6% of the total number of species of orchids included in the analysis). These host species were followed by the *Schima wallichii* tree, which hosted 11 species of orchids, and the *Bombax ceiba* and *Terminalia alata* trees, each hosting 9 species (Figure 1).



Host species

Figure 1. The number of orchid species occurring more frequently than expected by chance in a given host species with the phi coefficient (Φ) > 0.2 for the data obtained in Nepal, 2006–2011.

The rarefaction analysis of all host species predicts that trees such as *Sapium insigne*, *Lyonia ovalifolia*, *Schima wallichii*, *Rhododendron arboreum*, and *Alnus nepalensis* should potentially host the largest numbers of species of orchids, which differ from the actual numbers recorded in the previous paragraph. On the other hand, trees known as *Dalbergia* sissoo,



Pyrus pashia, and *Quercus incana* are predicted by the rarefaction analysis to host the lowest number of species of orchids (Figure 2, Supplementary Materials Figure S1).

Figure 2. The number of orchid species found on each host species was limited to 20 individuals sampled, as there was a sufficient number of orchid individuals for resample analysis found in all the host species for the data obtained in Nepal in 2006–2011.

3.2. Orchid-Host Relationship and Distribution Patterns

The total number of species of epiphytic orchids recorded on different species of host plant increased significantly with an increase in the number of individuals of their host plant species on a log–log scale (p < 0.001, $R^2 = 0.753$; see Figure 3).

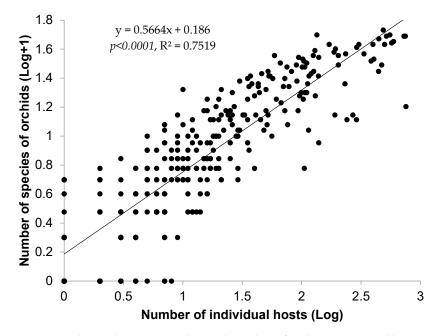


Figure 3. Relationship between the total number of orchid species and host species recorded from 2006–2011. Each dot represents the number of orchid species $(\log + 1)$ per host individual of a particular species (\log) in each region for the obtained data.

The relationship between species richness of host plants and altitudinal range indicates that the species richness of epiphytic orchids significantly increased with increasing altitude, reaching its maximum at 1500 m (n = 86 species), and then decreased with increasing altitude (p < 0.001 and $R^2 = 0.34$) (see Figure 4). The epiphytic orchids with the narrowest altitudinal range of 100–500 m were *Eria concolor* and *Micropera obtusa*. The species with the widest altitudinal range was *Pleione hookeriana*, with an altitudinal range of 2200–4400 m.

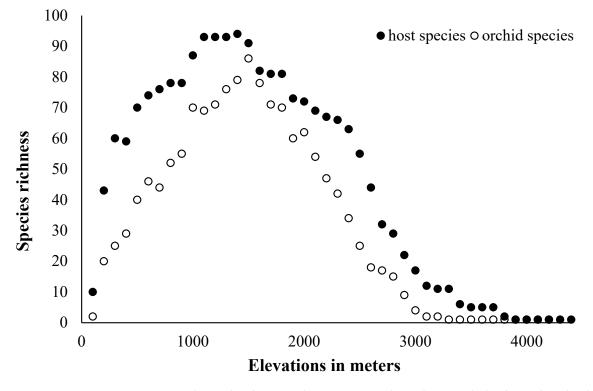


Figure 4. Relationship between host species and epiphytic orchids along altitudinal gradients recorded in the study in Nepal, 2006–2011. Each dot represents the species richness of host or orchid species at respective altitudes.

In the same way, the number of species in the host plants significantly increased with increasing elevation, reaching its highest point at 1400 m (n = 95 species). After that, it sharply decreased with an increasing elevation (p < 0.001, $R^2 = 0.50$) (Figure 4). The host plant species with the narrowest altitudinal range of 100–200 m was the *Madhuca longifolia* tree, and the species with the widest altitudinal range was the *Abies spectabilis* tree, with an altitudinal range of 2200–4400 m.

3.3. The Effect of Temperature Increases on the Number of Plant Species

If the temperature increases by 1.5 °C, it will affect three species of epiphytic orchids, while an increase of nearly 3.18 °C will affect 52 species (Figure 5). The most strongly affected species of epiphytic orchid species are those with narrow distribution ranges, namely *Cleisostoma simondii*, *Micropera obtusa*, *Phalaenopsis taenialis*, *Pinalia concolor*, *Porpax extinctoria*, and *Vanda tessellata*.

Similarly, of the total number of species of host plants, only nine species are affected if the temperature increases by 1.5 °C and 58 if the temperature increases by nearly 3.18 °C by the end of the century (Figure 5). The species that will be affected the most are those with narrowly distributed host plants, and they are *Alstonia scholaris*, *Careya arborea*, *Carissa macrocarpa*, *Ficus lacor*, *Ficus racemose*, *Madhuca longifolia*, *Stereospermum chelonoides*, *Syzygium formosum*, and *Syzygium nervosum*.

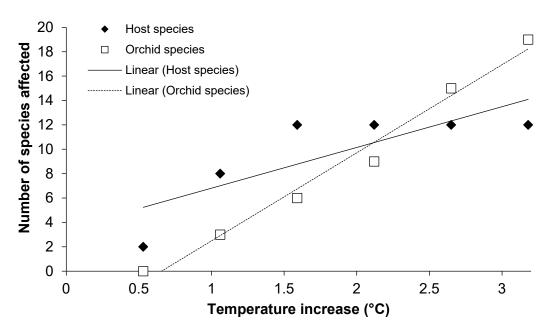


Figure 5. Relationship between the projected moderate increase in temperature by the year 2100 and the numbers of host plant species and epiphytic orchid species that will be affected in Nepal for the data obtained from 2006 to 2011.

4. Discussion

4.1. Associations of Epiphytic Orchids and Their Host Species

The results of the present study indicate that epiphytic orchids differ in their associations with different species of host plants. This is because the associations of orchids with host plants depend upon different characteristics of the host species, such as bark texture, host type (evergreen or deciduous types), and strata height of the host plant species, as shown by Timsina et al. [11]. A high number of species of orchids were associated with certain trees that are tall (such as *Shorea robusta* top canopy layer in the tropical region and *Sapium insigne* top canopy layer in the subtropical region). This could be due to the fact that tall trees receive more light and offer a larger surface area for epiphytic orchids compared to shrubs [21]. However, this study indicates that medium (*Terminalia alata, Bombax ceiba, Rhododendron arboreum, Lyonia ovalifolia, Schima wallichii*, and *Alnus nepalensis*) or big shrubs (*Lagerstroemia parviflora* and *Gaultheria fragrantissima*) that are very abundant may host many species of orchids.

Humus-rich *Rhododendron* forests are good habitats for most of the orchids [19,37–39]. The analysis indicates that *Schima wallichii* and *Alnus nepalensis* are the most preferred host species, most likely because of their high water-holding capacity [21]. A low number of species of orchids are associated with *Delberia sissoo*, *Pyrus pashia*, and *Quercus incana*, possibly because they are deciduous, and it is reported that deciduous trees host fewer epiphytes than evergreen trees [11].

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4.2. Orchid–Host Relationship and Distribution Patterns

The number of species of epiphytic orchids increased with the increasing number of host individuals, which was indirectly related to the species–area relationship [41], meaning

that a larger number of host individuals gave more space (observed as a proxy of area) for the growth of epiphytic orchids. The relationships between the species richness of epiphytic orchids and both host species richness and altitudinal range take the form of a hump-shaped curve with respect to altitude, similar to that reported in previous studies on flowering plants [31,42,43], ferns [32], liverworts and mosses [44], lichens [45], and orchids [14] in Nepal. The maximum species diversity of epiphytic orchids was recorded at 1500 m and that of their host plants at 1400 m. This is in accordance with the results of other studies in Nepal, which report maximum species diversity of vascular plants between 1500 and 2500 m [42] and orchids in the eastern Himalayas at 1600 m [14].

4.3. The Predicted Effect of an Increase in Temperature on the Number of Plant Species

Based on our analysis, three species of epiphytic orchids and nine host species will be affected if temperature increases by 1.5 °C (the predicted minimum increase in global temperature by the end of this century), and 52 orchid species and 58 host species that are narrowly distributed if temperature increases by 3.18 °C (the expected moderate value of the increase in global temperature by the end of this century). Subedi et al. (2015) predict very similar numbers for vascular plants in Manang in central Nepal, with seven species threatened by an increase in temperature of $1.5 \,^{\circ}$ C and at least 70 species threatened by an increase in temperature of 5 °C. As the effect of climate change in the Himalayan region is expected to be considerable, particularly in mountain ecosystems [46], many species will have to move to higher altitudes to survive future climate change [47]. Plants, however, are unlikely to spread vertically as fast as animals, so they will have to adapt to the changing environment [48]. The predicted rise in temperature in the Himalayan region may lead to contractions in distributional ranges and even to species extinctions [49]. In addition to this, other factors such as the abundance and rarity of host species [10], the microclimate [8], the height of host species [50], and pollination strategies [51] may play a role. Thus, the ecology of epiphytic orchids is complex and should be studied using an integrated approach, such as determining the distribution of different populations of specific species [52] and finding the factors affecting their population sizes.

5. Conclusions

Knowledge of the associations between epiphytic orchids and their host species allows us to predict the host species that support most epiphytic species. This will allow us to protect and manage important host species so that epiphytic species growing on them will also be protected. Our finding shows that there were 30 host species that supported the high number of orchid species, and the most important host species were Gaultheria fragrantissima, Lagerstroemia parviflora, Rhododendron arboreum, and Sapium insigne, as they supported the growth of 12 epiphytic orchid species on each of them. The altitudinal distributions of the different epiphytic orchids and their host species provide important information on how they are distributed spatially, which can also be used to estimate their future fates under climate change. Although many species are tolerant of increases in temperature, species with narrow distributions are expected to be less tolerant and, thus, might be affected in the future. It is, therefore, a matter of urgency that the less tolerant orchid species (Cleisostoma simondii, Micropera obtusa, Phalaenopsis taenialis, Pinalia concolor, Porpax extinctoria, and Vanda tessellata) and host species (Alstonia scholaris, Careya arborea, Carissa macrocarpa, Ficus lacor, Ficus racemose, Madhuca longifolia, Stereospermum chelonoides, Syzygium formosum, and Syzygium nervosum) need protection so that they will not go extinct in the future due to an increase in temperature. This can be achieved by planting them at high altitudes, which will have the same temperature as their previously distributed ranges.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/d16040252/s1. Figure S1: The associations between the number of orchid individuals growing on different host species in Nepal were estimated using a rarefaction analysis. The dots are mean values. **Author Contributions:** P.K. conceived and designed the experiments. Data collection: B.T., M.B.R., B.S., B.P.B. and B.B.R. Data analysis: B.T., M.B.R. and Z.M. Writing the paper: B.T., M.B.R., Z.M. and P.K. All authors have read and agreed to the published version of the manuscript.

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