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To Collect and Identify Orchid Species from Varied Climatic Zones of Himachal Pradesh for Population and Ecological Analysis

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Abstract: Currently, 39 species of orchids have been collected at 78 locations (643-3979 m amsl) in Himachal Pradesh. Among these species are two newcomers to the state flora, Habenaria aitchisonii and H. pubescens. The only two species that didn't grow on the ground were Gastrodia falconeri and Cymbidium macrorhizon, both of which were mycoheterotrophs. The other two, Gastrodia calceolaris and Rhynchostylis retusa, were ground-dwelling. The maximum density of Androcorys monophylla, a species found in a variety of environments including shaded woodlands, shrubby grasslands, grassy slopes, and road embankments, was determined to be 6.54±1.60. The mid-hill (sub-tropical-warm temperate) zone was the most densely populated by the species. There was a significant difference in the maximum frequency of Satyrium nepalense (90%) and Cypripedium cordigerum (20%), which may be explained by differences in environment and fruit set percentage. There was a 1–-1 range for FIS values. Overabundance of homozygotes or a limited population size may both contribute to minimal genetic variability across different morphotypes of a species.

Keywords: Orchid, Species, Climatic, Zones Himachal Pradesh , Ecological

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INTRODUCTION

There are some 25,000–35,000 species of orchids in the Orchidaceae family, making it the second biggest group of flowering plants (Mabberley, 1997; De and Singh, 2015; De and Medhi, 2017). The distinctive floral morphology, fluffy minute seeds, and relationship with mycorrhiza are distinctive features of this unusual collection of highly developed monocotyledonous plants. Theophrastus first recognized these plants in 285 BC, and ever since then, their beauty and enormous potential have fascinated humans. In reference to the paired tubers seen in terrestrial orchids, the name "orchis"—meaning "testicles" in Greek—is derived from. Orchids are very global, having been found on almost every continent except Antarctica. Almost every habitat on Earth is home to orchids. Orchids may be categorized as epiphytes, lithophytes, terrestrial, or mycoheterotrophs based on their habits, environment, and chances of survival. According to Cribb et al. (2003), around 73% of orchid species are known to be epiphytic. Epiphytes are plants that live on top of other plants or inanimate things; they get their water and nutrients from the air, stem-flow during rainstorms (Awasthi et al., 1995), and plant detritus. In contrast, you may find ground orchids, or terrestrial orchids, in grassland or woodland habitats. Orchids very often exhibit mycoheterotrophy, a natural phenomenon. Throughout their lives, mycohereotrophs rely on mycorrhiza, which are fungal partners, for growth and development (Leake, 2004).

Among the most prominent groups of monocotyledons in terms of size, diversity, and evolutionary history is the Orchidaceae family, which includes the most commercially significant flowering plants—the orchids. As a matter of fact, they are among the most beautiful plant species; they make up 8-10% of flowering plant species and 40% of monocots. Orchids were originally christened by Theophrastus (370–285 B.C.) in his book Enquiry into Plants, although they were referenced often in ancient Indian (Sushrut Sanhita, Charak Sanhita, Ashtangsangraha–600–200 B.C.) and Chinese (Shen-nung's Materia Medica) literature (cf. Bulpitt, 2005). The orchid family is known for its intricate flower structures that aid in pollination, its stifled endosperm development, and its abundance of tiny seeds with severely diminished embryos that can only germinate when they form a mycorrhizal relationship.

The medicinal and floricultural uses of orchids are well-documented. Orchids have long been hailed as the crown jewels of the cut-flower industry, thanks to their stunning variety of blooms and their ability to retain their beauty for an extended period of time. Nowadays, floriculture centered around orchids is a thriving industry in many countries, including Australia, Thailand, Japan, Singapore, USA, and Malaysia. Orchids are widely used in traditional medicine for a variety of conditions, such as dysentery, hemorrhage, worm infection, pain, and dendrobium teretifolium. This is due to the orchids' high phytochemical content (Toh, 1994; Husen and Rahman, 2003; Lai et al., 2004; Rao, 2004; Bi et al, 2005; Nayak et al., 2005; Jalal et al., 2008a). Cymbidium hybrid, Epipactis helloborine, and Liparis ovata were the sources of the HIV-inhibitory plant lectins (De Clercq, 1994; Balzarini, 2006). Moscatilin, a phytochemical isolated from Dendrobium loddigesii, shown modest anti-cancer effects in vitro against lung and stomach cancer cell lines (Ho, 2003). In addition to their culinary uses, gums, glues, narcotics, essences, fragrances, and even as poisons, orchids have many more practical uses (Lawler, 1984). Most people know orchids for their fragrant oil, vanillin, which is extracted from the green pods of the Vanilla planifolia plant.

REVIEW OF LITERATURE

Vélez, Leisberth& Jiménez et. al. (2023) - Orchids are among the most endangered plant species, however they are also among the most numerous and important in tropical ecosystems for things like water balance, nutrient cycle, and biomass. Because of the strong correlation between environmental factors and the variety of these organisms, we postulate that local elevation may affect their abundance. One sandstone plateau in the Cordillera del Cóndor contains a great variety of orchid species, and this research evaluated the diversity of orchid species at altitudes of 1200, 1400, and 1700 meters. For every level, three plots of 0.1 hectare were made. There were twenty-five subplots in all, with eight randomly selected from each plot. In these subplots, every species of orchid was counted and recognized. The results revealed a total of 867 individuals, representing 119 species from 54 genera. At an elevation of 1700 meters, there were 63 species, the most at 1400 meters with 52 species, and the fewest at 1200 meters with 39 species. Different orchid species were found at each of the three altitudes due to the much greater humidity levels at roughly 1700 m. The most promising markers of environmental status in this region were 21 species, including Maxillariagrayi, Elleanthusoliganthus, Maxillariamapiriensis, Stelispittieri, and Stelisortegae. In the sandstone plateau forests of the Cordillera del Cóndor's Shagmi Range, we find that height is the main factor restricting orchid populations.

Africa is home to a wide variety of ecosystems, according to Bakayoko, A. et al. (2022). Despite being home to the world's second-largest forest reserve, its flora has received little attention due to a lack of funding for botanical prospecting investigations. Not many families have had their members investigated extensively; for example, the Orchidaceae are mostly unknown. The purpose of this research is to add to our understanding of this family by examining its distribution and use throughout continental sub-Saharan Africa. West Africa, Central Africa, Southern Africa, and East Africa were the four main areas used for the orchid flora investigation, excluding South Africa and North Africa. The literature review provided the groundwork for this research. In order to narrow our focus, we reviewed literature on orchids, local floras, and distribution maps from the studied regions. We managed to compile a list of 1,373 species from 88 different genera. According to the findings, out of the four phytogeographic zones, Central Africa has the most species (708), followed by Southern Africa (637), and East Africa (583). Among the world's regions, West Africa has the fewest species, with 413. The list of uses is somewhat extensive. Orchids are most often used as decorative plants, in food, and in pharmacopoeia. Tanzania, Zimbabwe, the Democratic Republic of the Congo, Mozambique, Zambia, Malawi, Rwanda, Angola, Gabon, Central African Republic, Uganda, Burundi, and Kenya were among the nations where endemism was confirmed.

Vitt, P., Taylor, A., Rakosy, D. et al. (2023), Global conservation prioritizing for well-studied species, such birds, mammals, and amphibians, is based on quantitative estimates of endemism, evolutionary uniqueness, and extinction hazard. Nevertheless, the majority of the world's taxa do not have access to such data. An example of this is the Orchidaceae family, which is known for its extreme diversity and global reach yet has few data on its evolutionary history and potential dangers. We provide a framework for defining conservation priorities using phylogenetic and taxonomic criteria of uniqueness and rarity according to the number of regions and the area of habitation. The Neotropics are characterized as hotspots for richness for 25,434 orchid species with distribution data (89.3% of the Orchidaceae), whereas New Guinea is characterized as a hotspot for evolutionary uniqueness. Several islands are found to have several rare and unusual species. Although there are fewer monotypic genera in orchids than in other Angiosperms, a greater number of taxonomically unique orchid species are located in only one area. We locate 278 species that need conservation efforts right now, and we find that over 70% of those species are not protected in ex-situ collections at botanical gardens nor have an IUCN conservation assessment. Our research sheds light on orchid hotspots and species that are critically endangered, while also providing a foundation for future studies of other taxa with less data.

Morato and Danielle (2023) - The genus Euglossini includes the orchid bee as one of its subspecies. The variety and quantity of these bees are often higher in areas with more plant cover. The researchers in this study set out to figure out what effect deforestation has on euglossine bee populations in the Rio Branco municipality area of Acre State and the neighboring regions. Ten randomly selected forest sections of varying sizes were classified as either rural or urban for the purposes of the research. Bee samples were collected between 2005 and 2006. Of the 3,675 bees that were gathered, 36 were of different species and 4 were of different genera. When comparing bee populations in urban and rural areas, no statistically significant difference was found. An indicator of bee variety and abundance was the index of edge in fragments. Additionally, species richness might be reliably predicted by the projected connectedness. The faunal composition of pieces was more comparable when there was a significant degree of similarity in the landscape structure of those pieces.

Guy Chiron (2019) - Species endemism and species richness are two complementary concepts that are often used to assess biodiversity. When used to demarcate protected areas, they may prove to be useful tools. The present inquiry is based on these beliefs, which are based on the Baptistonia genus of orchids, which are peculiar to the Brazilian Atlantic Forest. From the species richness investigation, we can determine that there is one highly rich location (the Serra do Castelo, ES) and six wealthy places in general. There are three of these areas where the endemism index is more than 1. When it comes to protecting biodiversity, the eight spots on the list are crucial. Hierarchical endemism links across nine phytogeographical zones are evaluated using a parsimony analysis of endemicity. By examining the genus Baptistonia's phylogenetic connections, this study verifies two things: (a) the southern Atlantic Forest is home to two major endemism centers, and (b) the Serra do Castelo seems to represent the genus's center of diversification.

MATERIALS AND METHODS

The location of the present research in the state of Himachal Pradesh. The location in the northwest Himalayas, with its undulating terrain and heights ranging from 350 to 7000 meters above sea level, lies between the coordinates of 30°22′ and 33°12′ North latitudes and 75°47′ and 79°04′ East longitudes. From 2010 to 2013, researchers in Himachal Pradesh conducted periodic field surveys to identify orchids in four distinct climate zones: subtropical, warm temperate, cold temperate, subalpine, and alpine. A total of 78 locations spread over nine districts were sampled, with elevations ranging from 643 to 3979 meters. The data collection was carried out in 30 quadrats per location, with 10 1×1 m quadrats placed inside each transect at a distance of around 50 m. The data used to compile the findings in the following chapter came from at least 120 and no more than 180 quadrats. The moisture content of soil was determined by Oven dry method (Allen *et al.*, 1974). 10g of freshly collected soil sample was kept in a hot air oven at 105ºC for 24 hrs. Genetic similarities and distances were calculated between all pairs based on the method of Sneath and Sokal (1973). Isoenzyme genotypes of different morphotypes were compared at each locus to find out genetic variability.

RESULTS

Orchid species studied in different localities

From 2010 to 2013, a total of 39 orchid species belonging to 23 genera were gathered in Himachal Pradesh for the purpose of this research (Table 1).

Table 1: Orchid Species Collected from Himachal Pradesh (2010–2013)

Serial No.	Orchid Species	Genus	Subfamily	Distribution Zone
1	Androcorys monophylla	Androcorys	Orchidoideae	NW Himalaya
2	Brachycorythis obcordata	Brachycorythis	Orchidoideae	NW Himalaya
3	Dactylorhiza hatagirea	Dactylorhiza	Orchidoideae	Central Himalaya

4	Dithrix griffithii	Dithrix	Orchidoideae	NW Himalaya
5	Goodyera biflora	Goodyera	Orchidoideae	NW Himalaya
6	Habenaria aitchisonii	Habenaria	Orchidoideae	New Record - HP
7	Habenaria pubescens	Habenaria	Orchidoideae	New Record - HP
8	Habenaria edgeworthii	Habenaria	Orchidoideae	Eastern Himalaya
9	Habenaria intermedia	Habenaria	Orchidoideae	Eastern Himalaya
10	Habenaria stenopetala	Habenaria	Orchidoideae	Southern India
11	Habenaria ovalifolia	Habenaria	Orchidoideae	Central Himalaya
12	Habenaria plantaginea	Habenaria	Orchidoideae	Central Himalaya
13	Habenaria marginata	Habenaria	Orchidoideae	Central Himalaya
14	Herminium lanceum	Herminium	Orchidoideae	NW Himalaya
15	Pecteilis gigantea	Pecteilis	Orchidoideae	Eastern Himalaya
16	Peristylus goodyeroides	Peristylus	Orchidoideae	Central Himalaya
17	Platanthera edgeworthii	Platanthera	Orchidoideae	Southern India
18	Satyrium nepalense	Satyrium	Orchidoideae	Widespread
19	Spiranthes sinensis	Spiranthes	Orchidoideae	Plains (Haryana)
20	Crepidium acuminatum	Crepidium	Epidendroideae	Western Himalaya
21	Cymbidium macrorhizon	Cymbidium	Epidendroideae	Epiphytic (Subtropical)
22	Dienia cylindrostachya	Dienia	Epidendroideae	Eastern Himalaya
23	Epipactis helleborine	Epipactis	Epidendroideae	Eastern Himalaya

24	Eulophia dabia	Eulophia	Epidendroideae	Southern India
25	Gastrochilus calceolaris	Gastrochilus	Epidendroideae	Andaman & Nicobar
26	Gastrodia falconeri	Gastrodia	Epidendroideae	Eastern Himalaya
27	Liparis ovata	Liparis	Epidendroideae	Eastern Himalaya
28	Oreorchis indica	Oreorchis	Epidendroideae	NW Himalaya
29	Rhynchostylis retusa	Rhynchostylis	Epidendroideae	Andaman & Nicobar
30	Cypripedium cordigerum	Cypripedium	Cypripedioideae	NW Himalaya
31	Gastrochilus obliquus	Gastrochilus	Epidendroideae	Central Himalaya
32	Cymbidium aloifolium	Cymbidium	Epidendroideae	Central Himalaya
33	Eulophia nuda	Eulophia	Epidendroideae	Southern India
34	Epipactis royleana	Epipactis	Epidendroideae	Central Himalaya
35	Dienia ophrydis	Dienia	Epidendroideae	Eastern Himalaya
36	Liparis nervosa	Liparis	Epidendroideae	Eastern Himalaya
37	Oreorchis micrantha	Oreorchis	Epidendroideae	Eastern Himalaya
38	Crepidium resupinatum	Crepidium	Epidendroideae	Central Himalaya
39	Goodyera repens	Goodyera	Orchidoideae	NW Himalaya

Ten different tribes and three distinct subfamilies make them up (Cameron et al., 1999). The tribe Cypripedieae only had one species, Cypripedium cordigerum, representing the Cypripedioideae family. In the family Orchidoideae, there were twelve genera and twenty-four species: Androcorys, Brachycorythis, Dactylorhiza, Dithrix, Goodyera, Habenaria, Herminium, Pecteilis, Peristylus, Platanthera, Satyrium, and Spiranthes. The tribes Cranichideae and Diseae were furthermore included. In contrast, fourteen species belonged to 10 genera in the family Epidendroideae, which included the following: Crepidium, Cymbidium, Dienia, Epipactis, Eulophia, Gastrochilus, Gastrodia, Liparis, Oreorchis, and Rhynchostylis (Fig. 1).

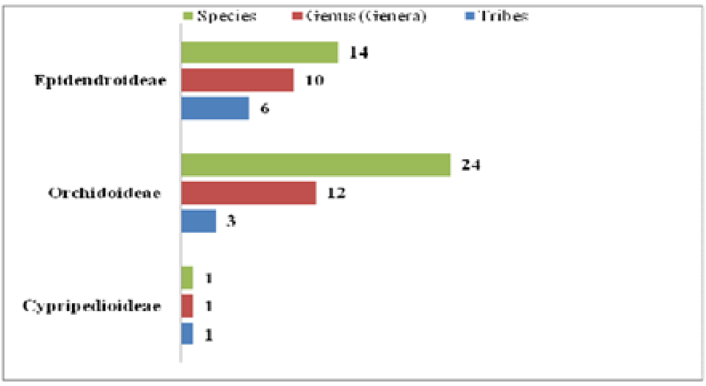

Figure 1. Numerical strength of different subfamilies in terms of tribes, genera and species

New to the state flora are two species of Orchidoid, Habenaria aitchisonii and H. pubescens. The 78 locations around the state were included in the collection, and the altitude range was from 643 to 3979 meters. Out of all the places that were examined at the moment, Taradevi (Shimla) has the most diversity. The largest genus was Habenaria, which had nine species. Fifteen genera with one species each were represented, including Androcorys, Brachycorythis, Crepidium, Cymbidium, Cypripedium, Dactylorhiza, Dienia, Dithrix, Gastrochilus, Gastrodia, Pecteilis, Peristylus, Rhynchostylis, Satyrium, and Spiranthes (Fig. 2).

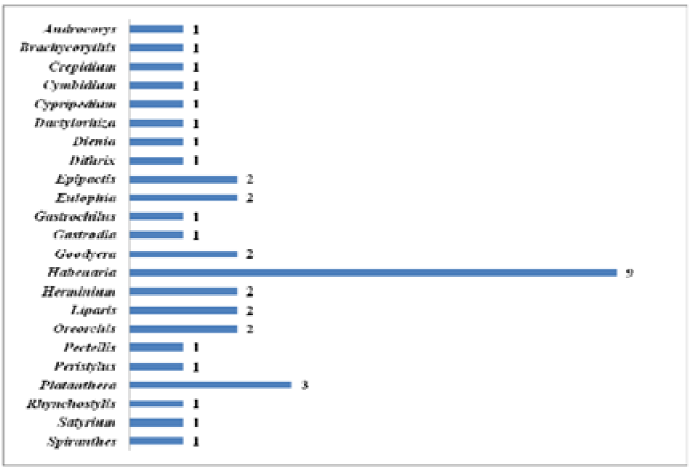

Figure 2. Numerical strength of different genera in terms of species

Species habits, distribution pattern and habitats

The ground-dwelling habits of all species except two were documented in Table 1: Gastrochilus calceolaris and Rhynchostylis retusa. The leafless mycoheterotrophic life form was shown by two terrestrial species, though: Cymbidium macrorhizon and Gastrodia falconeri.

Orchids in the study region had a wide variety of distribution patterns, each reflecting the specific climate needs of the plant species. The cold temperate (1800-3000m) and warm temperate (900-1800m) climates were home to the vast majority of the species, including both mycoheterotrophs. In most cases, they emerge during or just after monsoon rains, produce fruit and flowers, and then die off as winter draws near. Rhizomes, pseudobulbs, and tubers were some of the organs they used to endure bad times for a long time.

These two epiphytic species grow best on broad-leaved trees with fissured bark and are thus limited to the subtropical to mild temperate zones (up to 1800 m).

Geographical affinities of the species

The orchid species studied presently showed greater affinities with those of adjacent areas and none of these was found to be endemic to the state of Himachal Pradesh. All of them are on record from different parts of northwest Himalaya and adjoining plains.

Table 2: Frequency, Density and Abundance of Orchid Species

Serial No.	Orchid Species	Frequency (%)	Density (Individuals/mÂ²)	Abundance (Individuals/Plot)
1	Androcorys monophylla	39.96	3.14	4.23
2	Brachycorythis obcordata	86.06	1.23	2.04
3	Dactylorhiza hatagirea	68.56	3.47	8.77
4	Dithrix griffithii	57.89	0.71	6.61
5	Goodyera biflora	22.48	5.96	3.98
6	Habenaria aitchisonii	22.48	2.05	1.57
7	Habenaria pubescens	14.65	4.48	3.8
8	Habenaria edgeworthii	79.29	2.37	3.93
9	Habenaria intermedia	58.09	3.62	7.57
10	Habenaria stenopetala	66.65	3.78	6.74
11	Habenaria ovalifolia	11.65	1.61	8.98
12	Habenaria plantaginea	87.59	6.32	5.25
13	Habenaria marginata	76.6	5.15	2.08
14	Herminium lanceum	26.99	6.14	7.42
15	Pecteilis gigantea	24.55	5.87	7.85
16	Peristylus goodyeroides	24.67	4.09	6.05
17	Platanthera edgeworthii	34.34	6.03	7.94

18	Satyrium nepalense	51.98	1.03	5.44
19	Spiranthes sinensis	44.56	1.68	5.7
20	Crepidium acuminatum	33.3	0.77	4.85
21	Cymbidium macrorhizon	58.95	2.45	1.23
22	Dienia cylindrostachya	21.16	2.83	1.97
23	Epipactis helleborine	33.37	2.13	1.28
24	Eulophia dabia	39.31	5.47	6.73
25	Gastrochilus calceolaris	46.49	2.64	3.83
26	Gastrodia falconeri	72.81	2.19	5.58
27	Liparis ovata	25.97	3.76	9.17
28	Oreorchis indica	51.14	1.35	3.24
29	Rhynchostylis retusa	57.39	5.31	4.69
30	Cypripedium cordigerum	13.72	0.95	7.8
31	Gastrochilus obliquus	58.6	6.42	3.06
32	Cymbidium aloifolium	23.64	5.13	1.69
33	Eulophia nuda	15.2	1.69	3.61
34	Epipactis royleana	85.91	0.53	2.45
35	Dienia ophrydis	87.25	5.39	9.37
36	Liparis nervosa	74.67	4.74	8.27
37	Oreorchis micrantha	34.37	4.87	6.7
38	Crepidium resupinatum	17.81	5.13	8.84
39	Goodyera repens	64.74	0.94	8.23

Three species (*Androcorys monophylla*, *Goodyera biflora*, *Oreorchis indica*) are restricted in distribution to Northwest Himalaya, whereas the others extend their distribution to other adjacent regions as well. All of the species find distribution in adjoining state of Uttarakhand. Twenty-five species occur in Jammu and Kashmir and one of them (*Spiranthes sinensis*) in the adjacent plains of Haryana. Thirty-three species occur in the Central (Nepal) Himalaya, 36 in Eastern Himalaya/ Northeast India, and ten species find distribution in the southern (peninsular) region of the country; two of which (the epiphytic *Gastrochilus calceolaris* and *Rhynchostylis retusa*) are well reported from Andaman and Nicobar Islands. As many as 20 species extend to Bhutan, six to Bangladesh, 23 to Pakistan, four to Afghanistan, 10 to Myanmar, 19 to China, and 14 to Thailand (Fig. 3).

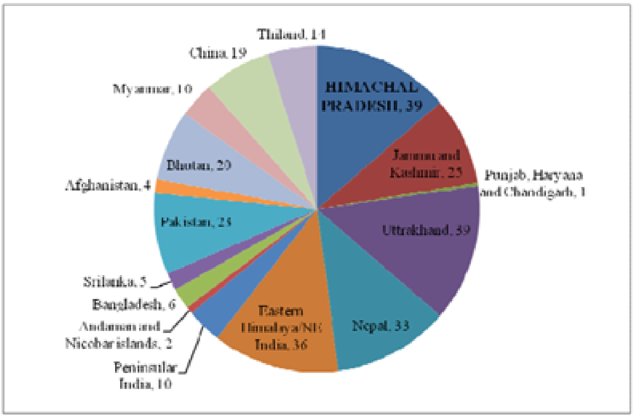

Figure 3. Geographical affinities of orchid species

Flowering and fruiting periods

All of the presently studied orchids possessed beautiful flowers (Figs. 4). Majority of them came into flower during the months of July (28 species) and August (26 species). The number of species in bloom progressively declines towards both sides of these months; none of the species was observed in flowering during the months of November, December, January and February. Fruit formation took one to one and a half month after the beginning of flowering; maximum fruit set (26 species) was seen during the month of August. The fruit set was not found similar in all of the presently studied species and it ranged between 60-90% in majority of cases. The lowest (26.45%) fruit set was seen in *Crepidium acuminatum* and the highest (96.26%) in *Rhynchostylis retusa*. Fruit dehiscence and seed dispersal was complete before the onset of winter season.

Figure 4: Orchids of Himachal Pradesh. A-B, Cypripedium cordigerum D. Don; C-D, Goodyera biflora (Lindl.) Hook. f.

Polymorphic species and biochemical studies

Some species of the study area showed intra-specific morphological variations both in vegetative (plant height, size and shape of tubers/ pseudobulbs, number and size of leaves) as well as floral (length of inflorescence, number of flowers per inflorescence and their arrangement, flower size and colour, lip structure, flowering period) characteristics. Isoenzyme studies were carried out in different morphotypes of three species (*Crepidium acuminatum, Herminium lanceum, Satyrium nepalense*) to reveal genetic relationship amongst them. Three enzyme systems, Esterase (EST), Glutamate oxaloacetate transaminase (GOT) and Peroxidase (PER) were employed for this purpose using Polyacrylamide gel electrophoresis (PAGE).

***Crepidium acuminatum* (D. Don) Szlach.**

Morphotypes were identified exclusively on the basis of their lip lobe feature and were named as C1 (slightly spreading lip lobes), C2 (widely spreading lip lobes) and C3 (overlapping lip lobes). These differences are illustrated in Fig.5. A total of nine accessions (three of each morphotype) were collected from following three localities (a, b, c) of different altitudes: a) Kasauli (1927 m) in Solan district, b) Karol (2135 m) in Solan district, and 3) Taradevi (2205 m) in Shimla district.

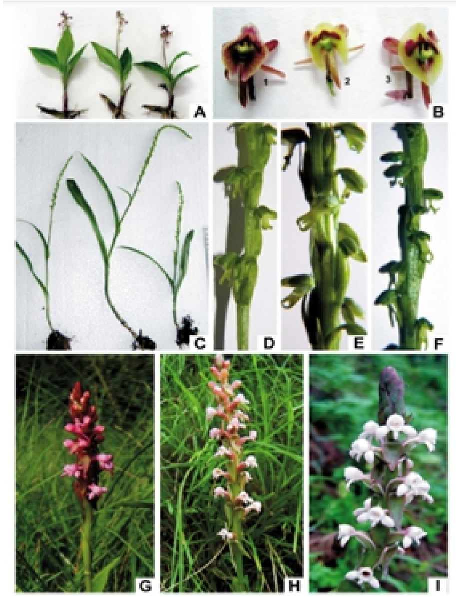

Figure 5: Morphotypes of the polymorphic species studied for isoenzyme analysis.

A-B, Crepidium acuminatum (1, slightly spreading lip lobes; 2 widely spreading lip lobes; 3, overlapping lip lobes); C-F, Herminium lanceum (D, widely spreading lip lobes; E, parallel lip lobes; F, overlapping lip lobes); G-I, Satyrium nepalense (G, pink flowers; H, pinkish white flowers; I, white flowers)

Esterase (EST). In electrophoretic pattern of EST isozyme studied for nine accessions, a total of 36 bands were observed (Fig. 6). These were grouped into four loci *viz.* EST I, EST II, EST III and EST IV and had Rm values 0.08, 0.26, 0.42 and 0.62 respectively. EST I phenotype was represented by moderately to darkly stained monomorphic zone near the origin. EST II and EST III loci were also monomorphic but gave lighter stained bands. Lightly stained EST IV phenotype was most anodal and was represented by two allozymic forms, EST IVa and EST IVb. Where EST IVa was observed in morphotypes C1 and C3, EST IVb was found to be absent in these both. Out of total four loci studied for esterase, EST I and EST IV were effectively considered for quantitative and qualitative difference respectively. As EST II and EST III had same band intensity and were monomorphic; they could not depict any significant genetic difference in various morphotypes.

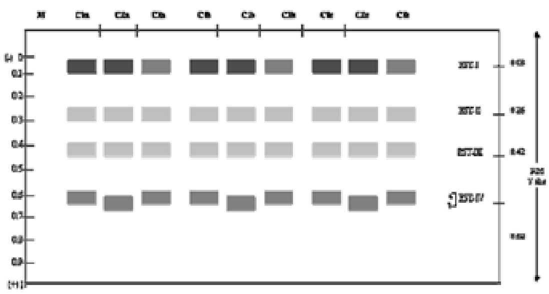

Figure 6. Isozymic pattern of esterase (EST) enzyme system in 3 morphotypes of Crepidium acuminatum (M=Marking dye; 1-3=Morphotypes; a, b, c=Localities)

Glutamate Oxaloacetate Transaminase (GOT). This enzyme system produced total 15 bands that were moderately to darkly stained (Fig. 7). Three loci, GOT I, GOT II and GOT III at Rm positions 0.48, 0.54

and 0.6 were observed. GOT I phenotype was moderately stained and was present only in morphotype C2. Phenotype GOT II, on the other hand was moderately to darkly stained allozyme and was present in two forms, GOT IIa and GOT IIb. GOT IIa phenotype was darkly stained and was observed only in only in morphotype C1.

Above results revealed that all loci (GOTI, GOT II, GOT III) can be considered very effectual for distinguishing various morphotypes of *Crepidium acuminatum* quantitatively as well as qualitatively.

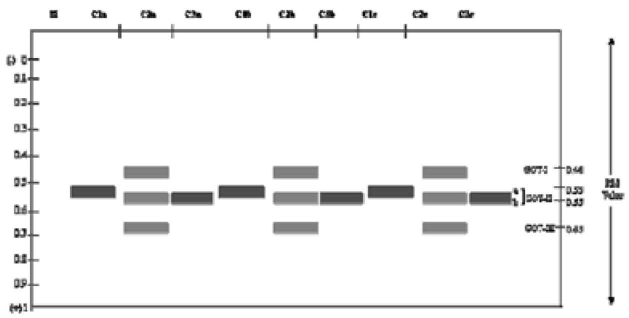

Figure 7. Isozymic pattern of Glutamate Oxaloacetate Transaminase (GOT) enzyme system in 3 morphotypes of Crepidium acuminatum (M=Marking dye; 1- 3=Morphotypes; a, b, c=Localities)

Peroxidase (PER). The elecrophoretic pattern of peroxidase isoenzyme gave total nine darkly stained bands. PER I was recognized as a darkly stained band near the origin with Rm values 0.12 (Fig. 8); it was present only in morphotype C2 and was found to be absent in case of other two (C1, C3). Phenotype PER II having Rm value 0.53 was also darkly stained, and was present in two (C1, C3) morphotypes. Since all bands were of same colour intensity, quantitative differences could not be seen. However, both GOT I as well as GOT II loci were effective for distinguishing morphotypes qualitatively.

So out of total nine loci studied for *Crepidium acuminatum* by three enzyme systems (EST, GOT, PER), two (EST IV, GOT II) were polymorphic, and were the resultant of heterozygous genotype i.e. AB. Other loci viz. EST I, EST II, EST III, GOT I, GOT III, PER I and PER II were monomorphic and were the resultant of homozygous genotypes AA.

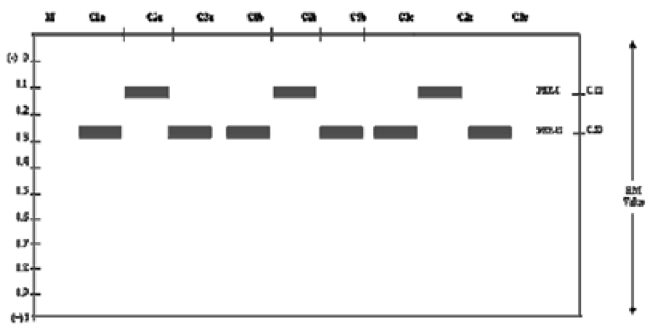

Figure 8. Isozymic pattern of Peroxidase (PER) enzyme system in 3 morphotypes of Crepidium acuminatum (M=Marking dye; 1-3=Morphotypes; a, b, c=Localities)

Genetic interpretation. The value of allele frequency in the present studies ranged from 0.5 to 1.0 at a polymorphic locus. The mean for allele per locus was observed (*A*) 1.14. Polymorphism (*P*) was 11.4% as observed in present investigation. Mean observed heterozygosity value (H_o) was 0.13. Fixation index i.e. F_{IS} (a parameter of F-statistics) showed negative values depicting more heterozygosity than observed. Morphotypes C2 (widely spreading lip lobes) and C3 (overlapping lip lobes) having F_{IS} = -1 value showed more heterozygosity than observed. The third one (C1, slightly spreading lip lobes) showed more observed heterozygosity than expected, having F_{IS} = +1. According to dendrogram contructed (Fig. 9), the nine genotypes were divided into three clusters. Morphotypes C1 and C3 were closely related and showed 71% similarity with each other; morphotype C2 was found to be distantly related having only 34% similarity with rest of the two.

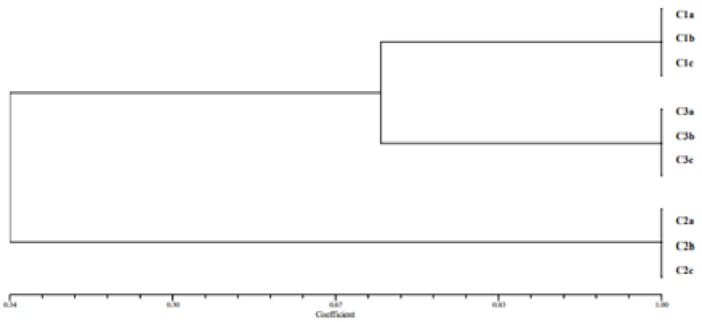

Figure 9. Dendrogram showing clustering of 9 accessions of Crepidium acuminatum (D. Don) Szlach.

CONCLUSION

The purpose of this research was to catalogue the orchid diversity found in Himachal Pradesh throughout its several climate zones. Quantitative study (density, frequency, abundance) of their populations and soil properties of natural habitats were conducted, together with information on the distribution pattern of various species, range of habitats, blooming and fruiting seasons, and potential threats. The research region did not support any of the species. The western boundary of the range of epiphytic orchids is Himachal Pradesh. From Jammu and Kashmir, the only species known to extend westward is Rhynchostylis retusa. Orchids often choose soils that have a low phosphorus concentration, and it is believed that the mycorrhizal partner satisfies this need. Dithrix griffithii had the lowest level (8.762±0.433) and Oreorchis micrantha had the greatest level (64.638±0.449). The soil potassium concentrations varied significantly, with Habenaria stenopetala having a range of 161.552±3.588 and Platanthera edgeworthii having a range of 656.532±1.073

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