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Full Length Research Paper

Morphometric analysis of *Dactylorhiza hatagirea* (D. Don), a critically endangered orchid in cold desert Ladakh region of India

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The morphometric study was conducted during 2009 to 2010. About 28 morphological characters were measured under 13 natural populations of *Dactylorhiza hatagirea.* Geographic variation in morphology reflects phenotypic responses to environmental gradients and evolutionary history of populations and species. At points, beside its broad geographic range (Nubra, Suru and Indus valley) characterization of *Dactylorhiza* phenotype is normally accomplished by use of morphological descriptors, hence as a first step, phenotype collection and its morphometric analysis was assessed. However, plant height, leaf length, lowermost leaf length, length of second leaf from base and mean length from lowest bract to the top of inflorescence are presented to account for the remarkable variation in morphological characters. Tirith population showed more values of this trait while Skurru showed less value. From this, it is concluded that Tirith showed great morphometric variation as compared to other population. Multivariate morphometric techniques, principal component analysis (PCA), multidimensional scaling (MDS) and cluster analysis were used to determine whether these populations can be reliably morphologically similar or dissimilar. The first two principal components encompass more than 75% variation among population. The results of PCA and MDS analysis were comparable to the cluster analysis, which shows considerable phenotypic variation in morphological and horticultural traits that can be utilized in its genetic improvement. To support this study, further constructive information were provided on the status of the populations of *D. hatagirea* which may increase the conservation value of this site and resolve the suitable areas with taxonomic and nomenclatural controversies.

Key words: Morphological characters, principal component analysis (PCA), multidimensional scaling (MDS), plant height, leaf length, leaf width.

INTRODUCTION

Dactylorhiza Necker ex Nevski (Orchidaceae) is a genus of about 75 named species, distributed throughout the world with 58 in Europe and North Africa (Averyanov, 1990). The greatest species richness is found in north-western Europe while, nine species of *Dactylorhiza* are endemic (Delforge, 1995). The distribution of *Dactylorhiza* covers most of Europe, temperate Asia, North Africa, Japan, the Aleutian Islands and northern parts of North America. *Dactylorhiza* is a very challenging genus and always present taxonomic difficulties (Pedersen, 1998; Hedren, 2001).

Dactylorhiza hatagirea syn. Orchis latifolia var. Indica (Lindley) is an indigenous species and is exclusively found in Ladakh region of Jammu and Kashmir. It has been identified as critically endangered (CAMP status), critically rare (IUCN status) and is listed under Appendix II of Convention of International Trade in Endangered Species (CITES) (Kala, 2000; Samant et al., 2001; Uniyal et al., 2002). Cold desert of Ladakh region is characterized by high wind velocity that continues throughout the year, which causes great variation in temperatures

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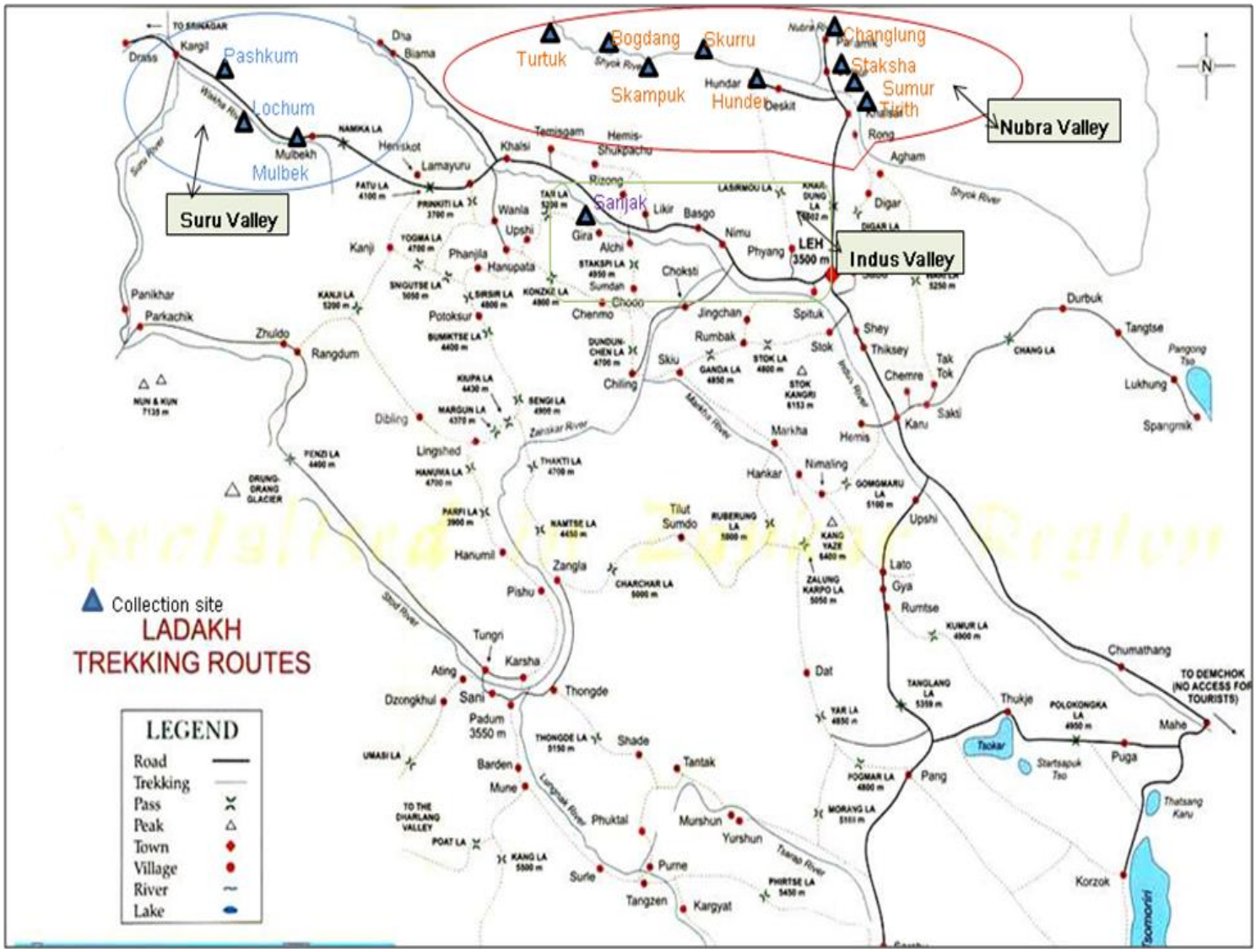

Figure 1. Map of Ladakh region of India.

(from -40°C in peak winters to 35°C in peak summers).

Geographical variation in plant morphology is a function of phenotypic changes in response to local environmental conditions, genetic variation and evolution among populations and the biogeographic history of a species (Thompson, 1991; Schlichting and Pigliucci, 1998). Morpho-logical variation and geographical separation among populations are also prerequisite to the formation of subspecies and variety (Losos and Glor, 2003). Morpho-metric analysis can be used to illuminate the interplay of climate, geographical history and evolutionary dynamics in generating new taxa (Avise et al., 1987; Templeton et al., 1995; Arbogast and Kenagy, 2001). They could also be caused by phenotypic variation using parameters, multiple observations and investigations of local variability. More recently, it has been documented that orchid systematics need to be based on a much broader establishment of morphometry. In particular, greater attention should be paid to the direct morphometric analysis of living plant (Dressler and Dodson, 1960) and to the characterization of the environment conditions at the same degree as that of the morphological variation in the plants (Sanford, 1974). Considering the size of Orchidaceae, there are a relatively small number of studies using morphometrics to estimate population variability; mostly, this has been focused on terrestrial temperate orchids (Bateman and Denholm, 1988; Bateman and Farrington, 1989; Dufrene et al., 1991).

The aim of this research was to study the morpho-logical variation of *D. hatagirea* using both quantitative and qualitative characters at variable altitude and in more emphasis, increased data and more powerful multivariate analysis methods to identify patterns of variation and

Table 1. Geographic localities of populations of *D. hatagirea.*

Serial no.	Valley	Population name	Longitude	Latitude	Altitude (ft)
1		Tirith	N 34°32'.378	E 77°38'.481	10443 ± 26.9
2		Sumur	N 34°31'.128	E 77°34'.481	10120± 12.7
3		Changlung	N 34°55'.884	E 77°28'.276	10982± 39.7
4		Staksha	N 34°55'.885	E 77°28'.276	11081± 49.2
5	Nubra	Turtuk	N 34°50'.849	E 76°49'.720	9240 ± 25.8
6		Bogdang	N 34°48'.198	E 77°02'.453	9240 ± 25.8
7		Hunder	N 34° 35'.043	E 77°28'.592	10357 ± 18.0
8		Skurru	N 34° 40'.229	E 77°18'.031	10295 ± 20.8
9		Skampuk	N 34°35'.238	E 77°34'.481	10490 ± 17.4
10	Indus	Sanjak	N 34°34'.458	E 76°31'.584	9607 ± 49.2
11		Mulbek	N 34°35.437	E 76°32'.673	9731 ± 32.5
12	Suru	Lochum	N 34°28'.064	E 76°15'.337	10032 ± 18.1
13		Pashkum	N 34°31'.326	E 76°10'.960	9475 ± 13.1

determining the characters related to these patterns.

MATERIALS AND METHODS

Sampling

About 20 plants from four different habitats (in farm, near water flow, under tree and open slope) in each population were studied for morphometric analysis. The study was based on *Dactylorhiza* orchid samples collected from 13 populations of Ladakh region (Figure 1). Number of samples taken from each population was based on the geographic distribution and area in order to cover, as much as possible, diverse growing habitat (Table 1).

Morphometric traits

Morphological measurements were taken from all parts of plant including stem, leaves and flowers (Bateman and Denholm, 1985); lobe of tuber was described somewhat different by using single characters according to populations. A total of 28 quantitative and qualitative traits were determined on all 260 individuals (Table 2). Measured traits included plant height (PLH), length of longest leaf (LFL), width of longest leaf (LFW), position of maximal width, the distance from leaf base to the place of maximal width (MAX W), leaf spot presence (1 = none, 2 = weak and 3 = heavy), leaf spot shape (1 = elongated and 2 = rounded), uppermost leaf length (ULFL), uppermost leaf width (ULFW), lowermost leaf length (LLFL), lowermost leaf width (LLFW), position from base, of the second leaf greatest width (POSFRB), length of second leaf from base (LSECLF), width of second leaf, (WIDOFSEC), number of cauline leaves (CAULF), spur length measured underneath the spur (SPULEN), lip color (1 = pink and 2 = dark pink), lip width (LIPW), length of middle lobe of lip, from the base to the top of lobe (LIPMID), length of lateral lobe of lip, from the base to the top of lobe (LIPLATE), bract length (BRAL), bract width (BRAW), length from lowest bract to the top of inflorescence (LOWBRA), length of inflorescence axis between the insertion points of first and fifth (INFAXIS), number of flowers (NOFLO), uppermost internodium length (UPPINT), stem diameter under inflorescence (STDI), stem diameter above lowermost leaf (STDLOW) and number of lobe of tubers (NOLOB) (one, two, three, four and five).

Data and statistical analysis

A range of univariate and multivariate statistical procedures were used to analyze the data. Descriptive analysis of the data was performed using SPSS 19 software. Analysis of variance (ANOVA) was performed and the mean of the results were compared by Duncan's multiple range tests at 5% significance level. To determine the degree of associations among the characters, Pearson's coefficients were used. Principal component analysis (PCA) and multidimensional scaling (MDS) was used to ordinate population means considering variance and covariance among characters within and among populations (Kim, 1975). Average Euclidean distance was calculated for each population and the resulting distance matrix was used to construct a phenetic dendrogram using average linkage method (Mohammadi and Prasanna, 2003).

RESULTS

Mean and standard error comparisons of each trait are based on Duncan's test as presented in Table 3. Results show the mean plants height (74.60 to 34.2), mean leaf length (13.4 to 8.4), mean lowermost leaf length (12.4 to 7.8), mean length of second leaf from base (13.2 to 10.5) and mean length from lowest bract to the top of inflorescence (16.5 to 9.7). The highest values of mean plants height, mean leaf length, mean lowermost leaf length, mean length of second leaf from base and mean length from lowest bract to the top of inflorescence were found in Tirith, while lowest values were found in Skurru. Therefore, Tirith population individuals were superior and significantly different as compared to other populations at 5% level. Figures 2 and 3 show the characters values plotted against the first two principal component variates from PCA and sample population with respect to their Euclidean distance from MDS. However, two patterns could be seen regarding the position of variable mean scores and Euclidean distance. First, traits are positioned

Table 2. List of morphological characters.

Serial no.	Acronym	Plant character
1	PLH	Plant height
2	LFL	Length of longest leaf
3	LFW	Width of longest leaf
4	MAX W	Position of maximal width, the distance from leaf base to the place of maximal width
5	LFSP	Leaf spot presence (1 none, 2 weak, 3 heavy)
6	LFSPSH	Leaf spot shape (1 elongated, 2 rounded)
7	ULFL	Uppermost leaf length
8	ULFW	Uppermost leaf width
9	LLFL	Lowermost leaf length
10	LLFW	Lowermost leaf width
11	POSFRB	Position from base, of the second leaf greatest width
12	LSECLF	length of second leaf from base
13	WIDOF	width of second leaf
14	CAULF	Number of cauline leaves
15	SPULEN	Spur length, measured underneath the spur
16	LIPC	Lip color (1 pink, 2 dark pink)
17	LIPW	Lip width
18	LIP MID	Length of middle lobe of lip, from the base to the top of lobe
19	LIPLATE	Length of lateral lobe of lip, from the base to the top of lobe
20	BRAL	Bract length
21	BRAW	Bract width
22	LOWBRA	Length from lowest bract to the top of inflorescence
23	INFAXIS	Length of inflorescence axis between the insertion points of first and fifth
24	NOFLO	Number of flowers
25	UPPINT	Uppermost internodium length
26	STDI	Stem diameter under inflorescence
27	STDLOW	Stem diameter above lowermost leaf
28	NOLOB	No. of lobe of tubers (1,2,3,4,5)

Source: Bateman and Denholm (1985).

relatively close to each other in the axis with respect to their population and secondly, sample population form three groups. The most closely related samples belong to group I which contains Changlung, Staksha, Skurru, Skampuk and Lochum population, while group II contains Sumur and Bogdang population and group III contains Tirith, Turtuk, Hunder, Sanjak, Mulbek and Pashkum population. The most important morphological characters distinguishing these three groups are reflected in their loadings on the first two principal components. In this case, the first two principal components encompass more than 75% variation among the population. The highest loadings characters are: plant height, length of longest leaf, position of maximum width, uppermost leaf length, uppermost leaf width, lowermost leaf length, lowermost leaf width, position from base of the second leaf, greatest width, length of second leaf from base, width of second leaf, number of cauline leaves, length of lateral lobe of lip, from the base to the top of lobe, bract length, length from lowest bract to the top of inflorescence, length of inflorescence axis between the insertion points of first and fifth, number of flowers and uppermost internodium length for PC1, spur length, measured underneath the spur, lip width and bract width for PC2 (Table 4). Dendrogram was drawn to display the phenetic relationships among different populations of Ladakh region based on Euclidean distances from the morphological data matrix. All populations were represented into cluster (Figure 4). Dendrogram based on average linkage (within group) analysis grouped the 260 phenotype into population group with main clusters A and B. Cluster A represents the phenotype of Skampuk, Lochum, Staksha, Skurru, Turtuk and Bogdang while, cluster B represents the phenotype of Tirith, Pashkum, Sanjak, Hunder, Mulbek, Sumur and Changlung. The results of PCA and MDS analysis were comparable to the cluster analysis.

DISCUSSION

Morphological traits are based on phenotype expression of the population and are influenced by different environmental factors (Heywood, 2002). Significant variation was observed for different morphological traits

Table 3. Duncan's test for mean comparisons of morphological characters among *D. hatagirea*.

Population	Mean plant height (SE) (cm)	Mean leaf length (SE) (cm)	Mean leaf width (SE) (cm)	Mean maximum width (SE) (cm)	Mean uppermost leaf length (SE) (cm)	Mean uppermost leaf width (SE) (cm)	Mean lowermost leaf length (SE) (cm)
Tirith	74.6^{e} (0.54)	13.4^{e} (0.10)	3.9^{e} (0.08)	5.7^{e} (0.10)	10.4^{e} (0.08)	2.5^{a} (0.04)	12.4^{e} (0.07)
Sumur	73.6^{e} (0.42)	13.5^{e} (0.06)	4.0^{e} (0.05)	4.9^{c} (0.08)	9.5^{d} (0.04)	1.7^{c} (0.03)	10.5^{d} (0.05)
Changlung	45.2^{c} (0.65)	10.6^{c} (0.06)	3.5^{bc} (0.04)	4.3^{a} (0.03)	8.1^{b} (0.05)	1.4^{b} (0.02)	8.6^{b} (0.45)
Staksha	35.2^{ab} (0.58)	8.4^{a} (0.04)	3.3^{a} (0.05)	4.3^{ab} (0.05)	6.7^{a} (0.02)	1.3 ^{b} (0.04)	7.8^{a} (0.03)
Turtuk	66.2^{d} (0.59)	12.5^{d} (0.07)	3.2^{a} (0.07)	4.1^{a} (0.05)	8.7^{c} (0.08)	1.2^{a} (0.03)	9.3^{c} (0.17)
Bogdang	65.9^{d} (0.55)	12.3^{d} (0.12)	3.2 ^{a} (0.06)	4.1^{a} (0.05)	8.6^{c} (0.07)	1.2^{a} (0.04)	9.2^{c} (0.18)
Hunder	65.2^{d} (0.81)	10^{b} (0.06)	3.6^{c} (0.06)	5.1^{d} (0.07)	8.6^{c} (0.04)	1.4^{b} (0.02)	10.2^{d} (0.04)
Skurru	34.2^{a} (0.73)	8.4^{a} (0.05)	3.2^{a} (0.05)	4.2^{a} (0.06)	6.7^{a} (0.02)	1.3^{b} (0.04)	7.8^{a} (0.04)
Skampuk	36.0^{b} (0.52)	8.5^{a} (0.03)	3.4^{ab} (0.04)	4.5^{b} (0.07)	6.7^{a} (0.02)	1.4^{b} (0.03)	7.8^{a} (0.03)
Sanjak	72.8^{e} (0.60)	13.4^{e} (0.10)	3.9^{e} (0.08)	5.7^{e} (0.10)	10.5^{e} (0.08)	2.5^{a} (0.04)	12.4^{e} (0.07)
Mulbek	65.9^{d} (0.55)	12.3^{d} (0.12)	3.2^{a} (0.06)	4.1^{a} (0.05)	8.6^{c} (0.07)	1.2^{a} (0.04)	9.2^{c} (0.18)
Lochum	36.0^{b} (0.52)	8.5^{a} (0.03)	3.4^{ab} (0.04)	4.5^{b} (0.07)	6.7^{a} (0.02)	1.4^{b} (0.03)	7.8^{a} (0.03)
Pashkum	74.3^{e} (0.54)	13.4^{e} (0.10)	3.9^{e} (0.08)	5.7^{e} (0.10)	10.4^{e} (0.08)	2.5^{a} (0.04)	12.4^{e} (0.07)

Population	Mean lowermost leaf width (SE) (cm)	Mean length of second leaf from base (SE) (cm)	Mean position from base of the second leaf greatest width (SE) (cm)	Mean width of second leaf (SE) (cm)	Mean number of cauline leaf (SE) (cm)	Mean spur length measured underneath the spur (SE) (cm)	Mean length of middle lobe of the lip from the base to the the top of the lobe (SE) (cm)
Tirith	2.7^{d} (0.06)	13.2^{c} (0.10)	2.6^{c} (0.09)	3.8^{e} (0.05)	9.7^{d} (0.11)	1.31^{d} (0.05)	1.39^{d} (0.04)
Sumur	3.0^{e} (0.07)	11.4^{b} (0.10)	2.7^{c}(0.05)	3.5^{d} (0.03)	9. 5^{d} (0.17)	1.14^{c} (0.04)	1.28^{c} (0.02)
Changlung	2.4^{c}(0.06)	11.2^{b} (0.08)	2.5^{b} (0.05)	3. 5^{d} (0.05)	8.1^{abc} (0.18)	1.38^{d} (0.0)	1.39^{d} (0.05)
Staksha	1.7^{a} (0.04)	10.4^{a} (0.01)	2.1^{a} (0.02)	2.5^{b} (0.03)	8.0^{a} (0.18)	1.02^{b} (0.05)	1.05^{ab} (0.04)
Turtuk	2.1^{b} (0.03)	10.5^{a} (0.02)	2.1^{a} (0.03)	2.4^{ab} (0.07)	8.5^{bc} (0.11)	0.75 ^{a} (0.03)	1.11^{b} (0.02)
Bogdang	2.1^{b} (0.02)	10.5^{a} (0.04)	2.1^{a} (0.03)	2.4^{a} (0.06)	8.6^{c} (0.11)	0.74^{a} (0.04)	1.12^{b} (0.02)
Hunder	2.3^{c} (0.02)	10.5^{a} (0.05)	2.4^{b} (0.04)	3.53^{d} (0.02)	9.4^{d} (0.15)	1.12^{c} (0.02)	1.28^{c} (0.01)
Skurru	1.7^{a} (0.03)	10.5^{a} (0.06)	2.0^{a} (0.02)	2.6^{c} (0.03)	8.1^{ab} (0.20)	0.89^{b} (0.05)	0.98^{a} (0.03)
Skampuk	1.7^{a} (0.04)	10.5^{a} (0.03)	2.1^{a} (0.02)	2.5^{c} (0.03)	7.9^{a} (0.18)	1.02^{b} (0.05)	1.05^{ab} (0.04)
Sanjak	2.7^{d} (0.06)	13.2^{c} (0.14)	2.6^{c} (0.09)	3.8^{c} (0.05)	9.7^{d} (0.11)	1.31^{d} (0.05)	1.40^{d} (0.04)
Mulbek	2.1^{b} (0.03)	10.5^{a} (0.07)	2.1^{a} (0.03)	2.4^{a} (0.06)	8.6^{c} (0.11)	0.74^{a} (0.04)	1.12^{b} (0.02)
Lochum	1.7^{a} (0.05)	10.5^{a} (0.06)	2.1^{a} (0.02)	2.5^{a} (0.03)	7.9^{a} (0.18)	1.02^{bc} (0.05)	1.05^{ab} (0.04)
Pashkum	2.7^{d} (0.06)	13.2^{c} (0.14)	2.6^{c} (0.09)	3.8^{c} (0.05)	9.7^{d} (0.11)	1.31^{d} (0.05)	1.40^{d} (0.04)

among population of Ladakh regions, but plant height was the only trait that showed great variation. Earlier report of *D. hatagirea* on taxonomy suggested that plant height is about 40 to 60 cm in height (Baral and Kurmi, 2006), but our result accomplished that it varies from 34.20 cm in Skurru to 74.60 cm of Tirith population at a significant level of 5. In this study, traits such as plants height, leaf length, lowermost leaf length, length of second leaf from base and length from lowest bract to the top of inflorescence were varied from population to population. Tirith

Table 3. Contd.

Population	Mean length of lateral lobe of the lip from the base to the the top of the lobe (SE) (cm)	Mean lip width (SE) (cm)	Mean bract length (SE) (cm)	Mean bract width (SE) (cm)	Mean length from lowest bract to the top of inflorescence (SE) (cm)	Mean length of inflorescence axis (SE) (cm)	Mean number of flower (SE) (cm)
Tirith	1.57^{e} (0.04)	1.22^{c} (0.04)	2.86^{e} (0.04)	0.48^{ab} (0.01)	16.5^{e} (0.24)	6.33^{e} (0.08)	13.2^{e} (0.1)
Sumur	1.43^{d} (0.04)	1.2^{c} (0.07)	2.57^{d} (0.04)	0.81^{e} (0.06)	14.2^{d} (0.14)	5.58^{d} (0.06)	10.6^{c} (0.35)
Changlung	1.33^{c} (0.04)	1.24^{c} (0.06)	2.39^{c} (0.05)	1.14^{f} (0.09)	11.4^{b} (0.1)	4.68^{b} (0.07)	10^{b} (0.16)
Staksha	0.97^{a} (0.04)	0.99^{b} (0.06)	1.43^{a} (0.03)	0.77^{de} (0.0)	9.7^{a} (0.2)	3.87^{a} (0.05)	9.5^{ab} (0.14)
Turtuk	1.21^{b} (0.01)	0.77^{a} (0.04)	1.99^{b} (0.04)	0.38^{a} (0.02)	11.2^{b} (0.21)	4.71^{b} (0.05)	9.75^{ab} (0.0)
Bogdang	1.21^{b} (0.00)	0.78^{a} (0.04)	1.99^{b} (0.04)	0.38^{a} (0.02)	11.1^{b} (0.20)	4.66^{b} (0.06)	9.65^{ab} (0.1)
Hunder	1.34^{cd} (0.03)	1.18^{c} (0.03)	2.55^{d} (0.02)	0.64^{bcd} (0.03)	13.1^{c} (0.03)	5.1^{c} (0.03)	11.4^{d} (0.23)
Skurru	0.97^{a} (0.03)	0.85^{a} (0.06)	1.47^{a} (0.02)	0.57^{bc} (0.03)	9.7^{a} (0.05)	3.94^{a} (0.07)	9.37^{a} (0.12)
Skampuk	0.97^{a} (0.04)	0.99^{b} (0.06)	1.43^{a} (0.03)	0.77^{de} (0.05)	9.7^{a} (0.03)	3.96^{a} (0.06)	9.5^{ab} (0.14)
Sanjak	1.57^{e} (0.04)	1.22 ^{c} (0.04)	2.86^{e} (0.04)	0.49^{ab} (0.04)	16.5^{e} (0.24)	6.33^{e} (0.08)	13.0^{e} (0.18)
Mulbek	1.22^{b} (0.01)	0.79 ^{a} (0.04)	1.99^{b} (0.04)	0.39^{a} (0.02)	11.1^{b} (0.20)	4.66^{b} (0.06)	9.65^{ab} (0.11)
Lochum	1.03^{a} (0.04)	0.96^{b} (0.06)	1.43^{a} (0.03)	0.78^{de} (0.06)	9.7^{a} (0.03)	3.96^{a} (0.06)	9.5^{ab} (0.14)
Pashkum	1.57^{e} (0.04)	1.22^{c} (0.04)	2.86^{e} (0.04)	0.49^{ab} (0.04)	16.5 ^{e} (0.24)	6.33^{e} (0.08)	13.0^{e} (0.19)

Population	Mean upper internodium length (SE) (cm)	Mean stem diameter under inflorescence (SE) (cm)	Mean stem diameter above lowermost leaf (SE) (cm)	Mean number of lobes (SE) (cm)
Tirith	9.03^{d} (0.1)	1.60^{b} (006)	1.75^{b} (0.01)	3.3^{bc} (0.1)
Sumur	7.68^{c} (0.4)	1.59^{b} (0.06)	1.75^{b} (0.03)	3.4^{bc} (0.1)
Changlung	7.5^{bc} (0.11)	1.57^{b} (0.04)	1.79^{b} (0.03)	3.1^{b} (0.1)
Staksha	5.67^{a} (0.0)	1.05^{a} (0.06)	1.25^{a} (0.02)	3.4^{bc} (0.7)
Turtuk	7.34^{b} (0.04)	1.59^{b} (0.04)	1.77^{b} (0.02)	3.5^{bc} (0.1)
Bogdang	7.35^{b} (0.03)	1.63^{b} (0.03)	1.79^{b} (0.02)	3.6^{bc} (0.15)
Hunder	7.6^{bc} (0.10)	1.88^{b} (0.02)	1.84^{b} (0.01)	3.6^{c} (0.11)
Skurru	5.66^{a} (0.04)	1.18^{a} (0.07)	1.34^{a} (0.01)	4.1^{d} (0.12)
Skampuk	5.73^{a} (0.04)	1.15^{a} (0.07)	1.36^{a} (0.07)	2.7^{a} (0.11)
Sanjak	9.03^{d} (0.18)	1.59^{b} (0.06)	1.75^{b} (0.07)	3.3^{ab} (0.16)
Mulbek	7.35^{b} (0.03)	1.63^{b} (0.03)	1.79^{b} (0.02)	3.4^{bc} (0.15
Lochum	5.73^{a} (0.04)	1.16^{a} (0.07)	1.36^{a} (0.07)	2.8^{a} (0.14)
Pashkum	9.03^{d} (0.18)	1.60^{b} (0.06)	1.75^{b} (0.07)	3.2^{ab} (0.16)

population showed more values of this trait while Skurru showed less value. From this, it is concluded that Tirith showed great morphometric variation as compared to other populations. It implies that Nubra valley population shows great morphological dissimilarity with population of Suru and Indus valley. This may be due to wide geographical range, species richness and environmental factor. Therefore, *Dactylorhiza*

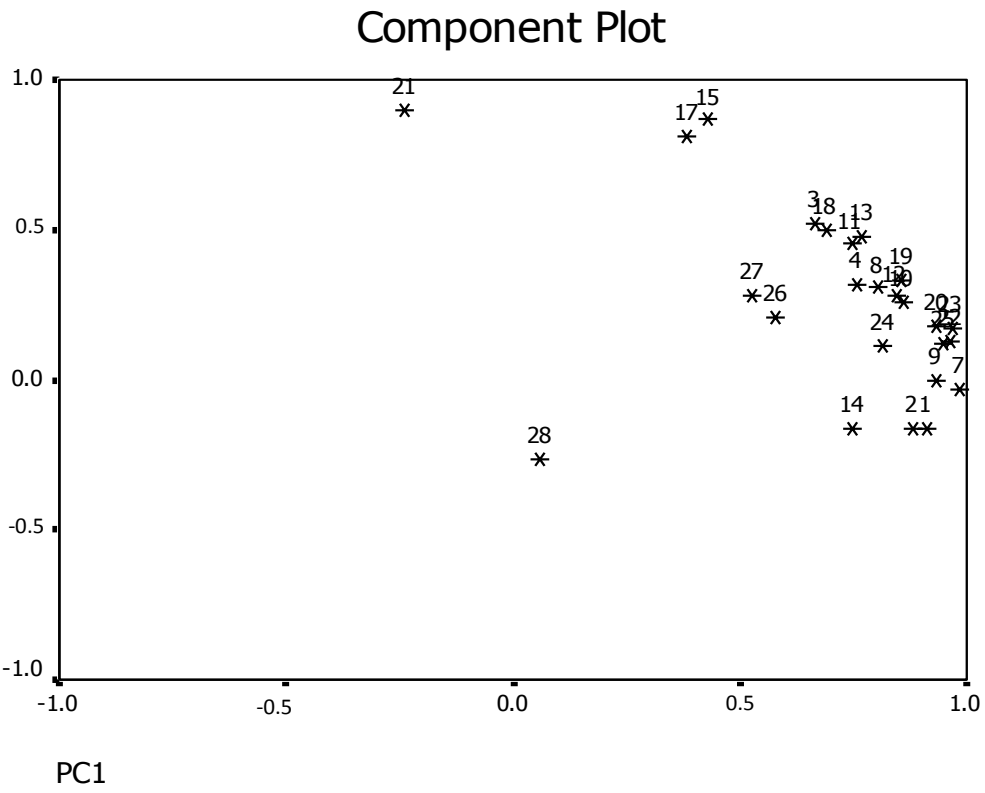

Figure 2. Principal component analysis of Morphological data of *D. hatagirea.*

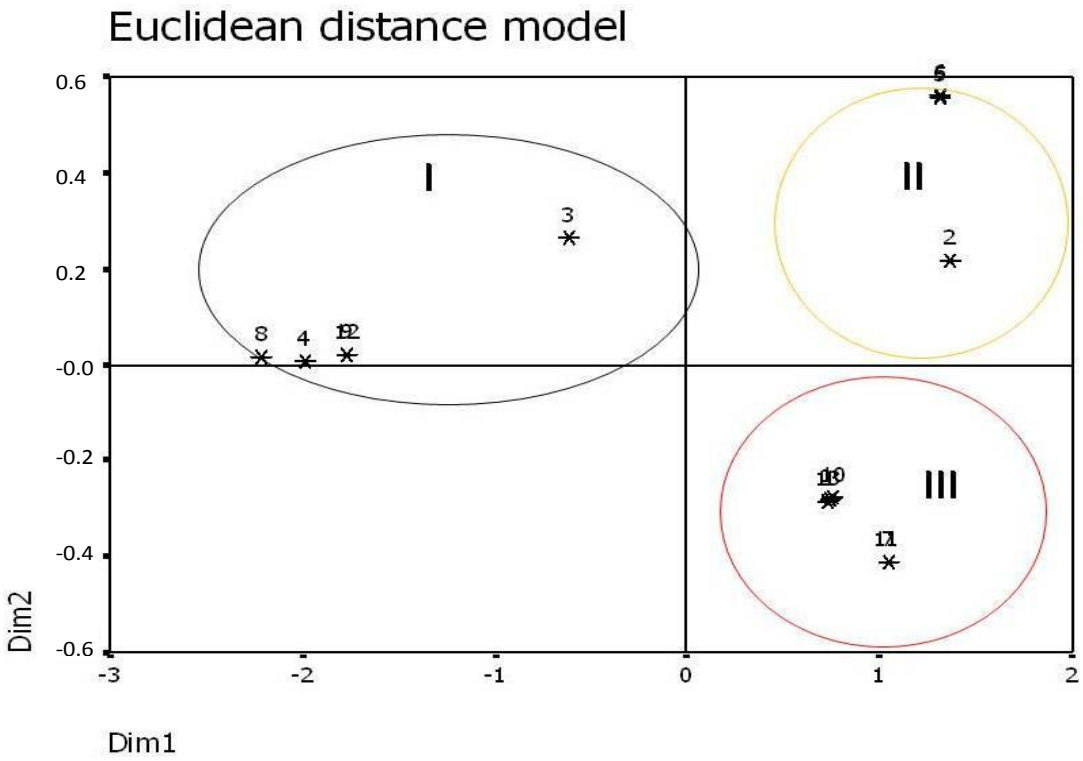

Figure 3. Multidimensional scaling of morphological data of *D. hatagirea* with their respective population (1, Tirith; 2, Sumur; 3, Changlung; 4, Staksha; 5, Turtuk; 6, Bogdang; 7, Hunder; 8, Skurru; 9, Skampuk; 10, Sanjak; 11, Mulbek; 12, Lochum; 13, Pashkum).

Table 4. Characters loadings in first two principal components for the analysis of *D. hatagirea* (morphological data only).

Character	Characters acronym	PC1 (63.49%)	PC2 (11.70% = 75.19)
Plant height	PLH	**0.911**	-0.165
Length of longest leaf	LFL	**0.883**	-0.163
Width of longest leaf	LFW	0.667	0.519
Position of maximal width, the distance from leaf base to the place of maximal width	MAX W	**0.760**	0.317
Uppermost leaf length	ULFL	**0.983**	-0.029
Uppermost leaf width	ULFW	**0.805**	0.305
Lowermost leaf length	LLFL	**0.930**	-0.005
Lowermost leaf width	LLFW	**0.859**	0.260
Position from base, of the second leaf greatest width	POSFRB	**0.747**	0.451
length of second leaf from base	LSECLF	**0.844**	0.277
width of second leaf	WIDOF	**0.768**	0.475
Number of cauline leaves	CAULF	**0.748**	-0.161
Spur length, measured underneath the spur	SPULEN	0.430	**0.865**
Lip width	LIPW	0.384	**0.814**
Length of middle lobe of lip, from the base to the top of lobe	LIP MID	0.688	0.496
Length of lateral lobe of lip, from the base to the top of lobe	LIPLATE	**0.857**	0.334
Bract length	BRAL	**0.933**	0.179
Bract width	BRAW	-0.238	**0.898**
Length from lowest bract to the top of inflorescence	LOWBRA	**0.964**	0.129
Length of inflorescence axis between the insertion points of first and fifth	INFAXIS	**0.967**	0.169
Number of flowers	NOFLO	**0.816**	0.112
Uppermost internodium length	UPPINT	**0.950**	0.118
Stem diameter under inflorescence	STDI	0.580	0.204
Stem diameter above lowermost leaf	STDLOW	0.527	0.278
Number of lobe of tubers (1,2,3,4 and 5)	NOLOB	0.060	-0.263

High loadings are highlighted in boldface type.

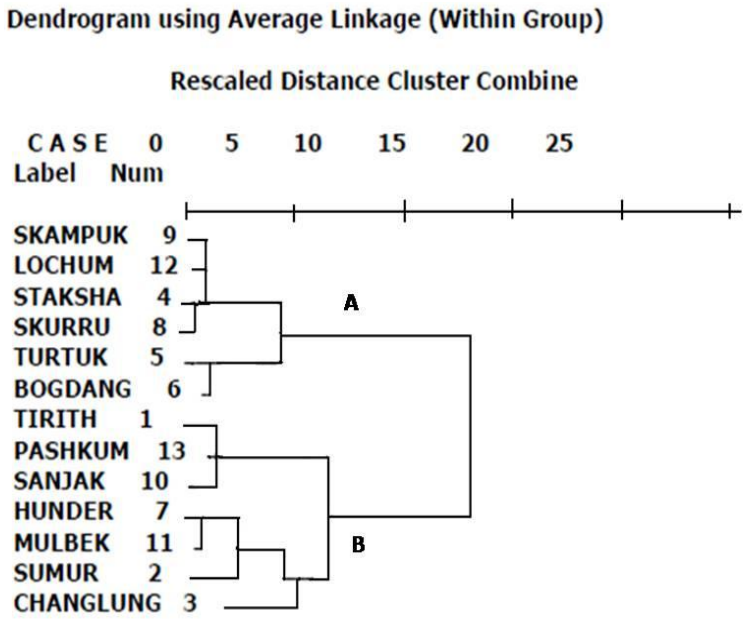

Figure 4. Dendrogram showing the phenetic relationships among 13 populations based on Euclidean distances from morphological data matrix.

populations deserve specific conservation attention as regards its habitat fragmentation. Conservation of its populations *ex-situ* and *in-situ* will have greater effects on population richness and status of such an endangered orchid.

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