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Research article

Morphometrics of selected *Dendrobium* spp. (Orchidaceae) in Sri Lanka

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Abstract: Morphometric analyses of five species of *Dendrobium* (Orchidaceae) found in Sri Lanka; *D. aphyllum*, *D. crumenatum*, *D. heterocarpum*, *D. nutans* and *D. panduratum* were carried out to infer their phenetic relationships. Thirty-one morphological characters were considered; six vegetative and twenty-five floral, of which five were qualitative and twenty-six were quantitative characters. The data were subjected to Cluster Analysis (CA) and Principal Component Analysis (PCA). Cluster analysis has produced two prominent clusters, *D. aphyllum*, *D. nutans* and *D. panduratum* as cluster 1 and *D. crumenatum* and *D. heterocarpum* as cluster 2. Both *D. nutans* and *D. panduratum* are producing small sized floral parts, compared to *D. aphyllum* hence exhibits close similarities in floral morphology which resulted in forming a cluster of most closely related species with least dissimilarity values. With respect to many characteristics, two species incluster 2, *D. crumenatum* and *D. heterocarpum*, are found to be similar except for the leaf thickness. Furthermore, PCA also in agreement with the relationships derived from the cluster analysis. The present taxonomic study has revealed that in inferring phenetic relationships of *Dendrobium* spp. both vegetative and floral characters are influential.
Keywords: Morphometrics - Phenetics - Cluster analysis - Principal component analysis.

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INTRODUCTION

Orchids inhabit every part of the globe except in the polar region (Jayaweera 1981). They belong to family Orchidaceae which is the largest family in angiosperms, consisting of approximately 850 genera and 17,000 to 35,000 species (Dressler 1993). Family Orchidaceae is classified into five subfamilies; Epidendroideae, Orchidoideae, Cypripedoideae, Vanilloideae and Apostasioideae. *Dendrobium* is the second largest genus of orchids representing 1184 species belongs to the subfamily Epidendroideae, subtribe Dentrobiinae (Begum *et al.* 2009; Leitch *et al.* 2009). *Dendrobium* orchids are of high economic value as ornamental and medicinal plants and are used in traditional medicine in China and several other Asian countries (Begum *et al.* 2009). Some *Dendrobium* species are used to treat the stomach ailments, promote the production of body fluids, moisten the lung, relieve coughing and as an antipyretic. Further, several species contain compounds with antioxidant activity and antitumor activity which can be used to inhibit cancer cell development (Yao *et al.* 2009, Takamiya *et al.* 2011).

Sri Lankan orchid diversity comprises 188 species placed under 78 genera, with one endemic genus; *Adrorhizon* Hook. f. and 55 endemic species (Fernando & Ormerod 2008). The most comprehensive treatment of Sri Lankan species of the genus *Dendrobium* was done by Jayaweera in 1981 and has revised the genus recognising eight species: *Dendrobium bambusifolium* Par. et Reichb.f., *Dendrobium crumenatum* Swartz., *Dendrobium diodon* Reichb.f., *Dendrobium heterocarpum* Wall. ex Lindl., *Dendrobium maccarthiae* Thw., *Dendrobium macrostachyum* Lindl., *Dendrobium nutans* Lindl., and *Dendrobium panduratum* Lindl. However, *D. macrostachyum* was synonymised with *D. aphyllum* Roxb. (Christenson & Wood 2003). Recently the world checklist of selected plant families (http://apps.kew.org) has proposed synonyms for *D. bambusifolium*, and *D.*

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nutans. Three species; *D. diodon*, *D. maccarthiae* and *D. panduratum* are endemic to Sri Lanka according to Jayaweera (1981) whereas Fernando & Ormerod (2008) has reported *D. maccarthiae* and few subspecies of *D. panduratum* and *D. diodon* as endemics to Sri Lanka.

Sri Lanka is an island, lies between 5º 55′ to 9º 51′ North latitudes and 79º 41′ to 81º 54′ East longitudes. It covers 64 453 km^2 of land area and 1 156.2 km^2 of inland waters from the total area of 65 609.8 km^2. The island is divided into three major climatic zones; wet zone, dry zone and intermediate zone considering the rainfall. Orchid diversity is highest in the wet lowland and montane forest while forests of the intermediate zone (submontane zone) harbour the country's richest orchid diversity (Fernando & Ormerod 2008).

Morphometric methods are used as valuable tools in systematics and have long been established for studying the development, population differentiation and systematics of plants (Bateman & Rudall 2006). Such studies utilized both quantitative and qualitative characters of taxa (Venhuis *et al.* 2007). Typically between 20 and 50 quantified characters are employed and generally these characters, consist of a heterogeneous mixture of metric, meristic, scalar and presence/absence characters. The role of floral and vegetative characters in orchid classification has been re-evaluated in several previous studies (Borba *et al.* 2002, Goldman *et al.* 2004, Bateman & Rudall 2006, Watthana 2006, Borba *et al.* 2007, Fischer *et al.* 2007, Pinheiro & Barros 2009). Although the genus *Dendrobium* was defined by Jayaweera in 1981, a detailed study of the morphological variation, using multivariate analysis of many traits, has not been undertaken in Sri Lanka. The present study was mainly focussed on the determination of the interspecific and intraspecific variation of *Dendrobium* species in Sri Lanka based on qualitative and quantitative morphometry.

MATERIAL AND METHODS

Plant material

Figure 1. Map showing localities of study area.

The localities recorded by previous authors were explored for the collection of plant material however, most of these localities are currently have undergone habitat destruction due to the human settlements. Therefore, the field collections that are conducted during the research period has managed to sample only 75% of species of the genus from 40 populations representing 25 localities in montane, submontane and lowland wet forests in Sri Lanka (Fig. 1 & Fig. 2). All the individuals were maintained in a greenhouse at Royal Botanical garden, Peradeniya, Sri Lanka.

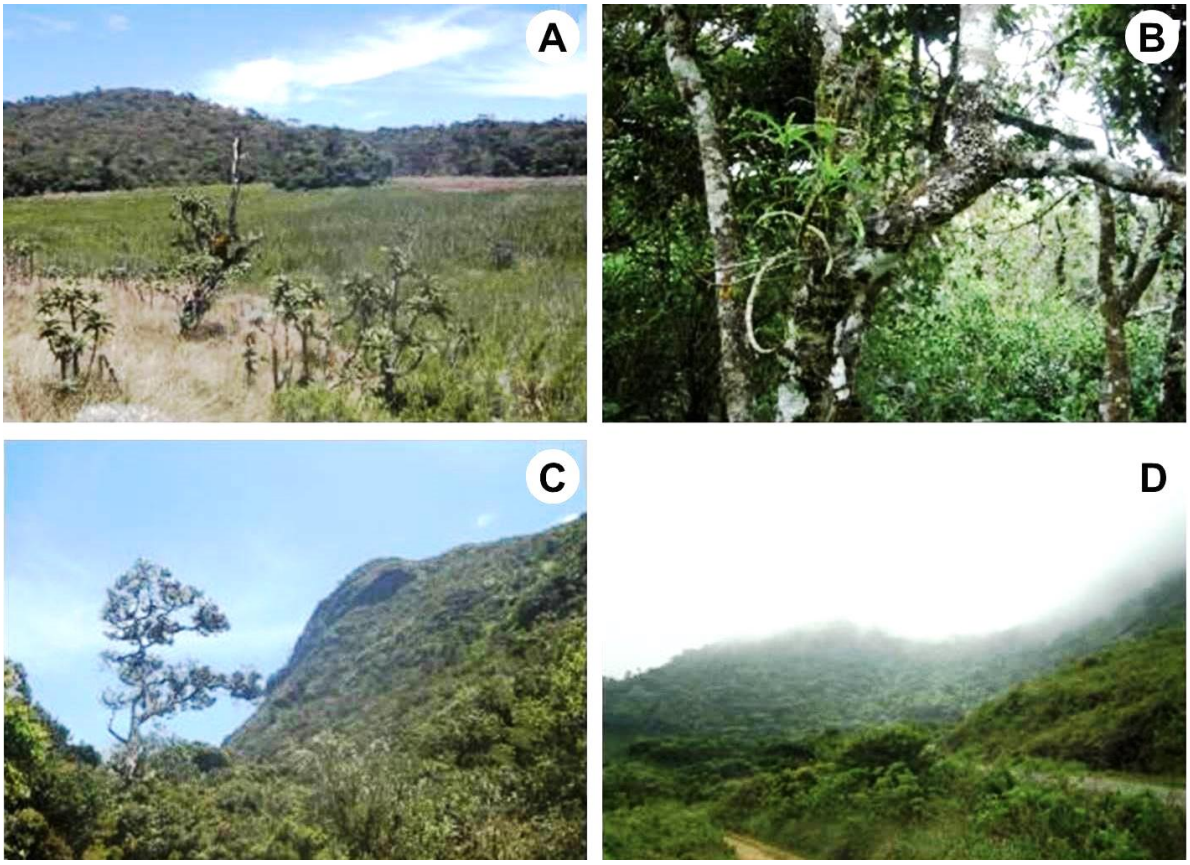

Figure 2. Photographs of sampling sites: **A & B**, Horton Plains National Park; **C**, Hakgala Strict Nature Reserve; **D**, Knuckles Forest Reserve

The collected specimens (Fig. 3) were identified using diagnostic keys, floral drawings and descriptions in Flora of Ceylon (Dassanayake & Fosberg 1981). The identified specimens were authenticated against the specimens in the National Herbarium, Peradeniya, Sri Lanka and voucher specimens were deposited in the National Herbarium, Peradeniya, Sri Lanka (Table 1).

Table 1. Voucher specimens deposited at National herbarium, Peradeniya, Sri Lanka.

Taxon	***Voucher number***
Dendrobium aphyllum Roxb.	003
Dendrobium panduratum Lindl.	158
Dendrobium nutans Lindl.	154
Dendrobium heterocarpum Wall. ex Lindl.	115
Dendrobium crumenatum Swartz.	075

Character observations

Thirty-one morphological characters; 6 vegetative and 25 reproductive/floral of which five were qualitative and 26 were quantitative, were recorded based on the measurements and observations of the samples collected from their natural habitats (Table 2).

Floral parts of dissected flowers and cross-sections at mid-region of the leaves were drawn with the aid of a stereomicroscope equipped with a camera lucida (Olympus SZX 7), and measurements were made from the derived drawings at the point of maximum dimension (Fig. 4). Each observation was made in replicates and the mean value was considered in the analysis.

Multivariate analysis

Principal Component Analysis (PCA): PCA on a correlation matrix was used as an objective method to deriven the interspecific variation of the taxa for the selected variables using Canoco for windows 4.5 software package. The number of axes, needed to consider for interpretation, was determined by comparing eigenvalues.

Cluster analysis (CA): Cluster analysis was performed for the five species by SYNTAX 2000 software package using the Euclidean distance treating with the Single Link Resolution and UPGMA as the sorting strategy. Four dendrograms were derived for floral and vegetative variables of population samples of each species as a pooled dataset and also as individual datasets.

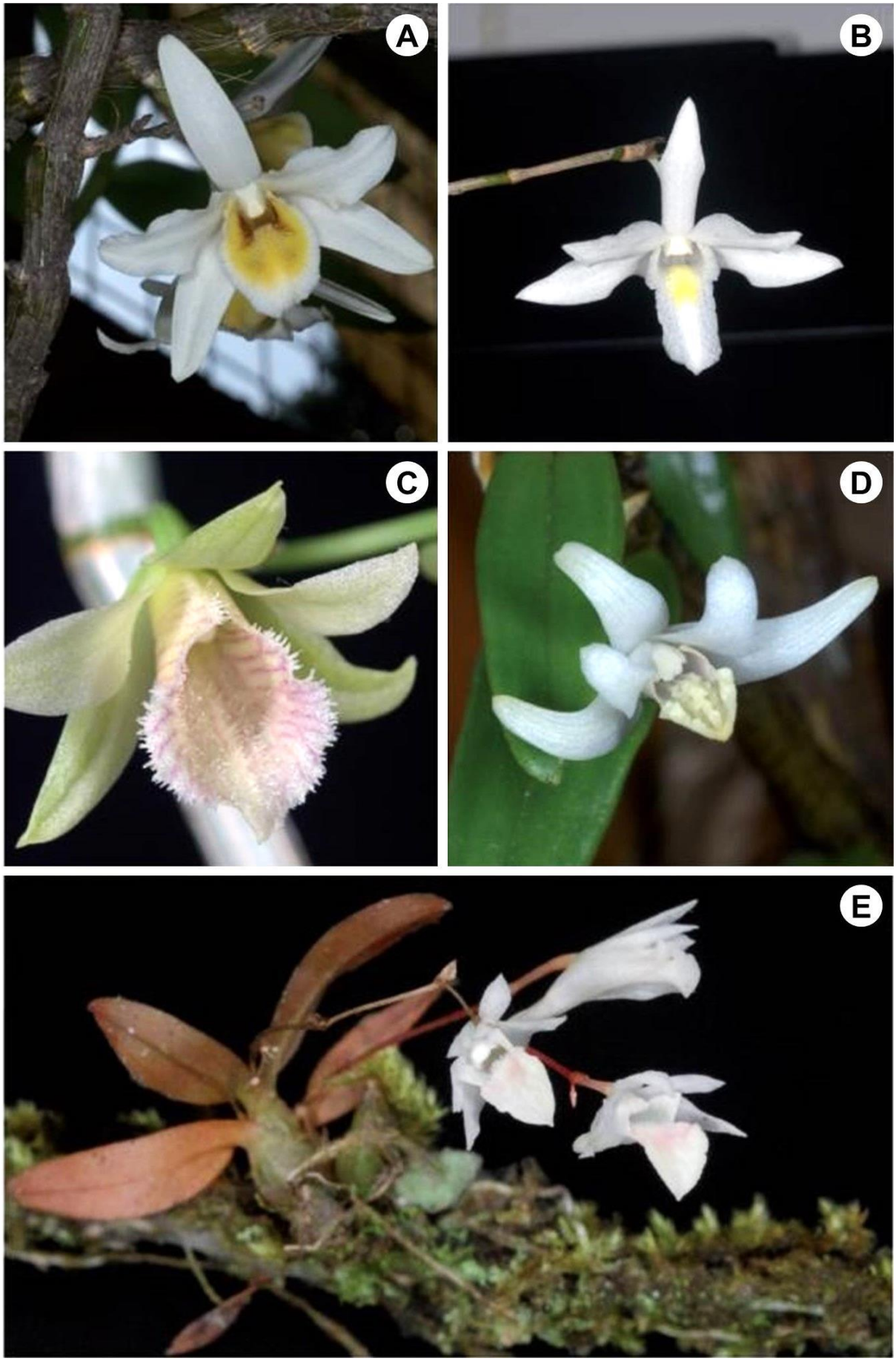

Figure 3. Photographs of flowers of collected field samples of *Dendrobium* spp.: **A**, *Dendrobium heterocarpum* Wall. ex Lindl.; **B**, *Dendrobium crumenatum* Swartz.; **C**, *Dendrobium aphyllum* Roxb.; **D**, *Dendrobium nutans* Lindl.; **E**, *Dendrobium panduratum* Lindl.

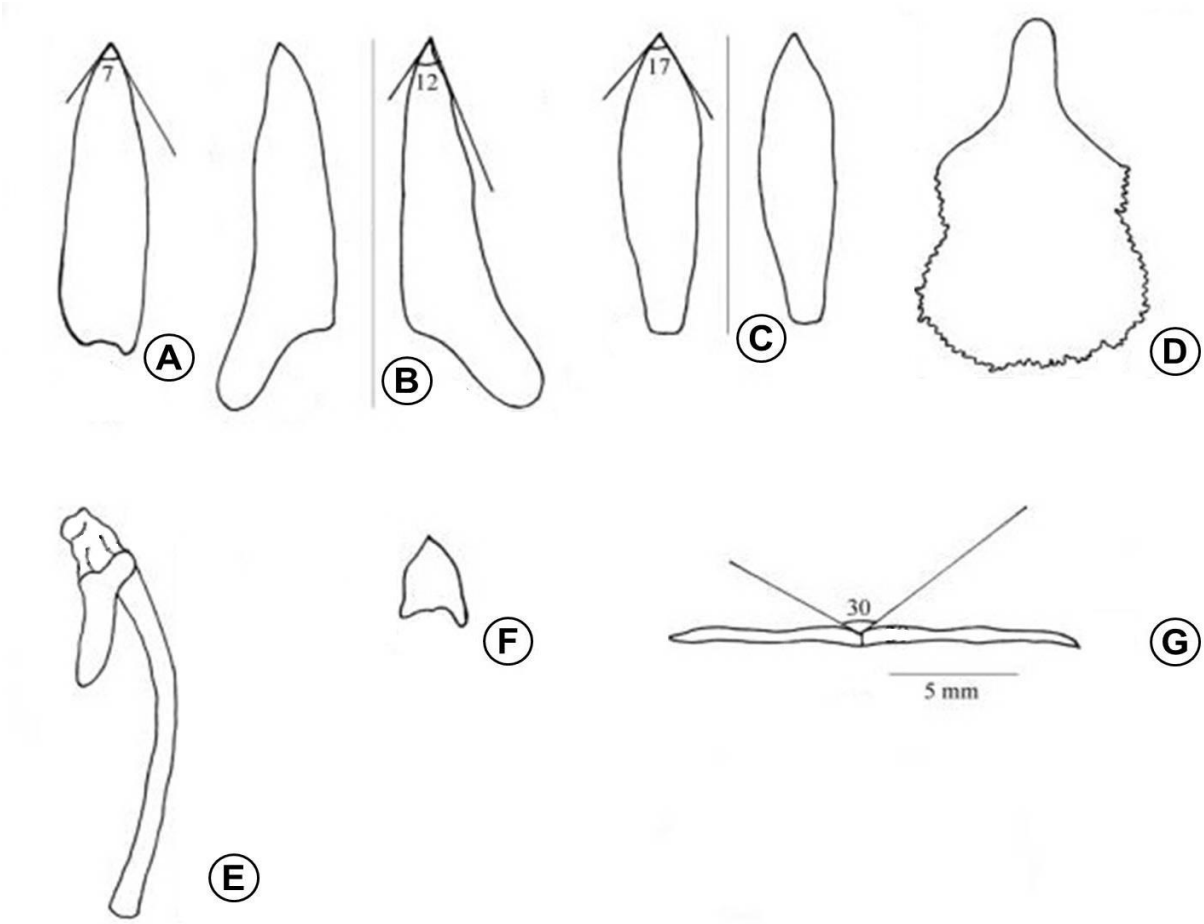

Figure 4. Line drawings of floral parts and cross-sections of leaves of *Dendrobium aphyllum* Roxb. showing floral and vegetative characters used in morphological analysis (except qualitative characters and some quantitative characters like number of veins): **A**, Dorsal sepals (spread); **B**, Lateral sepals (spread); **C**, Petals (spread); **D**, Lip (spread); **E**, Pedicel with column and spur (side view); **F**, Bract (spread); **G**, Cross section of leaf at mid-region. [Scale bar = 5 mm]

Table 2. Floral and vegetative characters used in morphometric analysis.

Character	Code	Character	Code
1. **Pedicel Length**	P-L	**Lip (Spread)**	
Flower		19.Length	L-L
2. Length	F-L	20.Width	L-W
3. Width	F-W	**Column**	
Dorsal sepal		21.Length	C-L
4. Shape	DS-S	22.Width	C-W
5. Length	DS-L	**Bract**	
6. Width	DS-W	23.Shape	B-S
7. Apex angle	DS-AA	24.Length	B-L
8. Number of veins	DS-NV	25.Width	B-W
Lateral Sepal		**Leaf**	
9. Shape	LS-S	26.Shape	LE-S
10. Length	LS-L	27.Length	LE-L
11. Width	LS-W	28.Width	LE-W
12. Apex angle	LS-AA	**Leaf (Cross-section at mid-region)**	
13. Number of veins	LS-NV	29. Thickness	LE-T
Petal		30. Inner angle	LE-IA
14. Shape	PE-S	31.Dorsiventral diameter	LE-DD
15. Length	PE-L		
16. Width	PE-W		
17. Apex angle	PE-AA		
18. Number of veins	PE-NV		

Median comparison

Median comparison was performed to describe the variation of the measurements of the quantitative characters of all the species and graphically presented in the form of box plots using SPSS ver. 16.0.

RESULTS

Principal component analysis (PCA)

PCA has shown 81.9% of the total variation whereas the first axis explained 68.5% of the total variation while the second axis showed 13.4 % . According to the plot (Fig. 5) and vector values, floral characters, *i.e.* LE-W, LE-L, P-L, F-L, F-W, DS-L, DS-W, DS-V, LS-V, PE-W, C-W, B-S and B-L are highly correlated with the first axis and confirmed the correlation as 0.99. Except for PE-S, the other characters are correlated with both first and second axes while the highest correlations are with the first axis in each case. PE-S showed its highest correlation with the second axis, which is 0.89.

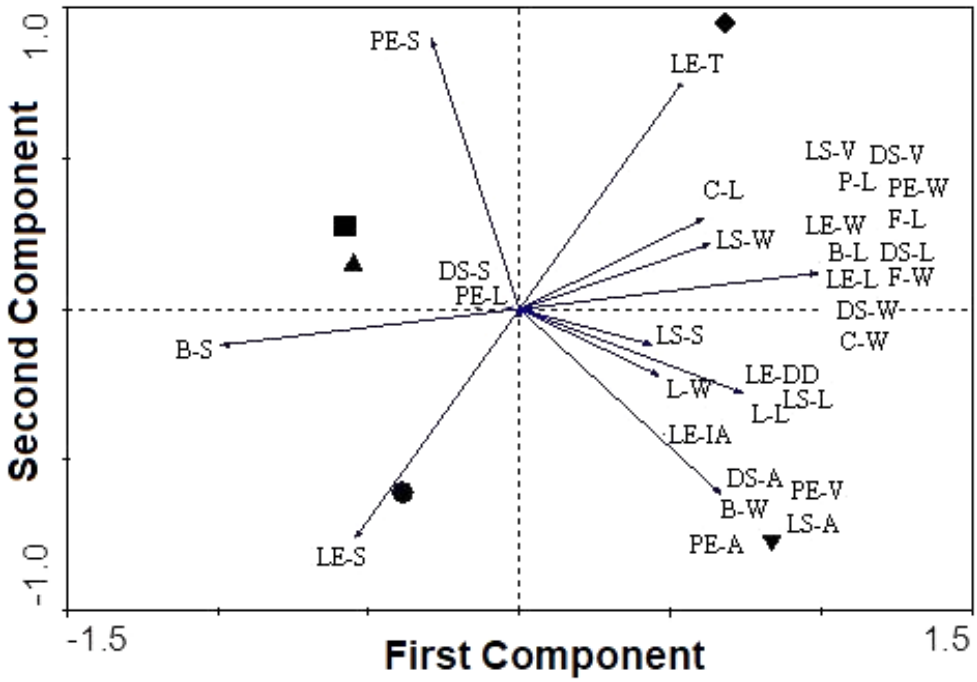

Figure 5. PCA of the five *Dendrobium* spp. based on 31 morphological characters (see Table 2). Axis 1 explains 68.5% and axis 2 explains 13.4% of the total variation. [▼=*Dendrobium heterocarpum* Wall. ex Lindl.; ♦= *Dendrobium crumenatum* Swartz.; ●= *Dendrobium aphyllum* Roxb.; ■= *Dendrobium nutans* Lindl.; ▲= *Dendrobium panduratum* Lindl.]

The bi-plot (Fig. 5) describes the species distribution and grouping pattern of observed morphometrics of the species. *Dendrobium crumenatum* and *D. heterocarpum* are located towards the right side of the plot while *D. nutans* and *D. panduratum* are placed in the left side. Whereas *D. aphyllum* is positioned in the bottom center of the plot. With respect to the distribution of morphological characters in the species, characters *i.e.* B-S, PE-S, LE-S, LE-T, LS-A, DS-A, B-W, PE-A and PE-NV are important in influencing variation of the species while DS-L and P-L have not shown significant contribution.

D. heterocarpum has shown a strong correlation with floral characters such as LS-A, DS-A, PE-A, PE-NV and B-W while a vegetative character, LE-T is with *D. crumenatum.* Both species have shown less correlation with characters such as LE-S, PE-S and B-S. *D. nutants* and *D. panduratum* have shown noticeable relation to characters like PE-S and B-S whereas *D. aphyllum* strongly correlated with vegetative character LE-S.

Cluster analysis

Both PCA (Fig. 5) and CA (Fig. 6) performed using 31 different morphological characters, have shown similar results. In the cluster analysis two prominent clusters were observed; *D. aphyllum*, *D. nutans* and *D. panduratum* as cluster 1 and *D. crumenatum* and *D. heterocarpum* as cluster 2 based on the dissimilarity values. Further, *D. nutans* and *D. panduratum* have clustered with least dissimilarity value indicating their close relatedness, especially in floral features. Compared to the large size floral parts of *D. aphyllum*, the other two species, *D. nutans* and *D. panduratum*, in the cluster 1, are with small-sized floral parts (Fig. 6A). Cluster analysis of the vegetative and floral characters of population sample datasets, has produced five main clusters with similar clustering pattern to the pooled datasets of the five species (Fig. 6A–B). However, when vegetative characters and floral characters were treated separately a different clustering pattern was observed. In the dendrogram of vegetative characters, a population sample of *D. nutans*, collected from Kandeela, has clustered with *D. crumenatum* and *D. heterocarpum* while the other *D. nutans* population sample, collected from Dothalugala, has clustered with *D. aphyllum* and *D. panduratum*. Similarly, different population samples of *D.*

aphyllum, collected from Gammaduwa, also have shown irregular clustering pattern (Fig. 6B–C). Further, a similar irregularity was observed in the clustering pattern of floral characters of different population samples of *D. aphyllum* (Fig. 6B–D).

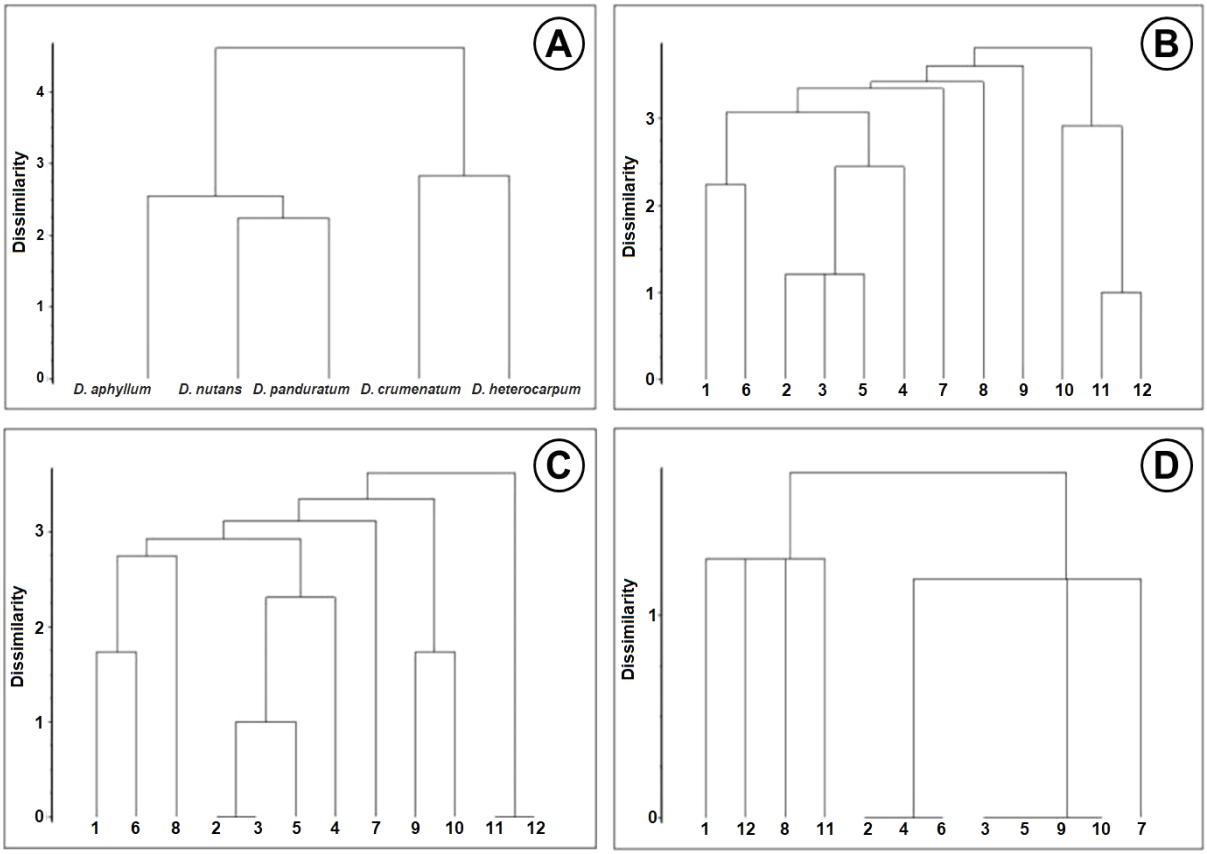

Figure 6. Cluster analysis of five *Dendrobium* spp.: **A**, Based on 31 morphological characters of pooled sample populations; **B**, Based on floral and vegetative morphometric data for each population sample; **C**, Based on floral morphometric data for each sample population; **D**, Based on vegetative morphometric data for each sample population.

Median comparison

Graphical tests (box plots) of the median comparisons of quantitative characters were performed to infer the variability of the most distinguished characters (Fig. 7). The results revealed that the characters LE-T, LE-IA, DS-L, DS-W, PE-A, L-W, B-L and B-W are distributed in overlapping patterns among the species while the other characters have shown efficient discriminant patterns. Further, the characters were distributed in a similar pattern among species pairs of *D. nutans* & *D. panduratum* and *D. crumenatum* & *D. heterocarpum* and have placed together as shown in figure 5.

DISCUSSION

With respect to the morphometrics, *D. crumenatum* and *D. heterocarpum* have shown clear separation from the other three species. The variations in the size of floral and leaf parts would be the reason for the difference which has evidently indicated in both CA and PCA performed in the present study. Nevertheless, *D. aphyllum*, *D. nutans* and *D. panduratum* have expressed their morphological similarity by clustering with less dissimilar values (Fig. 6A–C). However, *D. nutans* and *D. panduratum* were found to be highly related taxa by virtue of floral characters such as flower length, the shape of sepals and petals, width of the dorsal sepals and lateral sepals and the column width, despite the differences in vegetative morphology.

The clustering patterns (Fig. 6A) of these five taxa have clearly indicated the morphological differences, with respect to the qualitative characters like the shape of the dorsal sepals and petals, and quantitative characters like the number of veins in sepals and petals. Hence, these characters can be considered as the most influential characters in delimiting these five taxa in future taxonomic revisions. The differences in leaf thickness of *D. crmenatum* and *D. heterocarpum* may be the reason for the separation of these two species in cluster 2 as supported by the PCA. Further, cluster 1 has shown the difference, especially in the variations in the shape of leaves, petals and bracts. In the PCA, all the quantitative characters were interestingly combined to a distinct pattern.

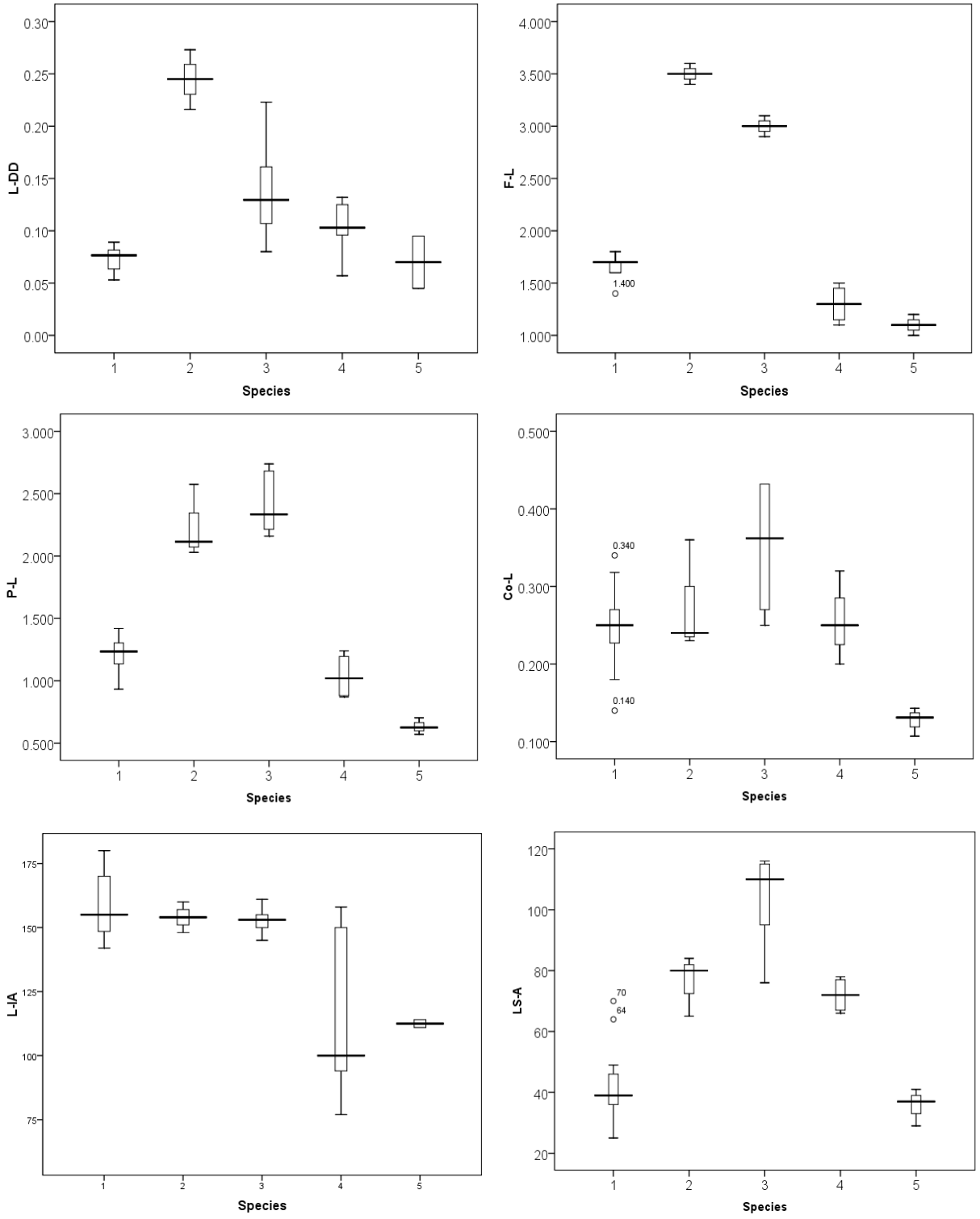

Figure 7. Variation in selected morphological characters of five *Dendrobium* spp. Each box shows the median, quartiles, and extreme values within a species. [Refer table 1 for character abbreviations. 1= *Dendrobium aphyllum* Roxb.; 2= *Dendrobium crumenatum* Swartz.; 3= *Dendrobium heterocarpum* Wall. ex Lindl.; 4= *Dendrobium nutans* Lindl.; 5= *Dendrobium panduratum* Lindl.

An intraspecific variation in *D. aphyllum* and *D. nutans* was indicated in the clustering pattern when the population samples were subjected to the cluster analysis for their vegetative and floral characters separately. Since the present study was limited to a few population samples due to the paucity of the distribution it is important to study more population samples representing different geographical regions to conclude the relationships of these taxa.

CONCLUSIONS

It can be concluded that the floral characters, compared to vegetative characters are significantly contributed

to inferring relationships of these species. Further, PCA revealed that the qualitative characters are more influential in deriving interspecific differentiation of the species than the quantitative characters. Also, the present study revealed the intraspecific variation in *D. aphyllum* and *D. nutans*, suggesting the need for taxonomic treatment of these taxa.

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REFERENCES

Bateman RM & Rudall PJ (2006) Evolutionary and Morphometric Implications of Morphological Variation Among Flowers Within an Inflorescence: A Case-Study Using European Orchids. *Annals of Botany* 98: 975–993.

Begum R, Alam SS, Menzel G & Schmidt T (2009) Comparative molecular cytogenetics of major repetitive sequence families of three *Dendrobium* species (Orchidaceae) from Bangladesh. *Annals of Botany* 104: 863–872.

Borba EL, Funch RR, Ribeiro PL, Smidt EC & Silva-Pereira V (2007) Demography, and genetic and morphological variability of the endangered *Sophronitis sincorana* (Orchidaceae) in the Chapada Diamantina, Brazil. *Plant Systematics and Evolution* 266: 129–146.

Borba EL, Shepherd GJ, van den Berg C & Semir J (2002) Floral and Vegetative Morphometrics of Five *Pleurothallis* (Orchidaceae) Species: Correlation with Taxonomy, Phylogeny, Genetic Variability and Pollination Systems. *Annals of Botany* 90: 219–230.

Christenson E & Wood H (2003) The Mystery of Limodorum aphyllum. *Orchids* 72: 774–777.

Dassanayake MD & Fosberg FR (eds) (1981) *A Revised Handbook to the Flora of Ceylon (Vol: II)*. New Delhi, Amerind Publishing.

Dressler RL (1993) *Phylogeny and Classification of the Orchid Family*. Cambridge University Press, U.K.

Fernando SS & Ormerod P (2008) An Annotated Checklist of the Orchids of Sri Lanka. *Rheedea* 18(1): 1–28.

Fischer GA, Gravendeel B, Sieder A, Andriantiana J, Heiselmayer P, Cribb PJ, Smidt E de C, Samuel R & Kiehn M (2007) Evolution of resupination in Malagasy species of *Bulbophyllum* (Orchidaceae). *Molecular Phylogenetics and Evolution* 45: 358–376.

Goldman SH, van den Berg C, & Griffith MP (2004) Morphometric circumscription of species and infraspecific taxa in *Calopogon* R.Br. (Orchidaceae). *Plant Systematics and Evolution* 247: 37–60.

Jayaweera DMA (1981) *A Revised Handbook to the Flora of Ceylon (Vol: II)*. New Delhi, Amerind Publishing.

Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW & Fay MF (2009) Genome size diversity in orchids: consequences and evolution. *Annals of Botany* 104: 469–481.

Pinheiro F & Barros FD (2009) Morphometric analysis of the *Brasiliorchis picta* complex (Orchidaceae). *Revista Brasileira de Botanica* 32(1): 11–21.

Takamiya T, Wongsawad P, Tajima N, Shioda, N, Lu JF, Wen CL, Wu JB, Handa T, Iijima H, Kitanaka S & Yukawa T (2011) Identification of *Dendrobium* Species Used for Herbal Medicines Based on Ribosomal DNA Internal Transcribed Spacer Sequence. *Biological & Pharmaceutical Bulletin* 35(5): 779–782.

Venhuis C, Venhuis P, Oostermeijer JGB & van Tienderen PH (2007) Morphological systematics of *Serapias* L. (Orchidaceae) in Southwest Europe. *Plant Systematics and Evolution* 265: 165–177.

Watthana S (2006) The *Pomatocalpa maculosum* Complex (Orchidaceae) Resolved by Multivariate Morphometric Analysis. *Taiwania* 51(1): 1–10.

Yao H, Jing-Yuan S, Xin-Ye M, Chang L, Ying L, Hong-Xi X, Jian-Ping H, Li-Sheng D & Shi-Lin C (2009) Identification of *Dendrobium* Species by a Candidate DNA Barcode Sequence : The Chloroplast psbA - trnH Intergenic Region. *Planta Medica* 75(6): 667–669.