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SPECIES DELIMITATION IN *VANDA* SECT. *CRISTATAE* LINDL. (ORCHIDACEAE): A MORPHOMETRIC APPROACH

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ABSTRACT. Species are arguably artificial units used for a variety of purposes, including classification, identification, and conservation, and authors have used different criteria for delimiting new species. With more than 25,000 species already named and described in the Orchidaceae (often based on single or very few characters) and limited time and funding for conservation, priorities must be set to target resources. Ideally, observable morphological differences should distinguish genetically meaningful species. A morphometric study of five species in *Vanda* section *Cristatae* (syn. *Trudelia* Garay) suggests three morphometrically distinct species groups.

Key words: Orchidaceae *Vanda, Trudelia,* species delimitation, morphometrics

INTRODUCTION

The species limits of the small group of orchids in John Lindley's *Vanda* section *Cristatae* (Lindley 1833), found in the Himalayas and Southeast Asia, are unclear. The species making up *Vanda* section *Cristatae* commonly are recognized as *Vanda alpina* Lindl. (Lindley 1853), *V. chlorosantha* (Garay) Christenson (Christenson 1992), *V. cristata* Lindl. (Lindley 1833), *V. griffithii* Lindl. (Lindley & Paxton 1851), and *V. pumila* Hook.f. (Hooker 1890). *Vanda griffithii,* however, sometimes is considered a synonym of *V. alpina* (M. Motes pers. comm., Roguenant & Chiron 2001, Senghas 1988). Another member of the section, *Vanda jainii* (A.S. Chauhan), was described by Chauhan (1984). Very little material of this species is available, certainly not in western European herbaria. *Vanda jainii,* described as closely allied to *V. cristata,* was not mentioned by L.A. Garay (1986), despite being described prior to his treatment of the group. It may be that *Vanda jainii* is a synonym of *V. cristata.* Both *V. jainii* and also *V. chlorosantha* are believed to be extremely rare, with only a few herbarium collections of either in existence.

This study investigates the confused species limits of the section, with particular attention to the status of *Vanda alpina, V. chlorosantha,* and *V. griffithii,* as separate species, a single species, or a 'complex'. A comparison between morphometric information on vegetative and floral characters in the group was made.

Species Concepts

With limited time and funding for conservation and more than 25,000 orchid species described, priorities are necessary to target resources. The conservation of the widest possible diversity of taxa deserves to be the main target, but taxonomists must be able to provide quantitative assessments of this diversity, if they are to provide policymakers with meaningful 'units' for conservation, be they species, populations, or whole ecosystems. Approaches based on a combination of morphological and molecular characters, incorporating an assessment of genetic diversity, need to be used in delimiting new species and reassessing existing classifications (Schuiteman & de Vogel 2003). The utility of the unit of 'species' would be increased, and the selection and conservation of the largest gene pool facilitated.

The species concept(s) used by authors when describing new species are rarely mentioned or discussed (Pridgeon 2003). The species concepts and criteria used by a taxonomist to distinguish separate species significantly impact the number of species recognized. Although some taxono-

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mists prefer to 'split' a group into different species on the basis of one or a few character differences, others prefer to 'lump' on the basis of similarities. For example, the Biological Species Concept (Mayr 1942, Donoghue 1985) considers entities that can and/or do interbreed (by 'ordinary means') to produce fertile progeny and that are reproductively isolated from other such groups, as belonging to the same species. Such concepts generally are not used in the Orchidaceae because of the ability of phylogenetically distant species to hybridize to produce fertile offspring.

A phylogenetic species concept, with all monophyletic, geographically distinct lineages regarded as distinct species (Brasier 1997, Donoghue 1985, Luckow 1995), cannot be applied to *Vanda* and its related genera, as a full molecular analysis of the group has yet to be undertaken.

More 'practical' species concepts applicable to groups not yet subject to molecular study include morphological species concepts, phenetic species concepts, and taxonomic species concepts; as well as more specifically, the Autapomorphic Species Concept (Mishler & Brandon 1987) and the Phylogenetic Species Concept sensu Nelson and Platnick (1981).

Taxonomic and morphological species concepts (Mayden 1997, Cronquist 1978) are intended to be practical, morphological concepts useable with all groups of organisms. Proponents of these concepts theorize that if an individual or a population of individuals can be repeatedly and consistently distinguished from others by a taxonomist using 'ordinary means', then it can be recognized as a distinct species. The diagnosis of a species may be based on only a few characters or even on a single character, such as flower color. Phenetic species concepts recognize species on the basis of overall similarity uniting taxa, in conjunction with their discontinuity from related taxa (Gornall 1997). Such concepts may involve only one or a few key diagnostic characters or a range of attributes may be taken into account. Concepts based on numerical taxonomy, such as the Phenetic Species Concept sensu Sneath and Sokal (1973), use a range of attributes, attempting to take all available characters into consideration to detect underlying patterns of similarity and discontinuity. Proponents of the concept find the species level is that at which distinct phenetic clusters can be observed. The Phenetic Species Concept is used in this study to examine species delimitation within *Vanda* section *Cristatae.*

Morphometric Analysis

Employing a morphometric approach to determine the species limits and number of species within *Vanda* section *Cristatae,* we analyzed both vegetative and floral characters using multivariate statistics. Numerical taxonomy, including morphometric techniques, traditionally has been used at or below species level; thus it is suited to species delimitation questions. Multivariate analysis can investigate underlying patterns and trends that may not be easily detectable to the taxonomist. Such analysis summarizes a range of variation in a relatively easily visualized and practical manner.

Classifications made using phenetic theory group entities are based on phenetic similarity, and the methods adhere to a series of neo-Adansonian principles, as described by Sneath and Sokal (1973). The more characters included, the more rigorous is the classification produced; and the greater the reduction in subjectivity, the greater is the increase in reproducibility of results. Characters, scored for individual operational taxonomic units (OTUs), are equally weighted and non-directional (i.e., no inference as to the probable direction of character evolution).

Morphometric methods have not been used extensively in the Orchidaceae, but a study by Bateman and Denholm (1983) used the multivariate technique to classify British and Irish marsh-orchids and recommended that the group be reduced to a single species with four subspecies. A study by Clifford and Lavarack (1974) compared the use of vegetative and reproductive attributes of 93 Orchidaceae taxa by means of group-average clustering and found both types of character "equally efficient predictors of the classification."

The most common multivariate statistical methods used in taxonomic processes are Cluster Analysis and the ordination techniques, which include the Principal Components Analysis (PCA) and its equivalent for handling continuous and discontinuous data—the Principal Coordinates Analysis (PCO or PCoA). The ordination techniques represent all data collected in multidimensional space and summarize it in 2- or 3-dimensional graphical plots.

Materials and Methods

A total of 30 spirit collections of flowers from *Vanda* section *Cristatae* from the University of Copenhagen (C), Royal Botanic Gardens Kew (K), and Royal Botanic Gardens Edinburgh (E) were scored for 39 floral characters (see Table 1). Flowers stored in preservative spirits unfortunately lose color characters, which therefore could not be scored. In all, 118 herbarium sheet specimens from Natural History Museum (BM), K, E, National Herbarium Nederland Leiden (L),

TABLE 1. Floral character list for a morphometric study of five species in *Vanda* section *Cristatae.*

No.	Floral diagnostic character	No.	Floral diagnostic character
1	Maximum breadth of flower (tip of tepal to tip of tepal)	20	Labellum epichile length (straight line along underside of labellum, from where epichile joins labellum midsection to end of labellum, excluding length of any apical projections)
2	Maximum height of flower (tip of dorsal sepal to tip of lowest lateral sepal)	21	Labellum hypochile midpoint width
3	Maximum distance from top of corolla tube to furthest tip of tepals	22	Labellum epichile midpoint width
4	Positioning of tepals around labellum and column: open (0), cupped (1), strongly cupped (2)	23	Labellum lateral lobes on hypochile: small (0), large (1)
5	Dorsal sepal—length	24	Labellum apical lip thickness
6	Dorsal sepal—midpoint width (perpendicular to sepal midline)	25	Labellum callus beneath epichile apex: none/indistinct (0), distinct (1)
7	Dorsal sepal—apical width (measured 1 mm from tip of sepal along midline)	26	Labellum callus length
8	Dorsal sepal—basal width (measured 1 mm along midline of sepal from point of attachment)	27	Labellum callus width
9	Lateral petal—length	28	Type of labellum apical projections: none (0), small bumps (1), large bumps (2), distinct horns/lobes (3)
10	Lateral petal—midpoint width	29	Number of labellum apical projections
11	Lateral petal—apical width	30	Maximum length of labellum apical projections
12	Lateral petal—basal width	31	Ribbing on labellum upper surface shallow (0), intermediate (1), deep (2)
13	Lateral sepal—length	32	Presence and type of spur on labellum hypochile: none/indistinct bowl-shape (0), pronounced deep hollow (1)
14	Lateral sepal—midpoint width	33	Depth of labellum spur (from base of sinuses of hypochilar lateral lobes to spur apex)
15	Lateral sepal—apical width	34	Midpoint width of labellum spur (side view)
16	Lateral sepal—basal width	35	Column length
17	Total labellum length (straight line measured from point of attachment to furthest tip of labellum)	36	Column midpoint width
18	Labellum hypochile length (straight line along underside of labellum, from point of attachment to rest of floral organs, to apex of 'spur')	37	Angle between midline of dorsal sepal to that of right-hand lateral petal
19	Labellum midsection length (straight line along underside of labellum, from apex of 'spur' to angled point where epichile starts)	38	Angle between dorsal sepal and lateral sepal
		39	Angle between line straight through column and central line of labellum

and Universität Wien (WU) were scored for 25 vegetative characters (see TABLE 2). Each individual plant (1 OTU) was assigned an identification code, and measurements were made using a metal ruler with 0.5 mm divisions, cotton thread for measuring curved structures, a grid of 1×1 mm squares, and a protractor for measuring angles.

The floral and vegetative data sets were analyzed with PCO (Principal Coordinates Analysis) in the statistical program MVSP (Kovach Computing Services 2002a). Analyses employed Gower's General Similarity Coefficient (Kovach Computing Services 2002b) and $\log_{10}$ transformed the data. Cluster analyses were performed on the floral data, using UPGMA as the clustering method, Gower's General Similarity Coefficient, and $\log_{10}$ transformation of the data.

RESULTS

Analyses of the floral morphometric data consistently showed three floral morphotypes, corresponding to all specimens previously identified as *Vanda cristata,* all *V. pumila* specimens, and a group of the *V. alpina, V. griffithii,* and *V. chlorosantha* specimens (see FIGURE 1). This pattern was repeated when labellum characters were excluded from analysis and when tepal characters alone were analyzed. Cluster Analysis of the floral data shows the OTUs split according to the same pattern, with *V. cristata* and *V. pumila* specimens dividing into two distinct clusters, similar more to each other than to the third cluster, composed of *V. alpina, V. griffithii,* and *V. chlorosantha* with no further intra-cluster groupings (see FIGURE 2). Analysis of the vegetative morphometric data showed no groupings at all (see FIGURE 3).

DISCUSSION

This study shows that the five currently recognized species of this section are not distinguishable on the basis of vegetative morpholo-

TABLE 2. Vegetative character list for a morphometric study of five species in *Vanda* section *Cristatae*.

No.	Vegetable diagnostic character	No.	Vegetative diagnostic character
1	Height of plant measured from base of stem (not incorporating roots) up to furthest point (usually tip of a leaf)	15	Leaf lobe height difference
		16	Average leaf length divided by average lobe height
2	Average root width measured 2 cm from emergence from stem	17	Leaves: lobes acutely ended (0), roundly ended (1), blunt (2), mixed (3), acute and blunt (4), rounded and blunt (5)
3	Stem length		
4	Stem width below first leaf	18	Leaves mucrone: absent (0), present narrowed (1), present expanded (2)
5	Stem midpoint width		
6	Stem width below 4 youngest leaves	19	Leaves: number attached to plant
7	Average leaf length	20	Number of leaves excised from plant
8	Average midpoint leaf width	21	Number of excised leaves divided by number attached leaves
9	Leaves: average apical leaf width measured 5 mm back from apex of shortest leaf lobe	22	Total number of leaves
10	Length of longest leaf	23	Height of leaf sheath bases
11	Midpoint width of longest leaf	24	Height of stem (sheath bases without leaves)
12	Midpoint width of widest leaf	25	Total stem height divided by height of stem (sheath bases without leaves)
13	Leaf length divided by midpoint width		
14	Leaf tapering: midpoint width divided by apical width		

gy; however, three distinct floral morphotypes, based on multiple characters, are *Vanda pumila*, *V. cristata*, and the *V. alpina* groups.

The floral study broadly supports the currently accepted classification of section *Cristatae*, with a few changes at the ranking level of the taxa. Using the Phenetic Species Concept sensu Sneath and Sokal (1973), three species can be recognized. They are *Vanda alpina*, *V. cristata*, and *V. pumila*, distinguishable on their floral morphologies. Intraspecific levels of variation occur within the species, corresponding to the

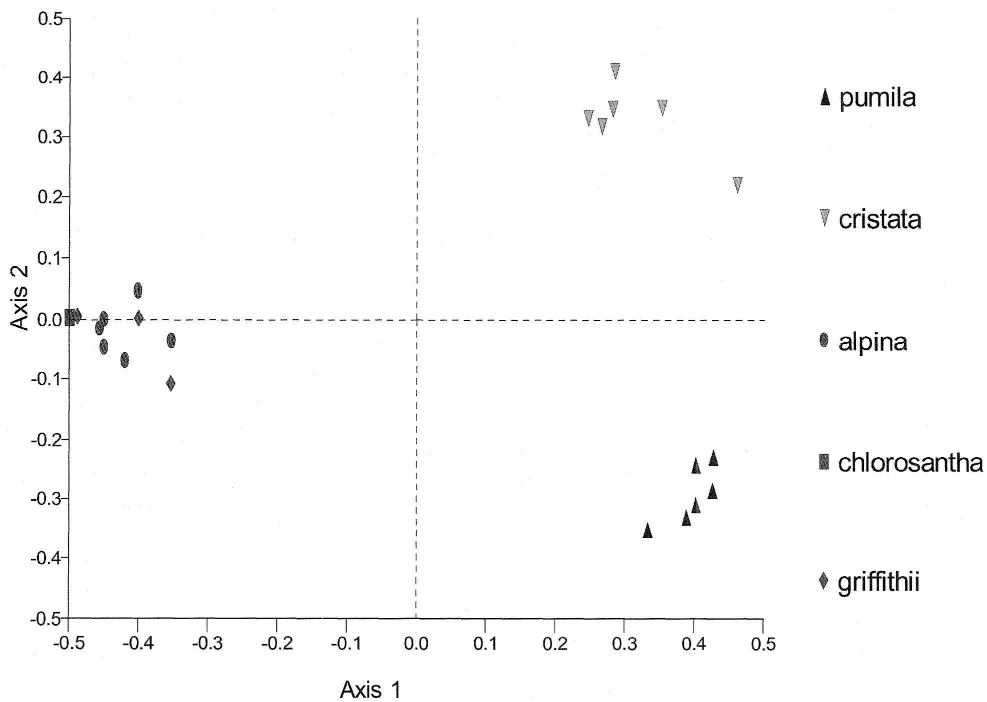

FIGURE 1. Analysis of floral characters: PCO axes 1 and 2 (Gower's General Similarity Coefficient used, data $\log_{10}$ transformed).

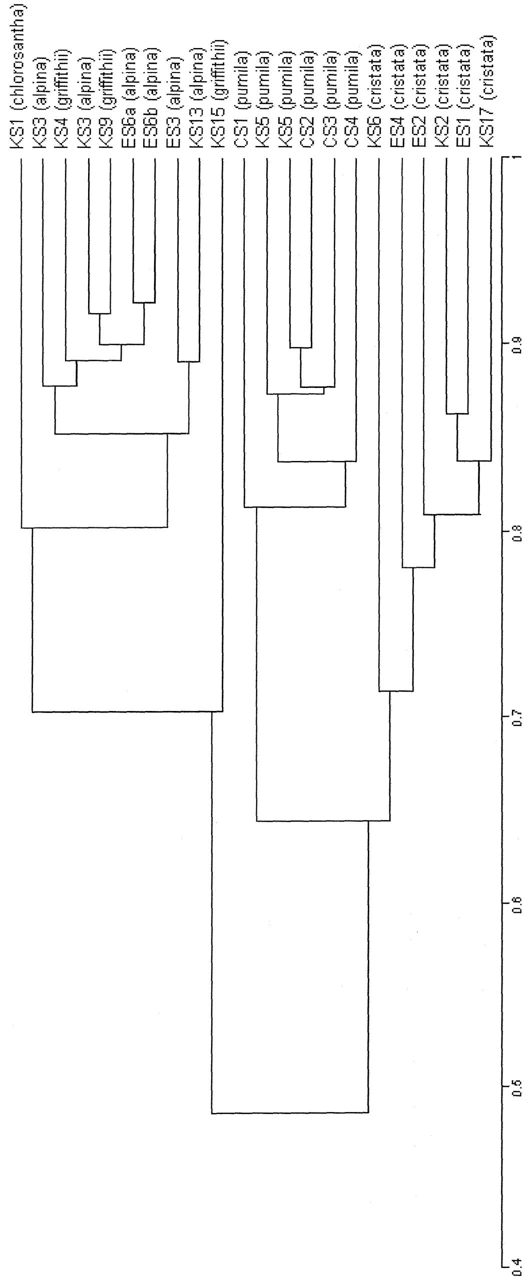

FIGURE 2. Analysis of floral characters: clustering analysis (UPGMA clustering method, Gower's General Similarity Coefficient used, data $\log_{10}$ transformed).

currently recognized species *V. griffithii* and *V. chlorosantha,* as subspecies of *V. alpina* divisible on the basis of one or a few floral characters (mostly related to flower color). If either the taxonomic or autapomorphic species concept was applied to the section, then all the taxa potentially could remain at the species level, as they are all distinguishable by a few unique characters.

Vanda jainii may be recognized as a subspecies of *V. cristata,* which shows an extremely high level of floral morphological variation, from the number and length of the apical projections on the labellum, to the color and number of striations on the labellum upper surface, even on the same plant (M. Motes pers. comm.). *Vanda jainii,* only collected once from the wild, may be part of the natural phenotypic variation of the species.

Where geographic distributions of species are very similar, where their distributions overlap, and where they share pollinators, we may not be looking at genetically distinct species. Instead we could be observing a complex of hybridizing entities with a shared gene pool. The taxa within the *Vanda alpina* complex may be interbreeding or, alternatively, may be gradually diverging.

If *Vanda* section *Cristatae* has speciated relatively recently to give the currently recognized species, and if few significant environmental differences exist between habitats, then the vegetative parts of the plants may not have diversified sufficiently to be discriminating at the species level by morphometric techniques. If recent speciation has been the result of isolation, perhaps because of a change in pollinator/pollinator behavior, radiation in floral structures would precede radiation in the vegetative parts.

The analyses presented here may suggest that *Vanda alpina, V. griffithii,* and *V. chlorosantha* are indistinguishable and therefore closely related, and it is not a priority to conserve all three. The three currently recognized species, however, are distinguished one from another by a few autapomorphies each—mostly characters related to the labellum and flower color. Since these are only a small number of characters out of the large number measured in this study, especially since color characters were not included because of the nature of the material studied, these differences among the taxa are not seen in the analyses performed. The species could be genetically closely related and in the process of diverging into three separate lineages, in which case, they may constitute relatively low priority for conservation. Alternatively, they could be distantly related, but morphologically similar either because of convergence or retention of the ancestral morphology of the group, in either case, constituting a higher priority for conservation. Phylogenetic study with wide sampling and surveys of distribution and pollinators—whether the species are sympatric or allopatric and whether gene flow is likely to occur between

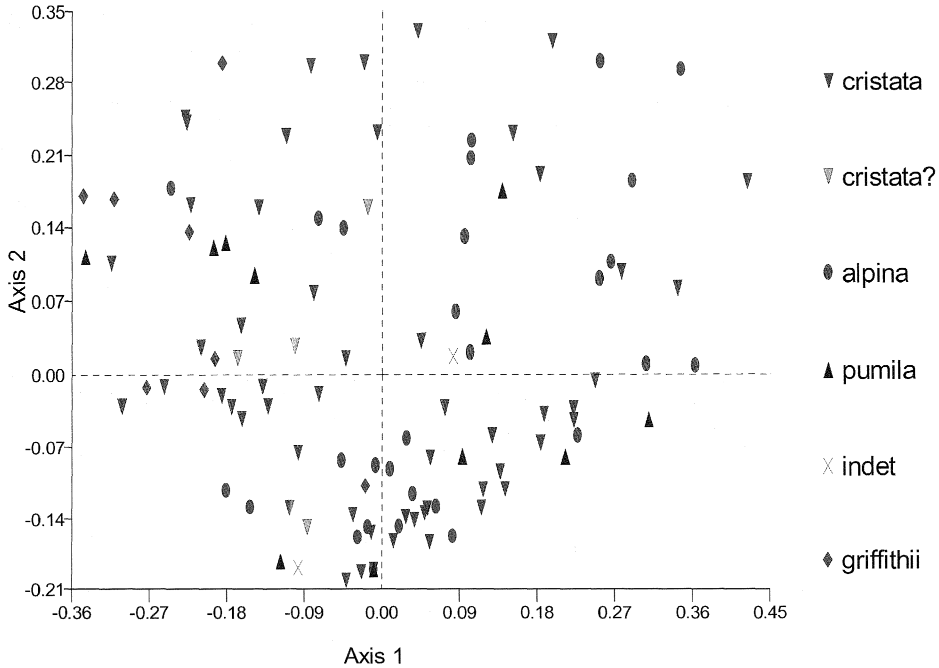

FIGURE 3. Analysis of vegetative characters: PCO axes 1 and 2 (Gower's General Similarity Coefficient used, data $\log_{10}$ transformed).

them—would provide greater insight into the relationships and dynamics of the group.

For *Vanda* section *Cristatae,* a morphological species concept based on floral characters appears to be an appropriate concept to distinguish species, with floral autapomorphies used to recognize intraspecific levels of variation. If such autapomorphic individuals consistently and persistently are identified and seen to form isolated populations with different distributions from the rest of the species, then grounds for the elevation of such taxa to specific level may be justified. *Vanda chlorosantha,* for example, may warrant remaining as a separate species from *V. alpina,* if it meets such criteria. In combination with genetic and biogeographic distribution studies, a revision of the section will be produced.

The whole genus *Vanda* needs considerable work in order to produce a phylogenetic framework around which to base a more meaningful classification of the group.

A simple identification key is presented, distinguishing the three floral morphotypes, *Vanda alpina, V. cristata,* and *V. pumila,* and the submorphotypes corresponding to the currently recognized species epithets, '*alpina*', '*cristata*', '*griffithii*', '*pumila*', and also '*jainii*'.

IDENTIFICATION KEY OF *VANDA* SECTION *CRISTATAE*

1. Labellum base open saccate hollow. Labellum equal in length or shorter than tepals. Small flowers, less than 2.5 cm diameter. . . . . *Vanda alpina* complex
    2. Strongly cupped flowers (tepals held closely around labellum). . . . . "*V. alpina*"
    2. More open flowers (tepals cupped slightly forward but not held closely around labellum).
        3. Flower pale yellowish green tepals, all same color. Labellum unstriped. . . . . "*V. chlorosantha*"
        3. Flower bright greenish. Labellum with purple striations. . . . . "*V. griffithii*"
1. Labellum base with distinct spur. Labellum longer than tepals. Large flowers, greater than 2.5 cm diameter.
    4. Strongly cupped flowers (tepals held closely around labellum). Thick, fleshy labellum without distinct callus below apex of labellum, slight apical swellings of varying size on edge of labellum. White flowers with reddish striations on labellum. . . . . *Vanda pumila* complex
    4. More open flowers (tepals cupped slightly forward but not held closely around labellum). Distinct callus below apex of labellum and distinct apical projections of varying size on edge of labellum. . . . . *Vanda cristata* complex

5. Apical projections very distinct horn-shaped lobes, commonly 2–3, often divaricating. Labellum longer than lateral sepals, 5–6 carunculate ridges on upper surface of labellum. Distinct, deep spur. . . . . "*V. cristata*"
5. Two erect lobules at apex of labellum. Labellum shorter than lateral sepals, 8–9 shallow purple ridges on upper surface of labellum. Spur obtuse and shallower. . . . . "*V. jainii*"

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