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About the taxonomic problems in *Orchis coriophora* (Orchidaceae)

Abstract

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Orchis coriophora s. l. includes a group of taxa that present a particular morphological and ecological variability which has led to disagreement as to their taxonomic status. With the aim of providing information to help clarify this situation, morphological data collected over two consecutive years in six populations belonging to the *O. coriophora* group were statistically analysed. Our results suggest that five of the characters studied are of taxonomic interest (lower leaf width, second leaf width, mid lobe length, spur length and diameter), some of which had not been previously considered. Finally, some considerations regarding the taxonomic status of these subgroups are discussed.

Introduction

Orchis coriophora s. l. is found throughout S., C. and E. Europe, N. Africa and the Near East and includes a group of taxa which present a particular morphological and ecological variability. This fact has led to some disagreement as to their taxonomic status.

Soó (1980) differentiated three subspecies, based mainly on lip and spur characteristics, as well as on flower odour: *O. coriophora* subsp. *fragrans* (Pollini) Sudre, *O. coriophora* subsp. *martrinii* (Timb.-Lagr.) Nyman (incl. *O. coriophora* var. *carpetana* Willk.) and *O. coriophora* s. str.

More recently, different proposals have been put forth which can be summarised into three tendencies. The first one recognises one polymorphic species without infraspecific categories (Buttler 1991). The second confers specific status upon the subspecies previously described (Delforge 1994; Benito Ayuso & al. 1999). And the third proposal maintains a variable number of subspecies or varieties (Davies & al. 1988; Bournerias & al. 1998).

Given the general tendency to accept the existence of different taxonomic levels within *O. coriophora* s. l. and the difficulty of establishing the taxonomic status for each one, this study was conducted in order to provide information to help clarify this situation.

Galicia (NW Iberian Peninsula) is a very interesting place in which to carry out this study because several subspecies are present in this area with allopatric populations, but there has been no agreement as to which of them may be included (Giménez de Azcárate & Amigo 1996; Cortizo & Sahuquillo 1999). Therefore, different populations were studied

with a view to detect their variability, to establish the relationship among samples analysed at different levels (population, subspecies or species) and to select the most interesting characters for the differentiation of the taxa.

Materials and methods

Morphological data from six populations collected over two consecutive years, 1999 and 2000, were used in this study (Table 1). Populations were selected to comprise the highest diversity in environmental conditions in which these taxa grow in Galicia (Cortizo & Sahuquillo 1999).

In each population morphological data from ten plants (eight from Trasigrexa in 1999) were measured *in situ* to minimize the impact. Twenty-three quantitative characters were checked in each specimen and several qualitative characters (flower odour and colours) were described as well.

First of all, a statistical analysis was done to calculate the Variation Coefficient (VC) in order to shed light on the behaviour of the characters in each population. Secondly, an ANOVA and Principal Components Analysis (PCA) were performed to select the most important characters and to establish different subgroups, independently of the classical taxonomic system. And in the third place, an Association Analysis was carried out to check their validity, using the Manhattan distance to determine the similarity between samples and the UPGMA method to elaborate the phenogram.

Table 1. Geographical origin, altitude, habitat, soil pH and number of specimens of the populations studied.

	Population	Taxon	Alt.	Habitat	pH	Date	n
1	O Estreito (Rubiá, Ou.)	<i>O. coriophora</i> subsp. <i>fragrans</i>	500 m	Dry scrub at hard slope, calcareous substrate	7,97	23/5/1999	10
27/5/2000						10	
2	Vilardesilva (Rubiá, Ou.)	<i>O. coriophora</i> subsp. <i>fragrans</i>	520 m	Grassland, calcareous substrate	7,59	23/5/1999	10
27/5/2000						10	
3	Forcadela (O Barco de Valdeorras, Ou.)	<i>O. coriophora</i> subsp. <i>fragrans</i>	600 m	Old vineyard with pines, calcareous substrate	8,1	12/6/1999	10
28/5/2000						10	
4	Trasigrexa (Vilardesilva, Ou.)	<i>O. coriophora</i> subsp. <i>martrinii</i>	620 m	Humid grassland, schist and slate substrate	5,31	15/6/1999	8
17/6/2000						10	
5	Vilavella (A Mezquita, Ou.)	<i>O. coriophora</i> subsp. <i>martrinii</i>	1120 m	Grassland and willow woodland, granitic substrate	5,71	9/7/1999	10
17/6/2000						10	
6	San Xil (Carballada de Valdeorras, Ou.)	<i>O. coriophora</i> subsp. <i>martrinii</i>	1250 m	Humid grassland, slate substrate	5,16	10/7/1999	10
1/7/2000						10	

Results and discussion

Based on ecological and morphological data, all the specimens from three populations were considered to be *O. coriophora* subsp. *martrinii*, and specimens from the remaining three populations to be *O. coriophora* subsp. *fragrans*. However, it is interesting to note that the "lip length" values found (6-8 mm in subsp. *fragrans* and 7-9 mm in subsp. *martrinii*) did not coincide with those cited by other authors in their descriptions of the subspecies studied (Soó 1980; Sanz & Nuet 1995). This fact was also reported by Molero & Rovira (1981) for other Iberian Peninsula populations.

Generally, vegetative characters presented a higher variation ($VC = 0,12-0,68$) than floral ones ($CV < 0,25$) in all populations and in both years. Vegetative characters were considered to be all those outside of the flower, in the strict sense. At the taxonomic level, populations identified as *O. coriophora* subsp. *martrinii* showed a higher variation than populations considered to belong to subsp. *fragrans*.

The results from the PCA pointed to a constant distribution of the characters considering the components obtained in both years, with the floral characters appearing consistently in the first component, while vegetative characters were distributed in the remaining components. Several characters were important in components 3, 4, 5 and 6: “number of cauline leaves”, “stem diameter below inflorescence”, “last internode length”, “stem length between the 1st and 5th flower”, “bract length”, “mid lobe base width”, “lateral lobe length from the base” and “lip width”. The results demonstrated that these characters were of little importance in the explanation of the variation observed, so their taxonomic utility was limited.

The three-dimensional distribution of the specimens based on the components extracted from the PCA shows a certain degree of overlapping among the populations in the two years studied (Fig. 1A). At the taxonomic level and taking into account *a priori* identifications, this analysis points to the existence of some morphologic continuity between subspecies (Fig. 1B), with specimens from the Vilavella population presenting intermediate values.

On the other hand, according to the result of the Variance Analysis, 19 of the 23 quantitative characters studied showed significant differences among populations, although seven characters did not present this behaviour in both years: “last internode length”, “stem length between the 1st and 5th flower”, “bract length and width”, “lateral lobe length from the base”, “mid lobe base width” and “lip length”. This result supported the findings from the PCA, corroborating their limited taxonomic interest.

A comparison of the averages among the populations using the Tuckey Test revealed that five characters were useful in the characterisation of these taxa: “spur length”, which clearly separated the populations into two groups coinciding with the subspecies in both years, “spur diameter” and “lower leaf width”, which followed suit but only in the year

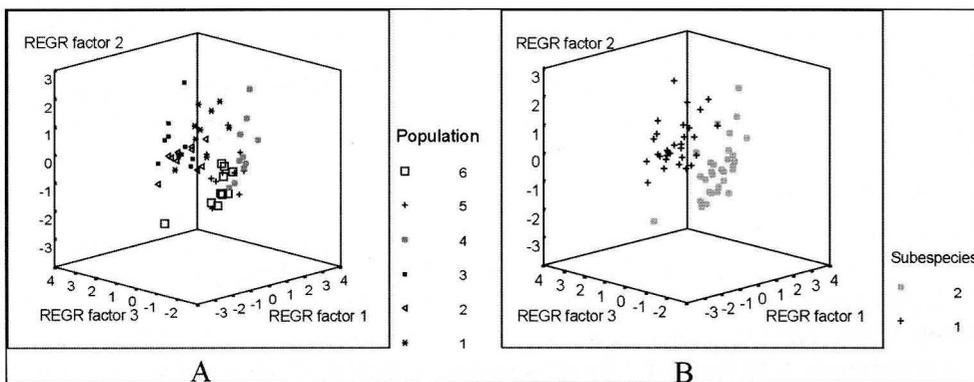


Fig. 1. Three-dimensional scatterplot from the Principal Components Analysis using data from the year 2000. A. populations; B. subspecies.

2000, as well as “second leaf width” and “mid lobe length”, which separated the subspecies but with some overlapping between the subgroups obtained in both years.

Finally, these five characters were used to carry out a Cluster Analysis to check their taxonomic validity. The resulting phenograms were similar in both years, therefore only one is shown in Figure 2. The first separation in the tree establishes two groups which coincide with the subspecies studied. However, three specimens belonging to the subsp. *martrinii* are included in the subsp. *fragrans* cluster. This fact corroborates the morphologic continuity cited above. “Spur length” stands out as the most important character in the differentiation of the taxa, while the remaining characters are less important, and appear at lower levels in the phenograms. On the other hand, all the characteristics were necessary to differentiate the subspecies using data from the year 2000. Even the elimination from the analysis of “mid lobe length”, which seems to have a low weight on the precedent analysis, led to a greater mixture among the specimens belonging to both subspecies. This result would indicate the existence of some variability in the behaviour of the characters, which makes their individual taxonomic use very uncertain.

We found no coincidence among the clusters obtained at the lower levels in the tree and the populations, so the range of variation of these characters was quite similar among the populations belonging to each subspecies. This may indicate that these characters were not affected very much by particular environmental conditions.

With regard to qualitative characters, personal field observations have shown that the low intensity of coloration or wide lip spots are not specific characters of *O. coriophora* subsp. *fragrans*. We have also found specimens belonging to *O. coriophora* subsp. *martrinii* which presented both characteristics. Odour is a highly subjective character and may not always be constant, as it is related to the degree of maturation of the flowers and the time of day. In fact, it is possible to find specimens with no odour belonging to subsp. *fragrans* (Molero & Rovira 1981) and fragrant specimens, that do not necessarily have a strong smell, belonging to subsp. *martrinii*.

Conclusions

Of the 23 quantitative characters analysed, floral characters seem to have higher taxonomic validity than their vegetative counterparts, with the most prominent being “spur length and width” and “mid lobe length” from the floral characters and “lower leaf width” and “second leaf width” from the vegetative ones, although the latter sometimes presented a high Variation Coefficient.

At the taxonomic level, there was a certain overlapping among the values found in the most discriminatory characters, in spite of the geographic isolation between the populations analysed. This finding questions the change in the taxonomic category of these subspecies and highlights the need for molecular studies to establish the phylogenetic relationships between them.

As a final conclusion, given the results obtained and following the biological criteria of species (Pedersen 1998), we consider that it is not suitable to change the taxonomic category to justify the variability observed within the *O. coriophora* group.

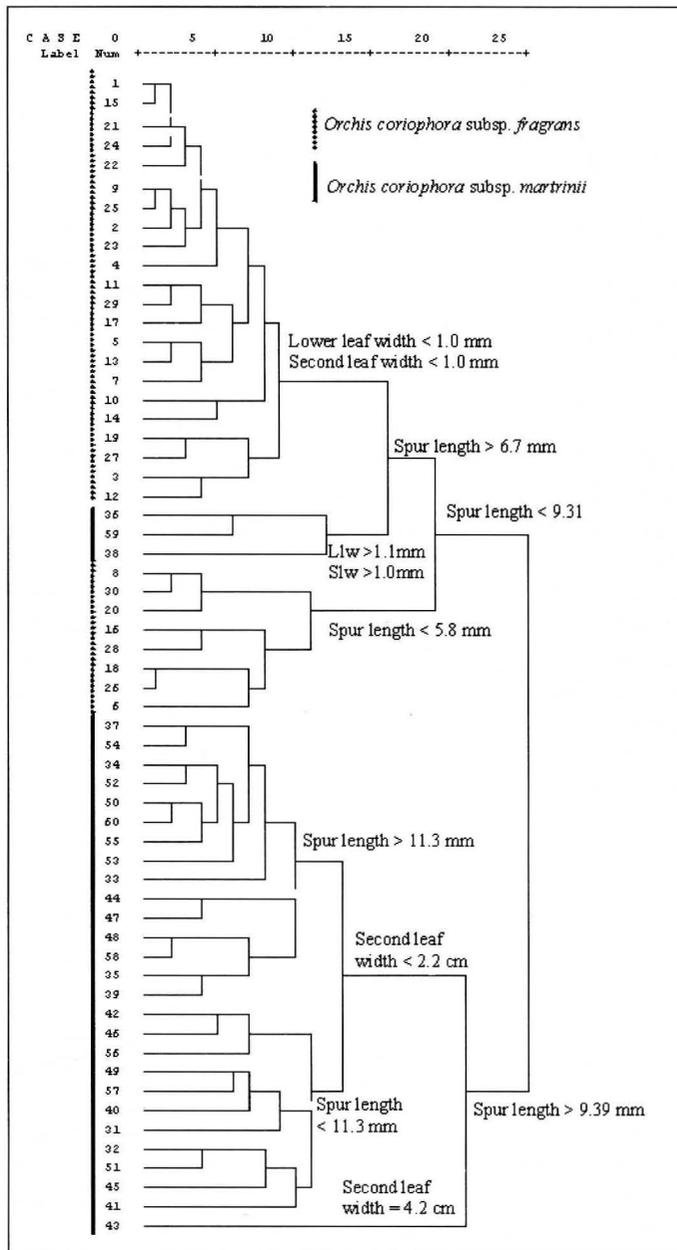


Fig. 2. Phenogram using data from the year 2000. LlW - Lower leaf width; Slw - Second leaf width.

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