doi: 10.1111/j.2008.0107-055X.00010.x,

Journal compilation © Nordic Journal of Botany 2007. No claim to US government works

Subject Editor: Stefan Andersson. Accepted 2 October 2007

Morphometric analysis of *Epidendrum secundum* (Orchidaceae) in southeastern Brazil

F. Pinheiro and F. de Barros

F. Pinheiro and F. de Barros (fdebarros@terra.com.br), Inst. de Botânica, Caixa Postal 3005, BR-01061-970 São Paulo, Brasil.

One of the largest genera of Orchidaceae with about 1125 species, *Epidendrum* L. presents several taxonomic problems, mainly due to the great variability of some of its species. The present study aims at evaluating the influence of different environments on the morphology of individuals from *Epidendrum secundum*, using morphometry to compare plants under cultivation and plants collected directly from the field. Eighty specimens maintained in cultivation at the Institute of Botany of São Paulo (Brazil) and 146 individuals originating from three natural populations, totaling 226 specimens, were analyzed with multivariate methods (PCA, CVA, DA). The fact that plants growing in rocky outcrops and plants from the Atlantic rainforest showed the largest morphological differentiation among themselves, indicates that phenotypes are strongly influenced by the habitat. Because plants collected from both habitats, maintained under cultivation for at least 20 years maintained their differentiation, such differentiation may have a genetic component. Because individuals collected in such environments present the largest morphological dimensions, new habitats generated by human activity, such as highway margins, seem to be quite favorable for these plants.

With about 1125 species (Chase et al. 2003), *Epidendrum* L. is the largest neotropical orchid genus. It presents many taxonomical problems, resulting mostly from its size, combined with the great variability of some of its species (Dressler 1967, 1984, Brieger 1976–1977, Hágsater 1984, Withner and Harding 2004).

Epidendrum secundum is one of the most variable species of the genus. This species belongs to the section Amphyglottidae, formerly recognized by some authors (Lindley 1852–1859, Pabst and Dungs 1975, Brieger 1976–1977, Hágsater 1984) as a group of highly polymorphic species, and is widely distributed in South America. The complexity of this group is reflected in the large number of names associated to it, sometimes treated as synonyms of E. secundum Jacq., sometimes as distinct species (E. elongatum Jacq., E. crassifolium Lindl., E. ellipticum Grah., E. ansiferum Rchb. f., E. versicolor Hoehne & Schltr., E. xanthinum Lindl., among others).

Epidendrum secundum is essentially characterized by being a caespitose plant, without pseudobulbs, with an elongated stalk bearing coriaceous and distichous leaves, and by inflorescences in densely flowered simple corymbs, with a variable number of small flowers (ca 2.0 cm in diameter), commonly lilac, with a 3-lobed lip with laceratedentate margin, and a complex callus in its center.

This work follows the assertion by Hágsater (1993) who considered *Epidendrum secundum* the oldest name for this group, and the delimitation of Vasquez and Ibisch (2004), that joined all the morphological and color forms into a single polymorphic species, like other authors (Dunsterville

and Garay 1961, Dunsterville 1979, Dressler 1989), based only on morphological characters. We follow the traditional taxonomical concept of species, mainly based on morphological discontinuities, because no other criteria to delimit species can be used in this group. Different kinds of biological information, employed by other species delimitation criteria, like reproductive biology and population genetics (Borba et al. 2002, Moccia et al. 2007), are not available for this species.

Apparently, no morphological discontinuities are present in this species, but the authors that studied the group did not employ objective methods or sample strategies that focused on identifying these discontinuities among specimens from between and within populations. Studies on the influence of habitat type on the morphological variation of the species are also absent. *Epidendrum secundum* can be found in numerous habitat types, like the Andean Cordillera, the central highlands from Brazil, the coastal Atlantic rainforest, dry inselbergs from Caatinga vegetation and the Venezuelan Tepuis. Even in populations located very close to each other, a few kilometers apart, the morphological variation both among populations and within populations is very high (Fig. 1).

Numerical methods are important tools in studies of taxonomy (Reinhammar 1995, Marhold 1996, Palestina and Sosa 2002, Goldman et al. 2004), mainly to evaluate morphological variation. Their advantage is the objectivity in the way they deal with the data, especially when many variables are involved (Manly 1994).

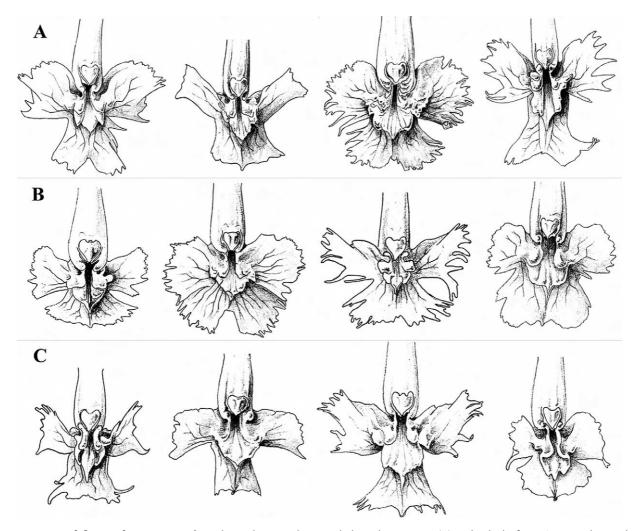


Fig. 1. Lips of flowers from *E. secundum* plants showing the morphological variation. (A) individuals from Santana do Riacho (population 3), (B) individuals from Cananéia (population 18), (C) individuals from São Bernardo do Campo (population 13). See Table 1 and Fig. 2 for provenance details.

Within Orchidaceae, multivariate methods have previously been applied as taxonomic tools on various groups and in various ways, in order to: (a) delimit taxa (Dufréne et al. 1991, Tyteca and Dufréne 1994, Reinhammar 1995, Tyteca 1995, Tyteca and Gerbaud 1998, Carlini-Garcia et al. 2002), (b) help recognize the hybrid origin of some taxa (Kallunki 1976, Du Puy et al. 1985, Catling and Catling 1997, Knyasev et al. 2000), (c) evaluate the morphological variability within populations (Shaw 1998, Cardim et al. 2001, Pedersen 2004), or (d) combine all these different approaches (Tyteca and Gathoye 1993). Multivariate methods frequently make it possible to recognize important morphological discontinuities and to enhance the selection of diagnostic characters (Tyteca and Dufréne 1994, Reinhammar 1995, Palestina and Sosa 2002). Nevertheless, in some instances, the use of morphometrics reveal gradients without clear morphological discontinuities, which can be explained as clinal variation (Kephart et al. 1999, Fritsch and Lucas 2000, Sapir et al. 2002).

This work aimed at analyzing the morphological variability within and among populations of individuals of the *E. secundum* complex by means of multivariate

analysis. We also aimed to evaluate the influence of different habitats on plant morphology, by comparing plants under cultivation and plants collected directly from the field.

Material and methods

A total of 226 living plants from 18 southeastern Brazilian populations were studied (Table 1, Fig. 2). One hundred and forty six individuals were sampled directly from the field in populations 11, 13 and 14 (Table 1, Fig. 2). Eighty specimens, collected in the populations listed in Table 1, have been cultivated for at least 20 years at the orchid collection of the Botanical Inst. of São Paulo (Brazil). Populations 6–13 and 15–18 grow surrounded by small trees and shrubs, at high elevations in the Atlantic rainforest mountains (Fig. 2). Population 13 grows in a disturbed habitat; a roadside cut. Populations 1–5 and 14 grow on rocky outcrops in both the central Brazilian plateau and the Atlantic rainforest (Fig. 2).

Twenty continuous morphological characters were measured (Table 2), of which three characters were vegetative

Table 1. The studied populations of *E. secundum*, together with the number of individuals (n) used in morphometric analysis and vouchers housed at SP. * denotes plants from Atibaia, São Bernardo do Campo and Salesópolis collected directly from the field in natural populations. The remaining populations were collected in natural populations. but maintained under cultivation for at least 20 years at the living collection from the Institute of Botany of São Paulo. Brazil. MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo states.

Population	n	Voucher
1. Pedra Azul – MG	1	Brólio and Silva s.n. (SP365921)
2. Diamantina – MG	2	Brólio and Silva s.n. (SP365931)
3. Santana do	4	Barros s.n. (SP365928)
Riacho – MG		
4. Caeté – MG	1	Bicalho s.n. (SP365896)
5. Mariana – MG	2	Brólio et al. s.n. (SP365904)
6. Miradouro – MG	4	Bicalho s.n. (SP365903)
7. Itutinga – MG	2	Brólio and Silva s.n. (SP365920)
8. Baependi – MG	3	Bicalho s.n. (SP365937)
9. Parati – RJ	14	Targa s.n. (SP365907)
10. São José dos	6	Ribeiro s.n. (SP365897)
Campos – SP		
11. Salesópolis – SP	50*	Pinheiro and Inês 214
12. Bertioga – SP	7	Bicalho s.n. (SP365912)
13. São Bernardo do	32*	Pinheiro and Inês 226
Campo – SP		
14. Atibaia – SP	64*	Pinheiro and Inês 202
15. Anhembi – SP	5	Bicalho and Kuhlmann s.n. (SP365932)
16. Mira-Estrela – SP	5	Kuhlmann s.n. (SP365895)
17. Apiaí – SP	11	Silva and Brólio s.n. (SP365902)
18. Cananéia – SP	13	Barros s.n. (SP365910)
Total	226	. ,

and 17 characters were floral (Fig. 3). Measures were taken at the point of the greatest dimension for each of the characters. Vouchers are deposited at the herbarium SP (Table 1).

The data were analysed with Systat (Wilkinson 2000) to provide the mean, standard deviation and 5 and 95 percentiles of each character. Multivariate analysis were carried out using the programs Systat (Wilkinson 2000) and Fitopac (Shepherd 1994). Two types of ordination analysis

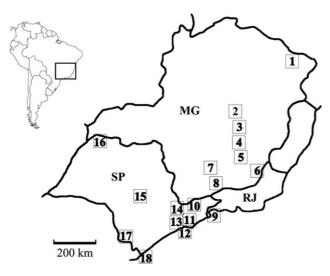


Fig. 2. Map with the localities of the populations studied in southeastern Brazil. See Table 1 for locality codes.

were employed to investigate possible distortions produced by a specific method (Everitt 1978); principal component analysis (PCA) was used to summarize variation when a priori knowledge of a population to which individuals belonged was not considered, and canonical variate analysis (CVA) was applied to ordinate pre-defined populations in a way that accounted for variance and covariance among characters within and among populations. In the PCA, the number of informative axes was determined by comparing eigenvalues with the random expectation in a broken-stick distribution (Frontier 1976). The CVA was based on the following groups: the three populations collected directly from the field (Atibaia, São Bernardo do Campo and Salesópolis) and all plants under cultivation (data pooled over populations). Discriminant analysis (DA) was performed with the same grouping variables as were used in the CVA. Wilks' lambda and jack-knife classifications, which assign unclassified specimens to groups, and provide F-toremove statistics, which give an indication of the relative importance of the variables used in the model, were also calculated.

Results

The results of the descriptive analyses show that the mean values of characters from cultivated plants and from individuals collected in a disturbed habitat at São Bernardo do Campo are larger than the values observed in the Atibaia and Salesópolis populations (Table 3). Although the cultivation conditions of the plants at the orchid collection of the Botanical Institute are homogenous, the standard deviations observed for these plants are high, revealing a great variability of all characters, as is also the case for the Salesópolis and São Bernardo do Campo populations (Table 3). The smallest values were observed in the Atibaia population (Table 3).

In the PCA, only the first two axes were considered as informative. Individuals under cultivation and those collected in São Bernardo do Campo and Salesópolis were mixed in the analysis, and occupied different regions of the scatter-plot (Fig. 4). Only the specimens from Atibaia showed a tendency to form a separate cluster. Many individuals collected in rocky outcrops and maintained under cultivation (populations 1–5) were grouped together with plants from Atibaia. Floral characters showed a high correlation with the first axis (Fig. 4, Table 2), and the five most important characters to define this axis were PT–L, LA–W, LA–L, CA–L and LS–L. Vegetative characters and IN–L were also correlated, but in a different direction, and did not define any relevant pattern in the scatter-plot (Fig. 4).

The first canonical axis in the CVA separates the Atibaia population from plants under cultivation which, together with the Salesópolis and São Bernardo do Campo populations, form a mixed group (Fig. 5), the same pattern as that observed in the PCA (Fig. 4). The five most important characters for the first axis were CO–L, LL–L, CL–W, LS–W and LA–W (Table 2). Individuals from São Bernardo do Campo showed a tendency to form a separate cluster along the second canonical axis (Fig. 5), and the five most

Table 2. Morphological characters used in morphometric analyses of *E. secundum* and results of the PCA (PC1 and PC2). the CVA (CN1 and CN2) and discriminant analysis (F-to-remove). PC1 and PC2: correlations between the original variables and principal components one and two. respectively. CN1 and CN2: correlations between the original variables and canonical discriminant axes one and two. respectively. F-to-remove: relative importance of the variables used in the model to discriminate groups in discriminant analysis (Table 4).

Characters	Abbreviation	PC1	PC2	CN1	CN2	F-to-remove
1. Leaf length	LE-L	0.506	-0.650	0.334	0.716	15.72
2. Leaf width	LE–W	0.253	-0.661	-0.424	-0.138	6.23
3. Stem length	ST–L	0.365	-0.642	0.037	-0.042	1.29
4. Inflorescence length	IN–L	0.521	-0.595	0.303	0.177	4.06
5. Pedicel length	PE–L	0.827	0.012	0.231	-0.752	14.27
6. Dorsal sepal length	DS–L	0.890	0.175	0.043	-0.226	0.73
7. Dorsal sepal width	DS–W	0.813	-0.022	0.143	0.361	3.22
8. Lateral sepal length	LS–L	0.855	0.158	-0.120	-0.253	0.77
9. Lateral sepal width	LS–W	0.843	0.026	-0.201	0.099	0.86
10. Petal length	PT–L	0.904	0.158	-0.130	0.152	0.59
11. Petal width	PT–W	0.802	0.015	-0.096	-0.245	9.28
12. Lip length	LA–L	0.884	0.191	0.108	-0.096	0.34
13. Lip width	LA–W	0.896	0.077	-0.480	0.026	3.71
14. Column length	CO-L	0.766	-0.127	0.458	0.393	9.08
15. Lateral lobe of lip length	LL–L	0.762	-0.010	0.425	0.178	4.49
16. Lateral lobe of lip width	LL–W	0.721	0.040	0.099	-0.359	3.69
17. Central lobe of lip length	CL–L	0.808	0.102	-0.103	0.086	1.58
18. Central lobe of lip width	CL–W	0.816	0.110	0.577	-0.101	11.04
19. Callus of lip length	CA-L	0.869	0.136	0.178	0.225	2.30
20. Callus of lip width	CA-W	0.722	0.176	0.132	-0.232	2.40

important characters on this axis were DS-W, CO-L, LL-W, PT-W and CA-L.

The Atibaia population showed the highest percentage of correct classification in the jack-knife procedure (Table 4). The five most important characters in the classification function, according to the F-to-remove values, were LE–L, PE–L, CL–W, PT–W and CO–L (Table 2).

Discussion

The PCA, the CVA, and the DA generated very similar results (Fig. 4, 5, Table 4). The Atibaia population, which grows on rocky outcrops, is separated from the other populations in all the analyses. Therefore, the habitat type

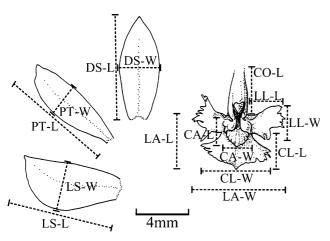


Fig. 3. Outline of flower segments and quantitative floral characters surveyed in this study (see Table 2 for character codes). Scale bar $=4~\mathrm{mm}$.

may be involved in this morphological differentiation from the other populations, because the Atibaia population in addition to populations 1–5 are rupiculous and more exposed to the sun, while individuals from the Atlantic rainforest populations grow on humus-rich soil, in a more shaded environment surrounded by small trees and shrubs (Fig. 4, 5, Table 4). This pattern may be related to a general size difference in floral characters, where the smallest values are those of the Atibaia population (Table 3), rather than a difference in shape: all the floral characters showed a strong correlation with only one axis, both in the PCA and in the CVA.

In the PCA, the CVA and in the jack-knife classification matrix (Table 2, 4), the floral characters were the most important to define the observed patterns, except for character LE-L, which was the most important in the jack-knife classification matrix. LA-W, CO-L, and CL-W appeared among the five most important characters in at least two of the three analyses performed (Table 2, 4). These characters are localized on the lip (Fig. 3), a structure traditionally used to delimit the species of the E. secundum complex (Pabst and Dungs 1975, Brieger 1976-1977, Sastre 1990a, 1990b), and are mainly qualitative, reflecting shape of the lip callus and the type of lip margins (denticulate, crenate, fimbriate, erose, etc.). Because these qualitative characters showed some relation with the groups obtained, displaying a high variation between populations and within a single population (Fig. 1), they must be used with caution as diagnostic characters in the taxonomy of the group.

Several authors (Dunsterville 1979, Dressler 1989, Hágsater and Arenas 2005) have already noted the ability of individuals of *E. secundum* to colonize new kinds of environments. Their commonly ruderal habit can have several implications for the group's evolution, as hybridization (Carson and Templeton 1984, Rieseberg 1997) and

Table 3. Summary of descriptive statistics for quantitative morphological characters measured on 226 individuals from E. secundum. (5 percentile-) mean±standard deviation (—95 percentile). CH= characters (see Táble 2).

Atibaia population	(59.44 -) 62.88 ± 13.74 (-66.31) (21,74 -) 23.03 ± 5.16 (-24.32) (279.27 -) 317.03 ± 151.18 (-354.80) (246.74 -) 267.81 ± 84.36 (-288.88) (15.38 -) 15.98 ± 2.43 (-16.59) (8.08 -) 8.26 ± 0.75 (-8.45) (3.20 -) 3.31 ± 0.41 (-3.41) (8.47 -) 8.64 ± 0.77 (-8.82) (3.75 -) 8.44 ± 0.77 (-3.93) (8.22 -) 8.40 ± 0.73 (-3.93) (8.22 -) 8.40 ± 0.73 (-3.93) (4.04 -) 4.17 ± 0.53 (-4.30) (7.65 -) 7.87 ± 0.87 (-8.08) (4.59 -) 4.68 ± 0.38 (-4.77) (2.30 -) 2.39 ± 0.36 (-2.48) (3.17 -) 3.30 ± 0.53 (-3.43) (2.66 -) 2.77 ± 0.43 (-2.87) (2.96 -) 3.05 ± 0.37 (-3.14) (2.90 -) 3.00 ± 0.42 (-3.11)
Salesópolis population	(78.32 -) 83.64 ±18.72 (-88.96) (22.13 -) 24.10 ±6.94 (-26.07) (325.84 -) 405.00 ±278.55 (-484.16) (354.04 -) 408.80 ±192.67 (-463.56) (21.38 -) 22.29 ±3.19 (-23.19) (9.97 -) 10.23 ±0.90 (-10.48) (3.72 -) 3.86 ±0.47 (-3.99) (10.33 -) 10.60 ±0.96 (-10.87) (4.29 -) 4.42 ±0.45 (-4.55) (10.15 -) 10.41 ±0.93 (-10.68) (3.56 -) 3.73 ±0.60 (-3.90) (5.15 -) 5.33 ±0.62 (-5.50) (9.21 -) 9.53 ±1.11 (-9.84) (5.48 -) 5.64 ±0.54 (-5.79) (3.30 -) 3.41 ±0.83 (-4.38) (3.30 -) 3.41 ±0.83 (-4.38) (3.38 -) 4.01 ±0.51 (-4.15) (3.28 -) 3.42 ±0.50 (-3.56)
São Bernardo do Campo population	(100.70 –) 109.47 ± 24.31 (-118.23) (23.55 –) 25.53 ± 5.49 (-27.51) (530.00 –) 598.13 ± 188.94 (-666.25) (492.67 –) 553.13 ± 167.67 (-613.58) (19.42 –) 20.66 ± 3.44 (-21.90) (9.35 –) 9.81 ± 1.30 (-10.28) (3.82 –) 3.98 ± 0.44 (-4.14) (9.78 –) 10.23 ± 1.25 (-10.68) (4.30 –) 4.74 ± 0.48 (-4.65) (9.75 –) 10.19 ± 1.24 (-10.64) (3.39 –) 3.60 ± 0.57 (-3.80) (4.87 –) 5.17 ± 0.83 (-5.47) (9.33 –) 9.75 ± 1.19 (-10.18) (5.76 –) 5.96 ± 0.55 (-6.15) (3.06 –) 3.25 ± 0.51 (-3.43) (3.37 –) 3.54 ± 0.62 (-3.76) (5.96 –) 6.32 ± 1.00 (-6.69) (3.71 –) 3.93 ± 0.61 (-4.15)
Plants under cultivation	(77.34 -) 80.60±14.66 (-83.86) (22.33 -) 23.39±4.75 (-24.44) (428.99 -) 465.44±163.80 (-501.89) (393.16 -) 23.88±3.66 (-24.69) (23.06 -) 23.88±3.66 (-24.69) (9.99 -) 10.24±1.09 (-10.48) (3.97 -) 4.07±0.43 (-4.16) (10.42 -) 10.71±1.29 (-10.99) (4.52 -) 10.50±1.16 (-10.76) (3.63 -) 5.51±0.70 (-5.67) (5.36 -) 5.51±0.70 (-5.67) (5.36 -) 5.51±0.70 (-5.67) (5.46 -) 5.59±0.62 (-5.73) (3.04 -) 3.17±0.58 (-3.30) (4.41 -) 4.61±0.88 (-4.80) (6.59 -) 6.83±1.04 (-7.06) (6.59 -) 6.83±1.04 (-7.06) (4.01 -) 4.14±0.56 (-4.26) (3.88 -) 4.03±0.69 (-4.19)
СН	

polyploidy (Levin 2001) often occur in such habitats. A significant morphological variation was observed by Shaw (1998) in individuals from different species of *Dacthylorhiza* (Orchidaceae) in industrial waste sites in England, with a high occurrence of hybridization. The differentiation of the individuals collected in the population of São Bernardo do Campo, in a highway margin (Fig. 5, Table 4), provides a circumstantial evidence of the importance of disturbed environments in the differentiation of populations of *E. secundum*. Highways can act as corridors that geographically connect isolated species, allowing hybridization between *E. secundum* and allied species (Pansarin pers. comm.).

Most characters of plants under cultivation are larger than those observed in specimens collected directly from the field (Table 3), as observed in species of Cerastium L. (Caryophyllaceae) by Brysting and Elven (2000), and in species of Pleurothallis R. Br. (Orchidaceae) by Borba et al. (2002). This could be the result of suitable growth conditions resulting from constant watering and fertilization. However, the morphological variation observed in the specimens under cultivation was as high as that observed in the specimens collected directly from the field (Table 3, Fig. 4, 5), indicating that the morphological variation has a genetic component in addition to an environmental one. The comparison of cultivated plants with individuals collected directly from the field was important in the study of Brysting and Elven (2000), since they were able to evaluate which characters that were more influenced by the environment, and which had a strong genotypic component. In some studies, all analyzed individuals were previously cultivated (Loos 1993, Borba et al. 2002, Goldman et al. 2004) to suppress the environmental influence on the characters. In this study, all measures taken from cultivated plants showed a variation similar to that of individuals collected directly from the field (Table 3), therefore, it was not possible to evaluate which characters that are more influenced by the environment. The morphological similarity between specimens from the Atibaia population and the individuals originating from populations 1-5 (and maintained under cultivation) can also be related to a genetic component. Molecular data used together with morphometric data have proved to be important tools to delimit species and/ or lineages in plants displaying high morphological variation (Kjaer et al. 2004, Pedersen 2004, Bernardos et al. 2005).

Because this study was a first attempt to understand the morphological relationships among populations of *E. secundum*, more questions than answers were raised. To date, it is not possible to judge if the morphological variation observed is sufficient to circumscribe more than one species in this sample, as the variation between populations may be due to phenotypic plasticity related to environmental conditions. The taxonomical doubts on how many species and/or lineages that do exist, due to the influence of habitat type on the morphological variation between populations, could be tested if more populations from rocky outcrops and the Atlantic rainforest are sampled, and if other methods like molecular markers (e.g. isozymes or microsatellites) are employed, to reveal patterns of genetic variability.

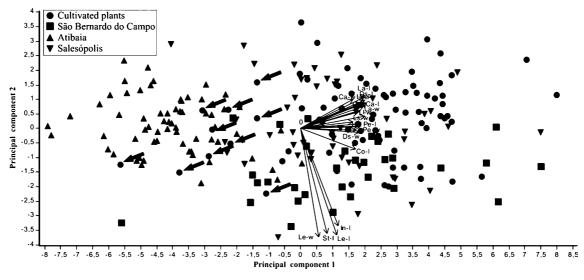


Fig. 4. PCA of 226 specimens from *E. secundum* based on 20 characters (Table 2). Specimens from populations 1–5 are indicated by arrows. Principal component (PC) 1 and 2 explain 58.1% and 9.2% of the total variation respectively.

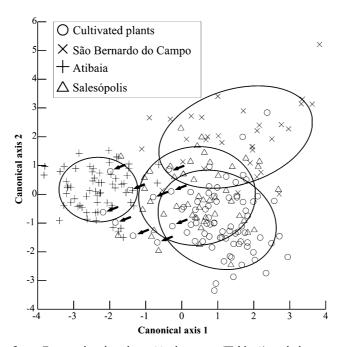


Fig. 5. CVA of 226 specimens from *E. secundum* based on 20 characters (Table 2) and the groups 'Cultivated plants'. 'Atibaia'. 'Salesópolis'. and 'São Bernardo do Campo' populations. Individuals from populations 1–5 are indicated by arrows. Axes 1 and 2 explain 63.5% and 24.3% of the total variation. respectively. The ellipses are centered on the sample means and comprise 70% of the sample from each group.

Table 4. Results of jack-knife classification analysis with plants under cultivation and natural populations from São Bernardo do Campo, Atibaia and Salesópolis as groups. Wilks' lambda =0.1147. p =0.00001.

Grouping variables	Cultivated plants	São Bernardo do Campo	Atibaia	Salesópolis	Percentage correct
Cultivated plants	54	6	8	12	68
São Bernardo do Campo	3	23	2	4	72
Atibaia	0	0	63	1	98
Salesópolis	11	7	4	28	56
Total	68	36	77	45	74

Acknowledgements – Thanks to the staff of the "State Orchidarium Section" of the Inst. of Botany, São Paulo, Brazil, for their technical support. Thanks also to Dr George J. Shepherd for his valuable comments on the manuscript. This work was supported by a grant from "Fundação de Amparo à Pesquisa do Estado de São Paulo" (Fapesp no. 00/07814–3) to the first author. The second author acknowledges the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq grant no. 303962/2004–6) for the grant received.

References

- Bernardos, S. et al. 2005. The section *Pseudophrys (Ophrys, Orchidaceae)* in the Iberian Peninsula: a morphological and molecular analysis. Bot. J. Linn. Soc. 148: 359–375.
- Borba, E. L. et al. 2002. Floral and vegetative morphometrics of five Pleurothallis (Orchidaceae) species: correlation with taxonomy, phylogeny, genetic variability and pollination systems. – Ann. Bot. 90: 219–230.
- Brieger, F. G. 1976–1977. Gattungsreihe Epidendra. In: Brieger, F. G. et al. (eds), Schlechter die Orchideen. Vol. 3. Paul Parey, Berlin, pp. 509–549.
- Brysting, A. K. and Elven, R. 2000. The *Cerastium alpinum–C. articum* complex (Caryophyllaceae): numerical analyses of morphological variation and a taxonomic revision of C. articum Lange s.l. Taxon 49: 189–216.
- Cardim, D. C. et al. 2001. Variabilidade intra-específica em cinco populações de *Oncidium varicosum* Lindl. (Orchidaceae– Oncidiinae) em Minas Gerais. – Rev. Bras. Bot. Suppl. 24: 553–560.
- Carlini-Garcia, L. A. et al. 2002. A morphometric analysis of floral characters in *Miltonia spectabilis* and *Miltonia spectabilis var.* moreliana (Maxillarieae: Oncidiinae). – Lindleyana 17: 122– 129
- Carson, H. L. and Templeton, A. R. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. – Annu. Rev. Ecol. Syst. 15: 97–131.
- Catling, P. M. and Catling, V. R. 1997. Morphological discrimination of *Platanthera huronensis* in the Canadian rocky mountains. – Lindleyana 12: 72–78.
- Chase, M. W. et al. 2003. DNA data and Orchidaceae systematics: a new phylogenetic classification. In: K. W. Dixon et al. (eds), Orchid conservation. Natural History Publications, Sabah, pp. 69–89.
- Dressler, R. L. 1967. The genera *Amblostoma*, *Lanium* and *Stenoglossum*. Brittonia 19: 237–243.
- Dressler, R. L. 1984. La delimitacion de géneros en el complejo Epidendrum. – Orquidea, Mex. 9: 277–298.
- Dressler, R. L. 1989. Will the real *Epidendrum ibaguense* please stand up? Bull. Am. Orch. Soc. 58: 796–800.
- Dufréne, M. et al. 1991. Biostatistical studies on western
 European *Dactylorhiza* (Orchidaceae the *D. maculata* group.
 Plant Syst. Evol. 175: 55–72.
- Dunsterville, G. C. K. 1979. Orchids of Venezuela *Epidendrum elongatum.* Bull. Am. Orch. Soc. 48: 447–454.
- Dunsterville, G. C. K. and Garay, L. A. 1961. Venezuelan orchids illustrated. Vol. 2. – Andre Deutsch, London.
- Du Puy, D. J. et al. 1985. A numerical taxonomic analysis of Cymbidium section Iridorchis (Orchidaceae). – Kew Bull. 40: 421–434.
- Everitt, B. S. 1978. Graphical techniques for multivariate data.
 North-Holland.
- Fritsch, P. W. and Lucas, S. D. 2000. Clinal variation in the *Halesia carolina* complex (Styracaceae). Syst. Bot. 25: 197–210.

- Frontier, S. 1976. Study of the decrease of eigenvalues in principal component analysis: comparison with the broken stick model.

 J. Exp. Mar. Biol. Ecol. 25: 67–75.
- Goldman, D. H. et al. 2004. Morphometric circumscription of species and infraspecific taxa in *Calopogon* R. Br. (Orchidaceae). – Plant Syst. Evol. 247: 37–60.
- Hágsater, E. 1984. Towards an understanding of the genus Epidendrum. – In: K. W. Tan (ed.), Proc. 11th World Orchid Conf., Miami, pp. 195–201.
- Hágsater, E. 1993. Epidendrum anceps or Epidendrum secundum? Orquidea, Mex. 13: 153–158.
- Hágsater E. and Arenas M. A. S. 2005. Epidendrum L. In: Pridgeon, A. M. et al. (eds), Genera Orchidacearum. Vol. 4. Oxford Univ. Press, pp. 236–251.
- Kallunki, J. 1976. Population studies in *Goodyera* (Orchidaceae) with emphasis in the hybrid origin of *G. tesselata*. Brittonia 28: 53–75.
- Kephart, S. et al. 1999. Varietal relationships in *Silene douglasii* (Caryophyllaceae): morphological variability at the population level. – Syst. Bot. 24: 529–544.
- Kjaer, A. et al. 2004. Investigation of genetic and morphological variation in the Sago Palm (*Metroxylon sagu*; Arecaceae) in Papua New Guinea. – Ann. Bot. 94: 109–117.
- Knyasev, M. S. et al. 2000. Interspecific hybridization in northern Eurasian *Cypripedium*: morphometric and genetic evidence of hybrid origin of *C. ventricosum*. – Lindleyana 15: 10–20.
- Levin, D. A. 2001. The recurrent origin of plant races and species.

 Syst. Bot. 26: 197–204.
- Lindley, J. 1852–1859. Epidendrum. In: Lindley, J. (ed.), Folia Orchidacea. J. Matthews, London, pp. 1–97.
- Loos, B. P. 1993. Morphological variation in *Lolium* (Poaceae) as a measure of species relationships. – Plant Syst. Evol. 188: 87– 99.
- Manly, B. F. J. 1994. Multivariate statistical methods, a primer. Chapman and Hall.
- Marhold, K. 1996. Multivariate morphometric study of the Cardamine pratensis group (Cruciferae) in the Carpathian and Pannonian area. – Plant Syst. Evol. 200: 141–159.
- Moccia, M. D. et al. 2007. The strength of reproductive isolation in two hybridizing food-deceptive orchid species. Mol. Ecol. 16: 2855–2866.
- Pabst, G. F. J and Dungs, F. 1975. Orchidaceae Brasilienses. Vol.1. Kurt Schmersow, Hildesheim.
- Palestina, R. A. and Sosa, V. 2002. Morphological variation in populations of *Bletia purpurea* (Orchidaceae) and description of the new species *B. riparia*. – Brittonia 54: 99–111.
- Pedersen, H. E. 2004. *Dactylorhiza majalis* s.l. (Orchidaceae) in acid habitats: variation patterns, taxonomy, and evolution. Nord. J. Bot. 22: 641–658.
- Reinhammar, L. 1995. Evidence for two distinctive species of Pseudorchis (Orchidaceae) in Scandinavia. – Nord. J. Bot. 15: 469–481.
- Rieseberg, L. H. 1997. Hybrid origins of plant species. Annu. Rev. Ecol. Evol. Syst. 28: 359–389.
- Sapir, Y. et al. 2002. Morphological variation of the *Oncocyclus* irises (*Iris*: Iridaceae) in the southern Levant. Bot. J. Linn. Soc. 139: 369–382.
- Sastre, C. 1990a. *Epidendrum* bambusiformes de Guadeloupe et de Martinique 1. L' Orchidophile 93: 149–158.
- Sastre, C. 1990b. *Epidendrum* bambusiformes de Guadeloupe et de Martinique 2. L' Orchidophile 94: 197–203.
- Shaw, P. J. A. 1998. Morphometric analyses of mixed *Dactylorhiza* colonies (Orchidaceae) on industrial waste sites in England. Bot. J. Linn. Soc. 128: 385–401.
- Shepherd, G. J. 1994. FITOPAC 1: Manual de usuário. Depto de Botânica, Univ. Estadual de Campinas, Brazil.

- Tyteca, D. 1995. Multivariate analyses of western European allogamous populations of *Epipactis helleborine* (L.) Crantz s.l., with special emphasis on *Epipactis tremolsii* Pau in southeastern France. Ber. Arbeitskrs. Heim. Orchideen. 12: 4–49.
- Tyteca, D. and Dufréne, M. 1994. Biostatistical studies of western European allogamous populations of the *Epipactis helleborine* (L.) Crantz species group (Orchidaceae). Syst. Bot. 19: 424–442.
- Tyteca, D. and Gathoye, J.-L. 1993. On the morphological variability of *Dactylorhiza praetermissa* (Druce) Soó (Orchidaceae). Belg. J. Bot. 126: 81–99.
- Tyteca, D. and Gerbaud, O. 1998. Nouvelles observations sur *Dactylorhiza lapponica* (Laest. ex Hartman) Soó en France. L. Orchidophile 131: 60–65.
- Vasquez, R. and Ibisch, P. L. 2004. Orquídeas de Bolívia. Vol. 2: Subtribus Laeliinae, Polystachinae, Sobraliinae. – Editorial FAN, Santa Cruz de la Sierra.
- Wilkinson, L. 2000. Systat Statistics ver. 10.0 for Windows. SPSS, Chicago.
- Withner, C. L. and Harding, P. A. 2004. The Cattleyas and their relatives. Vol. 7: the debatable Epidendrums. – Timber Press, Portland.