

Morphometric analysis of the *Brasiliorchis picta* complex (Orchidaceae)

FÁBIO PINHEIRO¹ and FÁBIO DE BARROS^{1,2}

(received: February 08, 2007; accepted: November 06, 2008)

ABSTRACT – (Morphometric analysis of the *Brasiliorchis picta* complex (Orchidaceae)). One of the largest genera of Orchidaceae in the Neotropics with about 450 species, *Maxillaria* presents several taxonomic uncertainties about its generic circumscription and the delimitation of species groups, mainly due to the large variability of some species. The present study aims at verifying the morphological variation and species delimitation in the *Brasiliorchis picta* complex, a recent new genus derived from *Maxillaria*, using morphometric multivariate analysis. A total of 340 specimens belonging to six species (*B. chrysantha* (Barb. Rodr.) R.B. Singer, S. Koehler & Carnevali, *B. gracilis* (Lodd.) R.B. Singer, S. Koehler & Carnevali, *B. marginata* (Lindl.) R.B. Singer, S. Koehler & Carnevali, *B. picta* (Hook.) R. Singer, S. Koehler & Carnevali, *B. porphyrostele* (Rchb. f.) R.B. Singer, S. Koehler & Carnevali and *B. ubatubana* (Hoehne) R.B. Singer, S. Koehler & Carnevali) were analyzed using multivariate methods (PCA, CVA, DA, and Cluster Analysis with UPGMA). *B. gracilis* shows the largest morphological discontinuity, mainly due to its smaller size. The other species tend to form distinct groups, but intermediate characteristics between pairs of species induce overlaps among the individuals of different species and thus confuse the distinction of each one. Hybridization and geographic distribution can be involved in the differentiation of the species and lineages in this complex. Because the species classified a priori in this work cannot be recognized by the quantitative characters measured here, such other tools as geometric morphometry and molecular data should be employed in future works to clarify species relationships in this complex.

Key words - morphometry, multivariate analysis, species complex, taxonomy

RESUMO – (Análise morfométrica do complexo *Brasiliorchis picta* (Orchidaceae)). *Maxillaria* é um dos maiores gêneros da região Neotropical com cerca de 450 espécies, apresentando diversas incertezas taxonômicas quanto à delimitação do gênero e de diversas espécies, principalmente pela grande variação morfológica que o grupo apresenta. Este trabalho tem por objetivo analisar a variação morfológica e a delimitação de algumas espécies no complexo *Brasiliorchis picta*, um gênero criado recentemente a partir de algumas espécies de *Maxillaria*, utilizando análise multivariada morfométrica. Foram analisados 340 indivíduos, distribuídos em seis espécies (*B. chrysantha* (Barb. Rodr.) R.B. Singer, S. Koehler & Carnevali, *B. gracilis* (Lodd.) R.B. Singer, S. Koehler & Carnevali, *B. marginata* (Lindl.) R.B. Singer, S. Koehler & Carnevali, *B. picta* (Hook.) R. Singer, S. Koehler & Carnevali, *B. porphyrostele* (Rchb. f.) R.B. Singer, S. Koehler & Carnevali e *B. ubatubana* (Hoehne) R.B. Singer, S. Koehler & Carnevali), empregando-se métodos de análise multivariada (PCA, CVA, DA e UPGMA). *Brasiliorchis gracilis* exibiu as maiores descontinuidades morfológicas, relacionadas principalmente ao seu porte reduzido. Entre as demais espécies, é possível notar apenas tendências de agrupamento, nas quais um grande número de caracteres com valores intermediários gera grande sobreposição entre indivíduos de diferentes espécies, não permitindo uma clara separação entre as espécies. Os padrões de distribuição geográfica e eventos como hibridização podem estar envolvidos na diferenciação de espécies e linhagens no complexo. Como as espécies classificadas a priori neste trabalho não podem ser reconhecidas através dos caracteres quantitativos utilizados, outras técnicas como morfometria geométrica e dados moleculares devem ser empregados em trabalhos futuros para esclarecer as relações de parentesco entre as espécies deste complexo.

Palavras-chave - análise multivariada, complexo de espécies, morfometria, taxonomia

Introduction

Maxillaria Ruiz & Pavón s.l. belongs to the subfamily Epidendroideae, tribe Maxillarieae, subtribe Maxillariinae (Dressler 1993). It is the largest genus of this subtribe, with about 450 species (Atwood & Mora-de-Retana 1999) distributed only in the Neotropics. According to Pabst & Dungs (1977), 94 species of the genus occur in Brazil. Because of the large morphological diversity of this genus,

its delimitation has become a controversial issue: Cogniaux (1904-1906), Hoehne (1953) and Butzin & Senghas (1996) consider *Camaridium* Lindl., *Marsupiaria* Hoehne, *Ornithidium* Salisb., and *Pseudomaxillaria* Hoehne as valid genera, segregated from *Maxillaria* mainly due to the structural variation of their vegetative characters. Conversely, Dunsterville & Garay (1961), Pabst & Dungs (1977) and Atwood & Mora-de-Retana (1999) place these genera within *Maxillaria*, possibly because they consider that the large variability of their vegetative structures is due to the wide geographic distribution of the group, which can be found from Florida to Argentina, as epiphytes or rupicolous (Holtzmeier *et al.* 1998).

1. Instituto de Botânica, Caixa Postal 3005, 01061-970 São Paulo, SP, Brazil.
2. Corresponding author: fdebarros@terra.com.br

Anatomical data revealed that *Maxillaria* is polyphyletic if *Mormolyca* Fenzl. is considered (Holtzmeier *et al.* 1998). Dathe & Dietrich (2006) and Whitten *et al.* (2007), whose sampling included different species of *Maxillaria* and such closely related genera as *Camaridium* Lindl., *Ornithidium* Saliob. ex R. Br., *Trigonidium* Lindl., *Chrysocycnis* Linden & Rchb.f., *Cryptocentrum* Benth., and *Mormolyca*, obtained a phylogeny based on molecular data and their results point out that *Maxillaria* is paraphyletic if these genera are maintained. Those studies supports a *Maxillaria* genus with a more restricted delimitation, and the recognition of some allied genera, many of them recently created based on phylogenetic results, such as *Brasiliorchis* R. Singer, S. Koehler & Carnevali (Singer *et al.* 2007), *Christensonella* Szlach., Mytnik, Górniak & Smiszek (Szlachetko *et al.* 2006) and *Sauvetrea* Szlach. (Szlachetko & Smiszek 2007). Taxonomic problems are common in the delimitation of species in *Maxillaria* s.l., as shown in the works by Illg (1977) on the section *Heterotaxis*, Onishi (1974) on the *Maxillaria madida* complex and Carnevali *et al.* (2001) on the *Maxillaria rufescens* complex.

The species of *Maxillaria* were divided in to 11 groups in the taxonomic treatment of Butzin & Senghas (1996). Group XI, which encompasses species with pseudobulbs in “cushion” form, joined by a short rhizome, was subdivided into three subgroups. One of these subgroups, the so called *Maxillaria picta* complex, in which most species have bifoliate pseudobulbs and occur exclusively in Brazil, is known by its extensive morphological variation, which entails several problems to delimit its species. Anatomical (Holtzmeier *et al.* 1998) and molecular (Dathe & Dietrich 2006, Whitten *et al.* 2007) data show a monophyletic group which includes the species of this complex, and occurs only in Brazilian atlantic and seasonal deciduous forests. This group was recently transferred for the genus *Brasiliorchis* (Singer *et al.* 2007), and here we adopt this criterion.

Many specific names are associated to this complex and it is not possible to delimit the species objectively, because of the high morphological variation (Hoehne 1953, Pabst & Dungs 1977). In this group, flowers have many colour patterns, even in a single population, mainly among the species with spotted sepals and petals, such as *B. picta* (Hook.) R. Singer, S. Koehler & Carnevali and *B. ubatubana* (Hoehne) R. Singer, S. Koehler & Carnevali, which can exhibit several pigmentation degrees (Hoehne 1953). Flower morphology is also variable in size and form, making species identification quite difficult.

Although Hoehne (1953) considered 15 species and 19 varieties in the *Brasiliorchis picta* complex, he admits

that mistakes may exist in species delimitation because of the large variations and that a large amount of collections needs to be investigated. Most varieties described by Hoehne (1952, 1953) illustrate specimens that present continuous morphological characteristics with no striking discontinuities. Pabst & Dungs (1977) have accepted most of the classification proposed by Hoehne (1953), but, based on vegetative characteristics, they split these species in three “alliances”: “*picta* alliance”, “*marginata* alliance” and “*gracilis* alliance”. Butzin & Senghas (1956) join together the “*picta*” and “*gracilis*” alliances of Pabst & Dungs (1977) and keep the “*marginata*” alliance as a different group.

This study aims at describing the morphological variation present in the *B. picta* complex using quantitative characters and multivariate analysis methods to identify patterns of variation, determining the characters related to these patterns, and explaining the species relationships within this complex.

Material and methods

A total of 340 living plants from 30 Brazilian localities were studied (table 1). The specimens were grown for at least 20 years at the “Instituto de Botânica” at São Paulo (Brazil), so that the influence of habitat conditions was minimized.

Because species recognition is complex, a broad delimitation was adopted, and the specimens were previously identified based on qualitative characters observed in living plants (table 2). The names used herein have been commonly employed in the taxonomic literature on this group (Hoehne 1953, Pabst & Dungs 1977, Butzin & Senghas 1996) and have nomenclatural priority over recent names avoided here. The specimens were split in six species (table 2). Line drawings of dissected flowers from representative specimens of these six species were made to show the morphological variation among and within species (figure 1). Because *Brasiliorchis consanguinea* (Klotzsch) R.B. Singer, S. Koehler & Carnevali, *B. heismanniana* (Barb. Rodr.) R.B. Singer, S. Koehler & Carnevali, *B. kautskyi* (Pabst) R.B. Singer, S. Koehler & Carnevali, and *B. schunkeana* (Campacci & Kautsky) R.B. Singer, S. Koehler & Carnevali are rare species whose morphological characters differ considerably from those of the individuals measured in this work and since no living material was available, they were not included.

Twenty continuous morphological characters were measured (table 3), four of which are vegetative and 16 floral (figure 1). All measurements of any of the considered character were taken at the point of maximum dimension. Three flowers for each specimen are measured, and the mean value among them was considered in the analysis. Figure 1 presents details of floral characters. Vouchers of representative individuals of each species and each population are deposited at the herbarium SP (table 1).

Table 1. Procecence, number of specimens analyzed and vouchers for each species. Vouchers were made from one specimen per population and are deposited in Herbarium SP (Institute of Botany, São Paulo State, Brazil). (BA = Bahia; ES = Espírito Santo; MG = Minas Gerais; PR = Paraná; RJ = Rio de Janeiro; SC = Santa Catarina; SP = São Paulo states).

Species/Origin	<i>n</i>	Voucher
<i>B. gracilis</i>		
Santana do Riacho – MG	27	<i>H.D. Bicalho s.n.</i> (SP373874)
<i>B. chrysantha</i>		
Mucuri – BA	2	<i>F. Pinheiro 407</i>
Teófilo Otoni – MG	7	<i>F. Pinheiro 319</i>
São Tomé das Letras – MG	3	<i>F. Pinheiro 411</i>
Linhares – ES	4	<i>F. Pinheiro 372</i>
Guaíra – PR	5	<i>F. Pinheiro 397</i>
<i>B. marginata</i>		
São Paulo – SP	12	<i>F. Pinheiro 277</i>
Blumenau – SC	15	<i>F. Pinheiro 419</i>
Orleans – SC	6	<i>F. Pinheiro 419</i>
<i>B. picta</i>		
Jacinto – MG	9	<i>F. Pinheiro 415</i>
Caldas – MG	23	<i>F. Pinheiro 416</i>
Parati – RJ	9	<i>F. Pinheiro 305</i>
Ubatuba – SP	27	<i>F. Pinheiro 285</i>
Atibaia – SP	14	<i>F. Pinheiro 317</i>
Salesópolis – SP	13	<i>F. Pinheiro 417</i>
Peruíbe – SP	8	<i>F. Pinheiro 293</i>
Cananéia – SP	15	<i>F. Pinheiro 279</i>
Jacupiranga – SP	20	<i>F. Pinheiro 332</i>
Batatais – SP	13	<i>F. Pinheiro 418</i>
Jaguariaíva – PR	28	<i>F. Pinheiro 386</i>
<i>B. porphyrostele</i>		
Teodoro Sampaio – SP	8	<i>F. Pinheiro 345</i>
Matos Costa – SC	15	<i>F. Pinheiro 331</i>
Lages – SC	8	<i>F. Pinheiro 358</i>
Bom Jardim da Serra – SC	5	<i>F. Pinheiro 364</i>
São Joaquim – SC	4	<i>F. Pinheiro 367</i>
<i>B. ubatubana</i>		
Itagimirim – BA	10	<i>F. Pinheiro 342</i>
Camanducaia – MG	5	<i>F. Pinheiro 343</i>
Petrópolis – RJ	3	<i>F. Pinheiro 294</i>
Campos do Jordão – SP	10	<i>F. Pinheiro 356</i>
Apiáí – SP	8	<i>F. Pinheiro 339</i>

The descriptive analysis of the data was performed with SYSTAT 10.0 (Wilkinson 2000) software and presented graphically as box plots. Multivariate analysis methods were carried out with the programs SYSTAT 10 and Fitopac 1.6 (Shepherd 2006) softwares. Two types of ordination analysis and one cluster method were used to avoid possible distortions produced by a specific method (Everitt 1978): principal component analysis (PCA) on a correlation matrix was used as an objective method to summarize variation when a priori knowledge of population to which individuals belonged was

not considered; canonical variate analysis (CVA) was used to ordinate population means considering variance and covariance among characters within and among populations; and a cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA), on an Euclidean distance matrix, to verify morphological discontinuities among species. Twenty plants of each species were randomly chosen from the total sample and analysed with the UPGMA method to improve the graphical representation of the dendrogram. In PCA, the number of

Table 2. Qualitative characters used to delimit the six species considered in this study. Species names considered synonyms are between parentheses.

Species	Qualitative characters
<i>Brasiliorchis chrysantha</i> (Barb. Rodr.) R.B. Singer, S. Koehler & Carnevali	Long rhizome, sepals and petals with vinaceous margins, vinaceous column, lip white with vinaceous spots.
<i>Brasiliorchis gracilis</i> (Lodd.) R.B. Singer, S. Koehler & Carnevali	Aggregated rhizomes, lip white with vinaceous spots, column pale yellow with vinaceous dashes.
<i>Brasiliorchis marginata</i> (Lindl.) R.B. Singer, S. Koehler & Carnevali (<i>Maxillaria murilliana</i> Hoehne) (<i>M. crassipes</i> Kraenzl.)	Long rhizome, lip pale yellow with central and lateral lobe apex dark purple, column yellow with vinaceous dashes.
<i>Brasiliorchis picta</i> (Hook.) R. Singer, S. Koehler & Carnevali (<i>Maxillaria rupestris</i> Barb. Rodr.) (<i>M. phoenicanthera</i> Barb. Rodr.) (<i>M. polyantha</i> Barb. Rodr.) (<i>M. hoehnei</i> Schltr.)	Aggregated rhizomes, sepals and petals with abaxial face pale yellow, adaxial face yellow, white lip with vinaceous dots, column yellow with vinaceous dashes, purple anther.
<i>Brasiliorchis porphyrostele</i> (Rchb. f.) R.B. Singer, S. Koehler & Carnevali	Aggregated rhizomes, sepals and petals yellowy green with vinaceous margins, lip white with vinaceous dots, column vinaceous.
<i>Brasiliorchis ubatubana</i> (Hoehne) R.B. Singer, S. Koehler & Carnevali	Aggregated rhizomes, pale yellow flowers with vinaceous dots, lip white with vinaceous dots, column vinaceous.

Table 3. Morphological characters used in morphometric analyses of *B. picta* complex and results of principal component analysis (PC1, PC2 and PC3), canonical variate analysis (CN1, CN2 and CN3) and discriminant analysis (F-to-remove). PC1-PC2: correlations between the original variables with principal components one and two, respectively; CN1-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 DA (see table 5).

Characters	Code	PC1	PC2	PC3	CN1	CN2	CN3	F-to-remove
1. Rhizome length	RI_L	-0.203	0.347	0.704	0.217	0.455	0.422	32.57
2. Pseudobulb length	PS_L	0.408	0.690	0.078	-0.089	0.178	-0.086	4.49
3. Leaf length	LE_L	0.524	0.279	-0.510	0.297	-0.362	-0.180	9.22
4. Leaf width	LE_W	0.436	0.524	0.265	-0.033	0.237	0.040	12.6
5. Inflorescence length	IN_L	0.507	0.659	-0.107	0.034	0.270	0.122	1.93
6. Pedicel length	PE_L	0.237	0.623	0.278	-0.292	0.119	0.242	5.75
7. Column length	CO_L	0.796	-0.236	0.367	-0.387	-0.009	0.132	3.21
8. Column width	CO_W	0.905	0.013	0.147	0.420	0.015	0.291	8.51
9. Dorsal sepal length	DS_L	0.877	0.085	-0.284	0.628	0.356	0.007	2.73
10. Dorsal sepal width	DS_W	0.891	0.019	-0.039	-0.012	0.097	-0.058	5.67
11. Lateral sepal length	LS_L	0.880	0.128	-0.300	-0.004	-0.296	-0.282	1.51
12. Lateral sepal width	LS_W	0.880	-0.021	0.021	-0.138	0.112	0.099	1.35
13. Petal length	PT_L	0.891	-0.002	-0.268	0.094	0.302	-0.566	3.43
14. Petal width	PT_W	0.848	0.123	-0.181	0.170	-0.023	-0.386	5.08
15. Lip length	LI_L	0.900	-0.225	0.124	0.088	-0.108	0.251	1.25
16. Lip width	LI_W	0.817	-0.329	0.282	0.397	-0.098	0.834	10.37
17. Lateral lobe of lip length	LL_L	0.877	-0.185	0.294	0.372	0.438	-0.113	3.16
18. Central lobe of lip length	CL_L	0.662	-0.195	-0.158	-0.667	-0.200	0.230	9.52
19. Central lobe of lip width	CL_W	0.741	-0.413	0.148	-0.406	-0.948	-0.185	17.91
20. Callus of lip length	CA_L	0.857	-0.129	0.168	0.226	-0.291	0.004	3.03

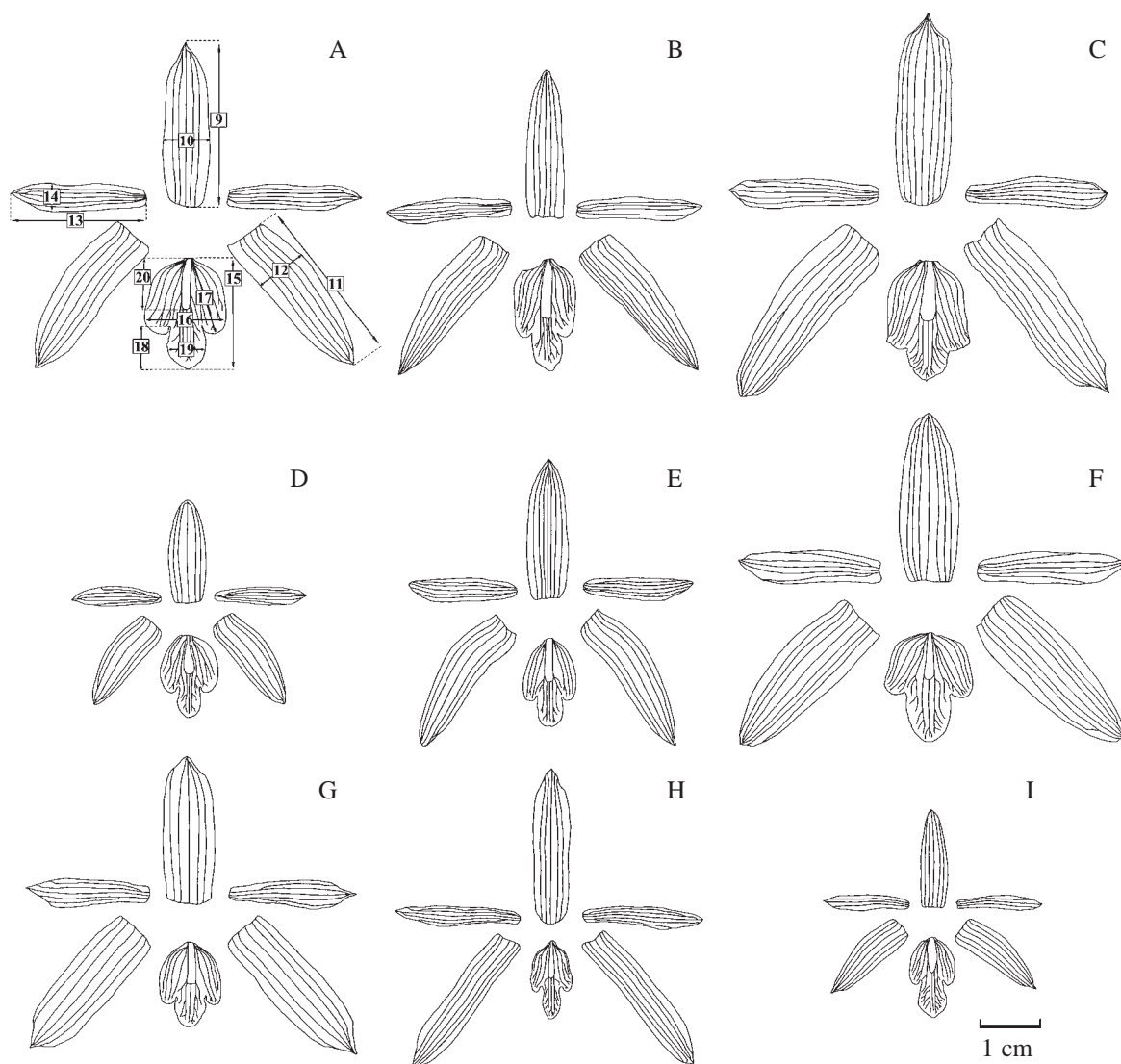


Figure 1. Line drawings of dissected flowers showing the morphological variation among and within the six species from *Brasiliorchis picta* complex. Vouchers of each flower are deposited at the herbarium SP. A. *B. chrysantha* (F. Pinheiro 294). B. *B. chrysantha* (F. Pinheiro 288). C. *B. ubatubana* (F. Pinheiro 385). D. *B. marginata* (F. Pinheiro 9261). E. *B. marginata* (F. Pinheiro 10438). F. *B. porphyrostele* (F. Pinheiro 328). G. *B. picta* (F. Pinheiro 320). H. *B. picta* (F. Pinheiro 341). I. *B. gracilis* (H.D. Bicalho s.n. SP373874). Details of measurements can be seen in A.

axes to interpret was determined by comparing eigenvalues to the random expectation in a broken-stick distribution (Frontier 1976). The six species were the grouping variables in CVA. Discriminant analysis (DA) was performed with the same grouping variables used in CVA. Wilks' Lambda, jackknife classification, which assigns unclassified specimens to groups, and F-to remove statistics, which give an indication of the relative importance of each variable, are also reported.

Results

The results of the descriptive analysis show that most characters overlap among the species (figure 2).

Brasiliorchis gracilis (Lodd.) R.B. Singer, S. Koehler & Carnevali is the species with lesser overlapping with the others. Asterisks (outside values) and circles (far outside values) are present in most box plots, showing specimens with extreme values in all species.

In PCA, the first three axes explain about 74% of the total variation (figure 3). The species show only a tendency to differentiation in the analysis, since individuals of different species are spread in many parts of the scatter plot, making the delimitation of patterns difficult. On the first axis, that explain 55.14% of the total variation, individuals of *B. gracilis*, *B. porphyrostele*

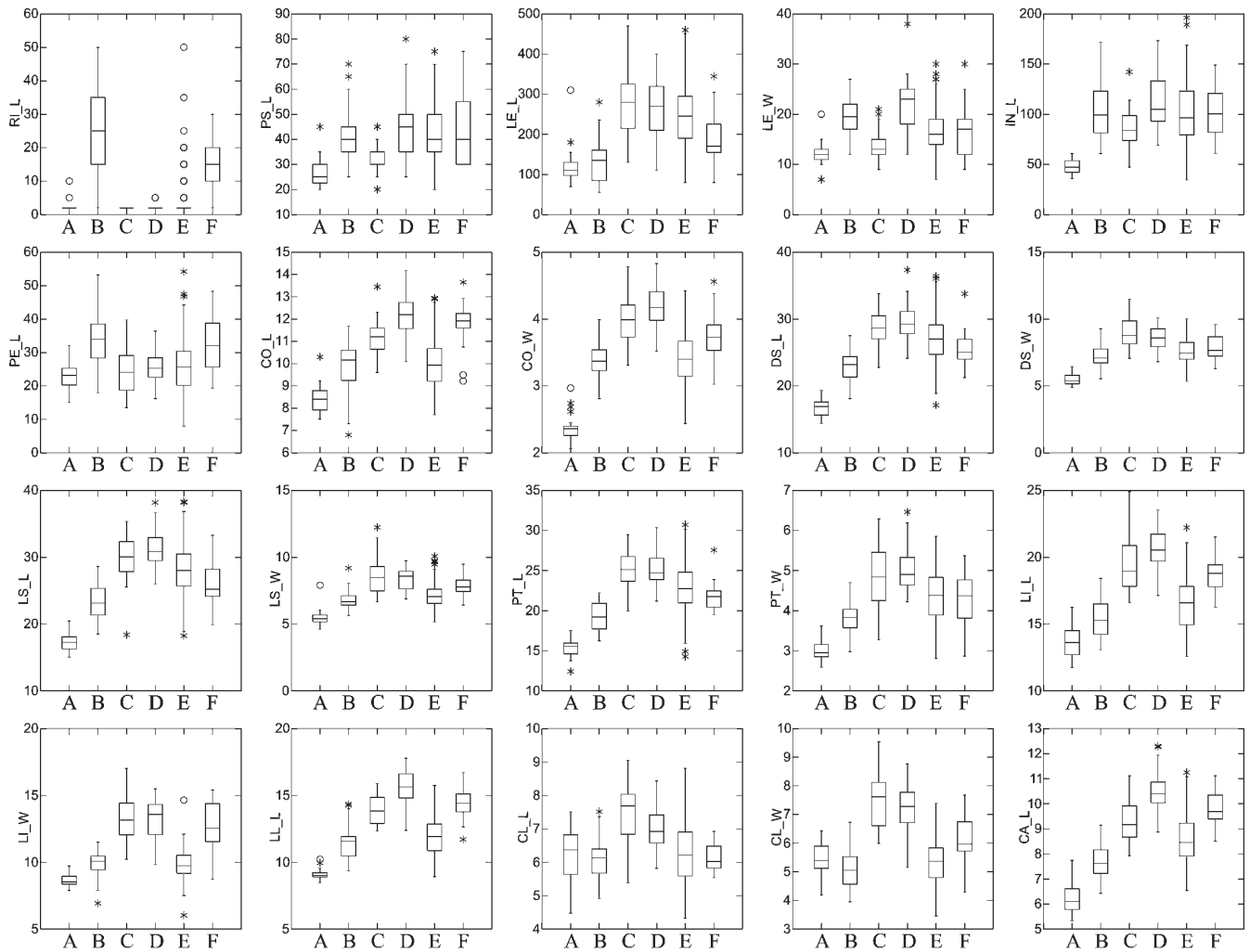


Figure 2. Box plots for the 20 quantitative characters. Rectangles define 25 and 75 percentiles; horizontal lines show median; whiskers are from 10 to 90 percentiles; asterisks and circles are extreme values. See table 4 for characters abbreviation. A. *B. gracilis*. B. *B. marginata*. C. *B. porphyrostele*. D. *B. ubatubana*. E. *B. picta*. F. *B. chrysantha*.

(Rchb. f.) R.B. Singer, S. Koehler & Carnevali and *B. ubatubana* (Hoehne) R.B. Singer, S. Koehler & Carnevali can be discriminated from the others. In the axis two, that explain 11.42% of the total variation, the majority of the individuals from *B. ubatubana* can be separated from *B. porphyrostele*. In this axis, *B. marginata* show a tendency to separate from the other species, but this pattern is best seen in axis three, where most of the individuals of this species are kept more apart from the others. The scatter plot with axes one and three show also a differentiation between specimens of *B. marginata* (Lindl.) R.B. Singer, S. Koehler & Carnevali and *B. chrysantha* (Barb. Rodr.) R.B. Singer, S. Koehler & Carnevali. Specimens of *B. picta* are spread along the three axes, showing no tendencies to split from

the other species (figure 3). Flower characters are correlated with axis one, and vegetative characters are more correlated with axes two and three. CO_W, LI_L, DS_W, PT_L, and LS_W are the five most important characters correlated with the first axis. PS_L, IN_L, PE_L, LE_W and RI_L are the five most important characters correlated with the second. Otherwise, RI_L, CO_L, LL_L, LI_W and PE_L are the five most important characters correlated with the third axis (see table 3 for abbreviations).

The first three axes in CVA explain about 86% of the total variation (figure 4). The first axis explain 39.1% of the total variation, and clearly separates specimens of *B. gracilis* from the other species. Specimens of *B. ubatubana* appear together in the analysis, showing

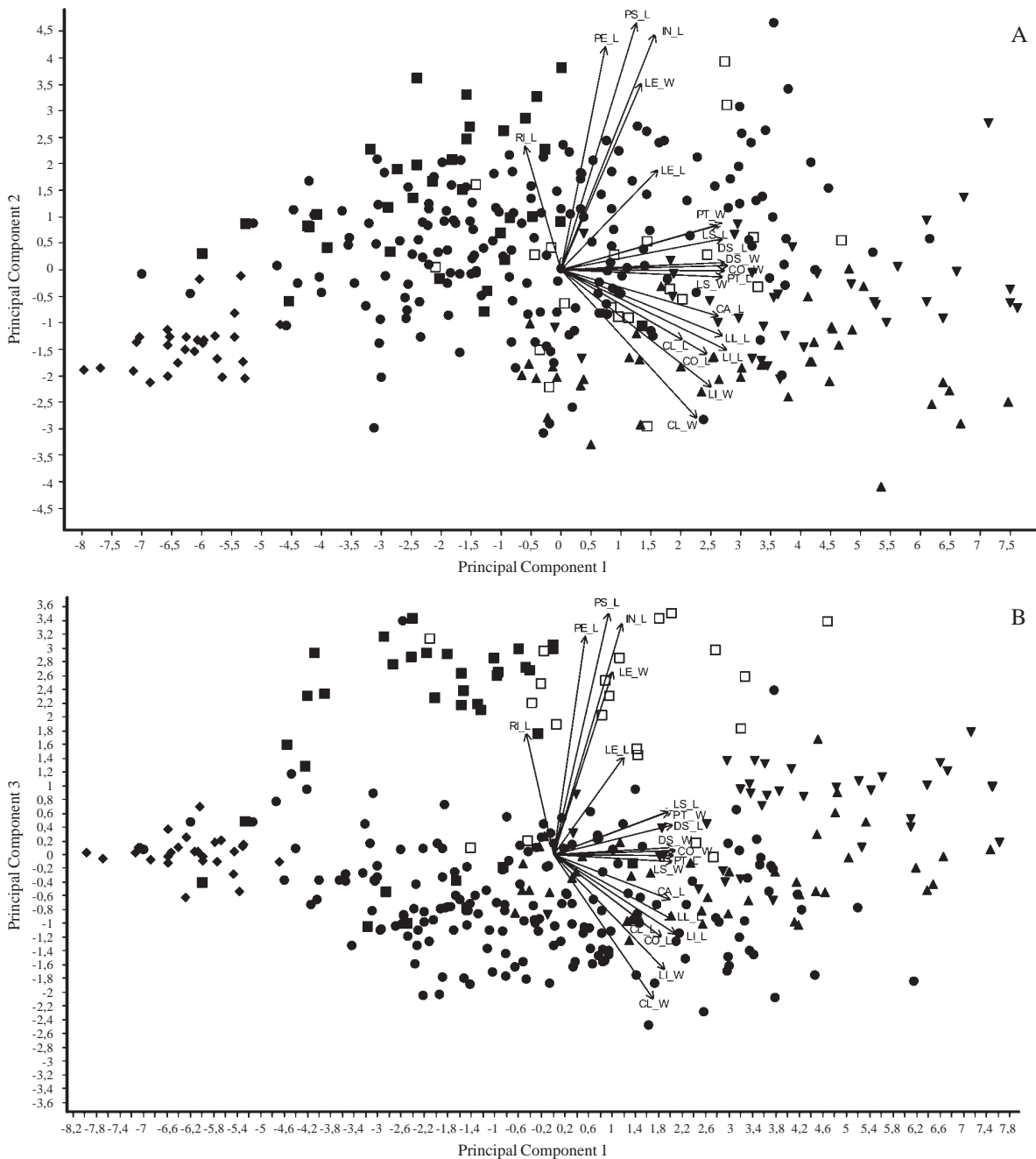


Figure 3. PCA of 340 specimens from the *B. picta* complex based on 20 characters (see table 3). A. Principal components 1 and 2. B. Principal components 1 and 3. Axes 1, 2 and 3 explain 55.14%, 11.42%, and 8.04% of the total variation, respectively. (◆ = *B. gracilis*; ■ = *B. marginata*; ▲ = *B. porphyrostele*; ▼ = *B. ubatubana*; ● = *B. picta*; □ = *B. chrysantha*).

also a tendency to separate from the other species. The second axis explains 26.7% of the total variation, and allows the discrimination of specimens of *B. porphyrostele* and *B. marginata*, both in the extremes of the scatter plot with axes one and two (figure 4A). The third axis explains 21.1% of the total variation and shows a more clear separation of *B. marginata* and *B. chrysantha*.

Most of the specimens of *B. picta* show a tendency to separate from the other species in the third axis, nevertheless they show some overlap with individuals of different species (figure 4B). CL-L, DS-L, CO-W, CL-W and LI-W are the five most important characters related to the first axis. RI_L, LL_L, DS_L, PT_L and IN_L are the five most important characters related to

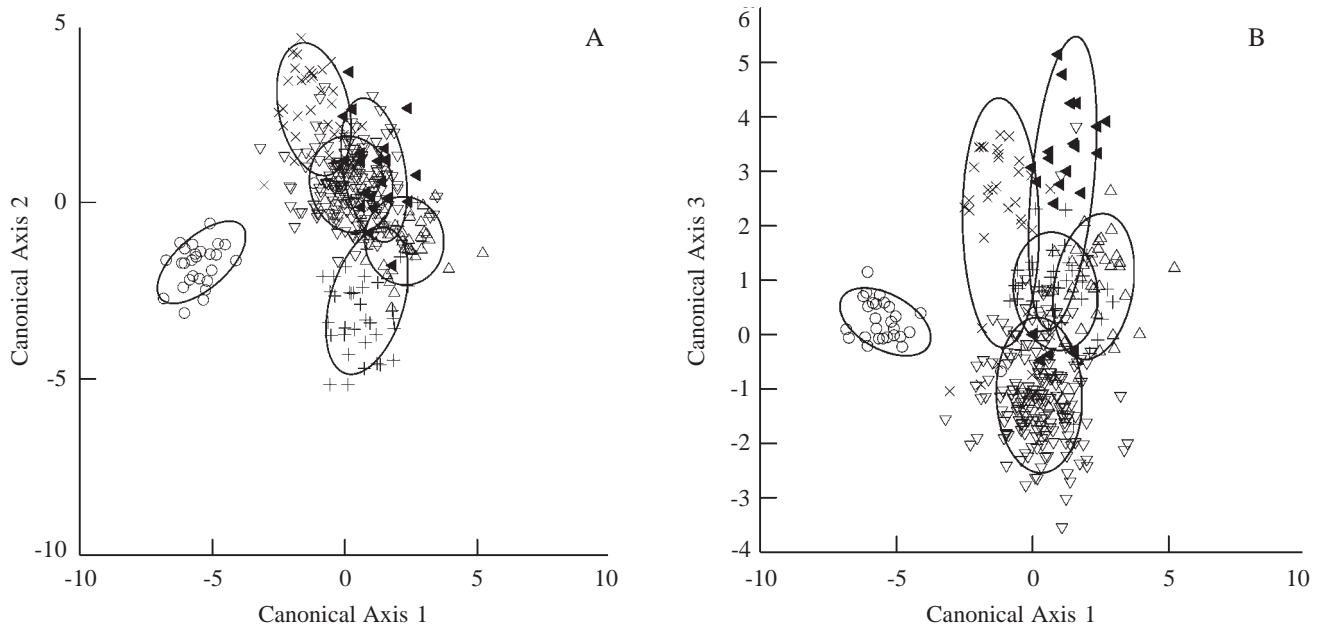


Figure 4. CVA of 340 specimens from the *B. picta* complex based on 20 characters (see table 3). Grouping variables are the six species considered in this study: *B. chrysantha*, *B. gracilis*, *B. marginata*, *B. picta*, *B. porphyrostele*, and *B. ubatubana*. Axes 1, 2 and 3 explains 39.1%, 26.7% and 21.1% of the total variation, respectively. The ellipses are centered on the sample means, and comprises 70% of the sample from each species. (\circ = *B. gracilis*; \times = *B. marginata*; $+$ = *B. porphyrostele*; \triangle = *B. ubatubana*; ∇ = *B. picta*; \blacktriangleleft = *B. chrysantha*).

the second axis and LI_W, RI_L, CO_W, LI_L and PE_L are the five most important characters related to the third axis.

The jackknifed classification matrix produced by DA shows 85% of correct classification of the individuals into the previously assigned species (table 4). *Brasiliorchis gracilis* showed the highest percentage of correct classification (96%), with one individual being misclassified as *B. picta*. *Brasiliorchis chrysantha* showed the lowest percentage of correct classification (67%),

sharing misclassified specimens with *B. picta* (4), *B. marginata* (2) and *B. porphyrostele* (1). *Brasiliorchis marginata* and *B. picta*, despite the high percentages of correct classification (74% and 89% respectively), have misclassified specimens into five other different species. *Brasiliorchis porphyrostele* and *B. ubatubana* showed 80% and 84% of correct classification, but have misclassified specimens into two and three other different species, respectively. A high level of morphological similarity can be noted between *B. ubatubana* and *B. porphyrostele*.

Table 4. Results of jackknifed classification matrix with 340 individuals and six species. (Wilks' Lambda = 0.011, $P = 0.0000$).

Grouping variables	<i>B. gracilis</i>	<i>B. marginata</i>	<i>B. porphyrostele</i>	<i>B. ubatubana</i>	<i>B. picta</i>	<i>B. chrysantha</i>	Percentage correct (%)
<i>B. gracilis</i>	26	0	0	0	1	0	96
<i>B. marginata</i>	1	25	1	0	6	1	74
<i>B. porphyrostele</i>	0	0	33	4	3	1	80
<i>B. ubatubana</i>	0	0	2	31	4	0	84
<i>B. picta</i>	0	4	5	9	161	1	89
<i>B. chrysantha</i>	0	2	1	0	4	14	67
Total	27	31	42	44	179	17	85

The patterns observed in the UPGMA dendrogram based on Euclidean distance (data not show) agree with the results of PCA (figure 3) and CVA (figure 4). Specimens of *B. gracilis* grouped together and their cluster are the farthest in relation to the other groups. The remaining species (*B. chrysantha*, *B. marginata*, *B. picta*, *B. porphyrostele*, and *B. ubatubana*) have individuals in many regions of the dendrogram, but tendencies to group together can be seen for all species, as most individuals of each species clustered together. The cophenetic correlation was 0.72.

Discussion

Brasiliorchis gracilis has the most evident delimitation among all the examined species, showing a morphological differentiation in all the analyses. Hoehne (1952, 1953) accepted six varieties for this species, however there are no evident morphological discontinuities among them, and it is quite impossible to recognize most of them. The small size of most of the characters in *B. gracilis* can be the reason for this strong differentiation.

Despite the high morphological similarity between *B. ubatubana* and *B. porphyrostele*, qualitative characters, like color of sepals and petals (white with red dots in *B. ubatubana*, yellow greenish in *B. porphyrostele*) allow a clear delimitation between them. Moreover, according to Hoehne (1953), these two species rarely happen sympatrically, and the geographical distribution is therefore one of the possible causes of the differentiation between them, as was also observed in some orchid species of the genera *Acianthera* (Borba *et al.* 2002) and *Pseudorchis* (Reinhammar 1998).

The long rhizome of *B. marginata* influenced the morphological diversity found in the performed analyses, since it allows the plant to spread on the tree branches or in flowerpots, when under cultivation: a same clump, originated from a single individual, can have quite different vegetative shapes. This phenotypic plasticity persists in the plants under cultivation, and it can hinder the identification of the species that exhibit individuals overlapping with *B. picta* and *B. chrysantha* in the analyses.

Brasiliorchis picta and *B. chrysantha* were the species showing the largest morphological variation, with some of their specimens scattered on PCA and CVA graphs, and individuals clustered with other species. Both have a wide geographical distribution (Hoehne 1953, figure 1) and grow in different habitats: they can be rupicolous, epiphytes or grow on organic matter

accumulated over the soil (Hoehne 1953). Extensive geographical distribution can be associated with extensive morphological variation in several genera of Orchidaceae, like *Dactylorhiza* (Tyteca & Dufréne 1994), *Acianthera* (Borba *et al.* 2002), *Pseudorchis* (Reinhammar 1998), and *Serapias* (Pellegrino *et al.* 2005).

Although they exhibit larger morphologic discontinuities, floral characters played a more important role in the analyses of PCA, CVA and DA than the vegetative ones. Traditionally, floral characters are more used in the taxonomy of the group (Hoehne 1953, Pabst & Dungs 1977, Butzin & Senghas 1996); sterile plants are quite impossible to identify. Papers using morphometric methods in the taxonomy of other Orchidaceae genera also evidenced the floral characters as the most informative ones (Reinhammar 1998, Borba *et al.* 2002, Bernardos *et al.* 2005).

As all these species grow in similar habitats, sometimes sympatrically, the divergence among them should be maintained by different mechanisms of pollination and/or post-mating barriers that warrant the isolation between species (Borba *et al.* 2002, Cozzolino *et al.* 2001, Mant *et al.* 2005). According to Singer & Koehler (2004), the species of the *B. picta* complex and allied genera like *Trigonidium* and *Mormolyca* (Singer 2002, Singer *et al.* 2004) do not offer resources for pollinators and are probably pollinated by deceit. As species pollinated by deceit do not have a high specificity with regard to pollinators, and since introgression and hybridization among sympatric species seem to be a common phenomenon (Soliva & Widmer 2003, Cozzolino *et al.* 2006), gene flow may occur between species in the *B. picta* complex, possibly giving rise to hybrid specimens, which contributes to the morphological overlapping among the species.

Since the use of morphometry may clarify the delimitation of close related species and the relationships of species complexes in Orchidaceae (Reinhammar 1998, Borba *et al.* 2002, Bernardos *et al.* 2005, Pellegrino *et al.* 2005), it is a crucial tool for an objective quantification of biodiversity (Henderson 2005). However, morphometry also has limitations that are evident in several groups of species with probable recent origin, which present an extensive overlapping of morphological characters between species, like the pattern observed in the *B. picta* complex. In these cases, the use of molecular data, mainly from markers able to reveal a great amount of polymorphisms, is fundamental to understand the species relationships. Works on a population level, using molecular markers like isozymes, AFLP or microsatellites, could identify the evolutionary processes involved in the

diversification of species complexes, as already evidenced for *Dactylorhiza* (Pillon *et al.* 2006), *Ophrys* (Soliva & Widmer 2003), *Orchis* (Cozzolino *et al.* 2006), and *Acianthera* (Borba *et al.* 2002). In these groups, the analysis of a large amount of individuals per population was fundamental to understand the diversification mechanisms of species and lineages, a trend that should be adopted in future works on the *B. picta* complex.

Acknowledgements – To the staff of the “Seção do Orquidário do Estado” of the Instituto de Botânica, São Paulo, Brazil, for their technical support. This work was supported by a grant from “Fundação de Amparo à Pesquisa do Estado de São Paulo” (Fapesp # 02/00078-5) to the first author. The second author acknowledges the Brazilian “Conselho Nacional de Desenvolvimento Científico e Tecnológico” for the grant received (CNPq # 303962/2004-6).

References

- ATWOOD, J.T. & MORA-DE-RETANA, D.E. 1999. Flora Costaricensis (Orchidaceae). *Fieldiana, Botany* 40: 1-182.
- BERNARDOS, S., CRESPI, A., DEL REY, F. & AMICH, F. 2005. The section *Pseudophrys* (*Ophrys*, Orchidaceae) in the Iberian Peninsula: a morphological and molecular analysis. *Botanical Journal of the Linnean Society* 148:359-375.
- BORBA, E.L., SHEPHERD, G.J., 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.
- BUTZIN, F. & SENGHAS, K. 1996. Subtribus: Maxillariinae. In Rudolf Schlechter, *Die Orchideen* (F.G. Brieger, R. Maatsch, K. Senghas, eds.). Blackwell, Berlin, p.1727-1792.
- CARNEVALI, G., MUÑOZ, J.L.T. & GOMEZ-JUAREZ, M. 2001. A synopsis of the *Maxillaria rufescens* complex in Mexico, Central America, and the Greater Antilles. *Brittonia* 53:454-465.
- COGNIAUX, A. 1904-1906. Orchidaceae. In *Flora brasiliensis* (C.F.P. Martius, A.G. Eichler & I. Urban, eds.), v.3, pars 6, p.1-604.
- COZZOLINO, S., ACETO, S., CAPUTO, P., WIDMER, A. & DAFNI, A. 2001. Speciation processes in Eastern Mediterranean *Orchis s.l.* species: molecular evidence and the role of pollination biology. *Israel Journal of Plant Sciences* 49:91-103.
- COZZOLINO, S., NARDELLA, A.M., IMPAGLIAZZO, S., WIDMER, A. & LEXER, C. 2006. Hybridization and conservation of Mediterranean orchids: should we protect the orchid hybrids or the orchid hybrid zones? *Biological Conservation* 129:14-23.
- DATHE, S. & DIETRICH, H. 2006. Comparative molecular and morphological studies in selected Maxillariinae orchids. *Willdenowia* 36:89-102.
- DRESSLER, R.L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland.
- DUNSTERVILLE, G.C.K. & GARAY, L.A. 1961. Venezuelan Orchids illustrated 2. Andre Deutsch, London.
- EVERITT, B.S. 1978. Graphical techniques for multivariate data. North-Holland, New York.
- FRONTIER, S. 1976. Study of the decrease of eigenvalues in principal component analysis: comparison with the broken stick model. *Journal of Experimental Marine Biology and Ecology* 25:67-75.
- HENDERSON, A. 2005. The methods of herbarium taxonomy. *Systematic Botany* 30:456-459.
- HOEHNE, F.C. 1952. Espécies e variedades novas de orchidáceas do Brasil. *Arquivos de Botânica do Estado de São Paulo* 2:121-136.
- HOEHNE, F.C. 1953. Orchidaceae. In *Flora brasílica* (F.C. Hoehne, ed.), v.12, fasc.7, p.1-397.
- HOLTZMEIER, M.A., STERN, W.L. & JUDD, W.S. 1998. Comparative anatomy and systematics of Sengha's cushion species of *Maxillaria* (Orchidaceae). *Botanical Journal of the Linnean Society* 127:43-82.
- ILLG, R.D. 1977. Revisão taxonômica da secção *Heterotaxis* (Lindl.) Brieg. do gênero *Maxillaria* Ruiz & Pavón. *Revista Brasileira de Biologia* 37:281-290.
- MANT, J., PEAKALL, R. & SCHIESTL, F.P. 2005. Does selection on floral odour promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution* 59:1449-1463.
- ONISHI, E. 1974. Sobre o polimorfismo do grupo *Maxillaria madida* (Orchidaceae, Maxillariinae). Tese de doutorado, Universidade Estadual de Campinas, Campinas.
- PABST, G.F.J. & DUNGS, F. 1977. Orchidaceae Brasilienses 2. Kurt Schmiersow, Hildesheim.
- PELLEGRINO, G., MUSACCHIO, A., NOCE, M.E., PALERMO, A.M. & WIDMER, A. 2005. Reproductive versus floral isolation among morphologically similar *Serapias* L. species (Orchidaceae). *Journal of Heredity* 96:15-23.
- PILLON, Y., FAY, M.F., SHIPUNOV, A.B. & CHASE, M.W. 2006. Species diversity versus phylogenetic diversity: a practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae). *Biological Conservation* 129:4-13.
- REINHAMMAR, L. 1998. Systematics of *Pseudorchis albida s.l.* (Orchidaceae) in Europe and North America. *Botanical Journal of the Linnean Society* 126:363-382.
- SHEPHERD, G.J. 2006. FITOPAC 1.6: Manual de usuário. Departamento de Botânica, Universidade Estadual de Campinas, Campinas.
- SINGER, R.B. 2002. The pollination mechanism in *Trigonidium obtusum* Lindl. (Orchidaceae: Maxillariinae): sexual mimicry and trap-flowers. *Annals of Botany* 89:157-163.

- SINGER, R.B. & KOEHLER, S. 2004. Pollinarium morphology and floral rewards in Brazilian Maxillariinae (Orchidaceae). *Annals of Botany* 93:39-51.
- SINGER, R.B., FLACH, A., KOEHLER, S., MARSAIOLI, A.J. & AMARAL, M.C.E. 2004. Sexual mimicry in *Mormolyca ringens* (Lindl.) Schltr. (Orchidaceae: Maxillariinae). *Annals of Botany* 93:755-762.
- SINGER, R.B., KOEHLER, S. & CARNEVALI, G. 2007. *Brasiliorchis*: a new genus for the *Maxillaria picta* Alliance (Orchidaceae, Maxillariinae). *Novon* 17:91-99.
- SOLIVA, M. & WIDMER, A. 2003. Gene flow across species boundaries in sympatric, sexually deceptive *Ophrys* (Orchidaceae) species. *Evolution* 57:2252-2261.
- SZLACHETKO, D.L., MYTNIK-EJSMONT, J., GÓRNIK, M. & SMISZEK, M. 2006. Genera et species Orchidaceae 15, Maxillarieae. *Polish Botanical Journal* 15:57-59.
- SZLACHETKO, D.L. & SMISZEK, M. 2007. Nouveaux genres dans le complexe *Maxillaria* (Orchidaceae). *Richardiana* 7:26-32.
- TYTECA, D. & DUFRÉNE, M. 1994. Biostatistical studies of Western European allogamous populations of the *Epipactis helleborine* (L.) Crantz species group (Orchidaceae). *Systematic Botany* 19:424-442.
- WHITTEN, M.W., BLANCO, M.A., WILLIAMS, N.H., KOEHLER, S., CARNEVALI, G., SINGER, R.B., ENDARA, L. & NEUBIG, K.M. 2007. Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based on combined molecular data sets. *American Journal of Botany* 94:1860-1889.
- WILKINSON, L. 2000. Systat Statistics version 10.0 for Windows. SPSS, Chicago.