

Contribution of Morphometry to the Taxonomy of *Baptistonia* Barb. Rodr. (Orchidaceae)

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Contribution of morphometry to the taxonomy of *Baptistonia* Barb. Rodr. (Orchidaceae)

Guy R. Chiron, Gaëtan Guignard & Georges Barale

Abstract

CHIRON, G. R., G. GUIGNARD & G. BARALE (2010). Contribution of morphometry to the taxonomy of *Baptistonia* Barb. Rodr. (Orchidaceae). *Candollea* 65: 45-62. In English, English and French abstracts.

The genus *Baptistonia* Barb. Rodr. (Orchidaceae) includes 23 species, all endemic to Brazil. As problems occur to differentiate some taxa of this genus, because of their hypothetical hybrid origine, the present study aims to use morphometry as an attempt to solve these issues. Twenty six floral morphometric characters were measured on 146 specimens, and analysed using multivariate analysis, such as Neighbour Joining Analysis (NJA), Principal Coordinates Analysis (PCoA) and Discriminant Analysis (DA). Morphometric data proved to be very useful for species delimitation, and a statistical tool here is presented in clearly separating taxa within the confusing groups. Hybrid nature of two species is presented. The contribution of morphometry in phylogeny for *Baptistonia* is discussed.

Key-words

ORCHIDACEAE – *Baptistonia* – Brazil – Morphometry – Multivariate analysis – Taxonomy

Résumé

CHIRON, G. R., G. GUIGNARD & G. BARALE (2010). Contribution morphométrique à la taxonomie de *Baptistonia* Barb. Rodr. (Orchidaceae). *Candollea* 65: 45-62. En anglais, résumés anglais et français.

Le genre *Baptistonia* Barb. Rodr. (Orchidaceae) comprend 23 espèces, toutes endémiques du Brésil. Comme des problèmes existent pour différencier certains taxons de ce genre, en raison de leur origine hybride probable, la présente étude entend apporter des réponses au travers de l'étude morphométrique. Vingt six variables morphométriques florales ont été mesurées sur 146 spécimens et étudiées suivant les analyses multivariées, tels que l'Analyse Neighbour Joining (ANJ), l'Analyse en Coordonnées Principales (ACoP) et l'analyse discriminante (AD). Les données morphométriques se sont avérées très utiles pour la délimitation des espèces, et un outil statistique est proposé ici pour séparer clairement les taxons à l'intérieur des groupes confus. L'origine hybride de deux espèces est démontrée. La contribution de la morphométrie à la phylogénie pour *Baptistonia* est discutée.

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Introduction¹

The two main aims of systematics are taxa delimitation, and an understanding of their phylogenetic relationships. These are also the goals of this study, devoted to the genus *Baptistonia* Barb. Rodr. The genus is endemic to the Brazilian Atlantic Forest and belongs to the subtribe *Oncidiinae* Benth. Preliminary molecular studies (CHASE & al., 2005) have shown that it is part of the clade *Gomesa*, a set of orchids comprising the genera *Baptistonia*, *Gomesa* R. Br., *Rodrigueziella* Kuntze and *Rodrigueziopsis* Schltr., as well as several species endemic to southeastern Brazil previously assigned to the genus *Oncidium* Sw. About fifty names have been validly published at the species rank within *Baptistonia* (or within *Oncidium* before the re-establishment of the genus *Baptistonia*). However, CHIRON & CASTRO NETO (2004a, 2004b, 2005b, 2006a, 2006b) showed that the genus comprises only 23 species. Three of these species are supposed to be from hybrid origin (CHIRON, 2008): *Baptistonia damacenoii*, *B. gutfreundiana* and *B. riograndensis* (all the names of the species, with their authors names, are given in the appendix 1).

The notions of species definition and delimitation have long been a source of controversy (QUEIROZ, 2007). Deciding whether to consider a taxon as a good species or to place it into the synonymy of another species is often a debatable issue. This is also the case in *Baptistonia* with the treatment of PABST & DUNGS (1975) which left four cases of confusion. CHIRON & CASTRO NETO (2005a, 2005b, 2006b) discussed these taxa and proposed, based on morphological characters, one synonymy (for *B. cornigera*) and three morphogroups, each one consisting of taxa with vegetative and floral traits similar enough to present a risk of confusion. These morphogroups are the pair *B. albinoi* and *B. riograndensis* (Fig. 1A, B) - the pair *B. brieniana* and *B. widgrenii* (Fig. 1C, D) - and the “*pubes*” complex *B. pubes*, *B. lietzei* and *B. damacenoii* (Fig. 1E, F, G). Besides, *B. lietzei* is a very widely distributed species, with several known populations (CHIRON, 2007b), i.e. Serra de Villa Rica (Paraguay), forests patches along the Parana River in Brazil, northern Parana state, Serra do Japi, north to São Paulo, Serra da Mantiqueira and Serra do Mar in the Rio de Janeiro state. More work about differentiation between these populations is needed. Nevertheless the Paraguayan population was raised to the sub-species rank (*B. lietzei* subsp. *guaiensis*).

The species concept has been amply discussed in the literature, especially in recent years by WHEELER & MEIER (2000), HEY (2001), MALLETT (2001), AGAPOW & al. (2004). SITES & MARSHALL (2003) reviewed the most frequently employed methods for delimiting species. Morphological data has usually been

used for species delimitation. More recently, molecular data has also been employed, most often within animal groups, even if not always easily: examples of such concerns are discussed in BROWER (2006). The species delimitation issue is particularly acute within the recently radiated groups (as it seems to be the case in *Baptistonia*), because recently derived species often have not had sufficient time to achieve monophyly, as discussed in SHAFFER & THOMSON (2007). Molecular data have been more rarely used within plant groups (e.g. BORDA & al. (2001) for *Pleurothallis* R. Br.; JOLY & BRUNEAU (2007) for *Rosa* L.; SPOONER & al. (2007) for *Solanum* L.).

The relationships between the *Baptistonia* species were addressed by CHIRON (2007a) based on a set of morphological characters and CHIRON & al. (2009) based on molecular and chemical data. However, in both studies, a few nodes in the resulting phylogenetic tree are poorly bootstrap supported. More investigation is needed to better resolve the genus phylogeny.

In the present study we deal with the potential of morphometry to resolve species delimitation and hybrid origin issues and, to a lesser extent, intrageneric phylogenetic relationships. Morphometry has been defined (see in particular ROHLF, 1990), as the quantitative description, analysis and interpretation of forms and their variations in biology. Using multivariate analysis of the data, patterns of variation can be investigated and the clustering of taxonomic units into homogeneous groups can be proposed (BATEMAN & FARRINGTON, 1989; SELIN, 2000; HONG-WA, 2008). The number of necessary variables depends on the organisms being examined, and on the nature of the data (discrete or continuous). Similar studies carried on the family *Orchidaceae* have used from 20 to 40 variables: TYTECA & DUFRÈNE (1994) for *Epipactis* Zinn used 28 variables; VAN DEN BERG (1996) for *Cattleya* Lindl. used 24 variables; CARDIM & al. (2001) for *Oncidium* used 22 variables; CARLINI-GARCIA & al. (2002) for *Miltonia* Lindl. used 32 variables; GOLDMAN & al. (2004) for *Calopogon* R. Br. used 40 variables). In the present study, 26 variables were used.

Material and methods

Material

Baptistonia species demonstrate a strongly consistent vegetative morphology, with only few perceptible interspecific variations (CHIRON & CASTRO NETO, 2005a, 2005b, 2006a). Consequently the study focused on reproductive characters and, more precisely, on floral dimensions. In the light of the small size of the flowers (usually about 15 mm for the largest dimension) and of the difficulties of precisely evaluating the chosen characters from dried material, all of the working specimens were flowers removed from living plants. The measurements were taken either from fresh flowers or

¹ Note of the editors: the thesis of the author (CHIRON, 2010) was published in February 2010 in French. The figures are reproduced from page 24 to 29 by permission of the editor.

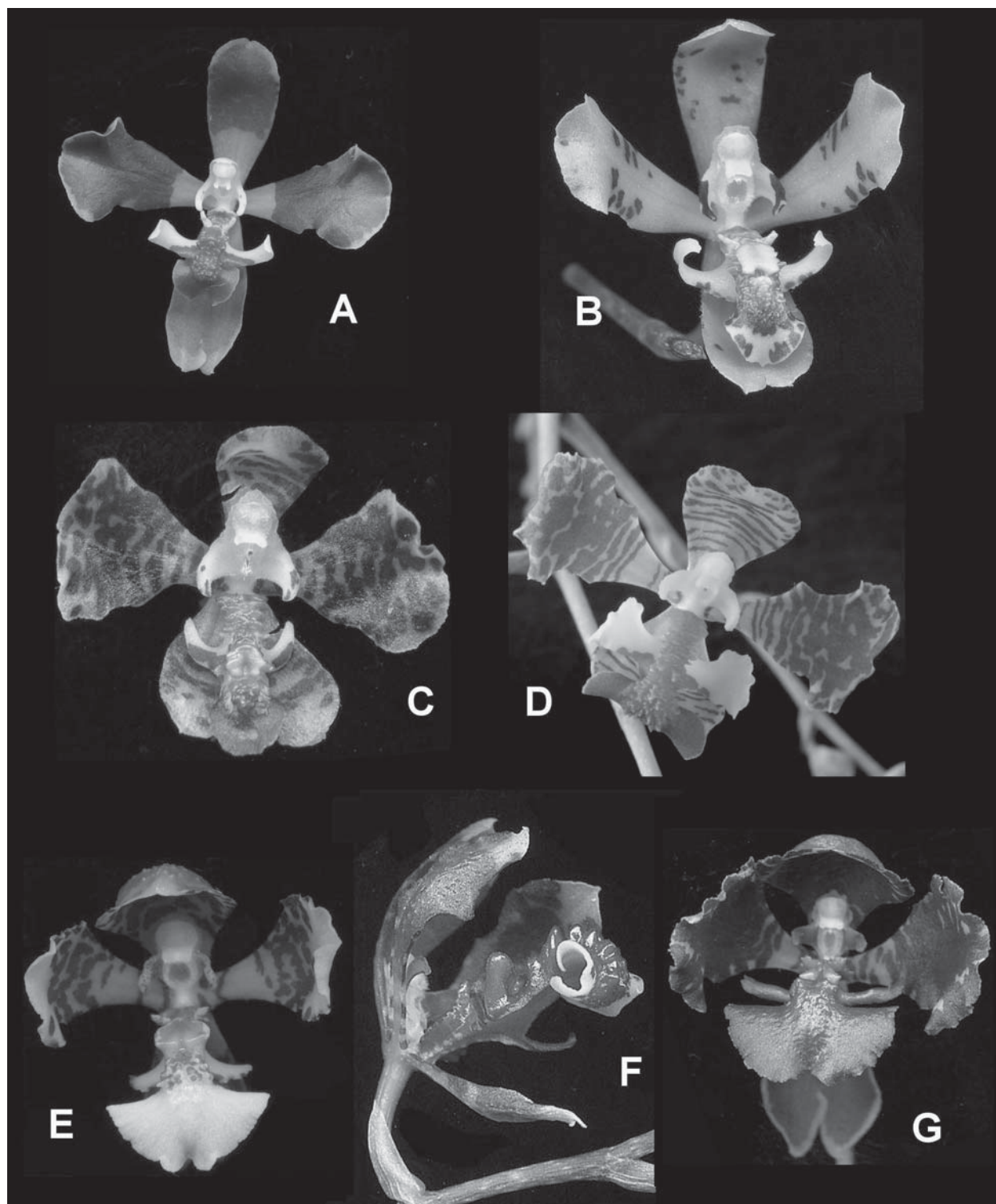


Fig. 1. – Flowers of some confusing species. **A.** *Baptistonia albinoi* (Schltr.) Chiron & V. P. Castro (GC2578); **B.** *B. riograndensis* (Cogn.) V. P. Castro & Chiron (GC3114); **C.** *B. brieniana* (Rchb. f.) V. P. Castro & Chiron (GC2676); **D.** *B. widgrenii* (Lindl.) V. P. Castro & Chiron (GC2577); **E.** *B. damacenoii* Chiron & V. P. Castro (Nego s.n.); **F.** *B. pubes* (Lindl.) Chiron & V. P. Castro (GC3046); **G.** *B. lietzei* (Regel) Chiron & V. P. Castro (GC3128).

[Photos: G. Chiron]

from flowers preserved in spirit, gathered either from wild plants or from cultivated plants. Before deciding to use flowers from our spirit collection, we checked on one specimen for *B. kautskyi* (Frey 1079) that no significant difference occurs between fresh flowers and spirit preserved flowers. In the same way, on some occasions (two *B. cornigera*, one *B. guttfreundiana*, one *B. lietzei*), flowers were first gathered from a wild plant and then, the following year, on the same plant placed in cultivation. In this way, we could check that, for any measurement, the variations observed between both types of flowers were equivalent to the variations observed between various flowers collected on one particular inflorescence.

When possible, a minimum of five different plants, collected within one or two different populations, of each species have been analysed. For *B. lietzei* and *B. cornigera*, the geographical distribution of which occurring from Rio de Janeiro to Paraguay (CHIRON, 2007b), we chose respectively more than 30 specimens from 4 regions: Paraguay and the Brazilian states Rio de Janeiro, São Paulo and Parana, and 13 samples from 3 states: Rio Grande do Sul, Parana, São Paulo (inland and coast). On the other hand, for some rare species, it has not been possible to find five samples because of the very small sizes of their populations and the even smaller number of flowering plants. Moreover, we were not able to collect any flower for *B. colorata* (Königer & J. G. Weinm. bis) Chiron nor for *B. velteniana* V. P. Castro & Chiron. Finally, 146 samples were examined: Appendix 1 gives the complete list and specifies, when possible, the geographical origin. Voucher specimens of flowers of all these samples are preserved, dried or in spirit, in Lyon University Herbarium (LY).

Data acquisition

Twenty six measurements (Fig. 2), generally used for orchid flowers (TYTECA & DUFRÈNE, 1994; VAN DEN BERG, 1996; CARDIM & al., 2001; CARLINI-GARCIA & al., 2002; GOLDMAN & al., 2004), were carried out on each of the flowers.

As for the measurement method, flowers were dissected, carefully flattened and scanned using a Perfection 2400 scanner from EPSON (Amsterdam, NL). Measurements were performed on the images obtained using SCION IMAGE software, version of NIH Images (see <http://rsb.info.nih.gov/ni-image>) from the Scion Corporation (Maryland, USA). Data has been analysed using the software PAST (HAMMER & al., 2007). Measurement ratios were avoided as they decrease the capability of the Principal Coordinates Analysis (PCoA) and the Canonical Variates Analysis (CVA) for discriminating between the effects of size and shape (GOLDMAN & al., 2004).

An index of variability (Iv_i) of the measured characters for all the samples and for each species was calculated. This index Iv_i is equal to the mean of the standardized variance of

each character (variance of the character divided by the square of its mean), calculated for each sample group (i.e. the complete genus and each species):

$$Iv_i = \frac{\sum_{j=1}^N (\sigma^2_{ij} / m^2_{ij})}{N}$$

Where: Iv_i = variability index of the species i , σ^2_{ij} = variance of the character j in the species i , m_{ij} = mean of the character j in the species i , N = number of characters.

Taxa discrimination

Regarding the separation of taxa, we began with PCoA (GOWER, 1966; DAVIS, 1986), as an exploratory investigation to check that all samples were correctly clustered within each species and, where this occurred, to detect any deviant samples. PCoA analyses were carried out using “Manhattan distance” (best distances and smallest horseshoe effect are often obtained using this method rather than Euclidian or Gower similarity index (PODANI & MIKLOS, 2002; ZILINSKAS & ZILINSKAS, 2006)).

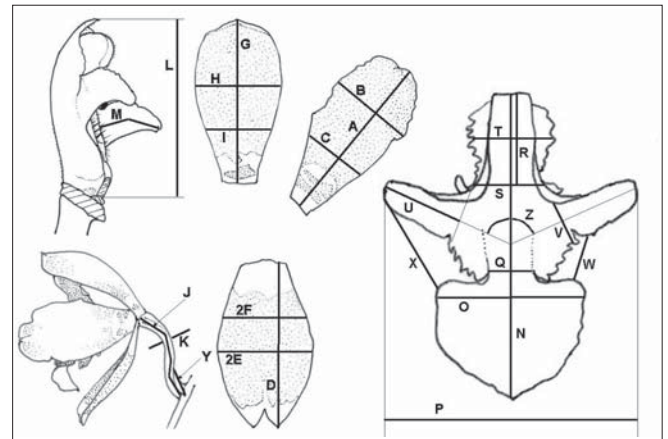


Fig. 2. – Sketch of measurements performed on a living *Baptiston pabstii* (Campacci & C. Espejo) V. P. Castro & Chiron. **A.** Petal length; **B.** Maximum petal width; **C.** Width of petal measured a third of the way along the petal from the base; **D.** Lateral sepal length; **E.** Maximum width of lateral sepal (or half the maximum width of the synsepal when the two sepals are fused); **F.** Width of lateral sepal measured a third of the way along from the base; **G.** Dorsal sepal length; **H.** Maximum width of dorsal sepal; **I.** Width of dorsal sepal measured a third of the way along from the base; **J.** Pedicel-ovary length; **K.** Ovary diameter measured at its base; **L.** Column length; **M.** Length of the column wings; **N.** Labellum length; **O.** Maximum width of the median lobe of the labellum; **P.** Labellum width measured at the level of the lateral lobes; **Q.** Isthmus width (minimum); **R.** Length of the labellum claw; **S.** Claw width measured at its base; **T.** Claw width measured at its mid-point; **U.** Length of a lateral lobe of the labellum; **V.** Lateral lobe width measured at its base; **W.** Width of the labellum sinus measured at its base; **X.** Sinus width measured from the end of a lateral lobe and the corner of the median lobe; **Y.** Length of the floral bract; **Z.** Angle made by the lateral lobes.

[GC3059, RJ]

As a preliminary operation we standardized the data by carrying out the following operation on each value X_{ij} (character j measured on sample i): $X_{ij} = (X_{ij} - M_j) / ET_j$, where M_j and ET_j are respectively the mean and the standard deviation of X_{ij} among all the samples.

The one-way multivariate analysis of variance (MANOVA) is the multivariate version of ANOVA and a simple extension of the Hotelling's test (HOTELLING, 1931) to more than two groups. It makes it possible to check the hypothesis that several data sets have the same mean (DAVIS, 1986; BROWN & ROTHERY, 1993). A similarity index P is provided by the software. However, as the multivariate normal distribution is not proven, we should use this index cautiously. CVA (e.g. FISHER, 1936) is an option under MANOVA: from a data set relating to several taxa, it consists of calculating, based on the multi-group discriminant, canonical axes producing maximal and second to maximal separation between all groups. These canonical axes are linear combinations of the original variables, and each associated eigenvalue indicates the amount of variation explained by the corresponding axis. This method has an important drawback: the number of samples should exceed the number of variables by two, which means that, in some cases, we need to exclude some characters in order to conform to this rule. Thus, for the pair *B. albinoi*-*B. riograndensis*, only 16 variables can be retained. For *B. brieniana*-*B. widgrenii*, only seven. We choose to exclude the less discriminating characters, as they appear in the PCoA result.

Specimen identification

Discriminant analysis (DA) of a data set relating to two groups of specimens is a classic method used to confirm or reject the hypothesis that two species are morphologically distinct, equality of the means being tested using the paired Hotelling's T^2 test. This method also makes it possible to sort a new specimen within one of the groups by means of a simple operation that consists of multiplying the characters measured on this specimen by the discriminant (scalar product) and subtracting from the result the offset value associated with the discriminant: the resulting sign indicates in which group the specimen is placed (HAMMER & al., 2007). Of course we should calculate the discriminant based on the original (not standardized) morphometric data, as only these are available from any new sample.

Testing the hybrid nature of a taxon

PCoA of a (standardized) data set relating to a taxon supposed to be from hybrid origin and to both presumed parents makes it possible to check the assumption. The values of at least one principal coordinate (PCO) relating to the "hybrid"

are expected to be placed in an intermediate position compared to the values of the "parents". Their variance is expected to be greater than the corresponding variance observed in the parents.

Phylogenetic inferences

According to HAMMER & al. (2007), the most appropriate tool for inferring phylogenetic relationships in PAST is the Neighbour Joining cluster analysis (NJ) using either correlation or the "Manhattan" coefficient, the most highly recommended for dealing with quantitative data. The reliability of the trees obtained in our case was significantly better in these conditions (NJ-correlation). This reliability was evaluated using the bootstrap test, with 2000 replicates. For bootstrap support, we considered bootstrap percentages of < 50% as poor, 50-70% as weak, 71-85% as moderate and > 85% as strong. Once again, preliminary standardization is required. Analyses were conducted at two different levels: 'specimen' level, where all specimens were used, and 'species' level, where an average specimen was calculated for each species, in which each character is the mean calculated from all the samples of this species.

Results

Data

Appendix 2 shows the original data matrix (146 × 26 quantitative values).

Table 1 provides the index of variability of the characters. The second series of figures shows the relative variability in relation to the genus ($Ir_i = Iv_i / Iv_B$). These values indicate that the measured characters are rather variable within any species. In some of them, the variability is almost as high as it is found in the entire genus: thus, the relative index value is 8.5% in *B. sarcodes*, and 5.5% in *B. leinigii*, while it is 12.8% for the entire genus.

Differentiating closely related taxa

The results relating to taxa differentiation, based on PCoA of morphometric data, are as follows.

B. albinoi-*B. riograndensis*. – The points that represent both taxa in a coordinate system given by the two most important eigenvectors show that these taxa are slightly but clearly different (Fig. 3A): PCO1 > 0 for *B. albinoi*, < 0 for *B. riograndensis*, without any separation according to axes PCO2 and PCO3. The percentage of variance explained by PCO1 is 49.5%, by PCO2 14.5% and by PCO3 9.5%. CVA, carried out keeping only the sixteen most significant variables (Fig. 3B), and DA (Fig. 3C) confirm the separation of these taxa ($p = 0.0454$).

Table 1. – Index of species variability.

Taxon	lv	lr
<i>Baptistonia</i> Barb. Rodr.	0,127609	100%
<i>B. albinoi</i> (Schltr.) Chiron & V. P. Castro	0,020243	16%
<i>B. brieniana</i> (Rchb. f.) V. P. Castro & Chiron	0,040814	32%
<i>B. cornigera</i> (Lindl.) Chiron & V. P. Castro	0,035279	28%
<i>B. cruciata</i> (Rchb. f.) V. P. Castro & Chiron	0,037516	29%
<i>B. damacenoii</i> Chiron & V. P. Castro	0,02495	20%
<i>B. echinata</i> Barb. Rodr.	0,012779	10%
<i>B. gutfreundiana</i> (Chiron & V. P. Castro) Chiron & V. P. Castro	0,050952	40%
<i>B. kautskyi</i> (Pabst) V. P. Castro & Chiron	0,050906	40%
<i>B. leinigii</i> (Pabst) V. P. Castro & Chiron	0,055026	43%
<i>B. lietzei</i> (Regel) Chiron & V. P. Castro	0,039239	31%
<i>B. lietzei</i> subsp. <i>guaiensis</i> Chiron	0,013179	10%
<i>B. nitida</i> (Barb. Rodr.) V. P. Castro & Chiron	0,028328	22%
<i>B. pabstii</i> (Campacci & C. Espejo) V. P. Castro & Chiron	0,01293	10%
<i>B. pubes</i> (Lindl.) Chiron & V. P. Castro	0,045854	36%
<i>B. riograndensis</i> (Cogn.) V. P. Castro & Chiron	0,030345	24%
<i>B. sarcodes</i> (Lindl.) Chiron & V. P. Castro	0,085362	67%
<i>B. silvana</i> (V. P. Castro & Campacci) V. P. Castro & Chiron	0,024236	19%
<i>B. truncata</i> (Pabst) Chiron & V. P. Castro	0,029734	23%
<i>B. uhlii</i> Chiron & V. P. Castro	0,044404	35%
<i>B. venusta</i> (Drapiez) Chiron	0,023854	19%
<i>B. widgrenii</i> (Lindl.) V. P. Castro & Chiron	0,021361	17%

[Abbreviations: lv = index of variability of a taxon; lr = relative value of lv]

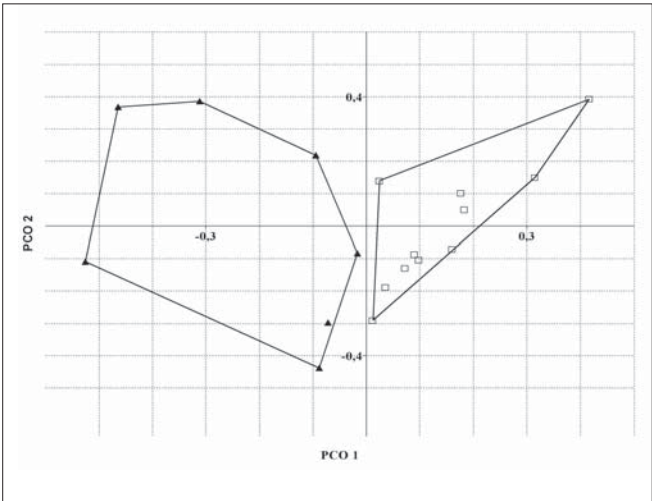


Fig. 3A. – Discrimination of *Baptistonia albinoi* (Schltr.) Chiron & V. P. Castro (□) and *B. riograndensis* (Cogn.) V. P. Castro & Chiron (▲) using morphometric data. Principal Coordinates Analysis with Manhattan distance (variance explained: PCO1: 49.5%; PCO2: 14.5%).

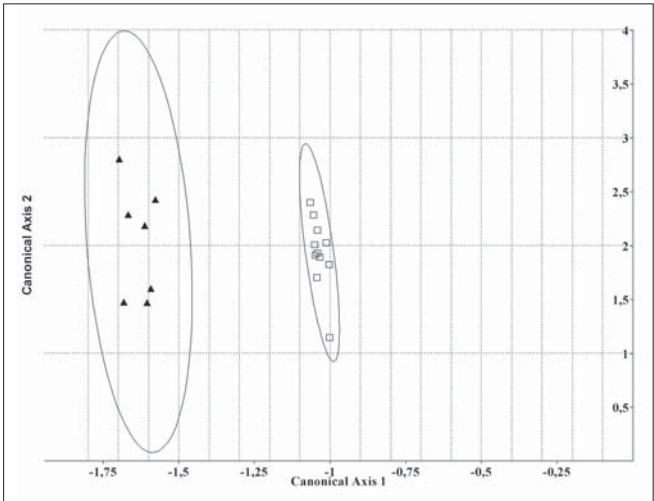


Fig. 3B. – Discrimination of *Baptistonia albinoi* (Schltr.) Chiron & V. P. Castro (□) and *B. riograndensis* (Cogn.) V. P. Castro & Chiron (▲) using morphometric data. Canonical Variates Analysis ($p = 0.0454$; ellipses = 95% confidence outlines; variance explained: PCO1: 99%; PCO2: 1%).

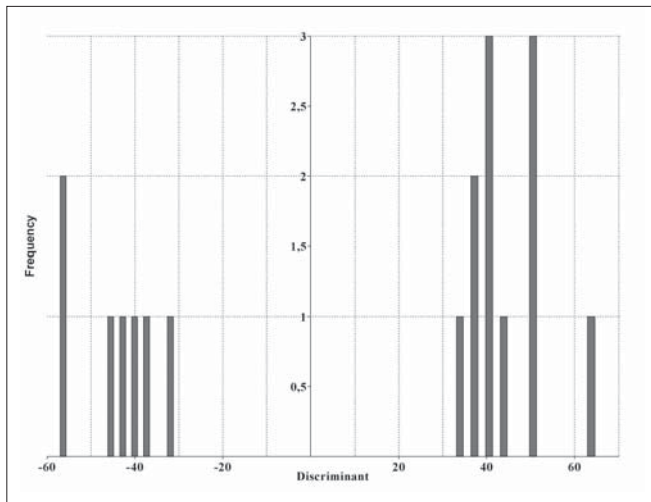


Fig. 3C. – Discrimination of *Baptistonia albinoi* (Schltr.) Chiron & V. P. Castro and *B. riograndensis* (Cogn.) V. P. Castro & Chiron using morphometric data. Discriminant Analysis.

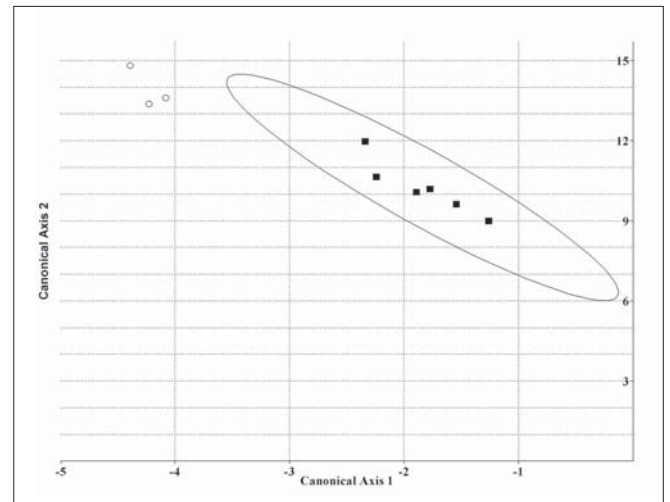


Fig. 4B. – Discrimination of *Baptistonia brieniana* (Rchb. f.) V. P. Castro & Chiron (■) and *B. widgrenii* (Lindl.) V. P. Castro & Chiron (○) using morphometric data. Canonical Variates Analysis ($p = 0.2$; variance explained: PCO1: 99%; PCO2: 1%).

B. brieniana-*B. widgrenii*. – These taxa are clearly distinguished in PCoA (Fig. 4A), with 70% of variance explained by PCO1, PCO1 < -0.25 for *B. widgrenii* and > -0.15 for *B. brieniana*. The CVA carried out keeping only the seven most significant characters confirms the separation of these taxa (Fig. 4B, $p = 0.2$).

B. pubes-*B. lietzei*-*B. damacenoii*. – PCoA clearly separates *B. pubes* from both other taxa (Fig. 5A), with 43% of the variance explained by PCO1, 10% by PCO2 and 7% by PCO3; *B. damacenoii* and *B. lietzei* are more slightly differentiated.

CVA carried out keeping all the variables confirms the separation between *B. lietzei* and each of the other two taxa (Fig. 5B, with p (for *damacenoii/lietzei*) = 0.000776, p (for *pubes/lietzei*) = 0.0111, p (for *damacenoii/pubes*) having failed). One *B. lietzei* sample (GC3128, bought in a Brazilian nursery under this name and from Salesopolis, SP, according to the vendor) is placed out of the 95% confidence ellipse of *B. lietzei* in an intermediate position between this ellipse and the ellipses of the other two species, without us being able to find an explanation.

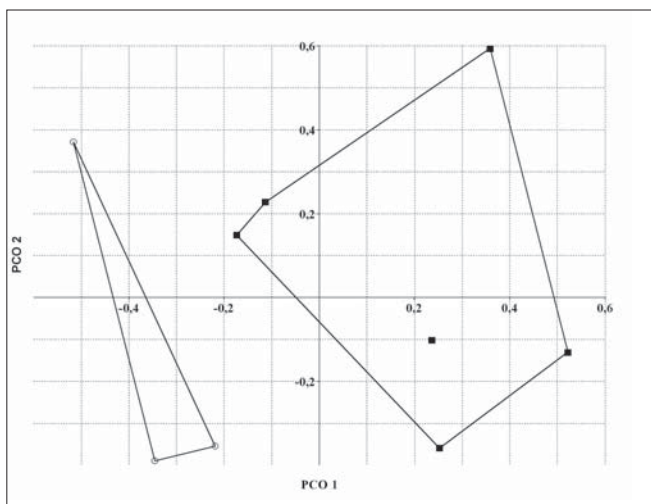


Fig. 4A. – Discrimination of *Baptistonia brieniana* (Rchb. f.) V. P. Castro & Chiron (■) and *B. widgrenii* (Lindl.) V. P. Castro & Chiron (○) using morphometric data. Principal Coordinates Analysis (variance explained: PCO1: 70%; PCO2: 9%).

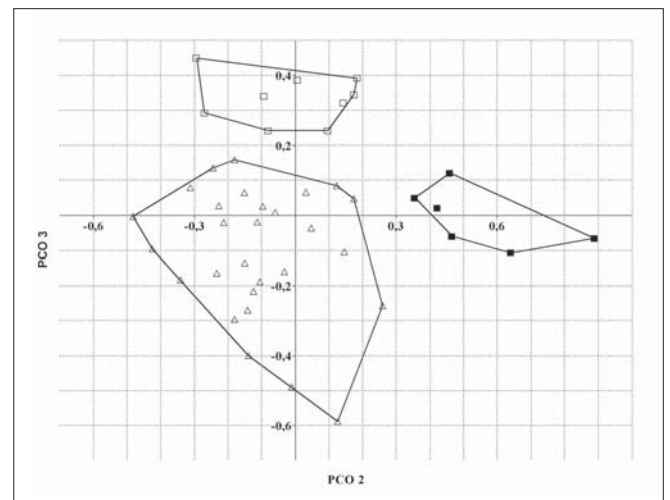


Fig. 5A. – Discrimination of *Baptistonia damacenoii* Chiron & V. P. Castro (□), *B. lietzei* (Regel) Chiron & V. P. Castro (△) and *B. pubes* (Lindl.) Chiron & V. P. Castro (■) using morphometric data. Principal Coordinates Analysis (variance explained: PCO2: 10%; PCO3: 7%).

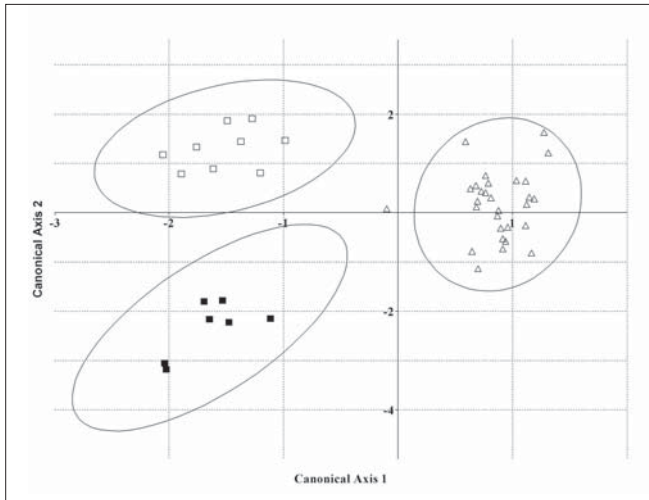


Fig. 5B. – Discrimination of *Baptistonina damacenoï* Chiron & V. P. Castro (□), *B. lietzei* (Regel) Chiron & V. P. Castro (△) and *B. pubes* (Lindl.) Chiron & V. P. Castro (■) using morphometric data. Canonical Variates Analysis [p (*damacenoï/lietzei*) = 0.000776; p (*pubes/lietzei*) = 0.0111; variance explained: PCO1: 80.5%; PCO2: 19.5%].

B. lietzei-*B. lietzei* subsp. *guairensis*. – The subspecies of *B. lietzei* from Villa Rica (Paraguay) is different from the Brazilian populations included in our study, from Rio de Janeiro (Nova Friburgo and Itatiaia), São Paulo (Serra do Japi, Águas da Prata, Cotia) and Parana states. The separation is weak in PCoA (Fig. 6A) and more strongly marked in CVA (Fig. 6B, $p = 0.058$).

B. cornigera-*B. fimbriata*. – PCoA failed to divide the thirteen samples into two different groups (Fig. 7 with 44% of the variance is explained by PCO1 and 22% by PCO2). It therefore supports the opinion that both names refer to one single species.

Tools for new specimen identification

For each pair of possibly confusing species, the discriminant and the offset value used to sort a new specimen within one of the species are shown (Tables 2a, 2b, 2c, 2d and 2e), respectively for the pairs *B. albinoi*-*B. riograndensis*, *B. brieni-ana*-*B. widgrenii*, *B. lietzei*-*B. damacenoï*, *B. pubes*-*B. lietzei*, *B. lietzei*-*B. lietzei* subsp. *guairensis*. The calculation of the discriminant for the pair *B. damacenoï*-*B. pubes* having failed, we are unable to propose for it such an identification tool.

Testing the hybrid nature of taxa

Along the first axis (PCO1) in the PCoA analysis of the data set for *B. riograndensis* and its “parents” *B. albinoi* and *B. cornigera* (Fig. 8), *B. riograndensis* is placed in an intermediate position. The variance percentage explained by PCO1 is 30%. The sample values along this axis show the following

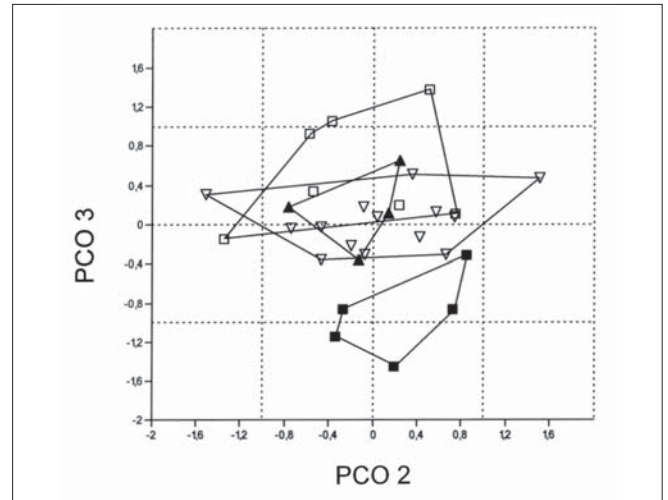


Fig. 6A. – Discrimination of various *Baptistonina lietzei* (Regel) Chiron & V. P. Castro populations using morphometric data (■: from Villa Rica (Paraguay), ▼: from PR, ▽: from SP, □: from RJ). Principal Coordinates Analysis (variance explained: PCO2: 8%; PCO3: 7%).

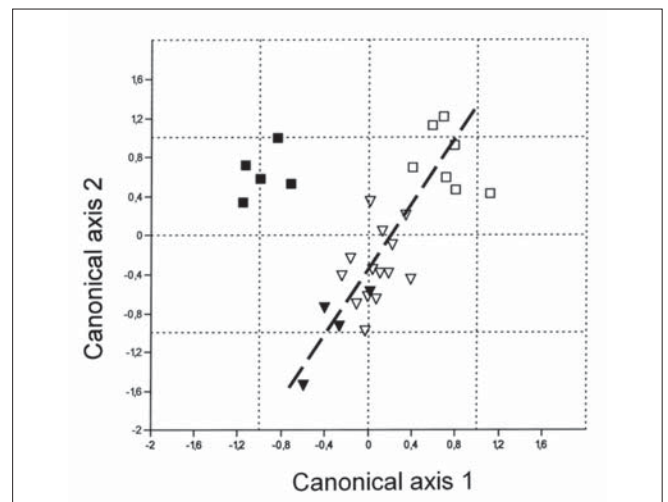


Fig. 6B. – Discrimination of various *Baptistonina lietzei* (Regel) Chiron & V. P. Castro populations using morphometric data (■: from Villa Rica (Paraguay), ▼: from PR, ▽: from SP, □: from RJ). Canonical Variates Analysis ($p = 0.058$).

means and standard deviations: for *B. albinoi*, 0.38 and 0.18, for *B. cornigera*, -0.62 and 0.16, and for *B. riograndensis*, 0.28 and 0.32. The values for one parent are clearly separate from the values for the other parent, with 95% confidence ellipses non overlapping. The *B. riograndensis* values are much more variable, as expected for an hybrid, and the 95% confidence ellipse is very large. Second and third PCO show no significant difference between the three species.

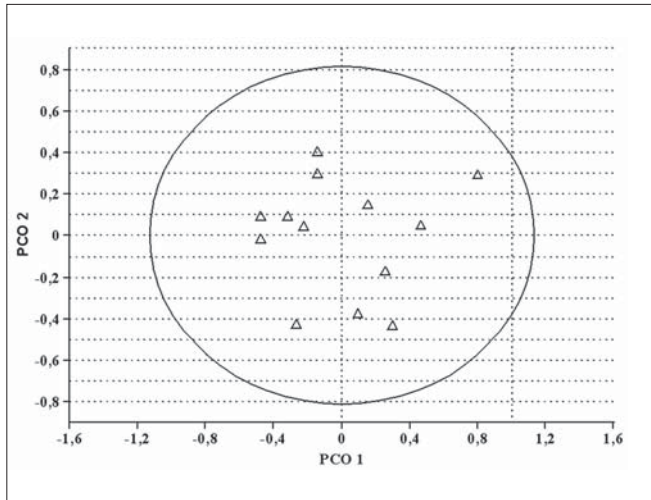


Fig. 7. – Non-discrimination of *Baptistonia cornigera* (Lindl.) Chiron & V. P. Castro samples using morphometric data. Principal Coordinates Analysis (variance explained: PCO1: 44%, PCO2: 22%; ellipse = 95% confidence outline).

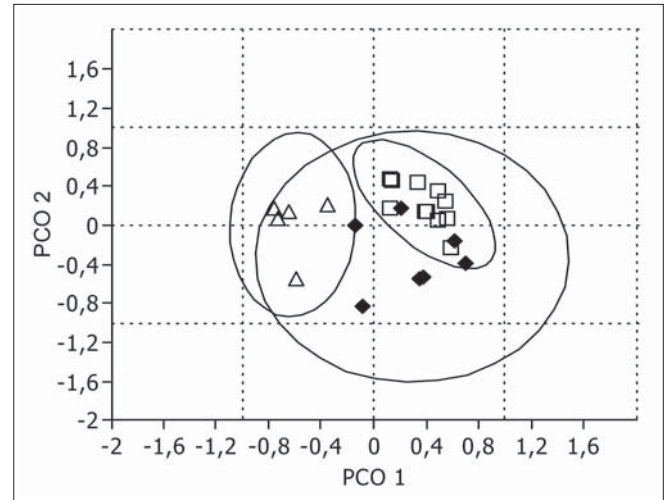


Fig. 8. – Discrimination of *Baptistonia albinoi* (Schltr.) Chiron & V. P. Castro (□), *B. cornigera* (Lindl.) Chiron & V. P. Castro (△) and *B. riograndensis* (Cogn.) V. P. Castro & Chiron (◆) using morphometric data. Principal coordinates analysis (variance explained: PCO1: 30%; ellipses = 95% confidence outline).

Table 2. – Results of discriminants data.

characters	a	b	c	d	e
A	-63.499	108.89	26.862	23.094	26.327
B			-45.691	35.489	3.172
C			-12.774	7.5658	26.227
D	15.705		-6.8154	12.287	14.004
E	152.16	44.585	14.663	7.518	-38.216
F	-93.085		-28.202	-8.8345	27.05
G	31.233		-20.742	-51.333	-27.692
H	-56.445	153.74	22.073	15.322	10.891
I	67.012		3.3322	7.1137	-28.962
J	7.639		6.5747	-16.612	2.542
K			11.899	-8.3692	1.712
L	-60.017	428.42	-13.485	-14.204	-21.771
M	-49.394		-6.9742	-26.49	-41.221
N	20.493	-163.51	18.951	-130.65	-19.396
O	13.549	-128.7	21.439	31.39	7.975
P	0.062	97.514	-14.573	69.216	4.221
Q	-95.211		0.65404	-1.1827	-6.768
R	-10.872		-0.26636	45.967	15.577
S			12.918	-13.325	11.262
T			-2.0562	-31.276	5.01
U	-73.579		3.8689	-29.998	-7.53
V			10.178	-1.8336	-6.88
W			12.578	19.552	-5.784
X			-15.636	32.454	2.485
Y			-0.60109	17.913	0.152
Z			-0.72319	0.8414	-0.656

[Abbreviations: **a** = *B. riograndensis*-*B. albinoi* (offset value: -748.59; result < 0: *B. riograndensis*; result > 0: *B. albinoi*); **b** = *B. widgrenii*-*B. brieniana* (offset value: 1055.98; result < 0: *B. widgrenii*; result > 0: *B. brieniana*); **c** = *B. damacenoii*-*B. lietzei* (offset value: 21.9632; result < 0: *B. lietzei*; result > 0: *B. damacenoii*); **d** = *B. pubes*-*B. lietzei* (offset value: 380.048; result < 0: *B. pubes*; result > 0: *B. lietzei*); **e** = *B. lietzei* subsp. *guaiensis*-*B. lietzei* (offset value: -192.093; result < 0: subsp. *guaiensis*; result > 0: Brazilian populations)]

Identically, *B. gutfreundiana* is in an intermediate position along axis 1 (42% of the variance being explained by PCO1) between its “parents” *B. cornigera* and *B. silvana*. The respective means and standard deviations are 0.01 and 0.34, 0.53 and 0.20, -0.68 and 0.30.

On the other hand, PCoA fails to clearly separate *B. damacenoii* from *B. cruciata* and *B. lietzei*, its presumed parents. The respective means and standard deviations of the PCO1 values (40% of the variance being explained by PCO1) are 0.016 and 0.12, 0.086 and 0.12, -0.03 and 0.17. The only discrimination is shown along axis 3. However the percentage of variance accounted for by PCO3 is very low (7%).

Phylogenetic relationships

In the NJ at ‘specimen’ level, it is not surprising given the variability, samples of a few taxa are mixed, and very poor bootstrap values are obtained regularly.

Data for the average specimens are shown in Table 3. At the ‘species’ level, NJ carried out with correlation coefficient brings out two moderately supported clusters: the pair *B. kautskyi*-*B. truncata* shows a 74% bootstrap support and the pair *B. pulchella*-*B. uhlii*, 73%. The bootstrap supports of the other clusterings are generally weak or poor: 51% for the group *B. pubes*-*B. lietzei*-*B. damacenoii*, 44% for the group *B. albinoi*-*B. brieniana*-*B. riograndensis* and 40% for the pair *B. echinata*-*B. sarcodes*, the remaining bootstrap values being even lower.

Discussion

Using a supertree-building method, CHIRON & al. (2009) combined results obtained from morphological characters, molecular data and floral oils composition, yielding rather clear relationships within the genus (although not entirely resolved). If we compare the relationships inferred from the morphometric study and the supertree topology, we observe that a few of them are compatible: the weakly to moderately supported groups exist in both topologies. However, the other relationships are too poorly supported in the morphometric analysis, as it is often the case (e.g. VAN DEN BERG, 1996). When looking at the variability index (Table 1), we realize that our morphometric data in several species are too variable to make it possible to infer reliable phylogenetic relationships throughout the genus. Thus, taking into account the topology obtained from morphometric data in the supertree-building method does not improve the final topology.

However, the analysis of the results obtained when excluding one or more variables shows that five morphometric data could be added to the characters set used in the morphological analysis to improve its result: column length, labellum length, maximum width of the median lobe of the labellum,

labellum width measured at the level of the lateral lobes (only the ratio between these two measurements was used), claw width measured at its mid-point.

CHIRON (2008) made the assumption that *B. damacenoii*, *B. gutfreundiana* and *B. riograndensis* are from hybrid origin, based on a careful observation of the floral traits. PCoA of morphometric data clearly supports this hypothesis for the two last-mentioned species. It proved unable to document the third case in spite of the intermediate position of *B. damacenoii*. Distances between each species are indeed lower than the samples dispersion: distance (*B. damacenoii*-*B. cruciata*) = 0.07 with a standard deviation of 0.17 and distance (*B. damacenoii*-*B. lietzei*) = 0.046 against 0.2. Finally morphometric test does not go in favour of the assumption nor against it.

As for the separation of taxa difficult to differentiate from a morphological point of view, the results obtained from the multivariate analyses of the morphometric data are fully operative (Fig. 3-6), in spite of a weak distinction of *B. damacenoii* and *B. lietzei* in PCoA.

The members of *B. lietzei* subsp. *guaiarensis* collected in the forests near Villa Rica, Paraguay, form a population too closely related to the Brazilian populations of this species to be easily distinguishable from them based on morphology, although sufficiently distinct to present morphometric differences (Fig. 6). These are mainly related to the pedicel length (8.9 mm vs. 6.5 mm for the Brazilian plants and the Paraguayan plants respectively), the shape of the lateral sepals (width/length ratio = 0.3 vs 0.36), of the dorsal sepal (0.68 vs 0.76), and of the lateral lobes of the labellum (width/length ratio = 0.34 versus 0.44). However, each individual morphological difference is weak and obviously not sufficient to guarantee a simple visual recognition. To separate the taxa it is best to use the discriminant proposed in Table 2. As Villa Rica is situated towards the South-West, more than 300 km far from the southern limit of the geographical range of *B. lietzei*, we are possibly witnessing a speciation process due to recent (i.e. late glacial period) geographical isolation, according to the refuge model (for a complete discussion of this model, see in particular HAFFER & PRANCE, 2002). Molecular and chemical data (CHIRON, 2008) also point out differences: five ISSR monomorph loci among the 183 loci observed are different; for the floral oils, the alkene and ester contents also show differences.

The other populations (from Parana, São Paulo and Rio de Janeiro states) are not separated by PCoA nor by CVA. In the latter (Fig. 6B), the variations seem to be continuous from Rio de Janeiro to Parana.

In order to check that the discriminant analysis of the data set relating to two groups of specimens is an effective tool, we used this method to ‘identify’ (in fact they were previously identified by other ways) four extra specimens (all of them

Table 3. – Specific average values of characters.

Species	Characters (A-Y [mm], Z [°])																										
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	
<i>B. albinoi</i>	12.46	6.5	3.5	11.5	2.85	2.38	11.95	4.91	3.54	9.09	0.91	5.78	2.13	9.93	4.45	9.99	2.11	2.67	2.21	1.76	3.95	1.55	2.02	4.38	2.83	89	
<i>B. brientana</i>	10.51	6.95	2.92	9.37	2.72	1.99	10.17	4.95	3.17	9.47	0.77	4.58	2.28	8.73	4.43	7.84	2.18	2.32	2.46	1.61	3.01	1.38	1.38	4.28	2.49	74	
<i>B. cornigera</i>	9.3	4.9	3.74	8.38	4.3	2.99	8.03	5.63	4.67	8.26	0.8	5.77	2.35	8.77	6.25	6.08	1.7	0.71	2.54	1.91	2.51	1.2	2.42	4.19	2.48	66	
<i>B. cruciata</i>	11.68	4.16	2.89	9.52	3.11	2.44	10.74	5.97	4.21	9.39	0.87	5.25	1.94	10.98	6.2	6.78	1.8	3.34	1.57	2.19	2.73	1.43	1.37	3.51	2.42	65	
<i>B. damacenoii</i>	12.14	6.29	2.88	8.92	2.71	1.81	10.53	7.42	4.47	8.83	0.93	5.06	1.9	12.96	7.89	7.18	1.65	3.81	2.28	2.4	2.53	1.46	2.05	3.36	2.97	92	
<i>B. echinata</i>	14.73	6.47	5.25	12.37	3.39	3.28	14.56	8.46	6.96	9.41	1.09	7.46	1.89	15.48	8.87	15.23	4.82	0	0	0	6.84	5.88	0	0	3.55	135	
<i>B. guiffrediiana</i>	10.11	5.33	2.53	7.56	2.83	2.02	9.04	6.1	3.71	8.03	0.91	4.76	2.52	10.06	7.77	6.23	2.22	2.95	1.92	1.63	1.77	1.32	0.89	1.5	3.07	98	
<i>B. kauskyi</i>	7.24	3.56	2.25	5.44	1.69	1.37	6.78	3.7	2.71	4.35	0.5	3.8	1.51	6.94	3.5	5.22	1.94	1.56	1.14	1.01	1.95	0.88	0.68	2.6	2.16	113	
<i>B. leingitii</i>	15.09	8.88	4.18	13.65	3.73	2.71	14.26	7.87	4.68	12.36	1.1	6.42	2.7	12.4	7.65	13.1	3.44	1.88	4.55	2.78	4.2	4.33	0.89	4.8	3.01	103	
<i>B. letzei</i>	12.58	7.27	3.32	9.55	2.84	2.07	11.16	7.65	4.38	8.92	0.96	5.23	2.16	11.12	6.75	7.64	1.87	3.21	1.86	2.18	2.98	0.99	1.91	3.77	3.78	86	
<i>B. letzei – guairi</i>	11.5	6.51	2.59	9.42	3.09	2.16	10.7	7.82	4.59	6.85	0.87	5.23	2.39	10.98	6.35	7.6	1.74	2.88	1.88	1.95	2.98	1.33	2.01	4.26	3.36	90	
<i>B. miida</i>	14.65	8.08	2.93	10.28	3.35	2.2	13.29	8.12	4.35	9.11	0.81	5.15	1.74	11.79	8.23	6.11	2.41	1.57	2.41	1.57	2.51	1.04	3.18	6	3.82	50	
<i>B. pabstii</i>	10.42	5	3.85	10.12	2.93	2.66	10	5.18	4.28	6.94	0.73	5.79	2.32	8.97	4.52	6.91	1.05	2.78	2.15	1.53	2.45	1.68	0.99	3.06	2.96	68	
<i>B. pubes</i>	13.57	5.85	2.81	10.77	2.27	1.67	12.82	6.75	4.14	11.21	0.99	5.12	2.6	13	6.74	8.26	1.81	3.29	2.76	2.44	2.79	1.55	35	4.58	3.57	84	
<i>B. pulchella</i>	10.75	6.39	4.53	9.88	2.33	1.86	11.04	3.84	2.82	10	0.88	5.77	3.86	8.9	4.59	5.63	1.95	0	0	0	1.8	3.72	0.76	2.58	3.68	107	
<i>B. riograndensis</i>	13.89	8.56	3.99	13.04	3.57	2.95	12.71	6.19	4.55	9.97	0.98	6.33	2.81	10.95	5.17	12.72	1.98	3.17	2.52	1.75	5	1.71	1.97	5.39	3.22	79	
<i>B. sarcodes</i>	17.51	12.94	8.13	12.63	5.96	3.9	13.34	11.58	8.03	17.89	1.54	7.35	1.76	18.31	18.81	11.04	5.6	0.3	2.7	2.32	2.94	2.9	2.31	2.41	4.83	93	
<i>B. silvana</i>	12.2	7.44	4.83	7.37	3.59	2.77	10.15	7.54	4.94	5.52	0.84	4.59	2.22	10.85	8.58	7.23	3.28	3.12	2.74	2.15	2.31	2.86	0	1.8	3.24	90	
<i>B. truncata</i>	8.73	4.03	2.12	6.57	1.78	1.35	7.78	4.4	2.8	6.32	0.71	4.62	1.79	9.43	4.17	6.23	2.99	3.01	1.99	2.04	2.05	1.24	0	1.66	2.12	125	
<i>B. uhligi</i>	11.5	6.79	4.97	9.69	2.24	2.03	10.92	4.27	3.48	13.84	0.76	7.05	3.47	10.56	5.82	7.37	2.95	0.6	3.38	1.81	2.1	3.33	1.23	4.27	4.48	94	
<i>B. venusta</i>	7.09	5.32	3.95	6.91	3.29	2.13	5.95	4.8	3.83	14.2	0.83	3.71	1.75	11.44	7.6	10.35	2.9	0.63	3.14	2.75	3.04	2.46	2.8	3.65	5.16	90	
<i>B. widgrenii</i>	13.19	9.15	3.39	8.97	4.09	2.93	11.28	7.65	4.72	8.6	0.89	5.44	2.63	12.5	6.88	10.62	2.62	2.89	2.66	2	3.73	1.8	2.04	4.42	2.59	76	

being preserved in LY as well) (see Table 2): *B. riograndensis* Chiron 07069 (against *B. albinioi*), *B. widgrenii* GC2243 (against *B. brieniana*), *B. damacenoii* GC3097 (against *B. lietzei*), *B. pubes* GC3036 (against *B. lietzei*), and *B. lietzei* subsp. *guaiensis* GC2695 (against *B. lietzei*, see Table 2). Each specimen was correctly identified.

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References

- AGAPOW, P.-M., O. R. BININDA-EMONDS, K. A. KRANDALL, J. L. GITTLEMAN, G. M. MACE, J. C. MARSHALL & A. PURVIS (2004). The impact of species concept on biodiversity studies. *Quart. Rev. Biol.* 79: 161-179.
- BATEMAN, R. M. & O. S. FARRINGTON (1989). Morphometric comparison of populations of *Orchis sitnia* Lam. (Orchidaceae) from Oxfordshire and Kent. *Bot. J. Linn. Soc.* 100: 205-218.
- BORDA, E. L., J. M. FELIX, V. N. SOLFERINI & J. SEMIR (2001). Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. *Amer. J. Bot.* 88: 419-428.
- BROWER, A. V. Z. (2006). Problems with DNA barcodes for species delimitation: ‘ten species’ of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperidae). *Syst. Biodivers.* 4: 127-132.
- BROWN, D. & P. ROTHERY (1993). *Models in biology: mathematics, statistics and computing*. John Wiley & Sons.
- CARDIM, D. C., L. A. CARLINI-GARCIA, M. MONDIN, M. MARTINS, E. A. VEASEY & A. ANDO (2001). Variabilidade intra-específica em cinco populações de *Oncidium varicosum* Lindl. (Orchidaceae, Oncidiinae) em Minas Gerais. *Revista Brasil. Bot.* 24: 553-560.
- CARLINI-GARCIA, L. A., C. VAN DEN BERG & P. S. MARTINS (2002). A morphometric analysis of floral characters in *Miltonia spectabilis* and *Miltonia spectabilis* var. *moreliana* (Maxillarieae: Oncidiinae). *Lindleyana* 17: 122-129.
- CHASE, M. W., L. HANSON, V. A. ALBERT, W. M. WHITTEN & N. H. WILLIAMS (2005). Life History Evolution and Genome Size in Subtribe Oncidiinae (Orchidaceae). *Ann. Bot. (Oxford)* 95: 191-199.
- CHIRON, G. (2007a). Phylogenetic analyses of the genus *Baptistonia* (Orchidaceae, Oncidiinae) sensu lato based on morphological characters. *J. Bot. Res. Inst. Texas* 1: 913-931.
- CHIRON, G. (2007b). Note sur la distribution géographique de *Baptistonia* Barbosa Rodrigues (Orchidaceae, Oncidiinae). *Richardiana* 7: 145-173.
- CHIRON, G. (2008). *Baptistonia* Barbosa Rodrigues (Orchidaceae, Oncidiinae) - Notes taxinomiques 2. *Richardiana* 8: 111-125.
- CHIRON, G. (2009). Notes sur l'évolution du genre *Baptistonia* (Orchidaceae, Oncidiinae). *Richardiana* 9: 101-118.
- CHIRON, G. (2010). *Les Baptistonia. Histoire naturelle et phylogénie*. Tropicalia.
- CHIRON, G. & V. P. CASTRO NETO (2004a). Contribution à la connaissance des orchidées du Brésil. III- Rétablissement du genre *Baptistonia* Barbosa Rodrigues. *Richardiana* 4: 109-120.
- CHIRON, G. & V. P. CASTRO NETO (2004b). Contribution à la connaissance des orchidées du Brésil. IV- Une nouvelle espèce de *Baptistonia* de Espírito Santo. *Richardiana* 4: 121-133.
- CHIRON, G. & V. P. CASTRO NETO (2005a). Révision du genre *Baptistonia* - 1. *Richardiana* 5: 113-128.
- CHIRON, G. & V. P. CASTRO NETO (2005b). Révision du genre *Baptistonia* - 2. *Richardiana* 5: 169-193.
- CHIRON, G. & V. P. CASTRO NETO (2006a). Révision du genre *Baptistonia* - 4. *Richardiana* 6: 1-30.
- CHIRON, G. & V. P. CASTRO NETO (2006b). Revision of the genus *Baptistonia* - 3. The “*Baptistonia brieniana*” complex. *Selbyana* 27: 34-43.
- CHIRON, G., R. P. OLIVEIRA, T. M. SANTOS, F. BELLVERT, C. BERTRAND & C. VAN DEN BERG (2009). Phylogeny and evolution of *Baptistonia* (Orchidaceae, Oncidiinae) based on molecular analyses, morphology and floral oil evidences. *Pl. Syst. Evol.* 281: 35-49.
- DAVIS, J. C. (1986). *Statistics and Data Analysis in Geology*. John Wiley & Sons.
- FISHER, R. A. (1936). The use of multiple measurements in taxonomic problems. *Ann. Eugenics* 7: 179-188.
- GOLDMAN, D. H., C. VAN DEN BERG & M. P. GRIFFITH (2004). Morphometric circumscription of species and infraspecific taxa in *Calopogon* R. Br. (Orchidaceae). *Pl. Syst. Evol.* 247: 37-60.
- GOWER, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 325-338.
- HAFFER, J. & G. T. PRANCE (2002). Impulsos climáticos da evolução na Amazônia durante o Cenozóico: sobre a teoria dos Refúgios da diferenciação biótica. *Estudos Avançados* 16: 175-206.
- HAMMER, O., D. A. T. HARPER & P. D. RYAN (2007). *PAST, Palaeontological Statistics, ver. 1.73*.
- HEY, J. (2001). *Genes, Categories and Species*. Oxford University Press.

- HONG-WA, C. (2008). Multivariate analyses of morphological characters of *Leptolaena Thouars* s.l. subgenera *Mediusella* and *Xerochlamys* (Sarcolaenaceae). *Bot. J. Linn. Soc.* 157: 559-574.
- HOTELLING, H. (1931). The generalization of Student's ratio. *Ann. Math. Statist.* 2: 360-378.
- JOLY, S. & A. BRUNEAU (2007). Delimiting Species Boundaries in *Rosa* Sect. *Cinnamomeae* (Rosaceae) in Eastern North America. *Syst. Bot.* 32: 819-836.
- MALLET, J. (2001). Concept of Species. In: LEVIN, S. A. (ed.), *Encyclopedia of Biodiversity* 5: 427-440.
- PABST, G. F. J. & F. DUNGS (1975). *Orchidaceae Brasilienses*. Brücke-Verlag Kurt Schmiersow.
- PODANI, J. & I. MIKLOS (2002). Resemblance coefficients and the horseshoe effect in principal coordinates analysis. *Ecology* 83: 3331-3343.
- QUEIROZ, K. DE (2007). Species concepts and species delimitation. *Syst. Biol.* 56: 879-886.
- ROHLF, F. J. (1990). Morphometrics. *Annual Rev. Ecol. Syst.* 21: 299-316.
- SELIN, E. (2000). Morphometric differentiation between populations of *Papaver radicum* (Papaveraceae) in northern Scandinavia. *Bot. J. Linn. Soc.* 133: 263-284.
- SHAFFER, H. B. & R. C. THOMSON (2007). Delimiting species in recent radiations. *Syst. Biol.* 56: 896-906.
- SITES, J. W. & J. C. MARSHALL (2003). Delimiting species: a Renaissance issue in systematic biology. *Trends Ecol. Evol.* 18: 462-470.
- SPOONER, D. M., D. FAJARDO & G. J. BRYAN (2007). Species limits of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes and the implications for species boundaries in *Solanum* sect. *Petota*. *Taxon* 56: 987-999.
- TYTECA, D. & M. DUFRÈNE (1994). Biostatistical Studies of Western European Allogamous Populations of the *Epipactis helleborine* (L) Crantz Species Group (Orchidaceae). *Syst. Bot.* 19: 424-442.
- VAN DEN BERG, C. (1996). *Estudo dos padrões de variabilidade intra e interspecífica em espécies brasileiras de Cattleya Lindley (Orchidaceae-Laeliinae)*. Unicamp, Campinas, SP (Brazil).
- WHEELER, Q. D. & R. MEIER (ed.) (2000). *Species concepts and phylogenetic theory: a debate*. Columbia University Press.
- ZILINSKAS, A. & J. ZILINSKAS (2006). On Multidimensional Scaling with Euclidian and City Block Metrics. *Technol. Econ. Development. Econ.* 12: 69-75.

Appendix 1. – List of samples studied.

Except where otherwise stated, all specimens are from Brazil.

Conventions for collectors: A = Vitorino Paiva Castro Neto, GC = Guy Chiron, JBL = Lyon Botanical Garden, JBSP = São Paulo Botanical Garden

All vouchers are deposited in LY.

Baptistonia albinoi (Schltr.) Chiron & V. P. Castro: GC2578, near Cotaes; A156, Valinho; A245, cult., s.l.; A118, cult., s.l.; A160, cult., s.l.; A162, cult., s.l.; A164, Santo André (SP); A165, cult., s.l.; A167, cult., s.l.; A246, Tapirai (SP); A247, Tapirai (SP); A248, Tapirai (SP).

B. brieniana (Rchb. f.) V. P. Castro & Chiron: *Castro Neto s.n.*, Villa Rica (Paraguay); GC2676, Villa Rica (Paraguay); A121, Paraguay; A122, Paraguay; A123, Paraguay; *Taborda s.n.* ex A114, Argentine.

B. cornigera (Lindl.) Chiron & V. P. Castro: GC2233, Maresias (SP); GC2456, Iguazu (PR); GC2457, Iguazu (PR); GC2460, Iguazu (PR); GC2462, Iguazu (PR); GC2846, Guarau (SP); GC3051, Japi (SP); GC3052, Japi (SP); GC3062, beach (SP); GC3077, cult., s.l.; GC3119, Porto Alegre (RS); GC3127, Porto Alegre (RS); GC3138, Sorrocaba (SP).

B. cruciata (Rchb. f.) V. P. Castro & Chiron: GC2876, Nova Friburgo (RJ); GC2866, Nova Friburgo (RJ); GC2867, Nova Friburgo (RJ); A116, cult. (SP); GCA159, Santo André (SP); A169, cult. (SP); *JBL s.n.*, cult., s.l.

B. damacenoii Chiron & V. P. Castro: GC2589, near Domingos Martins (ES); *Nego s.n.*, cult. (ES); A192, cult. (ES); A193, cult. (ES); A194, cult. (ES); A196, cult. (ES); A197, cult. (ES); A199, cult. (ES); A198, cult. (ES).

B. echinata Barb. Rodr.: *Campacci s.n.* ex GC2582, s.l.; GC3044, plagas (SP); GC3055, Cotia (SP); *Vico s.n.* ex GC3063, cult., s.l.

B. gutfreundiana (Chiron & V. P. Castro) Chiron & V. P. Castro: *Castro Neto s.n.*, Pau Brasil (BA); GC2781, cult., s.l.; GC2914, cult., s.l.; GC2952, Camacã (BA); *Vico s.n.* ex GC3037, cult., s.l.; A125, s.l. (BA).

B. kautskyi (Pabst) V. P. Castro & Chiron: GC2576, near Domingos Martins (ES); GC2572, near Domingos Martins (ES); GC2694, near Corrego da Fortuna (ES); GC2740, near Corrego da Fortuna (ES); GC2743, near Corrego da Fortuna (ES); GC3006, near Corrego da Fortuna (ES); *Frey1079*, near Venda Nova (ES).

B. leinigii (Pabst) V. P. Castro & Chiron: GC3152, Serra do Mulato (PR); GC3115, S. do Mulato (PR); GC3117, S. do Mulato (PR); GC3133, S. do Mulato (PR); A126, cult. (PR).

B. lietzei (Regel) Chiron & V. P. Castro: GC2273, cult., s.l.; A031, cult., s.l.; GC2241, cult., s.l.; *Vico s.n.* ex GC3034, cult., s.l.; *Vico s.n.* ex GC3036, cult., s.l.; A200, Nova Friburgo (RJ); A201, Nova Friburgo (RJ); A202, Nova Friburgo (RJ); A203, Nova Friburgo (RJ); A257, Itatiaia (RJ/SP); A259, Itatiaia (RJ/SP); A211, Cotia (SP); A212, Cotia (SP); A258, Cotia (SP); A53, Cotia (SP); A213, Jundiá (SP); A214, Jundiá (SP); A215, Serra do Japi (SP); A216, S. do Japi (SP); A206, Agua Prata (SP); A207, Agua Prata (SP); A208, Agua Prata (SP); A209, Agua Prata (SP); A210, Agua Prata (SP); A204, s.l. (PR); A205, s.l. (PR); A217, Cornelio Procopio (PR); GC3128, Serra do Mulato (PR).

B. lietzei subsp. **guaiensis** Chiron: GC2656, Villa Rica (Paraguay); GC2657, Villa Rica (Paraguay); GC2675, Villa Rica (Paraguay); GC2693, Villa Rica (Paraguay); GC2703, Villa Rica (Paraguay).

B. nitida (Barb. Rodr.) V. P. Castro & Chiron: *Nego s.n.* ex GC2597, cult. (ES); *Nego s.n.* ex GC2591C, cult. (ES); *Nego s.n.* ex GC2591E, cult. (ES); *Nego s.n.* ex GC2596, cult. (ES); *Dominguez s.n.* ex GC3100, cult. (ES); A002, s.l.

B. pabstii (Campacci & C. Espejo) V. P. Castro & Chiron: *Régent s.n.* ex GC3042, cult. (RJ); *Régent s.n.* ex GC3059, cult. (RJ).

B. pubes (Lindl.) Chiron & V. P. Castro: *Régent s.n.* ex GC3053, cult. (RJ); *Régent s.n.* ex GC3046, cult. (RJ); *Régent s.n.* ex GC3054, cult. (RJ); *Vico s.n.* ex GC 3038, cult., s.l.; *Vico s.n.* ex GC3040, cult., s.l.; *Vico s.n.* ex GC3048, cult., s.l.

B. pulchella (Regel) Chiron & V. P. Castro: GC2882, Nova Friburgo (RJ)

B. riograndensis (Cogn.) V. P. Castro & Chiron: GC3130, Forromecco (RS); GC3114, Forromecco (RS); GC3134, Forromecco (RS); GC3137, Forromecco (RS); A430, Forromecco (RS); *JBSP46*, Gramado (RS).

B. sarcodes (Lindl.) Chiron & V. P. Castro: GC2242, cult., s.l.; *Binot s.n.* ex GC2255, cult., s.l.; GC2683, Guaratuba (SP); GC3079, Serra de Itaperai (SP); GC3121, Serra do Mulato (PR).

B. silvana (V. P. Castro & Campacci) V. P. Castro & Chiron: GC2942, Serra Boa (BA); GC2922, Serra Boa (BA); GC2949, Serra Boa (BA); *Régent s.n.* ex GC3049, cult., s.l. (BA).

B. truncata (Pabst) Chiron & V. P. Castro: GC2262, cult., s.l.; GC2769, cult., s.l.; *Lauro s.n.* ex GC2899A, near Nova Friburgo (RJ); *Lauro s.n.* ex GC2899B, near Nova Friburgo (RJ).

B. uhlii Chiron & V. P. Castro: GC2689, near Pedra Azul (ES); GC2689A, near Pedra Azul (ES); GC3093, near Pedra Azul (ES).

B. venusta (Drapiez) Chiron: *Teobaldo s.n.* ex GC0108, cult. (RS); *Teobaldo s.n.* ex GC0109, cult. (RS); *Julio s.n.* ex GC0107, cult. (RS); *JBSP7028* ex GC0110, Mariana (MG).

B. widgrenii (Lindl.) V. P. Castro & Chiron: GC2577, sud MG; GC2581, sud MG; GC2574, sud MG.

Appendix 2. – Measurements of the 26 characters in the samples.

[Abbreviations: Samples: the first three letters stand for the species name]

Samples	Characters																									
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
alb2578	13.52	7.21	3.86	12.56	2.9	2.5	12.88	5.31	3.95	10.09	0.81	5.42	2.09	11.35	4.95	10.93	2.6	2.47	2.51	1.79	3.98	2.29	1.21	4.3	2.62	85
albA156	13.38	6.56	3.21	13.51	2.97	2.61	13.51	4.82	3.68	11.64	1.14	6.15	2.27	10.57	4.35	9.36	2.14	2.34	2.34	1.81	4.35	1.4	2.47	2.88	2.62	114
albA245	12.37	7.36	4	12.04	3.2	2.7	12.04	5.35	4.15	8.54	0.79	5.62	2.01	9.57	4.01	11.3	2.14	2.8	2.47	2.27	4.34	1.67	2.27	5.01	1.71	86
albA246	11.77	5.35	2.81	10.7	2.07	1.99	10.84	3.75	2.5	9.77	0.6	4.68	1.58	9.1	4.01	9.16	1.55	3.01	1.14	1.07	4.45	1.2	2.01	5.55	2.29	70
albA247	12.91	7.61	4.1	10.73	3.53	2.48	12.63	5.14	3.28	6.78	1.03	5.66	2.25	9.98	5.19	10.56	2.1	2.42	1.75	1.72	4.46	1.53	2.13	3.78	3.13	98
albA118	12.36	5.5	2.9	11.15	2.64	2.42	12.35	4.62	3.99	10.38	1	6.17	2.4	8.75	3.61	9.42	2.12	2.91	1.89	1.61	3.3	1.47	1.78	3.83	3.11	87
albA160	11.44	4.41	3	10.11	2.89	2.13	10.21	4.55	2.58	8.66	0.86	6.01	2	9.62	3.82	8.9	2.05	2.84	2.07	1.42	3.4	1.29	2.03	5.1	2.52	74
albA162	12.24	6.76	3.59	11.79	2.63	2.31	11.37	4.81	3.31	10.51	0.82	5.98	2.1	9.93	4.91	9.46	2.37	2.42	2.58	2	3.33	1.35	2.18	4.48	3.19	73
albA165	12.54	7.03	3.75	11.01	2.91	2.47	11.98	5.63	4.23	8.05	0.91	5.85	2.21	9.97	5.32	10.48	1.94	3.09	2.28	2.05	4.05	1.26	2.26	3.97	2.61	99
albA167	11.45	6.53	3.56	10.35	2.68	2.08	11.25	4.84	3.28	9.09	1.11	5.99	2.49	9.66	3.95	10.22	2	2.44	3.09	1.83	3.75	1.66	2	4.74	4.03	94
albA248	13.09	7.18	3.72	12.57	2.95	2.44	12.43	5.18	3.94	6.43	0.99	6	2.07	10.72	4.88	10.1	2.24	2.67	2.23	1.78	4	1.94	1.89	4.56	3.33	94
brivPCN	12	8.33	3.43	10.2	3.21	2.5	11.42	5.9	4.4	9.1	0.81	4.81	2.38	10.39	4.11	8.68	2.78	2.94	3	2.22	3.06	1.78	1.81	4.72	2.39	85
brvA121	8.15	4.8	2.63	7.21	1.78	1.48	7.5	3.79	2.63	7.09	0.72	4.32	2.1	7.56	3.48	7.19	1.84	2.18	2.21	1.45	2.8	1.1	0.97	4.48	2	62
brvA122	12.1	8.26	3.3	10	2.85	2.15	12.1	5.66	3.35	16.37	1.01	4.8	2.82	9.08	5.98	8.9	2.31	2.62	3.5	2.03	3.15	1.34	1.7	4.21	2.3	78
brvA123	9.62	6.29	2.66	8.75	2.28	1.59	9.12	4.1	2.79	8.83	0.77	4.62	2.39	9.04	4.68	8.12	2.36	2.35	2.12	1.5	3.2	1.5	1.3	3.92	2.9	80
brv2676	9.14	6.45	2.87	8.68	3.94	2.84	8.5	5.06	2.78	5.91	0.88	4.64	2.46	7.6	3.93	6.33	2.06	2.08	2.04	1.44	2.78	1.27	0.98	3.87	2.43	76
brvA114	12.02	7.56	2.64	11.37	2.26	1.4	12.36	5.21	3.04	9.49	0.43	4.28	1.54	8.73	4.37	7.83	1.75	1.75	1.91	0.99	3.08	1.27	1.5	4.5	2.91	60
cor2233	9.61	5.26	3.74	8.73	5.35	3.57	8.24	5.85	4.65	8.76	0.83	5.72	2.43	9.01	6.86	6.84	1.6	0.92	2.57	1.8	2.9	1.26	2.36	3.86	2.97	70
cor2456	9.17	4.49	4.01	8.03	2.52	2.04	7.37	4.73	4.19	8.39	0.78	5.57	2.04	8.99	5.57	5.49	1.86	0.6	2.52	1.92	2.22	1.26	2.58	4.91	1.6	60
cor2457	8.41	4	3.04	8.01	3.6	2.4	8.18	5.13	4.4	6.58	0.72	6.09	2.48	7.68	5.52	4.39	1.8	0.36	2.36	2.07	1.62	0.9	1.98	3.54	2	67
cor2460	9.26	5.23	4.03	7.76	4.09	2.89	8.36	7.22	5.95	6.98	0.78	5.77	2.41	8.78	5.89	6.93	1.68	0.48	3.28	2.63	2.53	1.2	3.13	5.08	3.1	63
cor2462	9.83	5.84	4.35	9.11	5.64	4.15	8.27	6.94	6.04	10.86	0.78	6.29	2.54	8.86	6.69	5.19	1.49	0.6	1.26	1	2.98	1.41	2.22	4.3	2.5	60
cor2846	9.55	5.18	3.96	8.66	4.65	3.3	7.99	5.74	4.81	9.25	0.81	5.82	2.35	8.97	6.48	6.03	1.65	0.77	2.2	1.6	2.74	1.3	2.36	4.23	2.45	65
cor3051	9.39	4.81	3.62	8.37	4.31	2.87	7.94	5.11	4.03	7.93	0.83	5.48	2.27	9.05	6.49	6.94	1.73	0.92	2.99	2.11	2.65	1.21	2.48	4.06	2.67	70
cor3052	9.14	4.65	3.3	8.37	4.67	2.99	8.21	5.24	4.1	7.33	0.81	5.66	2.41	8.61	6.47	6.57	1.71	0.84	2.94	2.16	2.45	1.09	2.28	3.61	2.8	73
cor3062	9.36	4.92	3.73	8.22	4.25	2.87	8.05	5.64	4.51	7.7	0.82	5.56	2.3	8.98	6.34	6.94	1.72	0.81	3.06	2.24	2.62	1.21	2.64	4.32	2.78	69
cor3077	9.11	4.4	3.29	8.46	4.14	2.7	7.88	4.41	3.52	7.8	0.81	5.59	2.29	8.68	6.36	6.09	1.77	0.88	2.68	1.92	2.35	1.11	2.1	3.55	2.3	72
cor3119	9.52	5.35	4.19	8.5	4.47	3.31	8.07	6.46	5.56	9.27	0.78	5.98	2.38	8.87	6.21	5.7	1.63	0.57	2.08	1.64	2.68	1.32	2.54	4.65	2.42	61
cor3127	8.95	4.57	3.69	7.93	3.4	2.44	7.97	5.69	4.85	7.32	0.76	5.81	2.31	8.48	5.66	5.6	1.78	0.48	2.72	2.21	2.12	1.12	2.56	4.51	2.23	63
cor3138	9.58	5.01	3.73	8.82	4.82	3.29	7.91	5.02	4.06	9.23	0.93	5.66	2.31	9.08	6.75	6.36	1.66	0.96	2.32	1.57	2.8	1.28	2.18	3.8	2.47	69
cru2876	14.88	5.63	3.81	11.79	3.14	2.63	14.5	6.75	4.64	12.09	0.99	5.42	1.64	13.3	7.87	7.6	1.95	4.04	1.47	2.57	2.76	1.47	2.05	4.17	2.4	65
cruA116	9.45	3.25	2.71	8.56	4.27	3.5	7.41	5.34	4.38	7.44	0.82	4.93	1.78	8.65	4.67	6.06	1.66	3.12	1.26	1.8	2.99	1.36	0.62	3.6	2.3	48
cruA159	9.14	3.18	3.16	7.85	2.6	2.16	9.28	4.67	4	8.63	0.88	4.99	1.97	8.78	5.33	5.64	1.36	2.69	1.35	2.86	2.44	1.5	1.12	3.07	2.7	68
cruA169	9.81	3.89	2.37	7.81	2.73	2.05	9.06	5.13	3.96	6.43	0.91	5.29	2.16	10.06	5.51	7.44	1.68	3.28	1.55	2.53	2.67	1.62	0.9	3.46	1.89	69
cruJBL	13.44	4.51	2.44	9.33	3	2.33	11.8	6.8	3.55	9.7	0.89	4.73	1.79	12.2	6.82	6.89	1.78	4.22	1	1.56	3	0.89	1.56	4	2.5	68
cru2866	13.96	5	3.04	11.85	2.98	2.32	12.74	6.93	4.78	13.15	0.91	5.48	1.91	13.17	7.12	7.04	1.88	3.49	2.9	1.95	2.64	1.63	1.72	2.9	2.1	72
cru2867	11.07	3.64	2.69	9.46	3.07	2.09	10.4	6.2	4.17	8.32	0.72	5.88	2.36	10.67	6.05	6.82	2.3	2.51	1.48	2.06	2.64	1.56	1.61	3.39	3.02	68
damA192	13.12	7.93	3.71	9.44	3.64	2.25	11.84	9.23	5.14	8.57	1.18	4.66	2.26	14.22	8.56	7.05	1.73	4.32	1.93	2.77	2.96	1.31	2.23	3.2	2.51	92
dam2589	10	5.32	2.15	6.71	2.02	1.33	8.23	5.38	4	11.25	0.45	4.39	1.86	11.4	7.59	5.98	1.9	2.9	1.88	2.37	2.03	1.52	2	3.2	3.03	92

Appendix 2 (cont.). – Measurements of the 26 characters in the samples.

[Abbreviations: Samples : the first three letters stand for the species name]

Samples	Characters																									
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
damNego	10.8	5.32	2.29	7.77	2.76	1.91	9.62	6.72	4.37	6.02	0.79	4.75	1.72	10.77	7.63	5.8	1.9	3.42	2.39	1.58	1.92	1.37	2.12	3.49	2.33	91
damA193	13.2	6.87	2.65	8.23	2.36	1.75	11.83	8.52	4.83	11.12	0.96	5.22	1.73	14.45	8.47	8.04	1.53	4.6	2.3	2.69	2.74	1.41	2.3	4.15	3	94
damA194	11.14	5.3	2.61	9.35	2.4	1.66	8.87	7.16	3.95	8.14	1	5.38	2.12	11.97	7.45	7.1	1.63	3.92	2.56	2.29	2.37	1.18	1.93	3.41	3.87	90
damA196	11.82	5.76	2.88	9.25	2.8	1.57	10.23	6.45	4.29	8.14	0.92	4.88	1.75	11.48	6.02	5.94	1.24	3.53	1.75	2.04	2.5	1.5	1.6	2.8	3	94
damA197	12.1	5.78	2.2	9.42	2.52	1.56	10.63	6.67	3.79	7.12	1	4.78	1.71	12.29	6.51	7.7	1.27	3.75	2.64	2.64	2.56	1.31	2	3.65	3.11	90
damA199	13.6	7.67	4.22	10.61	2.95	2.05	11.88	8.78	5.5	9.76	1.08	5.46	1.9	14.83	9.43	7.08	1.72	4.36	2.37	2.54	2.59	1.37	1.6	2.2	3.2	90
damA198	13.5	6.66	3.25	9.48	2.98	2.2	11.66	7.83	4.96	9.37	0.99	5.99	2.05	15.25	9.36	9.9	1.91	3.5	2.7	2.7	3.13	2.2	2.1	3.75	2.65	97
ech2582	12.3	5.06	4.46	11.25	3.27	3.15	12.1	6.9	6.41	9.68	1.05	7.46	1.73	12.43	7.36	11.5	3.58	0	0	0	5.3	4.35	0	0	3.53	130
ech3044	15.55	6.8	4.94	12.95	3.15	2.99	15.72	8.7	6.63	9.43	0.98	6.8	1.82	16.04	9.61	15.73	5	0	0	0	7.24	5.88	0	0	3.06	137
ech3055	15.83	7.43	5.4	12.35	3.16	3.06	15.77	8.63	6.65	10.29	1.05	8.22	2.12	16.76	9.87	16.32	5.38	0	0	0	7.23	6.47	0	0	3.86	138
ech3063	15.23	6.59	6.18	12.91	3.96	3.9	14.65	9.61	8.15	8.25	1.27	7.37	1.89	16.68	8.62	17.38	5.3	0	0	0	7.6	6.83	0	0	3.76	136
gufVPCN	8.96	4.93	2.3	5.62	2.44	1.96	7.39	5.49	3.14	11.13	0.97	4.48	1.48	8.6	7.21	5.36	2.12	2.4	0.75	1.16	1.9	1.11	1.17	0.76	3.87	105
guf2781	11.06	5.47	2.46	9.1	2.5	1.82	8.99	7	5.2	8.03	0.85	4.16	2.26	10.77	8.02	6.48	3.56	3.27	2.44	1.68	1.63	1.09	1.26	1.68	3.23	95
guf2914	9.4	4.9	2.11	8.3	2.48	1.88	9.05	5.63	3.58	5.44	0.84	5.08	1.8	9.82	7.66	5.75	1.48	3.31	2.06	1.38	2	1.19	1.02	1.94	3.72	96
guf2952	10.8	5.6	2.46	7.76	3.11	2.17	10.69	6.7	3.71	6.65	0.85	5.26	1.98	10.66	8.13	5.82	1.69	3.18	2.38	1.94	1.7	1.37	0.3	1.41	2.37	91
guf3037	12	6.4	3.41	7.52	3.19	2.48	9.7	6.72	4.03	9.35	1.05	5.74	5.1	11.86	9.3	8.58	2.24	3.29	1.86	2.26	1.97	1.72	0.92	2	3.3	95
gufA125	8.44	4.67	2.44	7.06	3.25	1.8	8.43	5.08	2.6	7.58	0.91	3.86	2.52	8.65	6.32	5.37	2.2	2.25	2.03	1.34	1.44	1.46	0.68	1.2	1.94	107
kau2576	5.8	3.25	2.45	4.45	1.84	1.71	5.1	3.7	2.35	5	0.5	3.5	1.44	5.1	2.7	2.84	2	1.15	1.14	0.88	0.89	0.85	0.5	1.07	2.86	135
kau2572	6.71	3.42	2.14	4.52	2.14	1.55	6	3.57	2.62	5.44	0.64	4.59	0.93	6.48	3.19	4.56	2.24	1.19	1.17	0.92	1.49	0.86	0.6	1.75	2.05	108
kau2694	9.24	4.3	2.56	7.1	1.8	1.5	8.31	4.22	2.93	4.17	0.62	4.67	2.03	9	4.92	7.07	2.86	2.08	1.23	0.93	2.85	0.92	0.7	2.9	2.04	126
kau2740	6.4	3.31	2.06	5.76	1.25	0.91	6.4	3.6	3.4	3.25	0.34	2.93	1.58	6.2	3.23	3.84	0.93	1.35	0.83	0.67	1.5	1.1	0.81	2.5	1.65	106
kau2743	6.97	4.31	2.55	5.14	1.92	1.58	6.35	3.99	2.83	3.9	0.67	3.96	1.59	7.67	4.08	6.68	2.13	1.62	0.96	0.89	2.3	1	0.91	4.23	2	93
kau3006	6.8	2.86	1.96	4.89	1.28	1.21	7.12	3.28	2.58	3	0.33	3.21	1.27	6.6	2.63	4.76	1.53	1.87	1.38	1.36	1.81	0.65	0.71	2.5	2.57	120
kau1079	8.75	3.45	2.01	6.19	1.6	1.16	8.16	3.57	2.29	5.71	0.42	3.75	1.72	7.56	3.76	6.8	1.91	1.67	1.25	1.43	2.83	0.79	0.5	3.28	1.98	103
lei3152	14.99	7.99	3.77	13.49	3.16	2.23	14.97	7.34	4.58	10.33	0.94	6.91	2.63	12.45	7.71	13.6	3.59	2.11	6.06	3.83	4.12	3.58	1.4	5.11	2.42	101
lei3115	16.66	9.63	4.6	14.55	4	3.33	14.96	9.11	6.06	13.76	1.11	6.44	3.14	13.51	7.33	12.72	4.3	1.86	3.99	2.84	4.19	5.29	1.26	6.4	2.52	103
lei3117	15.07	8.61	4.57	14.52	4.28	2.96	14.97	8.19	4.92	11	1.18	6.45	2.45	12.46	8	12.34	3.86	1.72	4.03	2.23	4.2	4.45	0.9	3.72	3.3	104
lei3133	17.62	11.05	4.85	16.75	4.68	3.5	17.21	9.75	5.47	19.14	1.35	7.06	2.95	13.07	8.82	15.45	2.72	1.23	4.16	2.72	5.08	5.55	0.4	5.63	4.81	103
leiA126	11.11	7.12	3.11	8.93	2.55	1.53	9.19	4.97	2.35	7.56	0.94	5.23	2.34	10.49	6.41	11.39	2.74	2.5	4.52	2.26	3.4	2.8	0.5	3.16	2	104
lie2273	15.11	9.62	3.83	11.2	3.72	2.41	14.29	12.33	5.11	9.92	0.91	4.48	2.5	14.06	8.04	10.9	2.41	4.89	2.63	2.78	3.83	0.98	1.9	4.11	4.3	68
lieA031	12.76	7.75	2.55	8.88	2.96	2.24	9.49	5.88	3.88	10.4	1.03	4.48	2.71	11.33	6.07	6.53	2.14	3.06	1.05	1.82	2.65	0.61	2.45	4.6	4	58
lieA200	11.9	7.58	3.69	9.69	2.32	1.84	11.05	9.17	4.89	9.21	0.94	5.55	1.92	10.69	6.27	6.8	1.66	3.4	1.27	2.35	2.96	1.18	1.98	2.8	3.6	91
lieA201	14.04	8.06	3.69	12.27	2.4	1.98	13.96	7.6	4.38	10.19	1.02	5.03	2.11	13.07	8.03	9.5	1.6	3.41	1.91	2.1	4.18	1.72	2.14	5.3	5.09	100
lieA202	14.28	9.02	3.37	10.66	3.75	2.2	12.58	8.69	4.46	12.21	1.1	5.53	1.89	11.76	7.5	10.45	1.72	2.48	1.88	2.83	4.38	1.85	1.96	4.21	4.25	97
lieA203	12.85	6.86	2.58	9.67	2.07	1.43	12.18	5.87	2.81	9.26	1.1	5.37	1.77	9.76	6.39	8.05	1.72	3.03	1.31	1.82	3.42	1.17	1.34	3.47	3.57	91
lie3128	14.71	10.9	4.61	12.58	4.58	2.6	12.89	9.85	5.72	11.9	1.08	5.72	2.85	14.26	11.43	10.98	2.64	3.31	2.8	3.57	3.45	1.08	2.2	4	5.7	72
lieA257	12.7	7.5	3.32	10.11	2.9	2.22	11.67	8.93	5.08	9.89	1.09	5.76	2.22	10.75	6.17	7.34	2.1	3.49	2.29	2.35	2.6	1.09	1.9	3.7	5.03	94
lieA259	12.55	7.39	3.64	9.96	2.57	2.21	10.99	9.21	5.69	7.48	0.76	5.14	1.95	10.79	6.82	7.62	2.23	2.96	2.2	1.79	2.83	0.98	1.2	2.75	3.93	102
lieA211	12.83	7.5	3.21	10.52	2.62	1.78	11.44	7.12	4.07	7.46	0.89	5.4	2.45	10.75	7.35	7.36	1.34	2.91	1.89	1.54	3.1	1.1	1.94	3.4	2.25	95

lieA212	13.88	7.6	3.06	10.99	2.65	1.89	11.37	8.72	5.06	6.19	0.95	5.08	2.48	11.74	6.09	7.35	1.7	3.67	1.37	2.14	3.29	1.24	2.09	4.3	3.08	93
lieA258	14.15	8.46	4.52	10.56	3.5	2.75	13.39	9.32	5.88	10.76	1.23	5.23	2.25	12.12	7.95	9.7	2.71	3.33	2.75	2.63	3.33	1.25	1.7	3.31	5.03	92
lieA53	12.5	7.25	3.8	9.02	3.03	2.25	9.23	6.56	4.4	9.2	1.05	5.66	2.22	11.55	6.76	8.42	1.94	3.38	1.7	1.75	3.33	1.05	2.08	4.01	2.85	88
lieA213	12.81	7.47	3.18	10.24	2.7	2.27	10.59	8.57	4.65	7.43	0.99	5.45	2.12	12.68	7.03	9.26	1.84	3.96	2.04	2.49	3.6	1.43	1.45	3.91	4.56	93
lieA214	8.79	5.25	3.35	6.7	2.4	1.95	7.41	5.49	3.62	4.74	0.84	4.5	1.69	9.1	5.38	5.09	1.56	2.59	1.55	1.69	2.28	0.7	1.23	3.48	2.69	74
lieA215	12.62	6.27	2.54	9.83	2.7	2.15	11.1	6.5	3.95	8.98	0.85	4.87	2.21	11.96	5.95	6.5	1.54	4.57	2.04	1.82	2.23	0.73	2.45	3.88	3.14	86
lieA216	12.43	6.46	3.88	10.1	2.83	2.22	11.25	7.08	4.49	10.15	0.9	4.71	2.11	11.78	5.82	8.15	1.44	3.9	2.02	2.21	3.42	0.7	2.32	3.97	4.2	103
lieA206	13.88	8.4	3.52	9.45	3.05	2.32	11.67	8.75	4.83	10.31	1.04	5.34	2.55	10.25	6.91	6.94	1.76	2.51	2.1	2.64	2.62	0.76	2.65	4.1	3.35	90
lieA207	9.25	5.19	3.43	7.18	2.72	1.65	7.55	5.99	3.64	5.46	0.85	4.39	2.33	8.77	5.53	5.84	2.01	2.14	1.89	2.01	2.55	1.25	1.28	3.76	3.71	73
lieA208	10.96	6.23	3.13	6.56	2.28	1.96	9.98	6.18	3.35	8.22	0.79	4.84	1.51	10.46	6.74	5.55	2.83	1.34	1.88	0.97	1.75	0.92	2	4.8	2.8	54
lieA209	13.84	6.79	2.68	10.13	2.32	1.7	12.12	6.2	3.53	8.14	0.94	5.64	2.16	11.01	6.87	7.52	1.73	3.17	1.41	2.34	3.27	0.91	2.55	4.7	3.48	76
lieA210	13.55	7.89	3.96	8.83	2.92	2.16	12.36	8.91	5.61	11.1	0.97	4.75	2.34	9.9	6.48	6.4	1.78	2.79	1.84	2.45	2.2	0.69	1.58	3.1	4.55	93
lieA204	13.76	9	3.59	10.93	3.1	1.88	13.55	8.65	3.82	9.26	0.99	5.44	2.16	11.65	8.59	7.37	1.84	3.09	1.73	1.97	3.08	0.84	2.12	3.71	4.64	82
lieA205	10	5.23	2.3	7.86	2.47	1.9	8.11	6.17	3.94	8.52	0.9	5.57	2.1	9.28	5.54	6.47	1.4	2.87	1.78	1.91	2.51	0.65	1.91	3.58	2.78	75
lieA217	11.31	6.3	3.05	7.86	2.58	2.2	10.2	6.75	4.36	9	0.92	4.62	2.1	9.58	5.86	8.52	1.66	2.88	1.98	2.2	3.3	0.83	1.72	2.8	3	90
lie2241	14.25	8.75	3.75	11.25	4.25	3.34	12.37	10	5.44	11.88	0.8	5.07	1.59	14	8	8	2.25	3.62	1.43	2.06	3.12	0.75	3.2	4.35	3.06	92
lie3034	15.26	8.17	3.74	12.06	3.25	2.61	15.22	11.32	5.68	16.6	1.13	5.43	2.38	13.4	8.1	10.32	2.66	4.13	3.82	3.22	3.3	1.8	2.71	4.48	4.71	89
lie3036	13.74	7.1	2.81	13.24	2.88	2.13	15.07	9.38	3.89	11	0.96	5.54	2.35	10.17	7.1	8.8	2.3	3.17	2	2.66	3.29	1.33	2.4	4.11	3.24	92
ni2597	17.57	9.78	2.93	12.71	4.25	2.64	16.29	10.5	5	9.07	0.93	5.1	1.78	12.86	8.71	7.25	3	2.38	2.69	1.79	2.57	1.21	3.89	6.16	3.22	68
niA002	15.41	8.29	3.9	8.88	2.98	2.49	12.68	7.9	4.97	13.85	0.78	4.8	1.27	12.2	9.6	4.32	2.47	1.61	1.71	1.36	2.41	1.1	3.75	8.93	4.44	47
ni2591C	13.95	8.54	3.22	9.74	3.62	2.22	11.38	8.52	4.64	8.9	0.77	5.19	1.92	11.25	8.4	6	2.57	1.25	2.38	1.59	2.58	1.01	2.43	4.8	4.44	36
ni2591E	12.49	5.95	2.37	9.73	3.15	1.89	12.23	6.92	3.79	5.87	0.77	5.02	1.87	10.67	6.87	5.76	2.06	1.53	2.57	1.57	2.25	1.14	2.78	4.19	3.75	62
ni2596	14.06	8.38	2.55	10.4	3.26	1.91	12.9	7.96	3.8	7.19	0.77	5.59	1.87	11.62	8.09	7.08	1.97	1.47	2.79	1.7	3	0.8	2.75	5.1	3	47
ni3100	14.41	7.54	2.62	10.22	2.86	2.02	14.28	6.89	3.88	9.79	0.85	5.19	1.75	12.13	7.69	6.26	2.4	1.17	2.31	1.4	2.22	0.96	3.5	6.82	4.08	41
pab3042	9.88	4.52	3.68	9.84	2.76	2.58	9.43	4.7	4.39	5.94	0.72	5.93	2.57	8.2	4.25	6.48	0.81	2.51	2	1.44	2.25	1.7	1.08	2.92	3.06	70
pab3059	10.95	5.47	4.02	10.4	3.1	2.74	10.56	5.66	4.16	7.93	0.73	5.64	2.07	9.73	4.79	7.34	1.29	3.05	2.29	1.61	2.64	1.65	0.89	3.19	2.85	66
pub3038	12.3	4.03	2.28	10.69	2.22	1.14	11.54	5.29	3.06	10.33	0.78	4.77	2.41	13.12	5.3	7.44	1.5	3.03	2.13	1.37	2.47	1.38	2.85	4.94	2.84	77
pub3053	14.89	6.33	2.48	10.57	2.16	1.52	13.46	6.93	3.55	11.44	0.97	5.61	2.8	14.44	8.42	9.12	2.73	3.21	3.38	2.65	2.81	1.6	3.5	5.5	4.26	84
pub3040	14.14	7.13	2.7	10.05	2.37	1.81	13.32	7.99	4.5	12.07	1.08	5.46	2.69	11.92	6.42	8.66	1.81	3.17	2.12	3.05	3.31	1.77	2.93	4.55	2.75	79
pub3048	17.27	8.78	4.29	15.71	2.97	2.18	17.34	10.02	6.78	14.8	1.22	5.81	2.74	14.22	8.62	10.21	2.44	3.67	4.27	2.95	3.35	2.21	2.83	4.5	3.93	85
pub3046	11.36	4.5	2.58	9.2	1.96	1.7	10.8	5.64	3.8	9.3	1	4.55	2.56	12.44	5.96	7.45	1.08	3.53	2.04	2.29	2.77	1.35	3.1	4	3.8	90
pub3054	11.45	4.3	2.51	8.38	1.95	1.68	10.46	4.6	3.12	9.29	0.9	4.53	2.37	11.85	5.7	6.68	1.28	3.15	2.61	2.35	2.05	1	3.1	4	3.82	94
pul2882	10.75	6.39	4.53	9.88	2.33	1.86	11.04	3.84	2.82	10	0.88	5.77	3.86	8.9	4.59	5.63	1.95	0	0	1.8	3.72	0.76	2.58	3.68	107	
rio3130	14.73	8.52	3.06	14.73	3.48	3	13.79	5.78	4.35	11.69	1.08	6.13	2.72	10.66	4.14	14.71	1.59	3.63	2.83	1.59	5.72	1.65	2.34	6.9	4.08	80
rio3114	15.2	9.58	4.67	14.04	4.02	3.07	13.69	6.22	4.61	11.1	1.21	7.22	2.86	11.3	4.77	13.94	1.18	3.82	2.77	1.56	5.64	1.38	2.63	6.72	3.83	78
rio3134	14.2	7.49	2.77	13.38	2.82	2.56	12.55	5.39	4.01	10.12	0.89	6.01	2.88	11.71	4.65	11.76	1.34	3.7	2.2	1.75	4.7	1.45	2.46	4.82	2.36	101
rioA430	13.04	8.34	3.57	12.16	3.23	2.56	10.83	5.72	4.29	10	0.96	6.18	2.3	11.8	6.62	11.6	2.93	3.34	2.26	2.04	4.3	1.85	1.41	3.5	3	78
rio3137	12.8	7.3	3.93	11.47	3.08	2.56	11.41	5.47	4.11	8.49	0.93	6.43	2.7	9.87	4.87	12.1	1.43	2.32	2.04	1.78	4.96	1.8	1.67	4.9	2.9	82
rioA164	12.76	8.38	4.17	11.75	3.91	2.81	12.74	6.9	4.15	8	0.88	5.96	2.77	9.64	5.27	10.9	3.07	2.21	3.41	1.85	4.1	1.8	1.2	4.6	3.37	69
rioSP46	14.5	10.31	5.79	13.76	4.43	4.09	13.94	7.84	6.36	10.39	0.9	6.38	3.42	11.7	5.88	14.01	2.29	3.19	2.14	1.68	5.57	2.06	2.06	6.27	3	66
sar2242	16.5	9.7	6.2	10.5	3	2.4	12.8	7.5	5.1	20	1.5	6.94	1.51	16.4	14	9.31	5	0.4	2.5	2.08	1.4	2.8	2.1	3.3	4.83	90
sar2255	15.55	12.33	6.66	11.62	3.08	2.04	11.23	11.79	8.9	9.7	1.42	7.47	1.5	18.81	21.56	10.61	5.8	0.2	2.51	2.23	2.57	2.84	2.21	2.3	4.55	90
sar2683	14.09	10.09	6.83	11.42	6.26	3.95	11.84	9.29	5.88	14.53	1.11	6.94	1.6	14.93	13.66	7.93	4.49	0.57	2.61	2.04	2.27	2.59	1.86	2.83	4.93	90
sar3079	21.77	16.95	11.5	15.06	7.75	4.5	14.3	14.24	9.66	24	1.82	7.5	2.02	22.65	24.18	15.61	6.83	0	2.12	2.12	5.3	4	2.74	2	4.52	96
sar3121	19.63	15.65	9.44	14.55	9.7	6.61	16.51	15.08	10.62	21.22	1.85	7.9	2.15	18.78	20.67	11.75	5.89	0.35	3.76	3.15	3.16	2.26	2.65	1.6	5.33	98

Appendix 2 (cont.). – Measurements of the 26 characters in the samples.

[Abbreviations: Samples: the first three letters stand for the species name]

Samples	Characters																									
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
sil2942	13.07	8.87	5.97	8.46	4.83	3.14	11.79	8.16	5.5	6.53	0.85	4.4	1.88	12.12	9.94	7.92	3.82	2.91	2.47	2.39	2.48	3.43	0	2.05	3.24	90
sil2922	11.77	7.09	5.26	7.21	3.66	2.95	10.4	8.46	5.85	4.27	0.86	4.7	2.21	10.63	8.93	8.03	3.91	3.27	3.06	2.23	2.17	2.53	0	1.4	2.63	90
sil2949	12.65	7.27	4.36	7.45	3.49	2.92	9.45	7.51	4.25	4.89	0.83	4.38	2.16	10.12	7.55	6.46	2.04	3.03	2.84	2.08	2.48	2.86	0	1.6	4.83	90
sil3049	11.3	6.54	3.74	6.34	2.38	2.06	8.96	6.04	4.15	6.37	0.82	4.87	2.61	10.53	7.88	6.52	3.36	3.25	2.59	1.91	2.1	2.62	0	2.13	2.26	90
ven0108	6.97	4.68	2.72	6.98	3.58	2.16	5.3	4.87	4.31	13.76	0.85	3.32	1.88	11.28	7.31	10.04	2.59	0.59	3.1	2.67	3.24	2.15	2.68	3.55	5.47	90
ven0109	6.95	5.9	4.36	6.96	2.53	1.49	6.82	5.6	4.18	15.05	0.85	4.37	1.93	11.63	7.58	10.2	3.32	0.85	3.59	2.96	2.61	2.48	2.67	3.63	7	90
ven0107	7.13	5.08	3.57	6.89	3.18	1.37	5.61	4.07	2.91	15.64	0.81	3.68	1.64	11.37	7.29	10.8	2.98	0.53	2.61	2.33	3.22	2.54	3.19	3.75	4.21	90
ven0110	7.29	5.61	5.14	6.8	3.88	3.5	6.05	4.64	3.91	12.34	0.79	3.46	1.53	11.48	8.2	10.35	2.72	0.56	3.25	3.02	3.07	2.65	2.64	3.66	3.94	90
trn2262	8.5	3.68	1.55	6.22	1.5	1.4	8.5	3.83	2.85	7.81	0.7	3.83	1.56	9.82	4.9	6.19	3.84	3.39	1.84	2.2	1.46	1.61	0	1.77	1.88	125
trn2769	9.22	4.35	2.29	6.98	1.82	1.43	7.31	4.04	2.6	5.83	0.68	4.81	1.65	9.87	4.09	7.1	2.53	2.44	1.74	1.32	2.69	0.83	0	1.52	1.5	126
trn2899A	9.22	4.62	2.58	6.99	2.29	1.41	8.01	5.82	3.25	8.11	0.73	4.67	2	9.6	4.05	6.09	2.9	3.44	1.63	2.38	2.31	1.35	0	1.81	2.76	122
trn2899B	7.97	3.47	2.04	6.1	1.5	1.16	7.31	3.91	2.5	3.52	0.71	5.17	1.96	8.42	3.63	5.53	2.7	2.78	2.76	2.25	1.75	1.17	0	1.52	2.32	127
uhl2689	12.64	8	5.79	11.93	2.46	2.25	12.64	4.89	3.93	16.07	0.71	8.43	3.03	11.29	6.89	7.93	3.21	0.6	3.16	1.8	2.29	3.5	2	4.69	3.83	84
uhl2689A	12.4	6.12	4.07	9.56	2.24	2.01	12.28	4.29	3.66	15.16	0.91	7	3.72	11.07	6.11	7.25	3.18	0.49	3.99	1.78	1.78	3.3	0.68	4.18	7.07	108
uhl3093	9.46	6.25	5.05	7.59	2.03	1.84	7.84	3.64	2.85	10.29	0.67	5.73	3.67	9.33	4.45	6.93	2.45	0.7	2.99	1.85	2.22	3.2	1	3.94	2.53	90
lie2656	11.75	7.04	2.88	8.74	3.5	2.08	10.58	8.99	4.91	6.45	0.85	5.02	2.29	10.71	6.27	7.01	1.65	2.44	1.59	2.01	2.6	1.23	2.15	3.97	3.54	90
lie2657	10.79	5.9	2.49	7.74	2.53	2.03	9.83	6.7	4.45	6.75	0.87	5.3	2.37	10.75	6.25	7.93	1.93	2.82	1.99	1.99	2.77	1.33	2.71	4.23	2.42	90
lie2675	11.65	7.17	3.03	9.97	3.72	2.64	10.8	8.3	4.71	7.19	0.84	5.14	2.41	12.29	7.13	7.23	2.03	3.11	1.72	1.61	3.43	1.02	1.84	5.46	3.4	90
lie2693	11.83	6.04	2.49	10.83	2.78	1.83	11.41	7.45	4.62	8.3	0.94	5.5	2.47	10.75	6.12	8.7	1.59	3.1	2.33	2.18	3.32	1.4	2.21	3.75	3.5	90
lie2703	11.5	6.56	2.37	9.64	3.13	2.27	10.77	7.73	4.85	5.51	0.92	5.11	2.27	10.56	6.12	6.65	1.53	3.06	1.76	1.98	2.45	1.36	1.72	4.02	4.39	90
wid2577	11.63	8.34	3.26	8.1	4.89	3.15	10.18	8	4.83	6.71	0.8	5.63	2.83	11.41	7.1	9.95	2.91	3.07	2.93	2.26	3.44	1.83	1.65	4.1	3.12	75
wid2581	13.49	9.26	3.43	8.86	4.11	3.06	10.39	7.16	4	8.49	0.97	5.51	2.3	12.41	7.06	9.4	2.93	2.65	2.27	1.74	2.99	1.46	1.78	3.92	2.06	76
wid2574	14.46	9.84	3.49	9.96	3.28	2.58	13.26	7.78	5.34	10.6	0.9	5.18	2.75	13.69	6.48	12.5	2.02	2.95	2.78	2	4.77	2.1	2.7	5.23	2.59	77