Consequences of the choice of landmarks for visualization and biological interpretation of shape changes in 2-D geometric morphometrics: a study case.

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Abstract. We compare shape changes in mandibles of marsupials, represented by 2-dimensional coordinates, using different subsets of landmarks. We show that use of Type III landmarks, often not recommended in geometric morphometric techniques, might permit a better interpretation of shape changes than use of Type I landmarks alone, or a combination of Types I and II. We suggest that the use of as many landmarks as necessary to cover the whole configuration of the studied structure, regardless of the type, will allow a better visualization of the biological forms without leading to a less clear discrimination among them.

1.Introduction

Ever since the beginning of the so-called "revolution in morphometrics" (Rohlf and Marcus, 1993), up to the widespread use of landmarks in 2-D and 3-D to describe shape changes of organisms (see for example Bookstein, 1998 for an historical overview), there has been some discussion about the appropriate choice of the landmarks and its implication for the outcome of the analysis, as well as for the biological interpretation of results.

Landmark-based methods of geometric morphometrics are based on the definition of shape space (Kendall, 1981, 1984). Configurations of digitized landmarks representing shape are plotted in a multidimensional space, after alignment by General Least Squares. As a consequence of this alignment the distance between the points are invariant to location, orientation and scale of the original coordinate system. The distances between points in Kendall's shape space are Procrustes distances, which are usually measured in radians. Being a high-dimensional sphere, Kendall's shape space has a non-Euclidean geometry, and therefore linear multivariate statistical methods do not apply. It is possible, however, to approximate shape space by projecting the points or specimens from the sphere to a linear tangent space. This Euclidean plane touches the surface of the sphere in a point called the "reference" form, usually the mean of all specimens or landmark configurations (Bookstein, 1996). It has been shown that even when there is great variability in shape, correlation among shape and tangent space is very high, allowing a good estimate of shape differences, represented by Procrustes distances in shape spaces and linear distances in the tangent space (Marcus et al, 2000). From the reference form (from its landmarks) is obtained a matrix of distances between all pair of points, called the bending energy matrix, since it summarizes the amount of energy needed to displace any pair of points projected in a theoretical infinite and negligibly thin plate. Points very close together need a high amount of energy to be displaced; inversely, points far apart need less energy. From this matrix are extracted a set of eigenvectors (called principal warps), which summarize all possible directions of shape change and the corresponding amount of energy needed to perform that transformation (their corresponding eigenvalues). Coordinates of the aligned specimens can be projected onto the principal warps vectors, decomposing shape variation in geometrically orthogonal components at different spatial scales called partial warps, or nonaffine shape components, as they describe localized changes. Rohlf (1998) demonstrates that the pattern of shape changes implied by each one of the principal warps, and hence the biological interpretation of each partial warp, seems to be very sensitive to the choice of reference, as well as the choice of landmarks.

Therefore we can conclude that the first step to be carefully taken in consideration before any geometric morphometrics study is choosing landmarks that will not only be present (and more or less evenly distributed) over all specimens under study, but could be considered morphologically and developmentally equivalent (homologous). Issues related to the choice and equivalence of landmarks in biomechanical, evolutionary and developmental studies are discussed, for example, by O'Higgins (1997). Here we will concentrate on the discussion of landmark types and their use.

According to Bookstein (1991), there are three principal types of points that frequently are usable as landmarks and correspond to different ways of grounding the epigenetic explanations motivating these measurements in the first place. These are: Type I – juxtaposition of tissues, or points in space at which structures meet, like bony sutures, branching points in tree structures of constant topology, centers of small structures, or intersections of extended curves with planes of symmetry. Deformations involving this kind of landmarks may have more than one valid functional explanation, but these are more commonly valid within single ontogenies than between different organisms; Type II – maxima of curvature, or other local morphogenetic processes, like tips of extrusions and concavities of invaginations. They often serve as points of application of biomechanical forces, and as reviewed in Bookstein et al. (1985) it is difficult to interpret the displacements of these landmarks; and finally the Type III – extremal points, whose definitions refer to information at separate locations. These are common in traditional multivariate morphometrics (dealing with linear distances), and according to this author are rarely meaningful landmarks, as their displacement is mainly in one direction representing the length of the defining segment. For this reason, they are often called "deficient". Still following Bookstein (1991), some landmarks can be hybrids: for example, landmarks upon the medial axis of an outline are hybrids of the second and third type.

A more operational definition based on these three types appears in Slice et al. (1996). Following these authors, the three types are: I. Mathematical point whose claimed homology is supported by strongest evidence, such as local pattern of juxtaposition of distinct tissues or a small patch of unusual histology. II. The claimed homology in this type is supported by geometrical instead of histological evidence, for instance, the sharpest curvature of a tooth. III. This type contains at least one deficient coordinate, and characterizes more than one region of the form. For instance either end of a longest diameter or the bottom of a concavity. O´Higgins and Jones (1998) add to these three the mixed type I/III.

It is easy to notice that both sets of definitions above may lead to doubt when classifying and choosing landmarks, but they also emphasize that Type III points are not as useful as the other two types, and their displacement must be interpreted carefully, if they are used at all. In fact, it has been said that what may be called "constructed" landmarks, or landmarks that are related to others previously chosen, or to selected planes projected over the form under study, should be avoided altogether because of their interdependence or covariation patterns.

On the other hand, sometimes we are dealing with structures or organisms in which we can not find discrete structures to use as landmarks, like smooth continuous surfaces. Therefore, depending on the form under study, type three landmarks are very useful tools to describe shape changes, as they allow the homogeneous distribution of points over the whole organism, facilitate visualization and lead to biological meaningful results.

In this paper, we used a dataset consisting of pictures representing lateral view of mandibles belonging to 7 species of marsupials (pouched mammals) from seven different genera, to test the utility and relevance of type three landmarks in the analysis of biological forms. We show that the use of Type III landmarks, might permit a better interpretation of shape changes than use of Type I landmarks alone, or a combination of Types I and II. We suggest that the use of as many landmarks as necessary to cover the whole configuration of the studied structure, regardless of the type, will permit a better visualization of the biological forms without leading to a less clear discrimination.

2. Material and Methods

2.1 Specimens studied

Seven species of New World marsupials were chosen, representing a variety of body sizes and feeding specializations. All specimens were fully grown adults, as determined by the full eruption of the third premolar and fourth molar. Species studied and samples sizes are presented in Table 1.

Table 1.: Studied species and sample sizes.

Species	N
Caluromys phulander	59
Didelphis albiventris	60
Lutreolina crassicaudata	54
Marmosa murina	59
Marmosops incanus	65
Metachirus nudicaudatus	57
Dromiciops gliroides	43

All species belong to the same order, Didelphimorphia, except for *D. gliroides*, which belongs to the order Microbiotheria. Of the remaining species, *Caluromys philander* belongs to the subfamily Caluromyiinae within the Didelphidae family, and all remaining species belong to the subfamily Didelphinae, within the same family.

Table2.: Landmark locations and types.

Lmk	Type	Location
1	I	Anterior basis of the first lower incisor (anterior extremity of the lower incisive series).
2	I	Posterior basis of the fourth lower incisor (posterior extremity of the lower incisive series).
3	I	Posterior basis of the lower canine.
4	I	Posterior basis of the third lower premolar and anterior basis of the first lower molar.
5	I	Posterior basis of the fourth lower molar (posterior extremity of the lower molar series).
6	II	Point of greater curvature at the end of the horizontal ramus and beginning of the coronoid process..
7	II	End of the anterior border of the coronoid process (point of higher curvature).
8	III	Higher point of the coronoid process (defined by a line parallel to landmarks 4 and 5).
9	II	Posterior extremity of the coronoid process.
10	II	Point of greater curvature between the articular process and the posterior border of the coronoid process.
11	II	Articular condyle.
12	II	Point of greater curvature at the beginning of the angular process.
13	II	Tip of the angular process.
14	II	End of the angular process and beginning of the ventral border of the horizontal ramus.
15	III	Point at ventral border of the horizontal ramus perpendicular to landmark 5.
16	III	Point at ventral border of the horizontal ramus perpendicular to landmark 4.
17	III	Point at ventral border of the horizontal ramus perpendicular to landmarks 3.
18	II	Antero-ventral extremity of the masseteric fossa.
19	I	Mental foramen.

2.2 Image acquisition and landmark determination

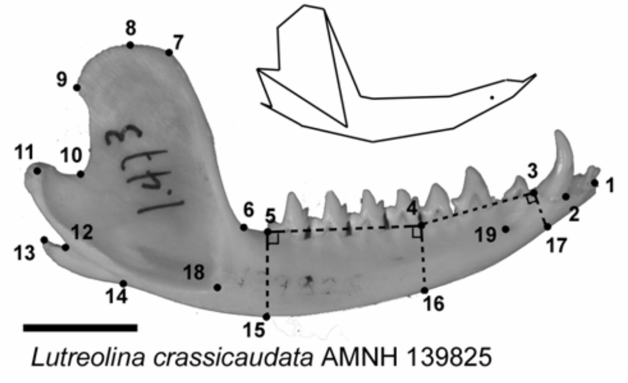

Figure 1.: Landmarks used in this study, on a mandible of *Lutreolina crassicaudata*. See Table 2 for landmark definitions. The outline represents landmarks linked for a better visualization, as used in the output graphs.

Photos of the right hemimandible were taken with a Nikon Coolpix 995 digital camera at a resolution of 1280x960 pixels. Mandibles are fairly flat structures, and previous studies (Astúa de Moraes, 1998) have shown the parallax is negligible. Nineteen landmarks of various types were defined and set in each image using the software TPSDig v. 1.37 (Rohlf, 2003) (Fig.1, Table 2). Landmarks 1 to 5 describe the relative positions and sizes of the teeth or tooth series (1-2: incisive series; 3: canine; 3-4: premolar series; 4-5: molar series). Landmarks 6 a 10 define the shape of the coronoid process. Landmarks 10-12 define position and relative location of the articular condyle. Landmarks 12-14 define the angular process. The angular process in marsupials is medially inflexed, but this inflexion is variable (Sánchez-Villagra & Smith 1997). Thus biological interpretation on these points should consider this fact. Landmarks 15-17 describe the shape of the ventral border of the horizontal ramus, and along with landmarks on the tooth series, describe its width. Their use was determined by the fact that traditional morphometrics analyses on a subset of these species (Astúa de Moraes 1998) had indicated some variation of width in the horizontal ramus. Since there is no discernible structure on the ventral border of the horizontal ramus, three landmarks were constructed relative to the positions of landmarks on its dorsal border. Those landmarks are used in the analysis to test the relevance and utility of this kind of “constructed” landmarks in capturing biological shape. Landmark 19, along with landmarks on the coronoid process, define shape and relative size of the masseteric fossa.

2.3 Shape analyses

Four landmark subsets were used to compare their effectiveness in discriminating the shape of the mandible of these species: (1) All 19 landmarks, types I, II and III; (2) 16 landmarks, of Types I and II; (3) 6 landmarks of Type I only; (4) 11 landmarks describing the horizontal ramus of mandible. This last subset was used to assess if the landmarks of the horizontal ramus could capture the information on the changes in its width that should reflect in the Uniform Component. All four subsets were submitted to a Relative warp analysis, which corresponds to a Principal Components Analysis on the Partial Warp scores, using the software TPSrelw, and the option alpha=0.

3. Results

Singular values and percentage of variation explained for the first three Relative Warps in each subset are presented in Table 3. The amount of variation represented by the first three RWs is roughly the same, regardless of the number or types of landmarks used.

Table 3. Singular values and percentage of variation explained for the first three Relative Warps, for the four subsets.

Subset	1	2	3	4
RW	SV (%)	SV (%)	SV (%)	SV (%)
1	0.89 (37.43)	0.90 (38.34)	0.75 (37.96)	1.01 (45.32)
2	0.73 (25.27)	0.71 (24.08)	0.60 (24.38)	0.76 (25.49)
3	0.47 (10.80)	0.49 (11.50)	0.42 (11.58)	0.53 (12.42)
Cumulative.	73.50	73.91	73.91	83.23

Figures 2 to 4 show the scores of the specimens for Subsets 1,3 and 4 on the relative warp axes. Scores for the Subset 2 (including only landmark types I and II) are very similar to the one obtained for the Subset 1, and so are not presented here.

For the first relative warp of the analysis including landmarks from Subset 1 (Figure 2A), *Caluromys philander* is on one extreme due to a posterior bending of the coronoid process, as represented in Figure 7a.

Along the second relative warp axis, *Didelphis* and *Lutreolina* are in one extreme and *Marmosops incanus* on the other. The first two are characterized by a more robust mandible, with a larger coronoid process (Figure 5B), while *Marmosops* shows a elongated and delicate mandible (Figure 5C).

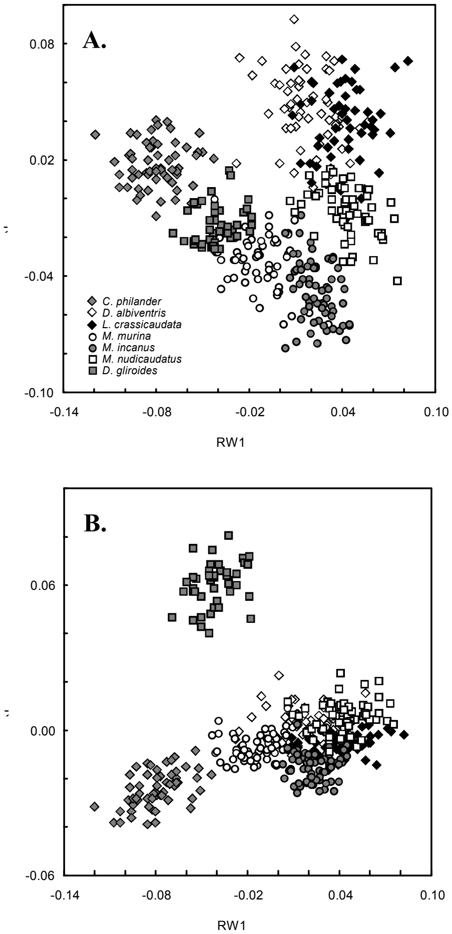

Figure 2. Plot of relative warps scores for Subset 1 (19 landmarks, including Types I, II and III). **A.** Relative warp 1 versus relative warp 2. **B.** Relative warp 1 versus relative warp 3. Legends in **B** are the same as in **A**.

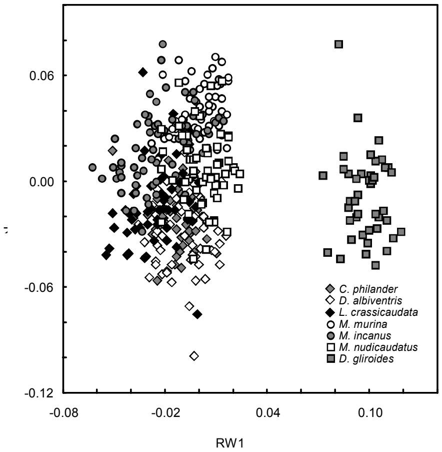

Figure 3. Plot of relative warp scores for Subset 3 (11 landmarks, including only Type I) on the first and second relative warp axes.

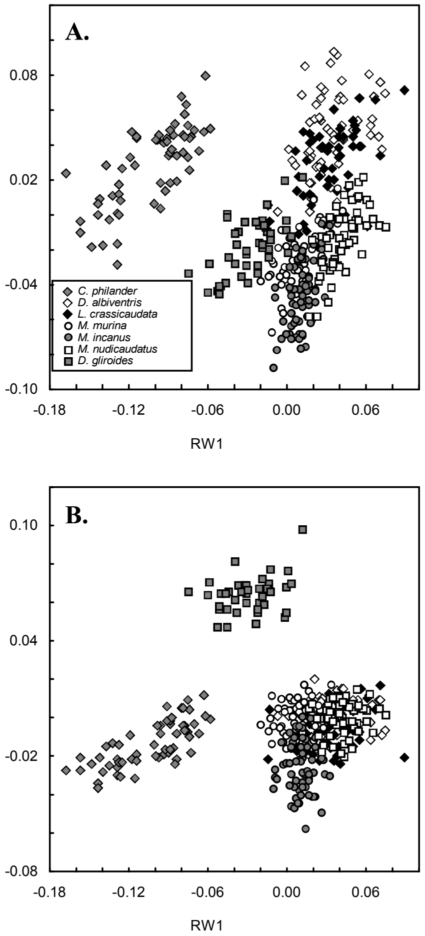

Figure 4. Plot of relative warp scores for Subset 4 (11 landmarks describing the shape of the horizontal ramus of the mandible). **A.** Relative warp 1 versus relative warp 2. **B.** Relative warp 1 versus relative warp 3. Legends in **B** are the same as in **A**.

The graph for the first and third relative warps shows a separation between *Dromiciops* and all Didelphinae (Figure 2B). This is due to a ventral displacement of the tip of the angular process and also of the anterior part of the mandible in this species, and also to related to differences in the position of the molar series, anteriorly displaced, shorter pre-molar series and longer incisive series turned down, all characteristics of *Dromiciops*. This species also presents the coronoid process inclined posteriorly (Figure 6A), while in the Didelphinae (Figure 6B) the angular process is medially inflexed and the tip of the mandible projected upwards, pre-molar series is longer and the coronoid process is larger and more vertical.

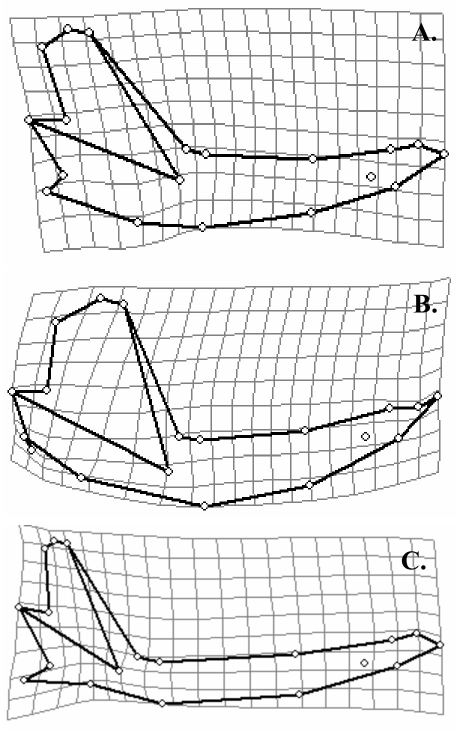

Figure 5. Splines representing deformation associated with the first and second relative warps for Subset 1 (Type I, II and III landmarks). **A.** *Caluromys philander*. **B.** *Didelphis albiventris* and *Lutreolina crassicaudata***.** **C**. *Marmosops incanus*.

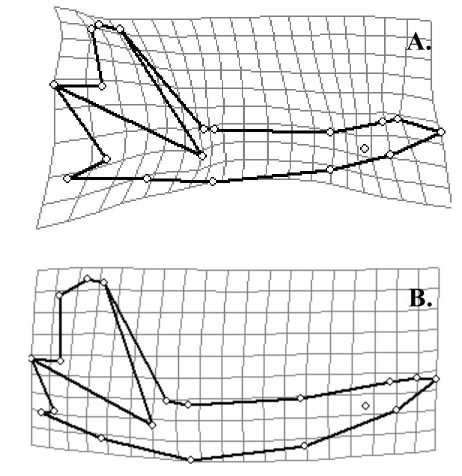

Figure 6. Splines representing deformation associated with the first and third relative warps for Subset 1 (Type I, II and III landmarks). **A.** *Dromiciops gliroides*. **B.** all Didelphinae.

This same trend can be seen, although in a less complete picture, examining the graph representing the first and second relative warps for the subset 3 (Figure 3), which includes only Type I landmarks. The deformation associated with the separation between *Dromiciops* along the first axis (figure 7A) and all Didelphidae (figure 7B) is related to the relative length of molar and pre-molar series, and to the bending in the anterior extremity of the mandible.

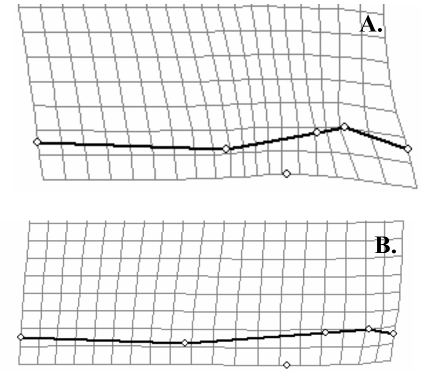

Figure 7. Splines representing deformation associated with the first and second relative warps for Subset 3 (Type I landmarks). **A.** *Dromiciops gliroides*. **B.** all Didelphidae.

Subset 4, including landmarks placed over the horizontal ramus of the mandibles, have the scores over the first , second and third relative warps represented on Figure 4A and 4B. The first relative warp is again separating *Caluromys* from all Didelphidae, based on a shorter and more robust ramus for the first (Figure 8A), while the didelphids in general present a slender ramus (Figure 8B).

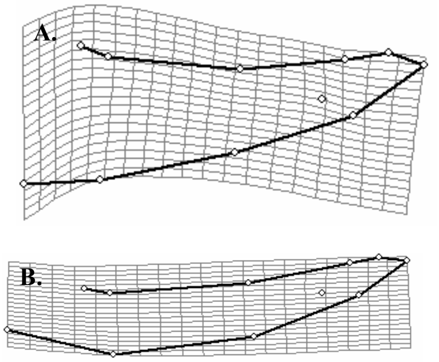

Figure 8. Splines representing deformation associated with the first and second relative warps for Subset 4 (horizontal ramus of mandible). **A.** *Caluromys philander*. **B.** all Didelphinae and *D. gliroides*.

The first and third relative warps together are separating three groups, formed by *Dromiciops* (Figure 9A), *Caluromys* (Figure 9B), and the Didelphinae (Figure 9C), based on the general robusticity of the ramus and on the orientation of the incisive series.

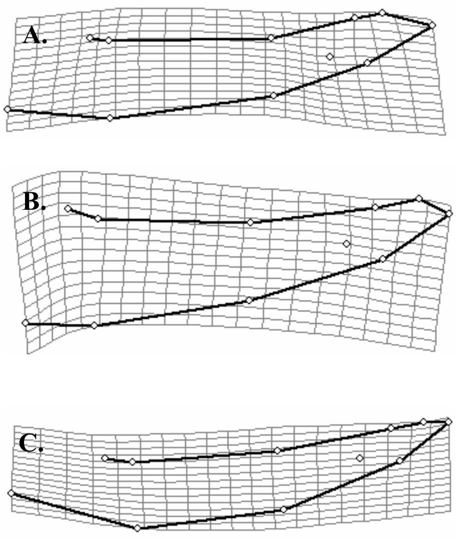

Figure 9. Splines representing deformation associated with the first and third relative warps for Subset 4 (horizontal ramus of mandible). **A.** *Dromiciops gliroides***.** **B.** *Caluromys philander*. **C.** all Didelphinae

These results presented on figure 2, indicating a separation based on the shape of the coronoid process, is suggesting that this part of the mandible have a strong variation among the species studied. However, the graphs including only landmarks over the horizontal ramus are showing some degree of horizontal compression and enlargement that are more well represented by the uniform component y, which is responsible for the horizontal compression present in the dataset as a whole. In fact, plotting the uniform component for the whole set of landmarks from subset 1 do not show any trend, as can be seen on Figure 10, while selecting only landmarks from Subset 4, representing the ramus, one can see a horizontal distribution suggesting a gradient of enlargement of the horizontal part of the structure (Figure 11), determined by the uniform y component. The x uniform component, representing the shearing of the mandible, is also present when considering only this subset.

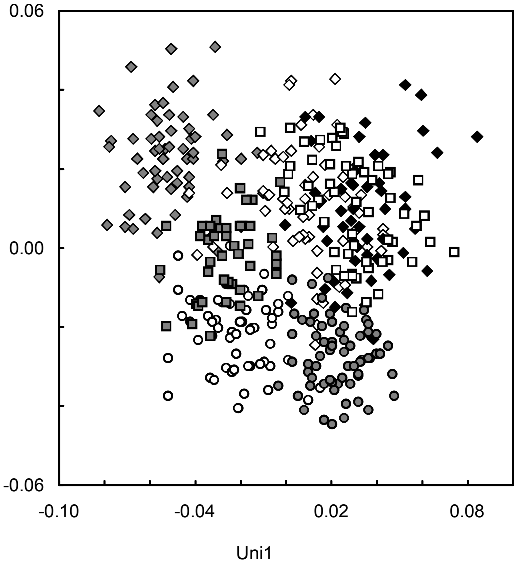

Figure 10. Plot of X and Y Uniform Components for the Subset 1 (19 landmarks, including Types I, II and III). Legends like in Figures **2A**, **3** and **4A**.

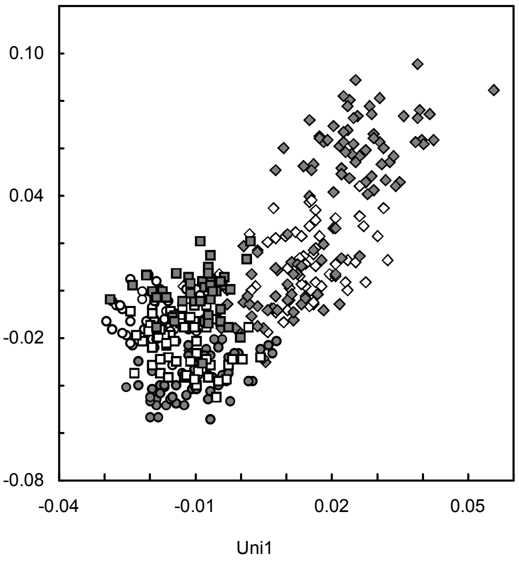

Figure 11. Plot of X and Y Uniform Components for Subset 4 (11 landmarks describing the shape of the horizontal ramus of the mandible). Legends like in Figures **2A**, **3** and **4A**.

4. Discussion

Results of our Relative Warp Analysis over subsets 1 (all landmarks, Types I, II and III) and subset 2 (landmarks Type I and II) have approximately the same singular values and percentages explained by each one of the first three relative warps, and moreover, the same ordination pattern along these axes. We considered this as one evidence against the widespread belief that the use of Type III landmarks will lead to problems related to the non-independence between them. One clear difference among the results for the different subsets, however, is the representation of shape changes and the interpretation derived from the thin-plate spline diagrams. Those diagrams containing all landmarks, including Type III, allow a far better visualization of the general shape of the mandible than all others. This is also evident when comparing the results obtained here with the ones presented by Astúa de Moraes *et al.* (2000) for the mandibles of six species of marsupials. These authors used a very similar set of landmarks, but excluding all Type III landmarks. Their plot for the two first canonical variates axes is separating only *Caluromys philander* from the other didelphids, and the diagrams presented do not lead to the perception of the structures involved. It is important to stress that the results for the ordinations presented in our paper are derived from an exploratory technique, the relative warps analysis, which will not allow a separation as clear as the canonical variates. Therefore, differences between the results obtained here and by Astúa de Moraes *et al.*, mainly our better separation among species and biologically more interpretable representation of shape changes, are due not only to our use of more numerous landmarks but also to the inclusion of the three Type III landmarks over the inferior part of the horizontal ramus.

Type I landmarks have been considered so far the most recommended type for geometric morphometric analysis, due to their non-dependence and for being biologically easier to interpret (for example, Bookstein, 1991). However, another conclusion that can be drawn from the graphs presented here is that Type I landmarks do not lead to results or interpretations as clear as the ones including the other two types.

Objections to the use of Type III landmarks based on their non-independence from other landmarks are mathematically valid. Nevertheless, landmarks of the first two types (I and II) can also show this kind of interdependence. One example of this is derived from ontogenetic studies. When studying a developmental series of mammal skulls, it is common to choose landmarks at the beginning and at the end of the molar series. During growth, these landmarks will be displaced far apart from each other, but at some point, and well before the end of growth, the teeth will attain their maximum size to exert their functional role, and so the two points will stop moving while most of the other landmarks over the skull will continue to change their configuration. Indeed, developmental studies usually presents a series of particular problems to the choice of landmarks and landmark types. For example, a Type I landmark placed over a three point suture over the skull of an adult can be transformed in a Type II landmark if placed on the equivalent structure over the skull of a very young specimen in which most sutures are not yet fused.

We conclude that the choice of landmarks to be used in studies of biological shape can not be purely based on mathematical considerations. We must consider carefully the organism under study and what kind of data we have (ontogenetic series, interspecific or intraspecific samples). Ideally, the whole structure should be represented by the landmarks, even if that implies the use of Type III landmarks. Constructed or pseudo-landmarks can not be entirely excluded from any analysis, as they might lead to a better visualization and therefore a better insight about the biological processes underlying shape changes.

Acknowledgements

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