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TOOLS AND TECHNIQUES

Description and Analysis of Spatial Patterns in Geometric Morphometric Data

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Abstract

The development of techniques for the acquisition of high-resolution 3D images, such as computed tomography and magnetic resonance imaging, has opened new avenues to the study of complex morphologies. Detailed descriptions of internal and external traits can be now obtained, allowing the intensive sampling of surface points. In this paper, we introduce a morphometric and statistical framework, grounded on Procrustes and Procrustes-like techniques as well as standard spatial statistics, to explicitly describe and incorporate the spatial pattern of these surface points into the analyses. We exemplified this approach by analyzing ontogenetic changes in a sample of human brain endocasts and inter-specific differences between primate skulls. An intensive sampling of points on 3D surfaces was performed by automatic techniques and the morphometric variation among specimens was measured by the residuals obtained after the alignment of points. Our results showed that shape changes in both examples are spatially structured. Different results were attained by using methods that incorporate or not the spatial structure in the evaluation of the effect of specific biological factors on shape variation. Particularly, these analyses indicated that the effect of biological factors acting at local scales can be confounded with more systemic factors (by example, the effect of the diet on the facial skeleton) if the spatial structure is not taken into account. Overall, our results suggest that the intensive description of shape differences among structures using densely sampled points on 3D surfaces combined with spatial statistical methods can be used to explore problems not widely addressed in morphological studies.

Keywords Pseudolandmarks · Semilandmarks · Intensive sampling · Spatial autocorrelation

The development of techniques for the acquisition of high-resolution 3D images, such as CT scans and MRI, has opened new avenues to the study of complex morphologies. Detailed descriptions of internal and external traits can be now obtained and visualized. However, the analytic tools for comparing and assessing morphological variation within a geometric morphometric framework have lagged behind, being mainly based on the description of discrete morphological traits by using a reduced number of anatomical points (landmarks; Klingenberg 2011; Adams et al. 2013). More recently, different methods have been developed for obtaining a better coverage of the structures by means of the semi-automatic or automatic placement of points on surfaces and curves, called semilandmarks and pseudolandmarks (Gunz et al. 2005; Gunz and Mitteroecker 2013; Boyer et al. 2015; Pomidor et al. 2016; Gao et al. 2018). These new methods allow the collection of an unprecedented volume of morphometric data, which constitute a promising alternative to assess the patterns of shape variation and covariation in evolutionary and developmental studies.

The intensive sampling of points not only provides a better description of morphological structures (Adams et al. 2013; Gunz and Mitteroecker 2013) but also allows the exploration of novel problems that cannot be adequately addressed by using sparsely distributed points. In this sense, the new methods for obtaining surface and curve points are suitable for studying patterns of variation

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and covariation at different spatial scales within anatomical structures. One of the most remarkable aspects that can be explored with these methods is the spatial pattern of morphometric data (Mitteroecker 2009; Márquez et al. 2012). Spatially neighbor points will likely have similar properties than more distant ones—i.e., spatial autocorrelation (Rohlf 1990)—as a result of the influence of factors with localized effects on subsets of close points, such as locally expressed molecules or local tissue interactions (Hallgrímsson and Lieberman 2008; Mitteroecker and Bookstein 2008; Mitteroecker 2009). The same as in ecological and biogeographical studies (Legendre and Legendre 1998; Diniz-Filho et al. 2009), the presence of spatial autocorrelation in morphometric data has important implications in the statistical methods used to describe morphological variation and assess its underlying factors. For example, when response variables (e.g. shape data) are modeled as a function of explanatory or independent variables (e.g. size), the spatial structure—or other dependence structures—in the data perturbs significance tests as well as the variance of parameter estimates of the standard (non-spatial) statistical techniques, which can led to a misunderstanding of the relationship between the variables of interest (Legendre and Legendre 1998; Rohlf 2006; Revell 2009). To date, however, most morphometric and statistical approaches do not evaluate neither take into account the spatial autocorrelation of the shape variables.

In this paper, we introduce a morphometric and statistical framework, grounded on Procrustes and Procrustes-like techniques as well as standard spatial statistics, to explicitly describe and incorporate the spatial pattern of morphometric data into the analyses. This approach uses densely sampled points on 3D surfaces to describe the morphological structures of interest and estimates the variation between specimens using point to point residuals of the entire surfaces after Procrustes or Procrustes-like alignments (Gunz et al. 2005; Boyer et al. 2015; Pomidor et al. 2016). The resulting shape data (i.e. Procrustes residuals or pseudolandmark residuals) are then analyzed using spatial statistical techniques (Legendre and Legendre 1998; Fortin and Dale 2005). Particularly, interpolation maps and the Moran's I correlogram are used to explore the spatial pattern of the residuals (Sokal and Oden 1978; Barbujani 2000; Diniz-Filho et al. 2009). Then, statistical techniques that take into account the spatial autocorrelation in the data are applied to evaluate the influence of independent variables on shape variation. This approach allows us both to describe patterns of spatial structure in morphometric variables and to test the relationship between these variables and biological factors hypothesized to account for phenotypic variation. In the following sections we describe how the approach proposed here can be applied in the analysis of semilandmarks and pseudolandmarks; and then provide examples based on 3D coordinates from skulls of two platyrrhine species and an ontogenetic sample of human brain endocasts.

Spatial Analysis in Geometric Morphometric

Acquisition of Surface Points and Morphometric Variables

Intensive sampling of points on 3D surfaces can be obtained by either manual and semi-automatic (Gunz and Mitteroecker 2013) or automatic techniques (Boyer et al. 2015; Pomidor et al. 2016). Once these points have been collected, the morphometric variation among specimens can be measured by the residuals obtained after the alignment of points to remove differences in position, orientation and—if needed—scale between configurations. These points are called pseudolandmarks or semilandmarks depending on the algorithm of alignment used.

Computation of Residuals from Pseudolandmarks

Pseudolandmarks are coordinates of points obtained by automatic procedures of sampling from 3D meshes (Boyer et al. 2015; Pomidor et al. 2016). The 3D meshes are aligned by using different implementations of the iterative closest point (ICP; Besl and McKay 1992) family of algorithms after an initial alignment based on the principal axis of variation of the surfaces. The correspondence between points is established by associating each point on one surface with its nearest neighbor on another surface. The best alignment is then obtained by minimizing the sum of distances between nearest neighbor points. This type of alignment has been recently implemented in two software applications, auto3dgm and GPSA (Boyer et al. 2015; Pomidor et al. 2016). Alternatively, the alignment can be obtained by first minimizing the Procrustes distances of a subset of landmarks manually digitized, and then using the Procrustes parameters obtained for aligning the vertices of the entire surface, such as in the algorithms implemented in programs for 3D analyses as Meshlab (meshlab.sourceforge.net). Finally, after the alignment, the residuals are estimated as the Euclidean distance between each point and its nearest neighbor. The residuals between surface–vertices (Gunz et al. 2012) are then used to quantify the morphological differences between specimens.

Computation of Residuals from Semilandmarks

Semilandmarks are points on homologous surfaces or curves obtained by manual or semi-automatic techniques, generally projecting surface points of a reference on a target surface (Gunz and Mitteroecker 2013). A generalized Procrustes superimposition, usually based on the least squares criterion,

is applied on these points. This procedure involves centering the coordinates at the origin, scaling the configurations to 1 by dividing the coordinates by the original centroid size of each specimen, and rotating the configurations, usually until the sum of squared distances among configurations is minimized (Rohlf and Slice 1990). The coordinates of points on surfaces are further slid in order to minimize the variance tangential to the surfaces or the bending energy (Bookstein 1997; Gunz and Mitteroecker 2013). Finally, the Procrustes residuals, defined as the Euclidean distance between corresponding semilandmarks, can be obtained to describe shape differences between specimens (Gower 1971; Gunz et al. 2012).

Visualization and Analysis of the Spatial Pattern in Shape Residuals

The residuals that measure differences between specimens have associated a spatial location, which is given by the x, y, z coordinates of each semilandmark or pseudolandmark. Consequently, the spatial structure of these residuals can be easily visualized by means of interpolation maps using algorithms such as the inverse of the distance, kriging and thin-plate spline, among others (Márquez et al. 2012; Pomidor et al. 2016). Among the available algorithms, the inverse distance weighting approach, which is frequently used in spatial statistics to generate interpolations based on the observations available from neighboring areas, it is especially suitable for morphological studies (Legendre 1993; Legendre and Legendre 1998). This algorithm estimates the unknown values assigning the weighted mean of the known observations in the neighboring area; $y_{\text{unknown}} = \sum w_i y_i$, where y_i is a known value and weight w_i is the inverse of the Euclidean distance between this value (e.g., semilandmark or pseudolandmark residuals) and the unknown observation. When the inverse distance weighting interpolation is applied to morphometric data, it is assumed that the interpolated values will be similar to the known values of a given anatomical region.

The spatial pattern of the differences between configurations of points can be further explored measuring the autocorrelation in shape residuals. Particularly, the pattern of spatial variation in the residual values can be efficiently explored using spatial correlograms or alternative techniques such as variograms (Legendre and Legendre 1998). In spatial statistics, the more frequently used technique measures the magnitude of spatial autocorrelation by the Moran's I autocorrelation coefficient:

$$I = \left(\frac{n}{S}\right)\left[\frac{\sum i \sum j (y_i - y')(y_j - y')w_{ij}}{\sum i (y_i - y')^2}\right]$$

where n is the number of spatial locations, y_i and y_j are the values of the residual measured in the spatial locations i and j, y' is the mean y, and w_{ij} is an element of a W matrix. In the W matrix the elements are equal to 1 if the pair i, j of spatial locations are within the class interval of a given distance or equal to 0 if the pair of locations are in different interval class. S is the number of cells in the W matrix. For morphometric data, the Moran's I coefficients can be plotted against the Euclidean distances between spatial locations or points in one surface used as reference, generating a spatial correlogram (Sokal and Oden 1978; Legendre and Legendre 1998).

An alternative to explore the spatial pattern of variation in the shape residuals is the use of non-parametric and iterative algorithms as the spatial K-means clustering, which estimates k different clusters that minimize the total error sum of squares in the residuals generating groups with the greatest distinction. This analysis can be run by taking into account or not the spatial localization of the residuals (Rangel et al. 2010).

Spatial Statistical Methods

The relationship between the pattern of variation in the shape residuals and independent variables can be explored by using statistical methods that incorporate the spatial localization of the shape residuals in the model. Several methods have been proposed to accomplish this, such as trend surface analysis, spatial eigenvector mapping and autoregressive method. The trend surface analysis is modeled as:

$$SR = XB + G + e,$$

where SR is the shape residual matrix; X represents a matrix containing one or several independent variables; B is the matrix of partial regression coefficients; $G = LS_L$, with L being a matrix with the localization of shape residuals and S_L the slopes of these localizations; and e is the error term (Legendre and Legendre 1998; Perez et al. 2010b). By using this approach we can explore changes in shape residuals at different spatial scales, similarly to the Factor analysis over a bending energy-partial warps variance matrix recently proposed for morphometric data (Bookstein 2017).

Other methods more frequently used, such as the generalized least squares (Dormann et al. 2007; Diniz-Filho et al. 2009; Perez et al. 2010a), take into account the spatial autocorrelation in the shape residuals by modeling the error term:

$$SR = XB + e.$$

The standard regression model employed in morphometric studies assumes that the covariance matrix (C) for the e term of the shape variables or shape residuals can be well described by $C = \delta^2 I$, where δ^2 is the variance of the residuals, and I is the identity matrix, with 1 in the diagonal and 0 in the off-diagonal terms:

$$I = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

Several local factors can generate non-independent neighbor shape residuals, violating the assumption modeled by the *I* matrix. Generalized least squares can account for this non-independence using a C matrix for the *e* term, which contains the resulting values from modeling the spatial structure of the shape residuals in the off-diagonal terms. For example, we can set the matrix $C = \delta^2 W$, where the zero values in the *I* matrix are replaced by w_{ij}, the inverse function of Euclidean distances (d_{ij}) between the spatial locations of all pairwise shape residuals, weighted by a factor α that controls the decay curve:

$$wij = \frac{1}{d_{ij}^{\alpha}}$$

$$W = \begin{bmatrix} 1 & w_{21} & w_{31} \\ w_{12} & 1 & w_{32} \\ w_{13} & w_{23} & 1 \end{bmatrix}$$

Alternative C matrices have been proposed in spatial analyses to model the non-independence of the residuals, such as simultaneous spatial autoregressive, conditional spatial autoregressive or moving average (Dormann et al. 2007; Diniz-Filho et al. 2009; Perez et al. 2010b). The significance of the regression model can be then assessed by the F-statistic.

The spatial analyses performed in the following examples were performed in SAM v4.0 (Rangel et al. 2010). There are also several R packages (e.g., rspatial, gstat, spdep) that perform the spatial analyses shown here (R Development Team 2019).

Examples

Example 1

We analyzed 3D meshes of the skull of two species of primates, one specimen of *Alouatta* and one specimen of *Saimiri*, as an example of the spatial pattern of shape variation at an inter-specific level. These 3D meshes were generated from CT-scans using Slicer 4 (www.slicer.org), saved in PLY file format and edited in MeshLab (meshlab.sourceforge.net), where the vertices were subsampled in a uniform way to ca. 30,000 points using the Mesh Element Subsampling filter. The CT-scans were obtained from the Digital Morphology Museum (DMM, KUPRI, Primate Research Institute, Kyoto University, Japan) repository.

The 3D surfaces extracted from the CT-scans were aligned using an approach based on reference landmarks implemented in MeshLab. The following landmarks were used with this aim: nasion, sub-spinale, prostion, bregma, lambda, opistion, basion, basioccipital, palatino, ectoconchion left and right, zigomaxillare left and right, asterion left and right, and porion left and right. The square difference between these landmarks was minimized and the *Saimiri* mesh was aligned to the *Alouatta* one. After the alignment of the surfaces, the shape variation between the two specimens was estimated by the residuals of the pseudolandmarks or homologized points, i.e. the 30,000 vertices. We calculated these residuals as the Euclidean distances between homologized points after the alignment procedure. These residuals represent the distance between vertices in one mesh to the closest points in the second mesh and were obtained by the Hausdorff distance implemented in MeshLab (Cignoni et al. 1998). In sum, by using this procedure we obtained the 3D coordinates of points used to describe the primate skulls and the Euclidean distances among the closest points between the meshes that represent the shape differences between specimens (i.e., shape residuals).

The spatial pattern of the residuals was depicted by color-maps interpolating the values of the residuals on one of the meshes. In this example, the interpolation map was displayed on the surface of the *Alouatta* skull (Fig. 1). As is shown in the colormap, the vault, anterior maxillary bone and zygomatic arch have large residual values, while the cranial base and the areas surrounding the orbitals have the smallest residuals (Fig. 1). This suggests that the morphometric differences between *Alouatta* and *Saimiri* are localized in specific areas of the skull that vary in a coordinate way. At this point, we applied the Mesh Element Subsampling filter of MeshLab to reduce the number of vertices to 2500 for subsequent analyses. The spatial correlogram of the residuals is characterized by positive autocorrelations at short distances coupled with a negative autocorrelation at large distances (Fig. 2a), corroborating the visual interpretation in the colormap. For the first distance class, the scatterplot of the residual values at each pseudolandmark against the average residuals of their neighbors shows a strong linear relationship (Fig. 2b).

The K means clustering performed on the residuals grouped the pseudolandmarks following a pattern similar to the one depicted by the interpolation map (Fig. 3). We then explored the fit of the shape residuals considering two previously hypothesized components or modules, the neurocranium and the facial skeleton, and taking into account the spatial structure of the shape variables. First, we performed a generalized least squares analysis with the shape residuals as dependent variables and the module as an explanatory factor and found that only 1.2% of the variance in the residuals was explained by this factor. Then, when the spatial structure was incorporated into the model, the percentage of shape variation explained by the predictor variable and the space increases up to 49.9%. These results indicate that a large amount of the shape residual variation between species is not

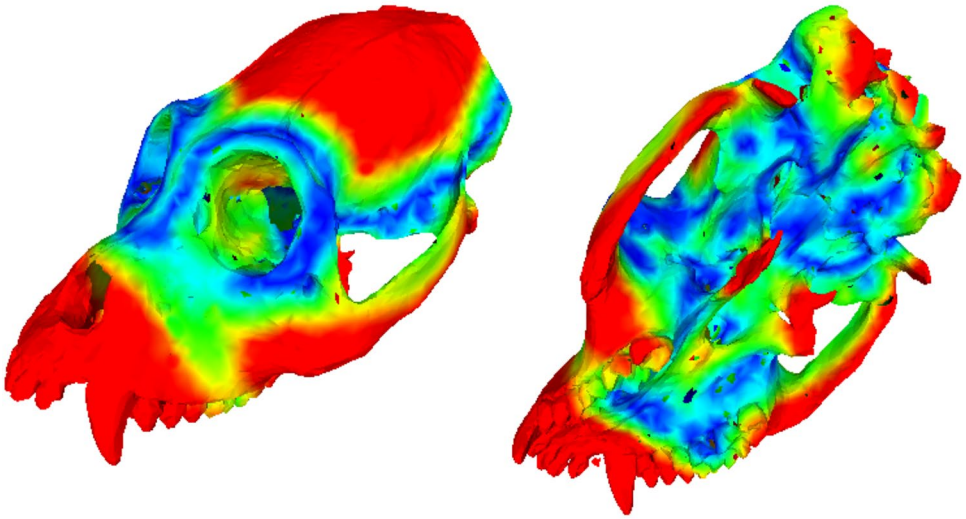

Fig. 1 Color-map representing the differences (pseudolandmark shape residuals) between the *Saimiri* and *Alouatta* specimens (Color figure online)

explained by the modules but it is associated to the spatial structure of shape changes between the two species.

Example 2

To exemplify the spatial structure of shape changes along the ontogeny we analyzed a sample of 19 human endocasts aged from 0 to 12 years old. The sample was obtained from the skull CT-scans of the Bosma collection (Shapiro and Richtsmeier 1997). The endocasts were manually segmented in Slicer 4 software to obtain the 3D meshes. Then, the Mesh Element Subsampling filter of MeshLab was used to reduce the number of vertices of each mesh to 5000. The 3D meshes obtained by such procedure were aligned by an automatic alignment procedure, the Generalized Procrustes Surface Superimposition (GPSA; Pomidor et al. 2016). Shape variation among specimens was estimated by the residuals of the pseudolandmarks obtained as the Euclidean distances between homologized points after the GPSA (Pomidor et al. 2016).

The interpolation map depicting the shape changes in the postnatal ontogeny of the brain endocasts, considering the youngest and oldest individual in the shape comparison, shows a strong spatial structure (Fig. 4). Shape changes are mainly located in the anterior region of the frontal lobe, the limit between frontal and parietal lobes, the inferior region of the temporal lobe and the occipital lobe. The spatial correlogram of the residuals between the youngest and the oldest specimen supports the existence of a spatial structuration of the shape changes between neonates and juvenile specimens (Fig. 5a). Residuals at short distances display moderate positive correlations (Fig. 5b) while there is no autocorrelation at larger distances.

For the dataset of human endocasts we explored the pattern of spatial autocorrelation in allometric shape changes. First, for each specimen we estimated the shape residuals between each specimen configuration and the mean shape. Then, the eigenvector of the first principal component obtained from the matrix of residuals was used to represent the allometric component of shape changes in brain endocasts. The first eigenvector is the only one highly correlated with the Log volume of the endocasts ($r = 0.81$). The analysis of autocorrelation for the values of the first eigenvector shows positive and high correlations at short distances, indicating that closer semilandmarks will tend to display a similar magnitude of change along the allometric axis (Fig. 6).

Discussion

Our results show that shape changes in the two discussed examples—the comparison of the skull between two species of primates and an ontogenetic series of human endocasts—are spatially structured, i.e. the shape residual are spatially autocorrelated. Some regions of both morphological structures display higher differences in shape than others and they tend to be near in terms of their anatomical position. We described and quantified this pattern by using methods that take into account the position of the shape residuals and were able to consider the spatial structure, particularly spatial autocorrelation, in statistical analyses applied to the study of modularity and allometry. As expected, different results were achieved when spatially explicit methods were used: in the first example, we found that part of the inter-specific variation in the skull that has been previously interpreted as evidence of functional modularity (Cheverud 1996; Lieberman 2011) can emerge by the influence of processes with local effects that generate the spatial autocorrelation observed in shape data; in the second example, the spatial regression showed that an important portion of

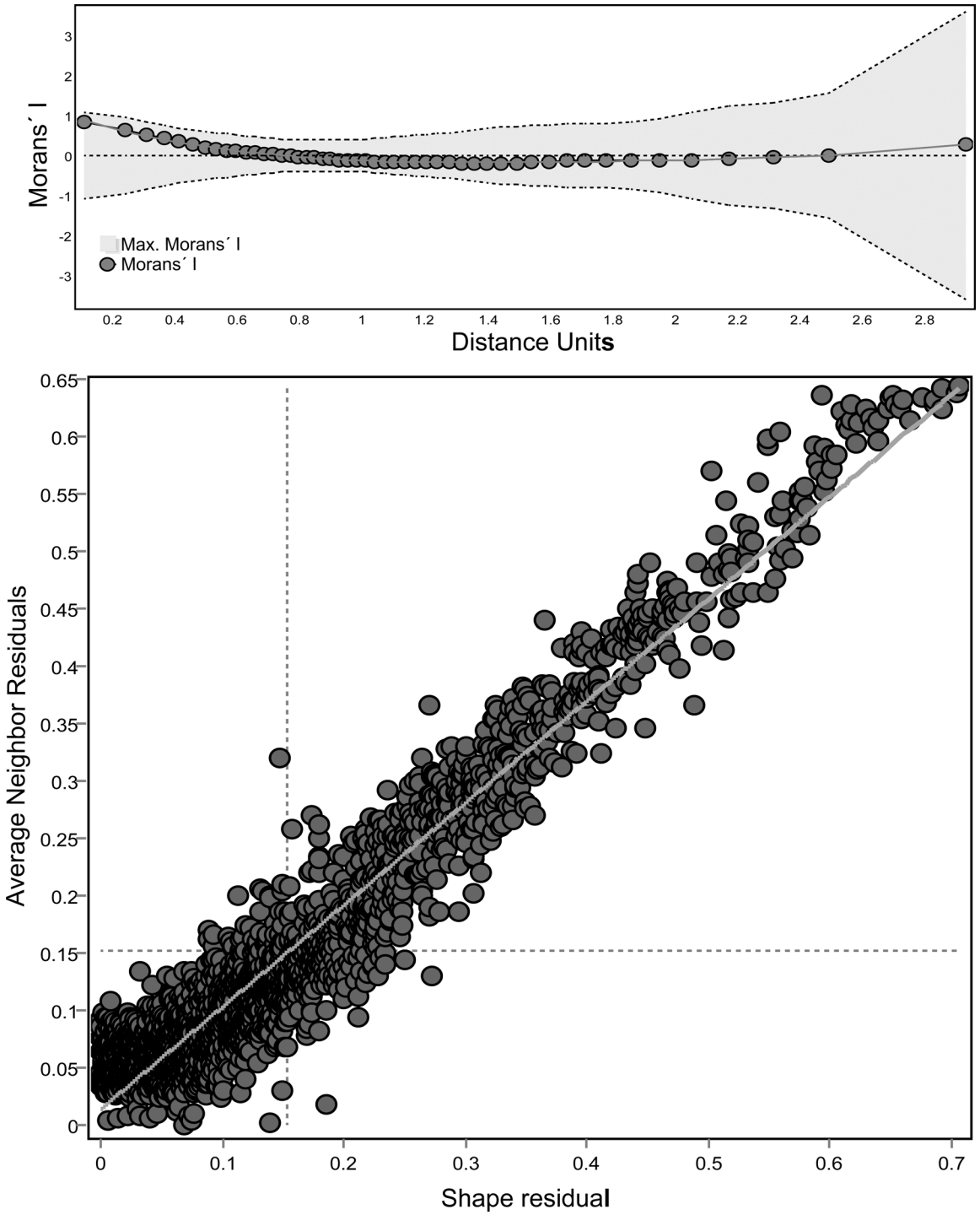

Fig. 2 Spatial correlogram of the residuals between the *Saimiri* and *Alouatta* specimens (above) and scatterplot of the shape residual values of pseudolandmark for the first distance class (below)

shape changes associated with ontogenetic allometry was localized even though a greater effect of global variation is expected as a consequence of growth (Durrleman et al. 2012). Therefore, our findings have implications for both the selection of morphometric variables and their posterior statistical analyses.

The high autocorrelation observed in the morphometric data suggests that the sampling design, in terms of the type and number of reference points (e.g. landmarks,

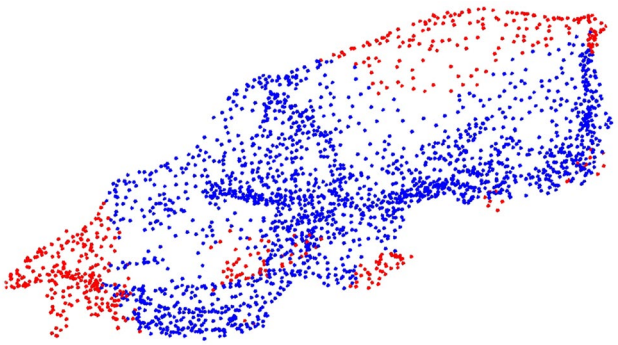

Fig. 3 K means clustering of the shape residuals of pseudolandmark between the *Saimiri* and *Alouatta* specimens

semilandmarks) digitized on the structure of interest, needs to take into account the spatial structure of shape variation, particularly when the interest is analyzing global and local shape changes as has been previously proposed (Rohlf 1993; Mitteroecker 2009). A recommended first step in such studies is to apply an intensive and non-directed sampling of surface points automatically extracted from the 3D meshes and then automatically align the structures to measure shape differences, such as in the methods proposed by Boyer et al. (2015) and Pomidor et al. (2016). The automatic sampling of surface points is particularly adequate for detecting areas of greater and lesser variation because the strategy of sampling is not biased by a priori assumptions of the observers (Gonzalez et al. 2016). These methods allow to explore the degree of dependence among shape residuals at nearby reference points, which can serve as a guide to design the sampling of points in a way that regions that are relatively independent are evenly represented. Then, one can decide whether the use of landmarks, a combination of landmarks and semilandmarks or pseudolandmarks is more suitable to answer the research questions under study. Additionally, the required number of points needed for an adequate assessment of shape variation could be also evaluated as it has been recently proposed (Watanabe 2018). The incorporation of the spatial information, and particularly the patterns of autocorrelation, in such analyses could contribute to improve the sampling strategies by applying informed rather than random removal of landmarks. In this context, the use of too few landmarks will preclude the description of shape variation at local scales, since these reference points tend to exclude local shape changes and better describe the variation at broad (global) spatial scales (Watanabe 2018).

Regarding the implications for statistical analyses, we show that different results are attained by using methods that incorporates or not the spatial autocorrelation in the evaluation of the effect of specific biological factors on shape variation. Particularly, these analyses indicate that the effect of biological factors acting at local scales can be confounded with more systemic factors (by example the effect of the diet on the facial skeleton; Cheverud 1996; Lieberman 2011) if the spatial autocorrelation is not taken into account. For these analyses, we incorporated the spatial autocorrelation structure using a generalized least squares technique, but different methods have been proposed in geographical and ecological studies for modeling and testing independent factors and taking into account the spatial variation, such as partial mantel, trend surface, autoregression and spatial eigenvector mapping (Legendre and Legendre 1998; Diniz-Filho et al. 2009; Perez et al. 2010a). These methods present advantages and disadvantages, depending the studied problem, and some of them have been criticized previously. By example, methods such as partial mantel, trend surface, autoregression and spatial eigenvector mapping have been criticized in phylogenetic studies (e.g. Rohlf 2001, 2006; Perez et al. 2010b), but the last two have shown to be efficient and flexible in spatial analyses (Dormann et al. 2007; Diniz-Filho et al. 2009). Conversely, there is an agreement that partial mantel and trend surface should be used with caution because they remove lineal trends or affine spatial variation and can eliminate the effect under study (Legendre and Legendre 1998; Perez et al. 2010b). However, the suitability of these methods will depend on the structure of the morphometric

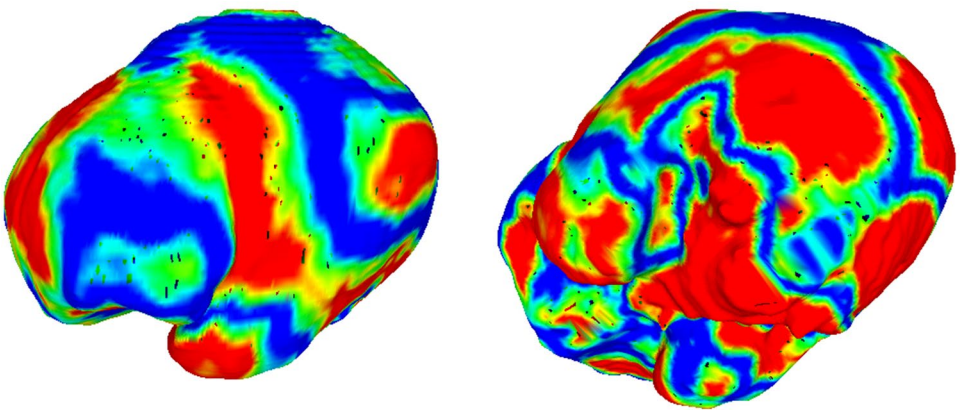

Fig. 4 Color-map depicting the shape changes in the postnatal ontogeny of the human brain (Color figure online)

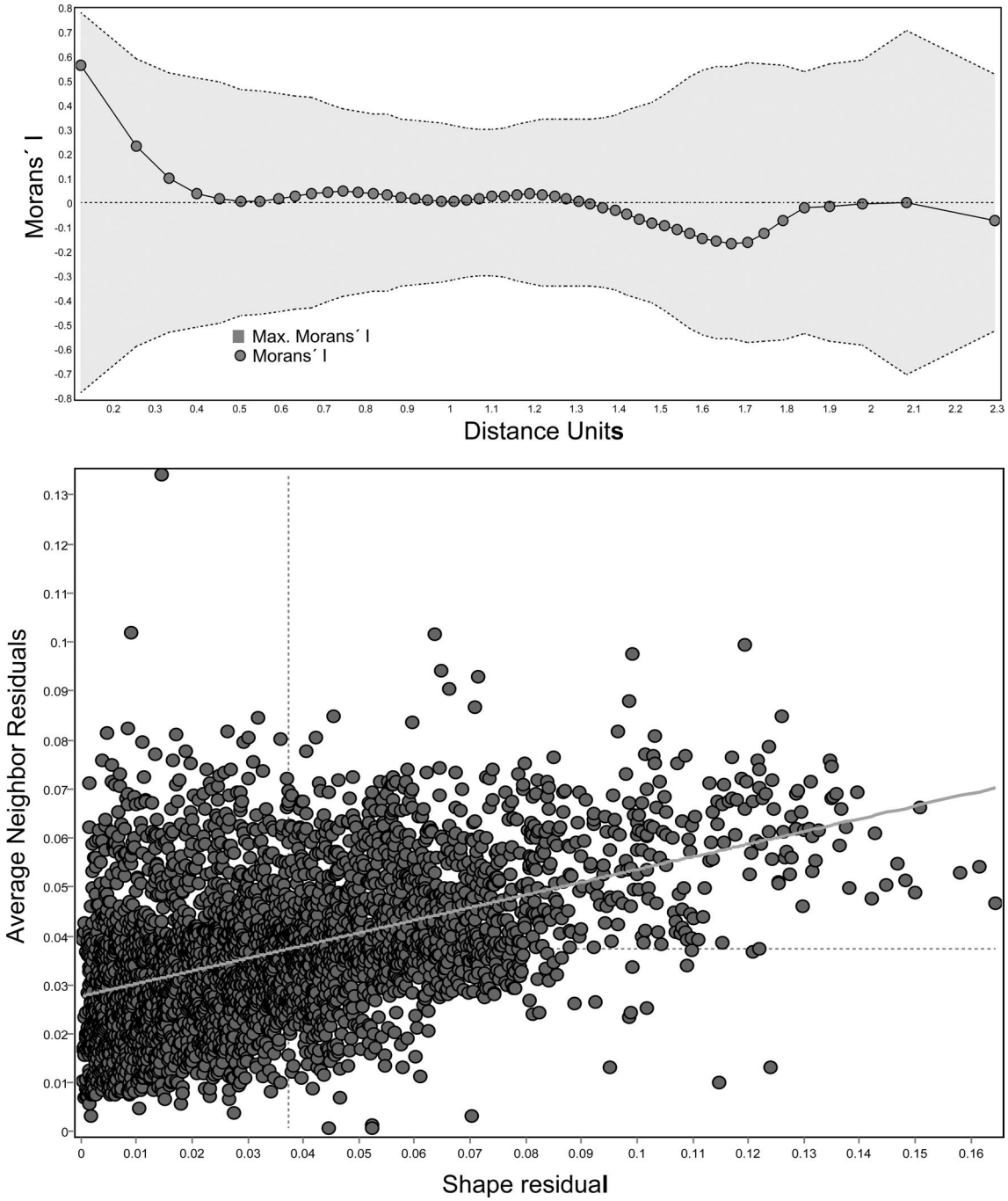

Fig. 5 Spatial correlogram of the residuals between the youngest and the oldest specimens (above) and scatterplot of the shape residual values of pseudolandmark for the first distance class (below)

spaces, such as the affine or non-affine characteristics, which might limit the meaningfulness of our biological statements (Huttegger and Mitteroecker 2011). In this context, the use of spatial regression techniques such as generalized least squares, autoregression or spatial eigenvector mapping have been recommended in spatial analyses because they are flexible and useful for modelling spatial variation and provide more accurate statistical estimations (Dormann et al. 2007; Diniz-Filho et al. 2009; Perez et al. 2010a, b).

Although we only analyzed two cases to exemplify the importance of spatial autocorrelation in morphometric studies, it is expected that they represent a common pattern if we take into account that developmental and functional factors can have local and global effects (Hallgrímsson and Lieberman 2008; Mitteroecker and Bookstein 2008; Mitteroecker

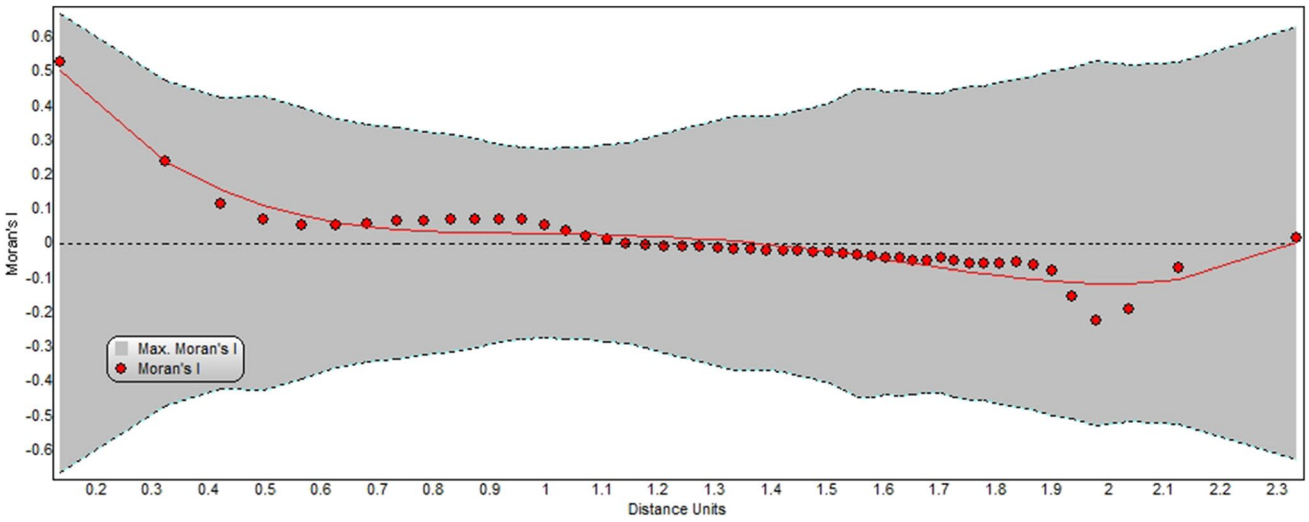

Fig. 6 Spatial autocorrelation for the values of the first eigenvector representing allometric changes

2009). Morphological traits arise during ontogeny under the influence of functional and developmental factors occurring in a sequential spatiotemporal manner (Hall 2003). Such factors can have a systemic effect influencing several traits across the organism (such as circulating hormones and environmental influences) or local effects on a subset of traits (such as locally expressed molecules with autocrine or paracrine actions and local tissue interactions) (Parker 2011). Additionally, closer traits tend to interact with each other and with their local surrounding environments being influenced by several common local process (Mitteroecker and Bookstein 2007). In consequence, characteristic patterns of variation and covariation in shape at different spatial scales within the anatomical structure studied are expected to emerge along individual ontogeny and evolve throughout generations. Therefore, the spatial variation need to be modeled and systematically incorporated in the statistical analysis of morphometric variation.

Overall, our results suggest that the approach presented here, which it is based on the intensive description of shape differences between structures using densely sampled points on 3D surfaces and employing spatial statistical methods to describe the pattern of differences and test biological hypotheses, can be used to explore several problems not widely addressed previously in modern morphological studies. The equipment for image acquisition, such as CTs and surface scanners, that allow for the acquisition and publication of 3D digital images (Davies et al. 2017), as well as the generation of high dimensional morphometric datasets (Gunz and Mitteroecker 2013; Pomidor et al. 2016), open new avenues to explore the shape variation beyond the use of landmark-based morphometric methods. However, whether landmarks or densely sampled points are used will depend on the research questions; if the interest is the study of specific anatomical regions or global shape characteristics, the use of a few anatomical points could be more adequate (Pomidor et al. 2016).

Finally, it is important to remark that more work is needed to explore which spatial regression techniques would be the most suitable for morphometric studies. The techniques used here are not new in evolutionary biology and macro-ecology (Dormann et al. 2007; Bini et al. 2009), but they have not been applied to morphometric data before. In addition, approaches based on principal components or factor analysis that have been successfully applied to take into account the phylogenetic structure in the data (Revell 2009; Tolkoff et al. 2017) could be also used to explore the variation in morphometric data at different spatial scales (Bookstein 2017). Within geometric morphometrics, we need to consider the characteristics of the shape space, because different approaches might be suited to morphometric residuals analyzed in the Tangent/Euclidean space or in the Procrustes/Kendall one. Techniques such as generalized least squares, autoregression or spatial eigenvector mapping are adequate for exploring and testing variation in the Euclidean space, although they have also been employed using spherical space, such as the Earth coordinates. Conversely, the phylogenetic eigenvector regression (PVR, Diniz-Filho et al. 1998) method, a phylogenetic version of the spatial eigenvector mapping has shown strong problems to work with the patristic phylogenetic spaces (Rohlf 2001). In this sense, we need to better explore the behavior of this methods in a non-Euclidean space, like Procrustes, where the spatial properties are more complex. In our view, because spatial autocorrelation and regression techniques are flexible enough and could contribute to add new information, they should be incorporated to the geometric morphometric tool-kit

(Mitteroecker and Gunz 2009), particularly to explore morphometric changes occurring at different spatial scales.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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