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Research progress of geometric morphometrics in animals: A review

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ABSTRACT

Morphological description is the basis of systematical classification and biodiversity research. Morphometry is the science of quantitative description, analysis and interpretation of morphology and morphological variation. With the development of statistics, it is necessary to analyze very complex data, which objectively leads to the emergence of multivariate morphometry. In the 1980s, the matching of geometric information of punctuation and punctuation relative position made an important breakthrough in data collection and analysis. As a result, the punctuation of multivariate analysis can be superimposed on the original biological map, which can not only generate scatter plot, but also objectively reflect the morphological characteristics of organisms. This research is called geometric morphometry, which is a revolution in morphometry. In the current researches, two kinds of punctuation methods are widely used, one is outline methods, the other is landmark methods. Compared with the traditional morphometric method, geometric morphometry (GMM) is a new type of morphometry, because it can not only accurately and quantitatively describe and analyze the morphological changes between biological samples and samples, but also analyze whether these changes are caused by growth and development, experimental treatment, or genetic evolution, etc., it is a commonly used method in morphological research at this stage. The present paper introduces the development process of geometric morphometrics (Outline method, Landmark method) on the basis of traditional morphometry, and puts forward in-depth summary and prospect on the application of geometric morphometrics in system development analysis, ecology and other aspects.

Keywords: *multivariate morphometrics; traditional morphometry; outline method; landmark method*

1. INTRODUCTION

For centuries, comparison of biological anatomical characters had become the core issue of biological researches, because the study of biological systematics and biodiversity were mainly based on morphological descriptions (Barlow et al., 1997; Savriama and Gerber, 2018). In the early 20th century, biology began to transition from descriptive and qualitative disciplines to quantitative studies, as did morphological analysis (Cardini et al., 2005; Chen et al., 2018). In morphological studies, the measurement of measurable traits and the comparison of different traits by means were found in morphological studies (Renaud et al., 2007; Maximiliano et al., 2020). The development of statistical methods provides more advanced and rigorous quantitative analysis. By the middle of the 20th century, the quantitative description of morphological traits integrated the statistical analysis of morphological changes within and between groups, which was also the beginning of modern morphometric methods (Renaud and Michaux, 2007).

Morphometrics was a science of quantitative description, analysis and interpretation of morphology and morphological variation. The term "morphometry" had appeared as early as the 1960s. People intuitively want to express shapes in a mathematical way, or as a basis for comparison. It was not until nearly 10 years ago that a breakthrough concept appeared, which changed and affected the development in the future. After that, the "new geometry" will be called "geometrics morphometrics" (Bookstein, 1991; Latif et al., 2019).

2. Traditional morphometry

In order to measure the length and width of the individual wing, we can use it to measure the length and width of the individual wing. After 1930, due to the maturity of multivariate statistics, scholars can explore multiple measurement variables at the same time and apply many new statistical methods to describe, identify, decompose, summarize and compare. At present, we generally call these analyses "multivariate morphometrics" (Cardini and Higgins, 2004). In the 1960s and 1970s, biostatisticians began to use multivariate statistical tools to describe morphological variation within and between groups, which is called traditional morphometry, or multivariate morphometry, including the application of multivariate statistical analysis methods to the analysis of morphological variables, such as the measurement of linear distance, quantity, proportion or angle. Since linear distance measurement is usually highly related to size, it is possible to analyze morphological variables of amorphous structures and study the patterns of morphological variation (Racz et al., 2005; Tarquini et al., 2019).

Although the traditional morphometric method combines multivariate statistics with numerical analysis of morphological traits, there were still some problems. For example: (1) the homology of linear distance is difficult to evaluate because many distances (such as maximum width) cannot be used as homologous traits. (2) The same distance data can be obtained from different shapes because the measurement points are not included in the data. If you measure the maximum length and width of ovals and teardrops, you may get the same data, but they are completely different shapes. Therefore, the ability of statistics to distinguish shapes is overestimated. (3) It is usually impossible to generate a graph to represent a linear distance because the geometric relationship between variables is not preserved (a series of linear distances are often difficult to capture the geometry of the original object). Therefore, some aspects of the properties of the object will inevitably be lost. These shortcomings bring great difficulties to the application (Table 1).

Table 1 Comparison between traditional morphology and geometric morphology

Methods	Advantages	Disadvantages
Traditional morphological methods	Easy to operate Methods mature Multivaeiate morphometrics	Inaccurate Homology of linear distances is difficult to assess Overestimate the power of statistics in shape discrimination Some aspect of an object inevitably loses its shape
Geometric morphological method	The actual size of the specimen is not taken into account It can thorough and complete description of the morphological structure	Unquantifiable data Program trival Multiple iterations are needed to remove the human error

3. The development and evolution of geometric morphometry

Due to the above problems, researchers try to overcome its shortcomings and explore other quantitative morphological traits and analysis methods. Their research interests mainly focus on the acquisition of geometric structure data (including contour data and punctuation data) and the analysis methods of these data (Braga et al., 2019).

3.1 Outline methods

Outline methods is the first geometric shape measurement method. When the edge of a structure or a region has homology, the sample points selected on the edge curve are not required to be completely consistent. This method is usually used to digitize the sample points on the contour, which conform to a certain mathematical function (usually Fourier analysis), and then analyze the shape variables as the multivariate of the function coefficients to compare the differences of the curves. Points in multivariable parameter intervals (such as Fourier coefficients) can be transformed into physical distance intervals of organisms and can be visualized as contours (Fig. 1).

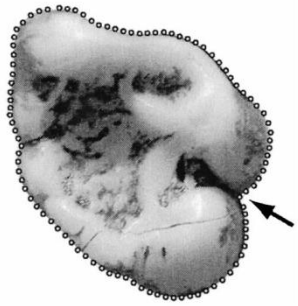

Fig. 1 One hundred outline points used to characterize the shape in occlusal view of the crown of the lower third molar of marmots. The arrow indicates the first point in the sequence (Caumul and Poll, 2005)

3.2 Landmark methods

As the name implies, ground punctuation refers to some obvious and easily recognizable points; these points can usually be defined or marked without doubt in organisms and sometimes even have specific nouns corresponding to traditional morphology. These methods are called landmark methods for morphometry. In the application of biology, landmark area can be divided into three categories: type I landmark refers to the intersection point between different tissues; type II landmark refers to the concave or protruding point in the structure; type III landmark refers to the pole of structure, such as the longest point, the widest point, etc (Murta-Fonseca et al., 2019).

There are two core problems in the new morphometry: one is the geometry of the form itself and the location of its points; the other is the problem of morphological deformation. In other words, we must make sure that the points of comparison are corresponding to each other in order to explain the meaning of deformation. The landmarks in the punctuation method are selected from the biological structure, and the structural changes in different specimens can be reflected by punctuation. Compared with the previous two methods, it has certain advantages: for example, the redundant nature of the multivariate method is difficult to remove, the shape information covered is not complete enough, it can only be used to check the difference of table values, and the reproducibility of data regression shape is poor However, the medium representation method of contour method can hardly overcome the consideration of homology in comparison among organisms, and it has insufficient analytical ability for regional deformation, etc. (Bookstein, 1991; Lopez et al., 2019).

Changes in the position, orientation and proportion of punctuation on the specimen will cause changes in the correlation of direct analysis, and these non morphological variations may interfere with the analysis, which can be done mathematically. Once the interference of non morphological variation can be removed, the variable of morphological variation can be used as a statistical tool to compare different samples, and graphical comparison is also possible. Although some methods have been applied to remove the interference of non morphological variation, the theory and optimization principle used by these methods are slightly different (Tozetto and Lattke, 2020). At present, overprint is commonly used. The shape information of creatures is represented by the coordinates of landmarks. By using this method, objects can be zoomed in and out without changing the original relative position of each point. Through rotation and overlap, the homologous landmarks can be as close as possible to explore the shape differences among different samples (Adams et al., 2004). Figure 2 illustrates the three main steps of overprint (Rüber and Adams, 2001): (1) quantification of raw data; (2) removal of the effects of non-morphological variation; (3) statistical analysis and graphical presentation of results (Fig. 2).

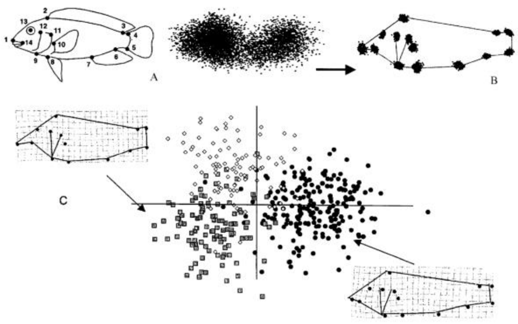

Fig. 2 Steps of data analysis of overprint punctuation (Rüber and Adams, 2001)

A. Quantification of raw data (Data from *Oreochromis mossambicus* (Cichlidae)); B. Remove the influence of non morphological variation; C. Statistical analysis and graphical results

Two point registrations is a simple overprint method, which is the basis of morphological analysis theory proposed by Bookstein in the late 1980s (Bookstein, 1991). This method eliminates the influence of non morphological variation by adding punctuation based on optimization principle. Considering that different base points may produce different results, the two-point method is still lack of objectivity. In addition, there are two methods, one is generalized procures analysis (GPA) and the other is generalized resistance fit (GRF). In the early days of GPA, the generalized least squares (GLS) were used to estimate the translation and rotation parameters using the smallest square, so as to overprint the landmark layout. In this process, the average value of the shape should be calculated repeatedly, but the overprint cannot be estimated in advance. When many morphological variables are limited by little punctuation, GRF can be used in this variable model. GRF can use the overprint parameter as the median instead of the minimum square estimation. The rotation angle and scale are used as the median of the set of points, and the translation is a simple coordinate median. In GPA, this step allows the sample to be overprinted repeatedly. Compared with GPA, GRF cannot be used for further statistical analysis (Chaiphongpachara, and Laojun, 2019).

In addition, there are also Euclidean distance matrix analysis (EDMA) and the finite element scaling analysis (FESA), etc. (Figure 3), but their theoretical basis is not mature and few studies have mentioned it (Nishimura et al., 2019).

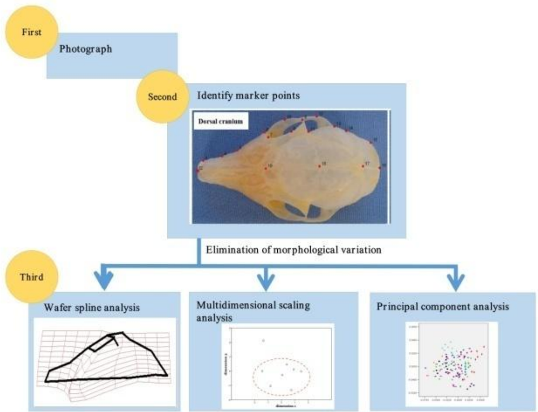

Fig. 3 The general procedure of geometric morphology analysis

4. Maturity of geometric morphometry

The sign of the revolutionary development of morphometry is that morphological research has changed from linear distance analysis to punctuation and contour analysis. In the early 1990s, geometric morphometry was widely recognized. Biologists gradually applied punctuation method and contour method to many fields on the basis of continuous skilled use. In addition, the work done by Kendall and other experts in the field of punctuation in the 1980s enabled the punctuation method to gain a deeper mathematical foundation (Kendall, 1984; 1985).

5. Application and Prospect

The data used in geometric morphometry describe the morphological structure deeply and completely, so these data can be used for cladistic phylogenetic analysis. But it is very difficult to combine the two. First of all, geometric morphometry should capture all possible morphological variations. The variables are continuous, but the characters in cladistic analysis are discontinuous. Secondly, the simplest phylogenetic tree is needed in cladistic analysis, but it is difficult to do this for geometric morphometry. Although the combination of the two is difficult, there are still some related research progresses. Based on certain theoretical standards, the original morphological data are encoded as discontinuous character states, which can be used as input data for cladistic analysis (Zelditch, 2000; Alvarez-Varas et al., 2019). But in the process of coding, some forms of information will be lost.

The application of geometric morphometry in ecology is mainly reflected in the study of ecological morphology. Ecomorphology is an interdisciplinary study on the interaction between morphological diversity and ecological diversity. Its foothold lies in the correlation between morphological characteristics and ecological distribution. It even thinks that the evolutionary characteristics and evolutionary patterns of organisms in different environments can be inferred from the ecological morphological characteristics (Lucas and Teaford, 1994). Due to the different geographical distribution, the living environment of the species may be different. The long-term adaptation results in the morphological differences of some small mammals living in them at the intraspecific or interspecific level (Yukibumi, 2002; Yoram and Shlomith, 2004). These morphological differences are reflected in the changes of bone and body size of the species to a certain extent (Zhu et al, 2008). Morphological characteristics have always been the basis of taxonomy and the main basis for exploring homology or heterologous phylogeny. The comparison of skull morphology not only has important reference value in the research of animal interspecific, intraspecific relationship and ecological characteristics, but also has very important significance in the study of animal geographical evolution, developmental biology and evolutionary ecology. The morphology of skull is closely related to its living environment, and the change of habitat may cause morphological changes.

According to Bookstein (1991), there are two main cores in geometrics: one is the geometry of the form itself and the location of its points; the other is the problem of morphological deformation. At present, geometric morphometry methods have been used by researchers in different fields. For example, Renaud and Michaux (2007) used *Apodemus* to understand the effects of island climate and genetic effects on island populations The results showed that mandible and molars of sylvaticus had different evolutionary patterns, which were related to island size, distance and competition level. Renaud et al. (2007) studied the mandible morphology of small rodents in the ancient continent and analyzed the IRBP sequence of photoreceptors The results showed that the morphological variation of *Marmota* could be distinguished in some groups whose molecular variation was not enough to distinguish; the skulls of marmots were studied by Caumull and Polly (2005) and Cardini et al. (2005). The results showed that there was a close relationship between the morphological differences of *Marmota* skulls and the environmental factors. Moreover, principal component analysis of the skulls of tree shrews from Yunnan, Guizhou and Guangxi Province showed that the samples distributed in Yunnan were completely separated from those in Guizhou and Guangxi in our previously study (Zhu et al., 2013).

At present, the method of geometric morphometry has been adopted by researchers in different fields. In principle, any research concerned with morphology and morphological variation can use this analysis to further understand the data. At present, we can see that there are various types of medicine, such as X-ray, ultrasound, tomography or magnetic resonance, MR imaging analysis and diagnosis, morphological disease analysis, study of the relationship between structural differences in the brain and mental disorders, and the application of morphology in dentistry, orthopedics or plastic surgery, etc.; the measurement of skull in anthropology, the reorganization and analysis of morphology by paleontology, and the application of other biological disciplines to functional morphology, ecological morphology, morphogenetic chemistry and systematic (Ellmouni, 2019).

6. Conclusion

Although morphometry is troubled by the so-called homology problem, it is still controversial in evolution and systematics. However, landmark morphometry can help to analyze many morphological changes. For example, recently scholars like to use morphometric data to explain species polymorphism, androgyny, functional differences or the impact of ecological and environmental factors, which is a very appropriate application However, these inferences still need to be based on sufficient evidence and understanding of the events to be explained, so as not to become a fallacy of over interpretation. In addition, the analysis and comparison of morphological variation found in some operational tests or surveys provide a more detailed identification approach. As for the application of biology, some scholars have tried to analyze the motion curve of objects, the analysis of movable joint bodies, and the combination of fossil morphological data and molecular evolution research. Of course, more scholars in different fields have brought more diverse materials and the feasibility of field detection methods. In fact, we can imagine that morphometry should have more applications, even in many non biological fields. In recent years, the problems that scholars are eager to solve include strengthening the application of contour method, integrating punctuation method and contour method, and overcoming missing data.

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