

# A New Statistical Method for Comparative Anatomy

## Nuevo Método Estadístico para Anatomía Comparada

Tales Alexandre Aversi-Ferreira

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**SUMMARY:** Rules, formulas and statistical tests have been widely used in studies that use continuous variables in the normal or Gaussian distribution or other tests whose data fit this type of distribution (*t* and *z* distributions) and whose parameters can be defined. However, there are cases in which the model and goal of the scientific work allow only the use of discrete or nominal variables. The absence or presence of a structure or organ (muscles, nerves, blood vessels, bones, and ligaments), characteristics associated with these organs, such as the source of nerves, vessels and muscles and the distribution of nerves and vessels are discrete and / or nominal variables that do not fit the Gaussian distribution, nor of *z* or *t*, unless the measures of these structures are taken. A simple comparative method within the non-parametric distribution has been proposed, (1) between two different samples from the same species and (2) for two different species, based on the concepts of normal and variation in anatomy and on modal class in statistics here called Comparative Anatomy Index (IAC). The main findings on the use of this method are: (1) to allow the relationship between data from discrete or nominal variables in comparative anatomy or in other areas of morphology, (2) to generate an objective method (numerical) of comparison between samples of structures from the same species and between different species and (3) to fill the lack of a specific method for the statistical work in comparative anatomy or in morphology, in general.

**KEY WORDS:** Morphology; Comparative anatomy; Statistical method.

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## INTRODUCTION

Rules, formulas and statistical tests are widely used in studies that use continuous variables in the normal or Gaussian distribution or other tests whose data fit this type of distribution (*t* and *z* distributions) and whose parameters can be defined.

Average and Standard Deviation are the most common statistical parameters used when the model used in the biological area allows the use of continuous variables, and for a population of continuous data. Average and Standard Deviation are measures derived from symmetric distributions and called as measures of central tendency (Centeno, 1982; Monteiro-Filho, 2004; Vieira, 2004).

However, other types of Averages can be calculated in non-normal distributions, as in the binomial distribution, but they do not allow central tendency analyses and require randomness among data.

Especially in Anatomy, the continuous variables are used to measures size (Absalão *et al.*, 2005; Cáceres,

2005), area or volume of structures or organs (Monteiro & Lessa, 2000; Christel & Billard, 2002). These variables, in biological samples, tend to have a symmetrical distribution and generally fit the Gaussian distribution.

However, there are cases in which the model and goal of the scientific work allow only the use of discrete or nominal variables. The absence or presence of a structure or organ (muscles, nerves, blood vessels, bones, and ligaments), characteristics associated with these organs such as the source of nerves, vessels and muscles and the distribution of nerves and vessels are discrete and / or nominal variables that do not fit to the Gaussian distribution, nor of *z* or *t*, unless the measures of these structures are taken.

These discrete variables do not generate symmetrical distribution; therefore, defining Average of population and standard deviation of this average or any other measure as the latter, with central tendency characteristics is not appropriate (Centeno; Vieira). In these situations, there is no way to define positions or classes for some non-parametric

distributions that are particularly used for measuring nominal and whole variables (Monteiro-Filho).

Non-parametric distributions are used when it becomes difficult to establish quantitative values for the data (Centeno).

Mode is the most appropriate measure to be considered in this study. Therefore, the percentage, which is one of the key measures in statistics (Monteiro-Filho) and indicates the frequency of a structure, is a good quantitative method to be used, sometimes the only appropriate, as shown in some scientific articles on anatomy (Ferreira *et al.*, 1997; Faria & Mariana, 2001; Murta *et al.*, 2002; Silva & Ferreira, 2002).

In morphology, the highest percentage of occurrence of a given structure is considered as normal, and the lowest percentage, consistent with the implementation of the function is called variation (Erhart, 1976; Moore & Dalley, 2001).

When the central tendency measures cannot be used, the non-parametric distributions are the most common choices (Vieira) and are useful in small samples (Centeno). The very concept of normality has the intrinsic concept of Mode.

From the non-parametric distribution, the best one proposed for anatomy is the Pearson's  $\chi^2$  distribution; however, its restrictions minimize its usefulness for this science, although this type of distribution is widely used for comparison between samples.

However, it is a basic condition that the  $\chi^2$  distribution should be used to measure whether the categorized variables are independent (Vieira), and the independence between data of biological samples is very limited because species from the same phylum, mainly of vertebrates, show the same body construction pattern, considering morphological, biochemical, physiological or even genetic aspects. Thus, the randomness condition is undone.

The binomial distribution also requires randomness and independence of discrete variables (Centeno) for morphological sciences, and considering discrete variables, the averages that are eccentric to average values of the sample better represent the reality, as in the binomial distribution, but the principles of calculating these averages are not appropriate due to the necessity of randomness of data in the sample, as occurs with the  $\chi^2$  distribution.

**Restrictions to the use of  $\chi^2$  in the analysis of data for the same sample.** The use of the Pearson's  $\chi^2$  distribution is not

appropriate for the analysis of data in the same sample of discrete morphological data due to some disadvantages, given the assumptions below:

1) This type of distribution requires the randomness of data as basic foundation. This does not occur in morphological studies of structures among individuals from the same species, as they have the same embryological origin pattern, thus, the study of anatomical structures within the same species does not create independence between events.

2) Based on premise (1), it is not likely that the structure to be studied occurs in 100% of cases in most samples, otherwise, there will be agenesis of the structure, and this event is rare or incompatible with life and should be considered separately as case description.

The hypotheses generated for the  $\chi^2$  test are the agreement between experimental and theoretical results (Centeno).

In studies on the structures of vertebrates, considering the same sample (within the same species), the hypothesis being tested is always that of equality between the frequencies of expected and observed values, i.e. the null hypothesis, therefore, for the presence of an organ and / or characteristic associated with this organ, the  $\chi^2$  value will be zero, almost always. In this case, the acceptance of the hypothesis could generate a type-II error.

3) Rarely, there will be more than five expected values in each category for the study on vertebrates, which, according to Monteiro-Filho, restricts the use of  $\chi^2$ , since it restricts the test associated with this type of distribution. This restriction is important in the study on higher vertebrates and / or wild animals, whose quantities to study are few and limited.

Thus, the events, which are the presence and characteristics associated with structures, are dependent for the same species and for different species, such as the presence of a structure in a region of the body that is repeated in almost all taxa of vertebrates, and this fact makes the use of such distribution in statistical analysis unviable, and if such use is coercive, a serious basic error will be committed.

As for the use of the  $\chi^2$  distribution, considering that the data observed in morphological sciences are independent, the statistical analysis is based on a false assumption with undesirable consequences in the responses found.

**Restrictions to the use of  $\chi^2$  in the analysis of data for different samples.** The hypothesis to be tested in the  $\chi^2$

distribution for comparison between different samples is the uniformity (Centeno). The  $\chi^2$  test is very useful in cases in which the variables are discrete, but the comparison between different samples (different species) violates the restrictions for the application of the distribution and the  $\chi^2$  test, based on the following premises:

1) The independence of variables (Vieira), and in the specific case discussed here, the variables of a morphological and anatomical study. This independence will not exist when close species are compared, such as species of primates between each other or with other mammals, especially, as expected, between homologous organs, since the formation of the general structure of vertebrates follows an embryological pattern approximately defined (Storer *et al.*, 2000; Hickman *et al.*, 2004).

It is important to note that comparisons of distant taxa are not suitable without intermediate comparisons between the closest ones.

2) The other restriction for this method is the sample size, which must be greater than 20 individuals (Centeno; Vieira), or has more than five expected values for each category (Monteiro-Filho) for further application of the  $\chi^2$  test. This condition is difficult to establish for higher vertebrates when the individuals are wild habitat and are at risk of extinction.

Considering the anatomy of wild animals, one can hardly expect so many individuals, also because the sacrifice of this amount of animals hurts the rules of COBEA (Brazilian College of Animal Experimentation) in Brazil and most of animal protection societies in several countries.

The  $\chi^2$  test and the binomial distribution are not suitable for anatomical analysis within the same species, or for comparisons between species, because both require independence between events.

## MATERIAL AND METHOD

**Material.** Papers on anatomy and comparative anatomy were used to justify the need for a simple and direct method to relate nominal variables, as well as texts on information on non-parametric statistics. Works that used inappropriate tests and not tests used in comparative anatomy were considered for examples.

**Method.** A simple comparative method within the non-parametric distribution, (1) between two different samples from the same species and (2) for two different species has

been proposed, based on concepts of normal and variation in anatomy and modal class in statistics.

Initially, the relative frequency (RF) is defined for each sample:

$$RF = (N - nv) / N;$$

where:

N is the total number of specimens of the sample,

nv is the number of individuals presenting variation of the normal pattern.

It is important to note that the difference between N and nv (N - nv) is the normal pattern.

When more than one parameter (location, nerve, blood vessel, origin and insertion of a muscle, for example) is considered, fact that should be considered in a more complete anatomical study, the Weighted Average of Frequencies (MPF) is carried out, generating specific weights according to the relevance degree of parameters to be considered, having as basis the aspects of less variation within these parameters, for example, the innervation of structures is the parameter of smallest variation, both for the same species and for different and close species.

Since the structure studied is a muscle and the consideration of parameters "innervation", "origin and insertion," and finally "vascularization", innervation should have weight 3 due to its higher comparative importance and to the fact that, in embryology, the ontogeny formation of muscles is dependent on the action of nerves that are inserted in them since their initial formation (Moore & Persaud, 2000), thus, innervation should be the parameter of less variability, while origin and insertion must have weight 2, since these standards have greater interference from the environment and greater variability than innervation; and vascularization, receives weight 1, since it is the factor of greatest variation between the correlated data, because within the same structure, in different antimeres in the same individual, the vascularization may be different, being the parameter of greatest variability among the other correlated parameters.

For each structure studied, considering the parameters, the researcher must decide the relative weights for these parameters and justify them with adequate knowledge on the subject, since according to Centeno, "The statistics only helps the researcher, but does not exempt the scientific and skeptic spirit, neither the depth knowledge on the material under study. A man with his head in a refrigerator and his feet on a stove is not living in a good average temperature".

The Weighted Average of Frequencies (MPF) should be made after the RF calculation.

$$MPF = [(RF_1 \cdot P_1) + (RF_2 \cdot P_2) + (RF_3 \cdot P_3)] / P_1 + P_2 + P_3$$

where:

RF<sub>1</sub> is the frequency of the muscle innervation and P<sub>1</sub> is the weight with value 3; RF<sub>2</sub> is the frequency of the muscle origin and P<sub>2</sub> is the weight with value 2, RF<sub>3</sub> is the frequency of the muscle vascularization and P<sub>3</sub> is the weight with value 1.

To consider the proximity between the structures studied, the difference in the relative frequency is calculated, or Comparative Anatomy Index (IAC) between samples, with or without the weighted average:

1. Without the weighted average:  $IAC = |RF_i - RF_{ii}|$ ;
  2. With the weighted average:  $IAC = |MPF_i - MPF_{ii}|$ ;
- where indexes i and ii represent samples 1 and 2.

From the above data, the closer to zero is the IAC value, the greater the similarity between samples will be, since zero will only be obtained if and only if, the values of relative frequency (RF or MPF) are equal, with the requirement of equality between the percentages of normal pattern values, and the closer to 1.0 is the ACT, more unequal the samples will be. The IAC acts as a non-parametric test whose intrinsic value defines the similarity degree between samples.

The highest possible value for the IAC is 1.0, since for extreme cases, the value will be 1.0 for the highest MPF value for a sample with 100% of normal pattern, and 0 for MPF, if the structure does not exist in the other sample, then, the largest possible module of the difference between MPF will be 1.0 or 100%.

For the comparative anatomy, it could be broadly considered that the more the values are closer to zero, the greater the morphological or perhaps phylogenetic proximity between species will be, because many morphometric data are used to generate taxonomic similarity data.

## RESULTS

The result will include the verification of data already published and the IAC will be applied to check the similarity between samples.

Case 1: Data without the use of the weighted average or for the determination of the IAC for only one structure.

Aversi-Ferreira *et al.* (2005) determined that the origin of the ulnar nerve in *Cebus* occurred from the medial fascicle of the brachial plexus in 7 out of the 8 animals studied. To make the comparison with humans, the description of Gardner *et al.* (1988) will be used, which does not cite that there are variations of this origin in humans. Therefore, to determine the IAC to make the comparison between the origins of the ulnar nerve in *Cebus* and man, the **FRi** for *Cebus* and **FRii** for Humans were calculated:

$$FRi = (8-7) / 8 = 0.875 \text{ and } FRii = 1.00$$

To determine the IAC:

$$IAC = |FRi - FRii|$$

$$IAC = |0.875 - 1.00| = 0.125$$

As the IAC value is closer to zero, it could be concluded that there are great similarities between the origins of the ulnar nerve for *Cebus* and man.

Case 2: Data using the weighted average or the determination of the IAC for more than one structure.

Using data for the coracobrachialis muscle of *Cebus* obtained from the articles of Aversi-Ferreira and colleagues (2005, 2007a, 2007b), the comparison of the similarity of this muscle between men and baboons in relation to its innervation, origin, insertion and vascularization will be performed.

To make the comparison with humans, the description of Gardner *et al.*, and for baboons, the description of Swindler & Wood (1973) will be used.

This muscle is innervated in humans by the muscle-cutaneous nerve, has roots in the coracoids process of the scapula, and is inserted in the anterior face of the middle third of the humerus, being vascularized by the brachial artery.

In *Cebus*, the coracobrachialis muscle is innervated by the muscle-cutaneous nerve, has roots in the coracoids process of the scapula, and is inserted in anterior-medial face of the middle third of the humerus, being vascularized by the ulnar artery.

The innervation with weight 3, the origin and insertion with weight 2, and vascularization with weight 1 will be considered, and the RF of the innervation as 1 for both the RF of the origin 1 as for both, the RF of the insertion as 1 for humans and as 0 for *Cebus*, and the RF of the vascularization

as 1 for humans and 0 for *Cebus* will be calculated. Since the discrete values are being represented, we will consider RF with value 1 as the existence of a factor and the absence of this factor as 0, by definition, since there is no way to set intermediary parameters for these calculations because they vary widely.

Therefore, the **MPFi** is the weighted average of frequency for *Cebus* and **MPFii** is the weighted average of frequency for humans:

So, **MPFi** is:

$$\text{MPFi} = [(1 \times 3) + (1 \times 2) + (0 \times 2) + (0 \times 1)] / 8 = 0.625$$

**MPFii** is:

$$\text{MPFii} = [(1 \times 3) + (1 \times 2) + (1 \times 2) + (1 \times 1)] / 8 = 1$$

Therefore, **IAC** is:

$$\text{IAC} = |0.625 - 1| = 0.375$$

It should be considered that, in general, this muscle is similar to humans, however, by such characteristics, the **IAC** between *Cebus* and baboons will be zero, which indicates high similarity between these muscles for both species, since the insertion and vascularization parameters for both are the same.

Therefore, if more distant species are considered, and since the comparison of the coracobrachialis muscle in *Cebus* and other species in which the origin, insertion and vascularization of the muscle in these animals is different in relation to *Cebus*, we must define RF parameters of 1 for *Cebus* and the different parameters in other species as 0, hence, the **MPFii** for this species is 0.375, therefore, the **IAC** between both will be 0.625, which indicates little similarity between them.

This work initially proposes that the **IAC** value of 0 indicates total similarity between the structures analyzed, from 0 to 0.25 as very similar structures, from 0.25 to 0.5 as similar, between 0.5 and 0.75 as somewhat similar, between 0.75 and 1.0 as unequal and with value 1 as completely unequal.

## DISCUSSION

Works on anatomy and comparative anatomy have shown the exclusive use of frequency for performing the statistics of discrete variables (Ferreira *et al.*; Faria &

Mariana; Murta *et al.*; Silva & Ferreira). Others use the frequency associated with standard deviation for this type of variable (Krill *et al.*, 1988; Barros *et al.*, 2003) and other authors use different statistical methods for continuous variables using or not statistics programs (Monteiro & Lessa; Christel & Billard; Lopes & Masunari, 2004; Absalão *et al.*).

There are cases in which the comparative analysis between structures from different species was performed only considering the descriptive aspect without statistical calculations (Câmara-Filho *et al.*, 2004; Mielke *et al.*, 2004, Bemvenuti, 2005; Izecksohn *et al.*, 2005).

In addition, in other studies, the authors used graphs with continuous straight lines when the ordinate variable had discrete value (Lopes & Masunari) and this discrepancy is inconsistent with the simplest rules of basic math, and instead of using a line, a column graph is rather used.

The above reports show, on the one hand, that the lack of a test for comparative anatomy leads researchers to disregard the statistical measures in its results, and on the other hand, they show that attempts to match data to other non-parametric tests can generate errors in the interpretation of results.

It is reckless to consider that in the formation of a being, in terms of species or individual, where the formation pattern is organized and harmonious, the data obtained on their structures are random. The simple concepts of embryology and phylogeny do not allow such opinion.

The use of undue distributions is due in part to the lack of a specific test for comparative anatomy. The **IAC** proposed here will allow quantifying nominal data, generating objective possibility of comparison between these data in a simple and direct way.

The  $\chi^2$  is a type of distribution used for comparison of nominal data, but its limitations for use in nominal variables derived from biological information minimize or prevent their use. The analysis of its basic fundamentals will allow this conclusion.

**Advantages and disadvantages of using IAC.** Other tricks have been used to analyze data derived from morphological characters, but methodological errors are common, especially in the attempt to adapt an inappropriate distribution or a test for discrete or nominal variables.

According to what has been shown above, the **IAC** was defined to compare nominal data based on fundamen-

tal anatomical concepts that alone allow comparison between data, since the concept of normal combines data derived from different samples. The **IAC** allows comparing the normal values between data from different samples, because in the analysis of the concept of normal, the researcher takes into account the higher occurrence percentage of the structure under study.

It is important to emphasize that this is a not very robust comparison index, as any other index based on a non-parametric distribution (Vieira), but the greater the sampling, the most reliable this index becomes.

Another consideration is that the severity degree of this index depends on the comparative method used by the researcher, for example, if the test is used to compare the mere existence of a given muscle between two mammalian species, the value may be zero, but it will not represent a significant proximity between species. However, if the researcher considers more specific points such as insertion and origin, innervation and vascularization of the muscles, for example, to be compared with their respective weights, the **IAC** value could provide better information on the similarity between samples.

Although the distributions and statistical tests for continuous variables that follow a Gaussian distribution are more reliable, the **IAC** has the advantage of generating a numerical value for comparison between samples from the same species and from different species without using tests for random distributions. However, the **IAC** value is relative

and provides an estimate of the morphological and / or phylogenetic proximity between different species and morphological proximity between individuals from the same species.

The **IAC** value for the several comparative studies will only have scientific value if the weights to be considered in the research are obtained based on well-defined phylogenetic parameters, taking into account that the largest weights are determined by factors of minor morphological variation.

This test allows that multiple parameters are considered and defined according to specific weights, in turn, defined by data already corroborated by evolutionary studies.

Further studies and new data associated with this method by other researchers will contribute for its improvement.

## CONCLUSIONS

The main findings on the use of this method are: (1) to allow the relationship between data from discrete or nominal variables in comparative anatomy or in other areas of morphology, (2) to generate an objective method (numerical) for comparison between samples of structures from the same species and between different species and (3) to fill the lack of a specific method for the statistical work in comparative anatomy or morphology, in general.

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AVERSI-FERREIRA, T. A. Nuevo método estadístico para anatomía comparada. *Int. J. Morphol.*, 27(4):1051-1058, 2009.

**RESUMEN:** Normas, fórmulas y pruebas estadísticas han sido ampliamente utilizadas en estudios que utilizan las variables continuas en el desarrollo de la distribución normal o de Gauss, u otras pruebas, cuyos datos se ajustan a esta tipo de distribución (t y z distribuciones), y cuyos parámetros pueden ser definidos. Sin embargo, hay casos en que el modelo y el objetivo de los trabajos científicos sólo permiten la uso de variables nominales o discretas. La ausencia o presencia de un estructura u órgano (músculos, nervios, vasos sanguíneos, huesos y ligamentos), las características asociadas a estos órganos, como la fuente de los nervios, los músculos y los buques y la distribución de los nervios y los buques son discretos y / o variables nominales que no encajan en el curva de Gauss, ni de z o t, a menos que las medidas de estos estructuras se toman. Un simple método comparativo dentro de la no-paramétrico de la distribución se ha propuesto, (1) entre dos muestras de la misma especie y (2) de dos especies diferentes, con base en los conceptos de normal y la variación en la anatomía y de la clase modal en estadísticas aquí llamado Índice Anatomía Comparada (IAC). Los principales conclusiones sobre el uso de este método son: (1) para permitir que la relación entre los datos de las variables nominales o discretos en la anatomía comparada o en otras áreas de la morfología, (2) para generar un método objetivo (numérico), de la comparación entre muestras de las estructuras de la misma especies y entre diferentes especies, (3) para cubrir la falta de un método específico para la labor estadística en la anatomía comparada o en morfología, en general.

**PALABRAS CLAVE:** Morfología; Anatomía comparada; Método estadístico.

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Correspondence to:

Dr. Tales Alexandre Aversi-Ferreira  
Universidade Federal de Goiás - Campus Catalão  
Departamento de Enfermagem  
Avenida Dr. Lamartine Pinto de Avelar, 1120  
Setor Universitário, Catalão/GO

Goiânia – GO  
BRAZIL

Phone: +55 64 3441-1500

Fax: +55 64 3441-1515.

E-mail: [aversiferreira@hotmail.com](mailto:aversiferreira@hotmail.com)

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