SHAPE AND CLUSTER ANALYSES FOR DISCRIMINATING POPULATIONS OF *RHIZOPRIONODON POROSUS* OF NORTHEAST COAST OF BRAZIL THROUGH CHONDROCRANIUM MORPHOMETRY

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▪ ABSTRACT: The genus Rhizoprionodon comprises seven species of sharks occurring in the Atlantic, Pacific and Indian Oceans. The sharks are small sized attaining about 150 cm, inhabiting coastal waters, estuaries and brackish waters. In Brazil, the two species found are Rhizoprionodon porosus and R. lalandii. The use of morphometric approaches has limitations for this group due to lack of anatomical landmarks, since they have a continuous form without angles. This study will analyze the chondrocranium, a structure that has species-specific shape, aiming at verifying the hypotheses suggested in the literature of two populations of R. porosus off northeastern Brazil. One population corresponds to the northern coast of the Northeast (RN) and, the other to the eastern coast of this region (PE). We performed a comparative morphometric study of the chondrocranium of R. porosus between areas using shape and cluster analyses. The results showed that there were differences for both adult and juvenile phases by areas of capture. In conclusion, there is indeed differences between chondrocrania from Pernambuco and Rio Grande do Norte.

▪ KEYWORDS: Elasmobranchs; geometric morphometry; anatomical landmarks; image J

1 Introduction

The genus *Rhizoprionodon Whitley* comprises seven species of the sharks distributed throughout of the Atlantic, Pacific and Indian Oceans. These species are small sized sharks that reach 150 cm, inhabit coastal waters, inlets and brackish waters, feeding mainly on mollusks, crustaceans and small fishes (SPRINGER, 1964; CERVIGÓN, 1999).

One of species of genus *Rhizoprionodon* is *R. porosus* (Poey, 1861) that is distributed from Bahamas to Uruguay (COMPAGNO, 1984). In Brazil *R. porosus* sharks is abundant in north and northeast coasts (MATTOS, 1998). The species has been object of several

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studies in Brazil, among them the study by Garcia (2008) that compares *R. porosus* and *R. terranovae* in three different areas (Pernambuco and Rio Grande do Norte, Brazil and Florida, USA) using geometric morphometry. Studies on age growth and demographic analysis of *R. porosus* were conducted in the Northeastern coast of Brazil (MONTEALEGRE-QUIJANO, 2002, LESSA *et al*., 2009).

In addition, Lessa et al (2011) found differences in age and growth for this species along the northern and eastern coasts of the Northeast Region. It was implied that differences between the two populations of *R. porosus* made up two groups, one group was formed by sharks on the eastern coast and the other on the northern coast of Brazil. Furthermore, Mendonça *et al*. (2011) analyzing the DNA variation detected two distinct patterns for the DNA of *R. porosus*:a pattern formed by specimens off the eastern coast and another relative to the northern coast of Brazil, thus corroborating the previously stated study.

Discrimination among fish species is a major issue in management and exploitation of fishing resources. On the other hand, this activity can present several difficulties, and its effective implementation requires investigations of new methodologies that are simpler and reliable. Morphometric studies are fundamental for species description (taxonomic application) as well as for the characterization of stocks. Geometric morphometry represents a relatively new research area, computationally intensive, whose development and application has significantly grown in recent years (ZELDITCH *et al*., 2004). A common approach of shape analysis, geometric morphometry (GM), use the reference points coordinates to record the relative positions of morphological points, contour curves, and surfaces as the basis for quantification. Analyses of geometric morphometry shapes are usually performed through a series of steps that can be called the Procrustes paradigm (ADAMS *et al*., 2013).

Geometric morphometry methods provide a comprehensive quantification of biological shape with increasing use in ecological and evolution research (KLINGENBERG *et al*., 2010; ADAMS, 2011; MARTÍNEZ-ABADÍAS *et al*., 2012), patterns of floating and directional asymmetry (KLINGENBERG *et al*., 2002; SCHAEFER *et al*., 2006), phylogenetics (SIDLAUSKAS, 2008; KLINGENBERG and GIDASZEWSKI, 2010; MONTEIRO and NOGUEIRA, 2011) and ontogenic patterns in human evolution (BOOKSTEIN *et al*., 2003; MITTEROECKER *et al*., 2004; MITTEROECKER and BOOKSTEIN, 2008), among other applications.

Thus, due to uncertainties regarding the identification of two populations of *R. porosus* on the northeaster Brazilian coast, the present work was motivated by the assumption that the two populations present some variability of shape, and that the analysis of shapes can be used to identify these populations. The use techniques as body-proportional approach, used in taxonomy, has limitations for this group due to lack of anatomical landmarks in elasmobranchs (REIS et al., 1987), since they have no angles in their shape as is required for use of geometric morphometry techniques. Thus, in present study, the chondrocranium - a species-specific structure (COMPAGNO, 1988) angularly shaped and located at the base of skull supporting and protecting the brain - was chosen.

Considering the morphological attributes of this structure, our goal is to morphometrically analyze the shape of *R. porosus* chondrocrania of the two populations as suggested in literature, one of these corresponding to north coast of Northeast Region (Rio Grande do Norte -RN) and other to the eastern coast of the Northeast Region (Pernambuco

-PE) of Brazil. Our objective is to characterize populations, so that this information can contribute to the management of this resource in Brazil.

2 Material and methods

2.1 Data

The chondrocranium is a cartilaginous structure that protects the brain and sensitive lateral capsules of olfactory, auditory and visual organs. The chondrocranium is divided into four regions: the ethmoidal region, including the face and nasal capsules; the orbital region (death-temporal or sphenoidal), including the orbits between the basal plate and the skull ceiling; the optical (auditory) region, including the optic capsules and the basal plate, and the occipital region at the posterior end of chondrocranium, (COMPAGNO, 2005), Figure 1.

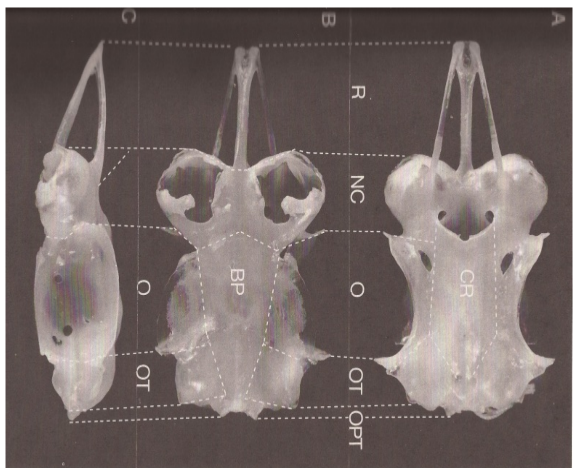

Figure 1- Chondrocranium region of *Rhizoprionodon porosus.*

The data set consists of 15 *R. porosus* chondrocrania from the Pernambuco region (PE), where 7 are adults and 8 juveniles, 13 from Rio Grande do Norte (RN), where 6 are adults and 7 juveniles. Sharks were classified by sex and age group (adults if their total length was greater than 65 cm if male and 70 cm if female) (COMPAGNO, 1984). The chondrocranium image was captured by a Sony camera model DSC-P200 and stored in jpeg image files. For standardization, a support was created, where the digital camera was coupled, thus establishing a standard height (45 cm) for all images, later the images were marked with 17 anatomical landmarks (set of characteristic coordinates of a shape) , as shown in Figure 2.

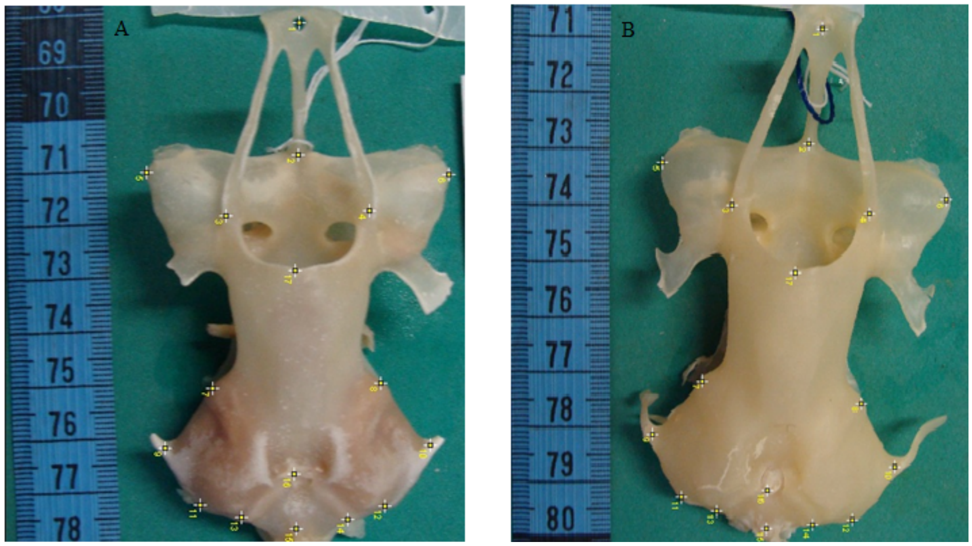

Figure 2 - Anatomical landmarks of chondrocranium of *R. porosus* from Pernambuco (A) and Rio Grande do Norte (B). 1-rostral fenestra (RF); 2-basirostral fenestra (BR); 3-lateral rostral cartilage left (LRL); 4-lateral rostral cartilage right (LRR); 5-nasal capsule left (NCL); 6-nasal capsule right (NCR); 7-orbit left (OL); 8-orbit right (OR); 9-postorbital process left (PTL); 10-postorbital process right (PTR); 11-glossopharyngeal foramen left (FGL); 12-glossopharyngeal right (FGR); 13-vagus nerve foramen left (FVL); 14-vagus nerve foramen right (FVR): 15-foramen magnum (FM); 16-pariental fossa (PRF); 17-anterior fontanelle (AF).

Through the Image J software, the values of pixel coordinates of the images were extracted. To guarantee the standardization, images were rotated, translated and staggered through the coordinates of Procrustes, forming 28 two-dimensional numerical matrices. These coordinates were used to generate the mean shape of chondrocrania of the two populations as well as to compare both populations. For evaluation of cluster analysis, the distance between some anatomical landmarks are listed in Table 1.

Table 1- Morphological variables of chondrocranium *R. porosus*

Variables	Code	Variables	Code
distance from RF to BR	D1	distance from PTL to FGL	D9
distance from RF to LRL	D2	distance from PTR to FGR	D10
distance from RF to LRR	D3	distance from FGL to FVL	D11
distance from NCL to NCR	D4	distance from FGR to FVR	D12
distance from NCL to OR	D5	distance from FVL to FM	D13
distance from NCR to OL	D6	distance from FVR to FM	D14
distance from OL e PTL	D7	distance from FM to PRF	D15
distance from OR tp PTR	D8	distance from PRF to AF	D16

Cluster analysis was used to evaluate the difference between groups of chondrocrania. The hierarchical clustering methods (method single linkage, complete linkage, method average, median, centroid and Ward) and non-hierarchical (*k-means*) methods were used (BUSSAB, 1990). For comparison between the hierarchical grouping methods, the cophenetic correlation coefficient (CCC) was used (SOKAL and ROLHF, 1962).

The CCC measures the degree of fit between the dissimilarity matrix (Phenetic Matrix F) and the resulting matrix of simplification provided by grouping method (cophenetic matrix C), (FARRIS, 1969).

$$r_{cof} = \frac{\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n}(C_{jj'} - \bar{C})(f_{jj'} - \bar{f})}{\sqrt{\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n}(C_{jj'} - \bar{C})^2}\sqrt{\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n}(f_{jj'} - \bar{f})^2}}$$

where,

$$\bar{C} = \frac{2}{n(n-1)}\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n} C_{jj'} \text{ and } \bar{f} = \frac{2}{n(n-1)}\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n} f_{jj'}$$

The higher the value obtained for r_{cof} the less distortion caused by grouping of individuals. Rohlf (1970) evaluated inadequacy of the grouping method when rcof < 0,7.

Fisher discriminant analysis was used to discriminate the groups according to the studied variables. In order to compare the chondrocranium shape of adult and juvenile sharks of two populations studied, the statistical shape analysis was used, using T^2 of Hotteling test, James and Lambda (SILVEIRA, 2008) using 17 anatomical landmarks described in Figure 2.

3 Results and discussion

Through the analysis of hierarchical cluster, the distinction of two chondrocrania stocks of *R. porosus* is observed, one of them referring to eastern coast (PE) and other of Brazilian northern coast (RN) for both adult and juvenile phases, Figures 3 and 4.

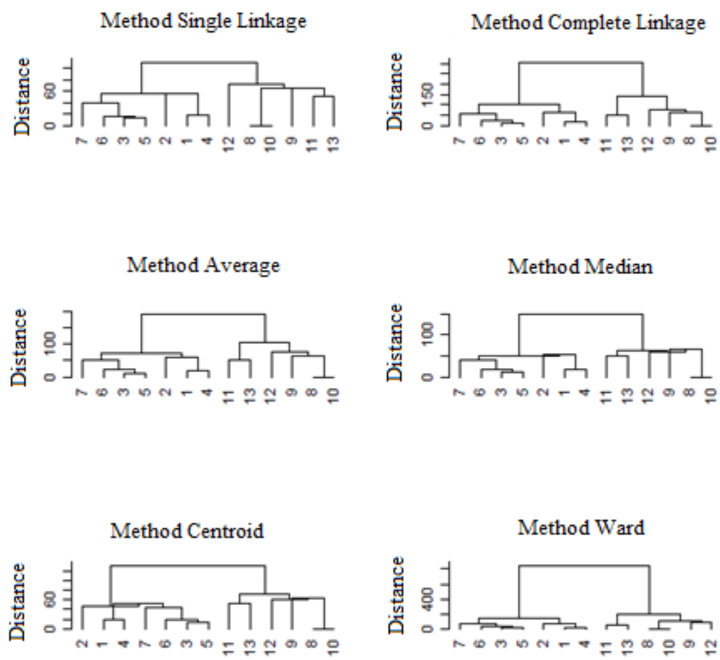

Figure 3 - Dendrogram of cluster methods for chondrocranium of adult *R. porosus* (Pernambuco - 1 to 7, Rio Grande do Norte - 8 to 13).

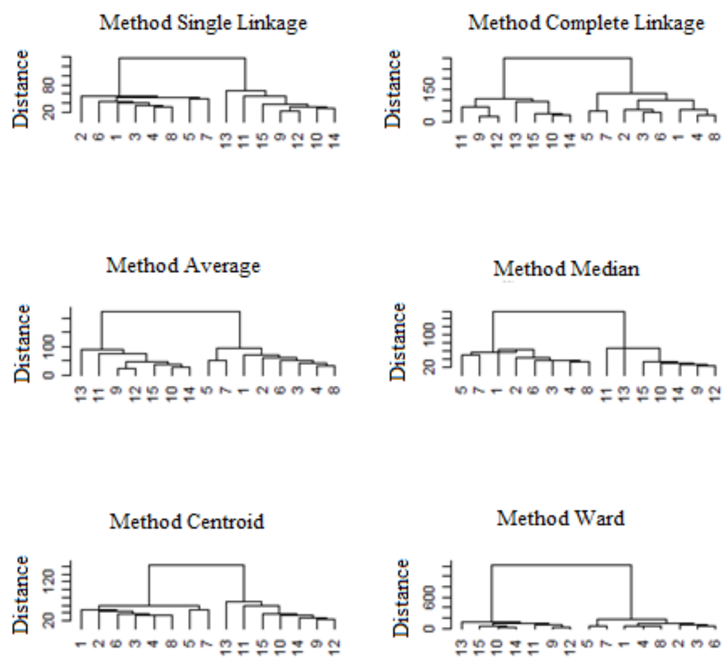

Figure 4 - Dendrogram of cluster methods for chondrocrania of juveniles *R. porosus* (Pernambuco - 1 to 7, Rio Grande do Norte - 8 to 13).

The CCC of grouping of *R. porosus* chondrocrania from adult phase was of 83% to 85%, whereas for the juvenile phase the correlation was of 92% and 93%, thus indicating adequacy of cluster methods for both age groups, Table 2.

Table 2 - Cophenetic Correlation Coefficient (CCC) by cluster methods of *R. porosus* chondrocranium in relation age groups

Cluster Methods	(CCC) Adult	(CCC) Juvenile
Single linkage	0,83	0,93
Complete linkage	0,84	0,93
Average linkage	0,85	0,93
Median linkage	0,84	0,93
Centrod linkage	0,84	0,93
Ward linkage	0,83	0,92

In k-means analysis, the separation of two chondrocrania stocks from *R. porosus* is observed again for both adult and juveniles. *K-means* success rate by was 100% for both populations, Figures 5 and 6.

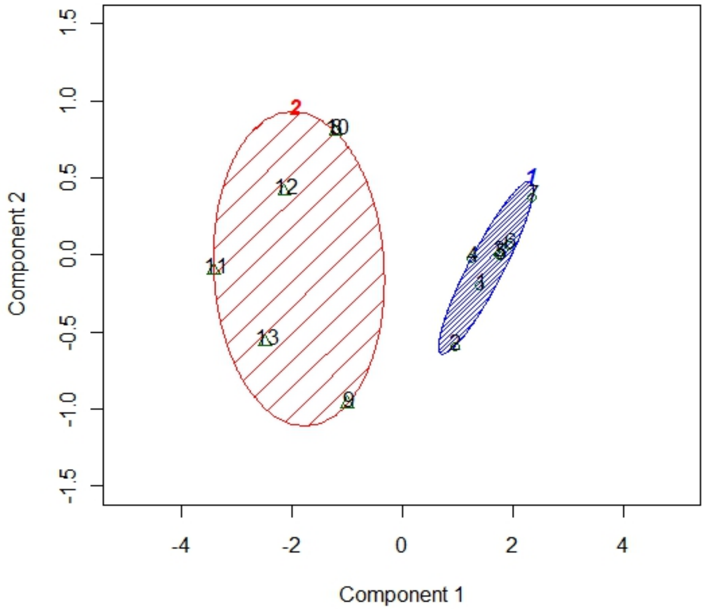

Figure 5 - Non-hierarchical cluster of chondrocranium of adult *R. porosus* (in blue - Rio Grande do Norte, red - Pernambuco).

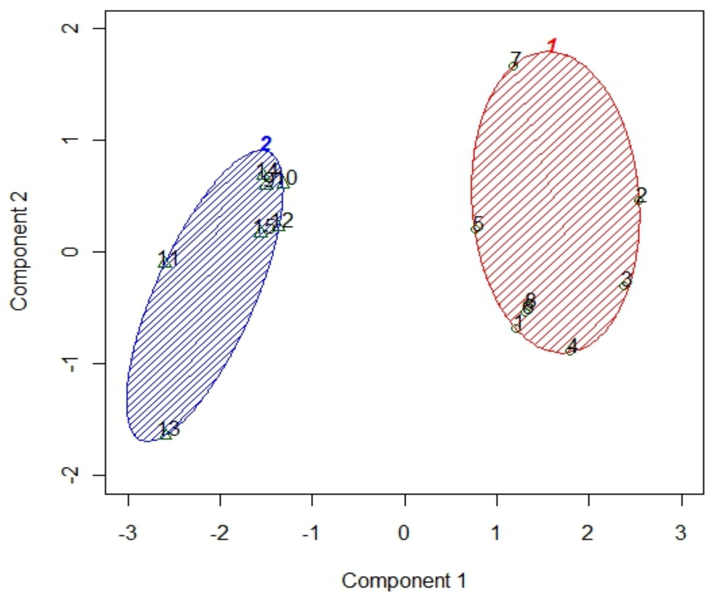

Figure 6- Non-hierarchical cluster of chondrocranium of juvenile *R. porosus* (in blue - Rio Grande do Norte, red - Pernambuco).

Through the discriminant analysis, the Fisher discrimination functions were obtained for adult and juvenile sharks as shown in Table 3.

Table 3- Discriminates function Fisher for adult and juvenile sharks

Variables	Discriminants function Fisher	
	Adults	Juveniles
D1	-5.38	-0.026
D2	-43.27	0.046
D3	-10.47	-0.004
D4	-37.83	0.033
D5	4.88	0.0003
D6	-16.36	-0.002
D7	-22.2	-0.005
D8	-40.37	0.005
D9	277.11	-0.215
D10	-115.77	0.007
D11	23.74	0.107
D12	99.52	-0.215
D13	-102.3	0.035
D14	-10.75	-0.047
D15	-28.53	0.075
D16	62.86	0.044

When compared to chondrocranium shapes of adult and juvenile of PE and RN, difference in averages between the shapes of the two populations were obtained when evaluated through the T-test of Hottelling, James and Lambda, Table 4 and Figures 7 and 8.

Table 4 – Tests, statistics of tests and p-value for comparison between the shape of two populations of chondrocrania of adult and juvenile sharks

Test	Test statistics	p-value
	Adults	
T^2 of Hottelling	37581110	<0.0001
James	211383409	<0.0001
Lambda	567.86	<0.0001
	Juveniles	
T^2 of Hottelling	51467953	<0.0001
James	191094771	<0.0001
Lambda	348.92	<0.0001

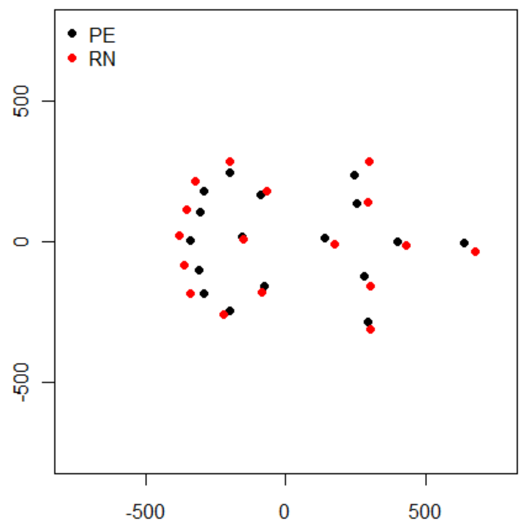

Figure 7 - Average chondrocranium shapes of individuals of coast eastern (PE -point black) and northern coast (RN - point red) of adult sharks.

The Figure 7 showed that the mean shape of the chondrocrania of PE sharks presents a more flattened and less broadened chondrocranium than the average shape of RN, whereas the average shape of juvenile sharks population in RN is smaller and closer to PE juvenile sharks population, Figure 8.

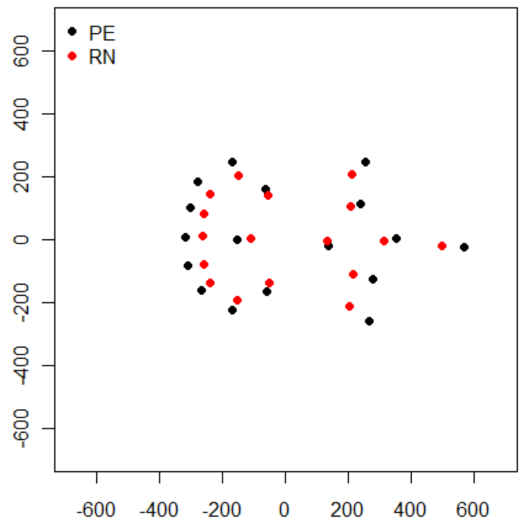

Figure 8 - Average chondrocranium shapes of populations of coast eastern (PE -point black) and northern coast (RN - point red) of juvenile sharks.

The results presented corroborated the hypothesis by Lessa *et al*. (2011) of two populations of *R. porosus* off North and Northeast Regions of Brazil. The results showed morphometric differences in chondrocrania of *R. porosus* from Pernambuco and Rio Grande do Norte states, corroborating the hypothesis of the two stocks of sharks. Furthermore, our results agree with the outcomes by Mendonça *et al*. (2011) concerning their study on the DNA variation for *R. porosus*, which demonstrated differences between the eastern and northern coasts of the Northeast Region of Brazil.

Conclusions

The difference in the shape of chondrocranium of *R. porosus* of Pernambuco and Rio Grande do Norte was verified for both adult and juvenile when using cluster and shape analysis techniques.

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- *RESUMO: O gênero Rhizoprionodon compreende sete espécies ocorrentes nos oceanos Atlântico, Pacífico e Índico, espécies de tamanho pequeno e médio atingindo cerca de 150 cm, habitando águas costeiras, estuários e águas salobras e alimentando-se principalmente de moluscos, crustáceos e pequenos peixes. No Brasil as duas espécies encontradas são Rhizoprionodon porosus e lalandii. O uso de abordagens morfométricas tem limitações para este grupo devido à falta de marcos anatômicos, uma vez que eles têm uma forma contínua sem ângulos. Este estudo analisará o condrocrânio que é uma estrutura considerada específica da espécie. O estudo objetiva verificar a hipótese sugerida na literatura de duas populações da espécie R. porosus no nordeste do Brasil. Um grupo corresponde à costa norte do Nordeste (RN) e o outro na costa oriental desta região (PE). Para isso, foi realizado um estudo morfométrico comparativo do condrânio de R. porosus entre as áreas indicadas, utilizando a análise de forma e de cluster. Assim, houve diferenças tanto para as fases adulta quanto juvenil por regiões de captura. Em conclusão, há de fato uma diferença entre os condrocrânios de Pernambuco e Rio Grande do Norte.*
- PALAVRAS-CHAVE: *Elasmobrânquios; morfometria geométrica; marcos anatômicos; imagem J*

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