Differentially expressed morphological characters depend on sex and ontogenetic stage in the crab *Goniopsis cruentata* (Crustacea: Grapsidae)

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Summary: The hypothesis of this study was that significant variations in the growth rates of *Goniopsis cruentata* are related to intersexual and age-related characteristics, particularly in areas identified as secondary sexual (pleon, carapace and chelipeds). From February 2019 to January 2020, a total of 713 individuals were captured, 378 males and 335 females, with a carapace width ranging from 0.42 to 6.59 cm. Males and females showed negative allometry ($\beta 0<1$) for all linear morphometric relationships, with an ontogenetic difference (p=0.0001) for all comparisons. In geometric morphometry, the dorsal view showed a significant difference in sexual comparisons (p=0.0001), in which males were wider in the posterior region than females. Furthermore, there was a significant difference in the abdominal region between young and adult females (p=0.0001), with the young ones being triangular and the adults oval, a characteristic brachyuran behaviour. We observed morphological separation in cheliped shape in sexual comparison (F×M) and laterality (R×L) (p=0.0001), with no ontogenetic variations (A×J). *Goniopsis cruentata* showed a noteworthy ontogenetic and sexual dimorphism. In addition, the discovery of cheliped variation was recorded for arboreal species that do not show a high degree of heterochely. Understanding these ontogenetic variations is crucial for effective conservation strategies because it allows specific life stages and their corresponding needs to be identified.

Keywords: Brachyura; geometric morphometry; linear morphometry; shape; size; tree crab.

Los caracteres morfológicos diferencialmente expresados dependen del sexo y la etapa ontogenética en el cangrejo *Goniopsis cruentata* (Crustacea: Grapsidae)

Resumen: Una hipótesis de este estudio sugiere que existen variaciones significativas en las tasas de crecimiento de *Goniopsis cruentata* relacionadas con características intersexuales y relacionadas con la edad, particularmente en áreas identificadas como sexuales secundarias (carapacho, pleon y quelas). Se capturaron un total de 713 individuos, 378 machos y 335 hembras, con un ancho de caparazón (CW) que oscila entre 0,42 cm y 6,59 cm de febrero de 2019 a enero de 2020. Al analizar las relaciones morfométricas lineales, tanto los machos como las hembras mostraron alometría negativa ($\beta 0<1$) para todas las relaciones lineales con diferencia ontogenética (p=0.0001) en todas las comparaciones. En la morfometría geométrica, la vista dorsal mostró una diferencia significativa en las comparaciones sexuales (p=0.0001), en las que los machos son más anchos en la región posterior que las hembras. Además, hubo una diferencia significativa en la región abdominal entre las hembras jóvenes y adultas (p=0.0001), siendo las jóvenes triangulares y las adultas ovaladas, un comportamiento característico de los braquiuros. Respecto a la forma de los quelípedos, observamos una separación morfológica en la comparación sexual (M×H) y lateralidad (D×I) (p=0.0001), sin variación ontogenética (A×J). Este estudio muestra que *Goniopsis cruentata* presenta un notable dimorfismo ontogenético y sexual. Además, se registró el descubrimiento de variaciones en los quelípedos para especies arbóreas que no presentan un alto grado de heteroquelía. Comprender estas variaciones ontogenéticas es crucial para estrategias de conservación efectivas, ya que permite la identificación de etapas específicas de la vida y sus necesidades correspondientes

Palabras clave: braquiuros; morfometría geométrica; morfometría lineal; forma; cangrejo arbóreo.

INTRODUCTION

Crustaceans have a different growth pattern from other animals because they undergo several stages of ontogenetic development through consecutive changes (Lobão et al. 1996). Along with this growth pattern, some body parts often change, especially those directly related to reproduction: the primary sexual characters and the secondary sexual characters (pleon, carapace and chelipeds), which are commonly used as a morphological maturity indicator (Souza and Pinheiro 2012). Brachyura are considered an interesting model for assessing variations in size using geometric morphometry (Alencar et al. 2014) because of the ease and accuracy of identifying homologous landmarks in the exoskeleton (Rufino et al. 2004).

Geometric morphometry is the study of shape covariation with underlying geometric factors, which are used to determine the "pure shape" in an individual or structure through discrete and well-defined points known as anatomical landmarks. These landmarks are used to verify significant shape and size differences in specific body structures, answering questions regarding intra- and interspecific variation and evolutionary and ecological issues, such as ontogenetic allometry and dimorphism (Bower and Piller 2015). This technique has increased the ability to discriminate sexes by identifying minimal morphological differences in body structures (Alencar et al. 2014). Furthermore, understanding the morphological variation of homologous characters makes it possible to discuss the directional sexual selection based on each sex morphology and to infer ecological pressures (Tatsuta et al. 2004).

Commonly known as the mangrove aratu, maria-mulata or red aratu, the red mangrove crab, *Goniopsis cruentata* (Latreille, 1803), is an important fishery resource for traditional communities located along the northeastern coast of Brazil (Pinheiro et al. 2016), especially in the estuaries of Pernambuco, Sergipe and Bahia, where it has a high economic value (Moura and Coelho 2004). This species also plays an important ecological role in the mangrove, affecting the muddy substrate's biogeochemistry, decomposition rates and nutrient cycling (Lima-Gomes et al. 2011).

G. cruentata belongs to the suborder Pleocyemata, whose females incubate eggs in the pleon until larval release, while males use the gonopod to pass their spermatophores to the female during copulation (Hartnoll 1983). It is considered an in situ generalist feeder that feeds on organic components of sediments, algae, plants and materials from molluscs and other invertebrates. It wanders through the mangrove substrate during low tide. During high tide, it migrates to the emersed region at the bottom of the canopy to avoid predation by fish and carnivorous marine crabs (Lima-Gomes et al. 2011). It also uses branches and burrows as refuges, occupying virtually all potential habitats in the mangrove ecosystem (Cobo and Fransozo 2003).

A great deal of information on the species is available, including larval description (Fransozo et al. 1998), ecological aspects (Sousa et al. 2000), population structure (Botelho et al. 2004), sexual maturity (Moura and Coelho 2004), reproduction and growth, morphological and physiological sexual maturation, reproductive period, fecundity and the influence of environmental factors on reproduction (Cobo and Fransozo 2003, 2005), reproductive aspects (Lira and Calado 2013), reproductive biology (Garcia and Silva 2006), growth (traditional morphometry) (Lira et al. 2015) and mangrove environmental quality (Ferreira et al. 2019). However, there is still a lack of studies exploring and describing the shape of *G. cruentata* and information stemming from it using the geometric morphometry technique.

This study aimed to use linear and geometric morphometry tools to investigate and quantify shape variation according to ontogenetic development in a species of tree crab, *Goniopsis cruentanta*. Furthermore, it aimed to search for intersex and age variations in the growth ratios of the species in the regions characterized as secondary sexual characters (pleon, carapace and chelipeds) to differentiate the specimens fit for reproduction through morphology and morphometry.

MATERIALS AND METHODS

Study area and laboratory procedures

Goniopsis cruentata specimens were collected in a tropical estuarine mangrove system at Tamandaré beach (08°78'28"S 035°10'52"W), northeastern Brazil, a floodplain (Duarte 1993), with semidiurnal tides and mean amplitudes of 2.4 m and an adjacent seawater temperature ranging between 24°C and 31°C (Moura 1991). The study area is within a marine protected area, the "Costa dos Corais" (Fig. 1). Specimens were captured monthly between February 2019 and January 2020 at their display and/or food capture time, always during the daytime low tide, using nylon line and hookless bait. After capture, the crabs were packed in plastic containers and transported alive to the laboratory.

In the laboratory, the specimens were cryoanesthetized, identified and sexed according to the identification guide proposed by Melo (1996). Afterwards, they were measured with a precision caliper (0.01 cm) for the dorsal and ventral regions and the cheliped (Fig. 2) and weighed with a precision scale (0.01 g). After the measurements, the specimens were placed on a modelling bed (to remove the influence of their bulging on the image parallelism) opposite a camera with a 50 mm focal length lens attached to a tripod parallel to the foreground and photo-documented in their dorsal, ventral, and cheliped (right and left) regions for further geometric morphometry analysis, as shown in Figure 2, which also illustrates their anatomical landmarks. Images of individuals with malformations or injuries (4% of the images) were discarded from the analyses. Ten landmarks were scanned on the carapace, ten on the pleon and five on the chelipeds (Fig. 2). The landmarks were strategically distributed to better obtain the shape of the animal using the TPSDig program
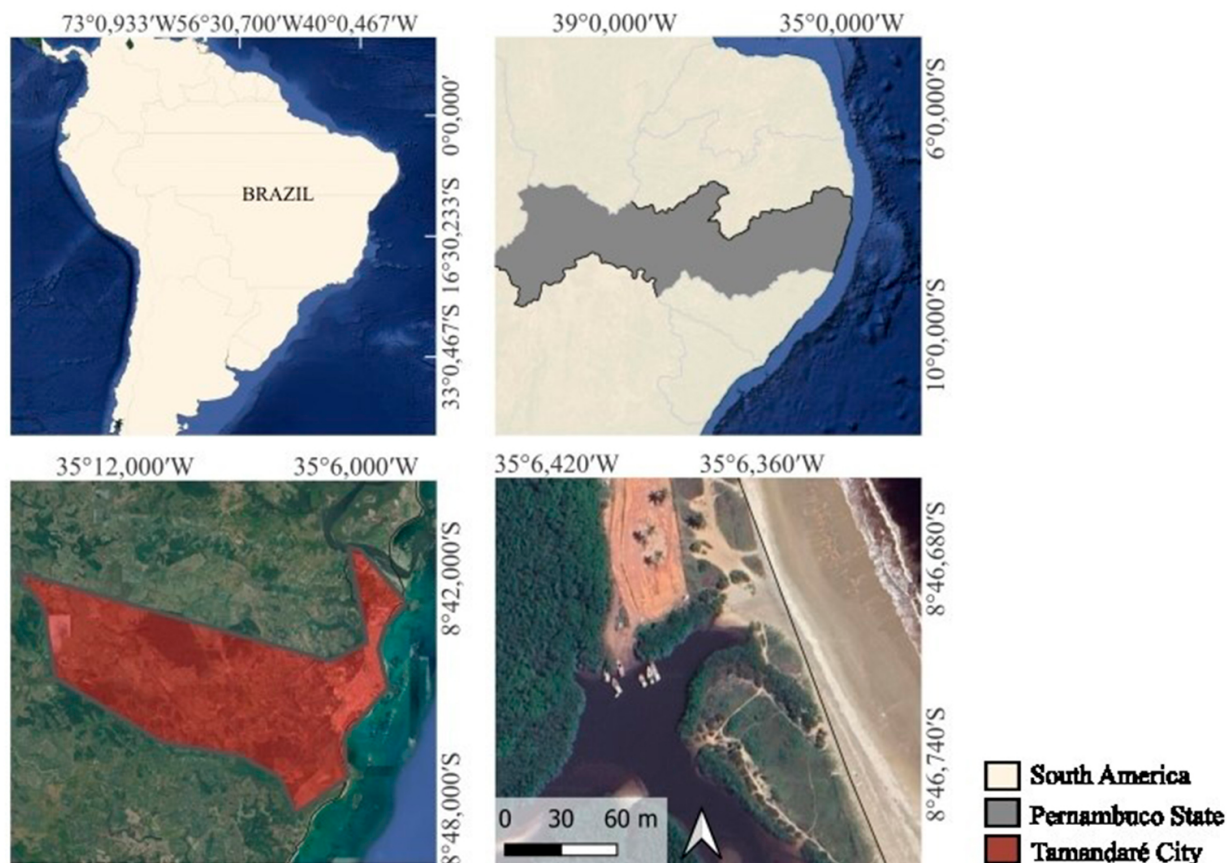
Fig. 1. – Location map of the tropical estuarine mangrove in the city of Tamandaré (northeast Brazil), the collection site of the crab *Goniopsis cruentata* between February 2019 and January 2020. Source: Google Earth.

(version 2.10, Rohlf 2006), based on previous work with other Brachyura (Silva et al. 2018, Marochi et al. 2018).

Statistical analyses

Initially, the specimens were separated into four categories: juvenile female (JF), adult female (AF), juvenile male (JM), and adult male (AM). The determination of morphological maturity (juveniles vs. adults) was based on the maximum inflection point (linear regression) of the relationships between the CW×GL (carapace width × gonopod length) structures for males, and CW×W5 (carapace width × width of the fifth abdominal segment) for females, considering that the GL and W5 variables were taken as dependent variables. Thus, males with sizes greater than 2.59 mm CW and females with sizes greater than 2.69 mm were considered adults.

To determine the growth allometry, the linear function $y = \beta0 + \beta1x$ and the potential function $y = \beta0x^{\beta1}$ were used, where $\beta0$ is the intercept constant on the ordinate axis, $\beta1$ is the allometric growth constant, x is the independent variable and y is the dependent variable.

Linear relationships were considered negative allometric when $\beta1<1$, isometric when $\beta1=1$, and positive allometric when $\beta1>1$. In the potential relation-

ships, the reference value for $\beta1$ was 3. The following relationships were analysed: CW×CL, CW×W4, CW × pleon length (PL), CW × carapace height (CH), CW × right chelipede height (RCH), CW × left cheliped height (LCH), CW × right cheliped length (RCL), CW × left cheliped length (LCL), CW×GL (the latter only in males) using linear regression; and CW×W using potential regression. The first variable (CW) was considered the independent variable for all relationships for all groups. The parameters $\beta1$ and $\beta0$ were compared using the *t* test of model comparison between and among species for all linear relationships. The relationships were considered different when at least one parameter showed a statistical difference. The coefficient of determination (r^2) was used as an indicator of the quality of linear regression.

To analyse the influence of linear measurements on the morphometric variation of the different groups, a principal component analysis (PCA) was conducted. To remove the influence of ontogeny on the data, all body dimensions were treated as proportional to CW (a variable less sensitive to distortion between size classes) (Harrison and Crespi 1999). To test for statistical differences between the group separations, a one-way PERMANOVA test with Bonferroni's correction was conducted ($p<0.05$).
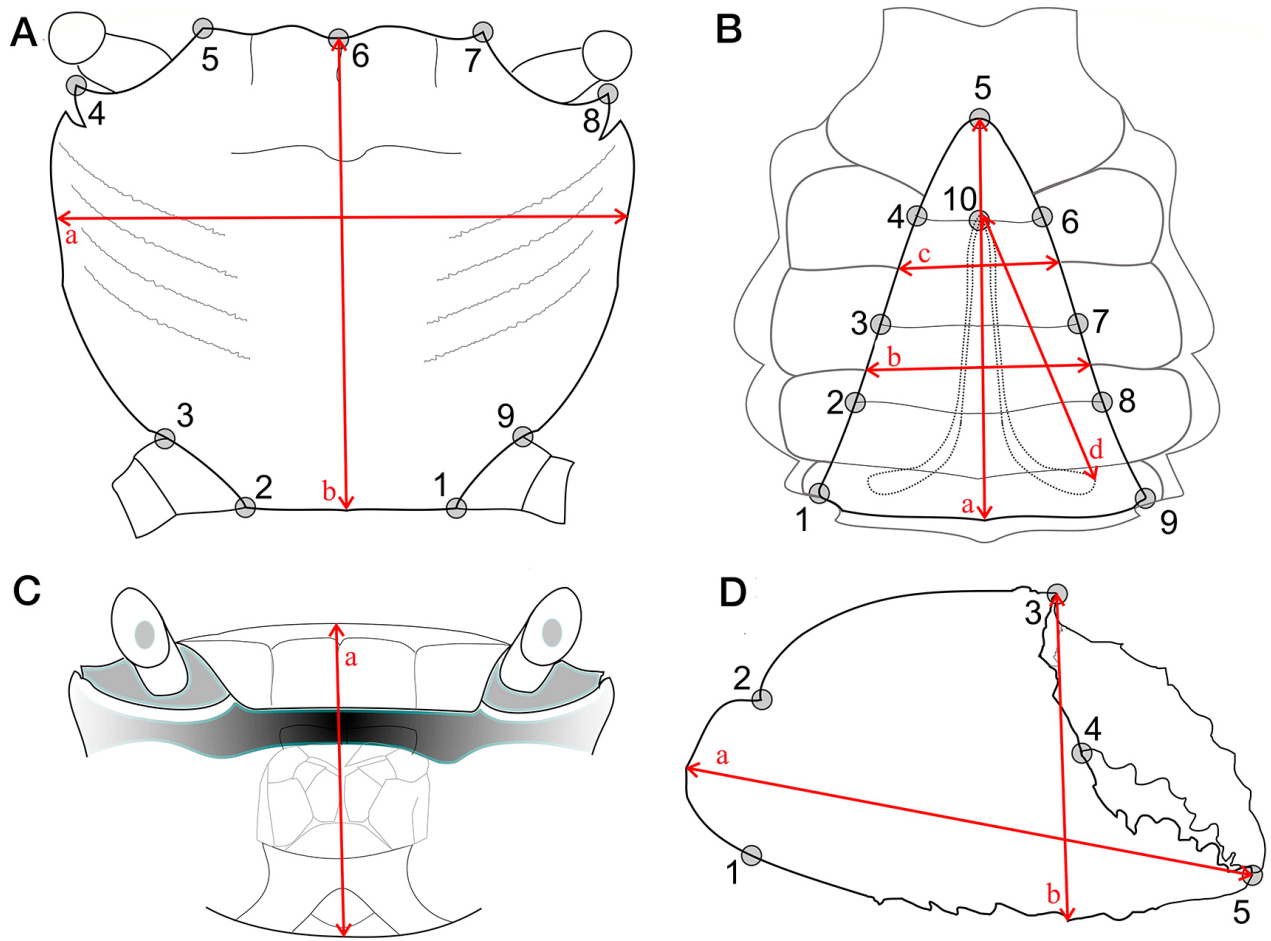
Fig. 2. – Distribution scheme of anatomical landmarks and linear measurements of *Goniopsis cruentata* captured between February 2019 and January 2020 in a tropical estuarine mangrove at Tamandaré-Pernambuco. I, cheliped region; II, carapace region, III, pleon region, IV, frontal region. A: a, carapace width; b, carapace length; 1, 2, posterior margin; 3, 9, posterolateral margin; 4, 8, anterolateral margin; 5, 7, front end; 6, forehead centre. B: a, pleon length; b, width of the fourth abdominal segment; c, width of the fifth abdominal segment; d, gonopod length; 1, anterior centre margin of the pleon; 1, 9, anterior margin of the pleon; 2-8, margin of the fourth abdominal somite; 3-7 margin of the fifth abdominal somite; 4-6, margin of the sixth abdominal somite; 5, top of the telson, ten centres sixth abdominal somite. C: a, carapace height. D: a, right and left cheliped length; b, right and left cheliped height; 1, lower attachment point of carpus with manus; 2, upper attachment point of carpus with manus; 3, upper point of dactylar joint with manus; 4, lower point of dactylar joint with manus; 5, polex tip.

For morphological exploration with the geometric morphometry technique, 475 images of the dorsal view, 469 of the ventral view, and 861 of the chelipeds were analysed. The landmarks' coordinates were aligned to the centroid, and generalized procrustes analysis (GPA) was performed using the program MorphoJ version 2.0 (Klingenberg 2011), where differences in orientation, position, and scaling were removed (Bookstein 1991, Adams et al. 2004). From the waste matrix generated in the GPA, a PCA was conducted to identify the main characteristics of the shapes. Then, canonical variance analysis (CVA) was performed, with 10000 permutations, to find the shape characteristics that best distinguished and separated the groups, using Procrustes distance. Differences between the forms were tested applying the Hotelling test (T²) with Bonferroni correction in discriminant analysis. Thin-plate splines functions

(Klingenberg 2008) were generated between groups using the MorphoJ program. A multivariate analysis of variance (MANOVA) with probability adjustment for Bonferroni multiple comparisons (Fornel and Cordeiro-Estrela 2012) was also conducted to identify possible differences in shape (pleon, carapace and chelipeds) and centroid size. Past software, version 3.07 (Hammer et al. 2001), was used for these analyses. Finally, CV1 and CV2 regression scores were related as a dependent variable of centroid size to demonstrate the ontogenetic trajectory graphically.

RESULTS

A total of 713 individuals were captured, 378 males and 335 females, with CW ranging from 0.42 to 6.59 cm. The individuals were distributed according to maturity stages, as shown in Table 1.

Table 1. – Number of *Goniopsis cruentata* crabs captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020. N, number of specimens analysed; JF, juvenile female; AF, adult female; JM, juvenile male; AM, adult male.

Sexo/sexual stage	N°	Carapace width
JF	114	0.42 a 2.69 cm
AF	221	2.71 a 6.13 cm
JM	118	1.47 a 2.59 cm
AM	260	2.61 a 6.59 cm

Linear morphometry

Males and females showed negative allometry ($\beta 0 < 1$) for all linear morphometric relationships, and there was a difference (p=0.0001 for all comparisons) in ontogeny (Table 2). Adult males showed a higher growth rate in CL, CH, RCH, LCH, RCL and LCL than juveniles. Adult females showed a higher growth rate than juveniles in the variables CL, W4, W5, PL, RCH, LCH, RCL and LCL. For potential ratios (CW×W), young specimens of both sexes showed negative allometry ($\beta 0 < 3$) and adults an isometric ratio ($\beta 0 = 3$). All models were statistically different when model parameters were compared using the *t* test (p=0.0001). For the coefficient of determination (R^2), the juvenile males showed the lowest values (CW×GL–R^2=0.355), whereas the adult males showed the highest values (CW×CL–R^2=0.936) (Table 2).

In the PCA, the first two components explained 75.16% of the variation, accounting for 58.46% and 16.69%, respectively. The variables of greatest influence in separating the groups were RCH, LCH, RCL and LCL for component 1 (PC1) and PL, CH and CL for component 2 (PC2). Note that PC1 showed a tendency to separate individuals according to the stage (juveniles and adults), thus appearing as an age dimorphism, while PC2 separated them according to sexual dimorphism, as can be seen in Figure 3.

The one-way PERMANOVA test for comparison between groups showed a statistical difference (p<0.005) between all groups.

Geometric morphometry

When the Procrustes distance from the CVA of the dorsal view was compared, no statistically significant difference was observed for the ontogenetic comparisons: AF×JF (p>0.05), and AM×JM (p>0.05). However, for the sexual dimorphism comparisons, all of them showed a statistically significant difference (p=0.0001) (Fig. 4). The same result was observed in the discriminant analysis, with a correct allocation of 59.75% for AF and 70% for JF and of 58.62% for AM and 68.18% for JM. The results obtained by MANOVA with Bonferroni correction for the dorsal view also showed no significant difference in centroid size between the AF×JF and AM×JM groups (p-value ranging from 0.41 to 0.92).

The morphology of the abdominal region showed a difference in AF×JF interactions (p=0.0001), with no difference between AM×JM (p=0.800). The correct al-

Table 2. – Linear relationships of the species *Goniopsis cruentata* captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020. F, female; M, male; a, adult, j, juvenile. CW, carapace width; CL, carapace length; W4, width of the fourth abdominal segment; W5, width of the fifth abdominal segment; PL, pleon length; CH, carapace height; RCL and LCL, right and left cheliped length; RCH and LCH, right and left cheliped height; W, weight; GL, gonopod length.

	M		F	
	j	a	j	a
CW×CL	y=0.915x-0.214 R^2=0.781	y=0.918x-0.272 R^2=0.936	y=0.840x-0.077 R^2=0.830	y=0.893x-0.194 R^2=0.928
CW×W4	y=0.336x+0.025 R^2=0.769	y=0.308x+0.124 R^2=0.868	y=0.548x-0.285 R^2=0.818	y=0.870x-0.958 R^2=0.843
CW×W5	y=0.359x-0.170 R^2=0.529	y=0.252x+0.134 R^2=0.852	y=0.521x-0.340 R^2=0.763	y=0.921x-1.182 R^2=0.905
CW×PL	y=0.609x-0.107 R^2=0.772	y=0.528x+0.166 R^2=0.835	y=0.657x-0.127 R^2=0.703	y=0.873x-0.723 R^2=0.882
CW×CH	y=0.474x+0.006 R^2=0.767	y=0.520x-0.091 R^2=0.920	y=0.545x-0.193 R^2=0.870	y=0.534x-0.133 R^2=0.795
CW×RCH	y=0.448x-0.471 R^2=0.562	y=0.568x-0.818 R^2=0.882	y=0.296x-0.118 R^2=0.814	y=0.358x-0.239 R^2=0.749
CW×LCH	y=0.431x-0.432 R^2=0.557	y=0.555x-0.793 R^2=0.873	y=0.284x-0.094 R^2=0.729	y=0.392x-0.346 R^2=0.791
CW×RCL	y=0.610x-0.118 R^2=0.528	y=0.976x-1.136 R^2=0.934	y=0.623x-0.190 R^2=0.908	y=0.837x-0.791 R^2=0.853
CW×LCL	y=0.66x-0.228 R^2=0.626	y=0.956x-1.094 R^2=0.928	y=0.650x-0.248 R^2=0.837	y=0.833x-0.771 R^2=0.853
CW×W	y=0.963x$^{2.065}$ R^2=0.696	y=0.406x$^{3.051}$ R^2=0.916	y=1.002x$^{2.023}$ R^2=0.775	y=0.405x$^{3.025}$ R^2=0.865
CW×GL	y=0.326x-0.091 R^2=0.355	y=0.308x-0.030 R^2=0.755	-	-

Score splot PCA

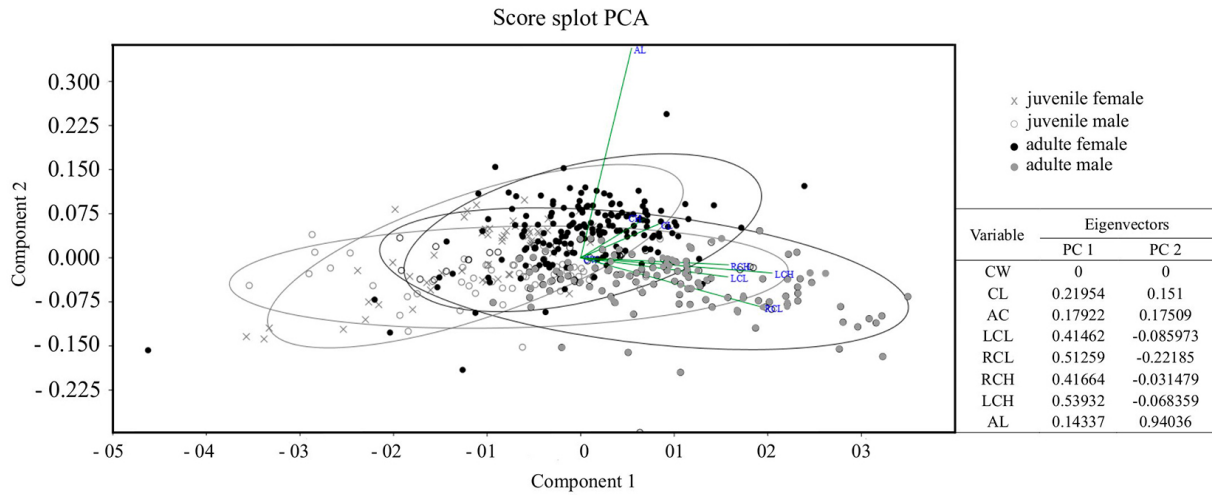| Variable	Eigenvectors	
	PC 1	PC 2
CW	0	0
CL	0.21954	0.151
AC	0.17922	0.17509
LCL	0.41462	-0.085973
RCL	0.51259	-0.22185
RCH	0.41664	-0.031479
LCH	0.53932	-0.068359
AL	0.14337	0.94036

Fig. 3. – Principal component analysis and eigenvectors of each variable that contribute to the principal components (PC1 and PC2) of the species *Goniopsis cruentata* captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020. CW, carapace width; CL, carapace length; CH, carapace height; RCL and LCL, right and left cheliped length; RCH and LCH, right and left cheliped height; PL, pleon length. Ellipse 90%.
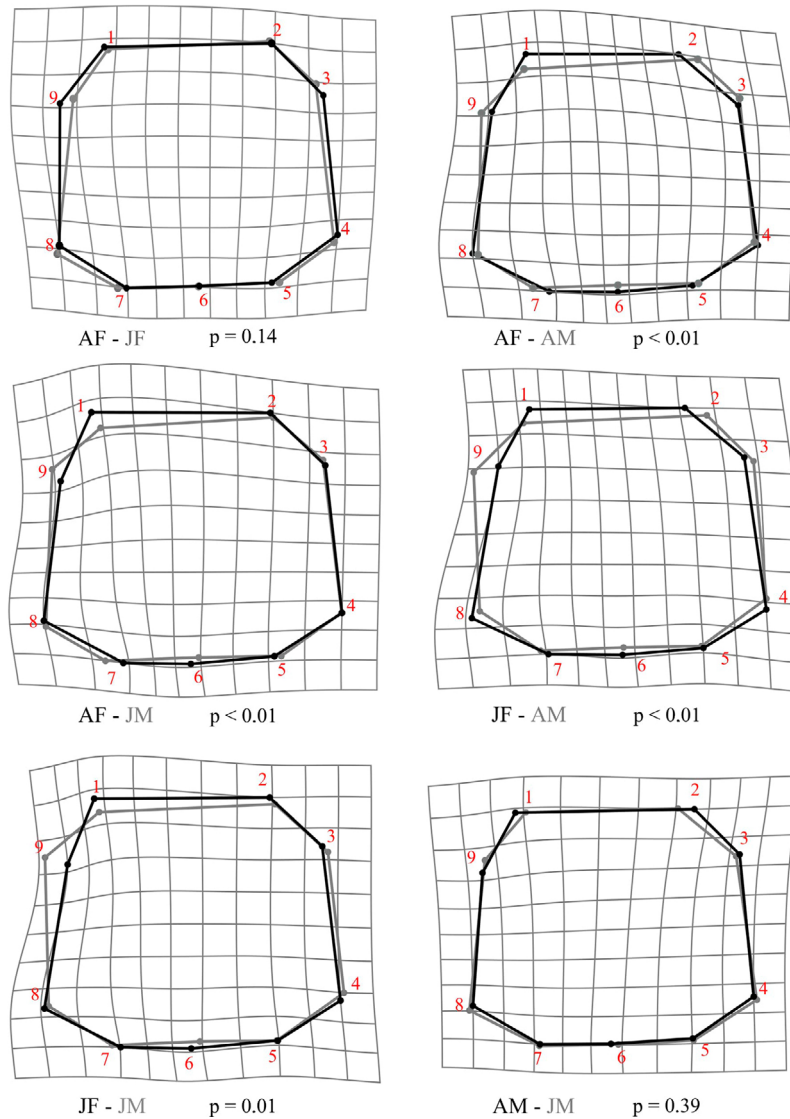

Fig. 4. – Thin-plate splines of morphological variation based on discriminant analysis of males and females of *Goniopicis cruentata* captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020. Deformation scale 5.0.

Fig. 5. – Morphological variation based on canonical variable analysis and ontogenetic trajectory based on multivariate symmetric component regression on the transformed centroid size of the pleon of female *Goniopsis cruentata* captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020. A, adult (grey); J, juvenile (black).
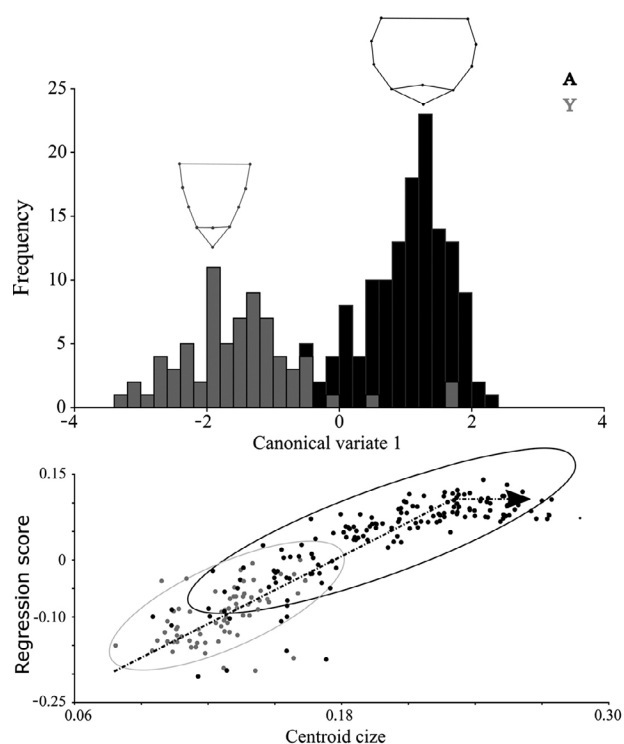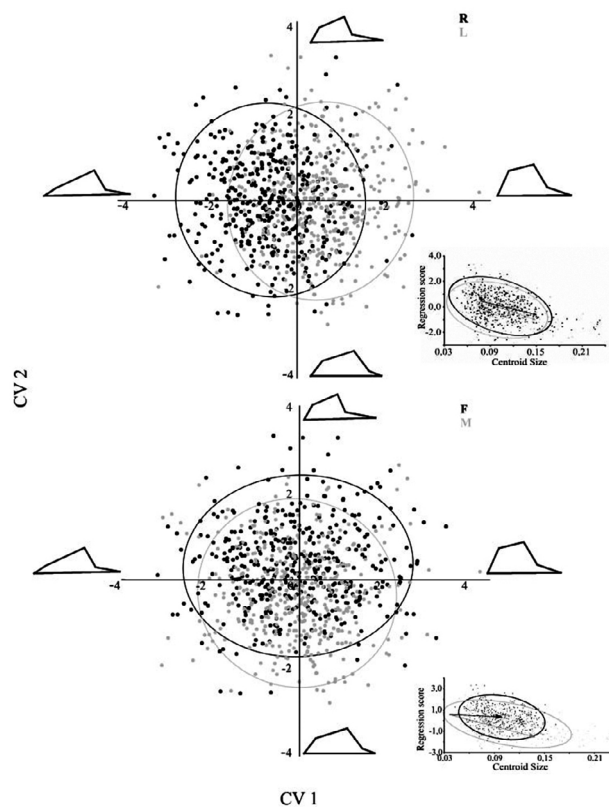

Fig. 6. – Morphological variation based on canonical variable analysis and ontogenetic trajectory based on multivariate symmetric component regression on the transformed centroid size of the cheliped of females and males of *Goniopsis cruentata* captured in a tropical estuarine mangrove (Tamandaré-PE) from February 2019 to January 2020.

location for AM×JM was 63.0% and 63.6% for AM and JM, respectively. The results obtained by MANOVA with Bonferroni correction also showed a significant difference in shape and size for AF×JF, as shown in Figure 5, and only for centroid size (p=0.0001) in the AM×JM comparison (Fig. 5).

When cheliped shape was analysed, a morphological separation was observed through CVA regarding sexual comparison (F×M) and laterality (R×L), with p-value=0.0001 for both interactions. However, no statistically significant morphological separation was observed when ontogenetic comparison (A×J) was conducted (p=0.72). These differences were also observed in the discriminant analysis of F×M and R×L (p=0.0001) and A×J (p=0.59) (Fig. 6). For the A×J comparison, the allocations were 52.75% for adults and 59.39% for juveniles in females. In males, they were 52.44% and 52.29% for adults and juveniles, respectively.

In chelipeds, the results obtained by MANOVA with Bonferroni correction also showed no significant difference in the ontogenetic comparison form (A×J), with p>0.05. However, in the sexual (F×M) and laterality (R×L) interactions, significant separation was observed, with p=0.0001.

DISCUSSION

The present study showed that the morphometry and morphology of *Goniopsis cruentata* exhibit a note-worthy ontogenetic and sexual dimorphism, with relative growth differing according to the morphometric relationship between males and females and juveniles and adults. These results are important events in the reproductive cycle of Brachyura crabs resulting from morphological, physiological and behavioural changes in the evolutionary process (Hartnoll 1983). Taxonomists have employed several characters within species boundaries to track evolutionary progress (Mayr and Ashlock 1991). For example, in systematic studies morphometric and morphological variables are reliable; they are targets for selection, detectable and applicable, and generally have been shaped under polygenic control (Garnier et al. 2005). However, traditional morphological studies are limited to measuring a few characteristics, while geometric morphometry allows the shape of a particular structure to be measured. (Mutanen and Pretorius 2007). The present study points to quantitative variations in body shape in an important estuarine tree crab species, including changes throughout ontogenetic development.

Linear morphometry

The allometric analysis showed that males acquired a larger average body size than females for the *G. cruentata* population captured in a tropical estuarine mangrove, and the same growth pattern was also described by Botelho et al. (2004). Accord-

ing to Masunari et al. (2017), the size variation between the sexes can be explained by differentiated reproductive needs, as size dominance in male crabs increases opportunities for victory during intraspecific competition, courtship and handling the female during copulation.

The body size of male specimens of *G. cruentata* captured on the southern coast of Pernambuco seems to be determinant in the sexual dimorphism of the population because the variables pleon length (PL), CH, and carapace length (CL) were responsible for the separation of males and females in both juveniles and adults. These morphometric characteristics suggest that both sexes of *G. cruentata* have a life history that prioritizes reproduction and survival, as rapid growth may provide selective advantages. For example, larger females of this species show a higher egg production per clutch (Cobo and Fransozo 2003), while larger males probably intimidate smaller ones in competition for receptive females and are accepted more often. This behaviour is commonly observed in other Brachyura species (Brockerhoff and McLay 2005), indicating a natural selection pattern.

As for the differentiation between juveniles and adults (ontogenetic variation), this study revealed that adult individuals of *G. cruentata* grew in carapace width (CW) and weight in the same proportion (isometry). In contrast, juveniles grew proportionally more in CW than weight (negative allometry). This difference may result from a differentiated diet influenced by success in capturing food, which is higher in large-sized individuals because the presence of larger undesired individuals inhibits the presence of smaller ones, causing them to dismiss or give up capturing the baits (personal observation). Moreover, the crab's weight after maturity increases as a result of the action of the androgenic gland (Pinheiro and Fiscarelli 2009) and the development of reproductive cells. This change in the weight/growth model through ontogeny in Brachyura species was also found by Mantelatto and Fransozo (1992) in *Hepatus pudibundus*, by Pinheiro and Fransozo (1993) in *Arenaeus cribarius* and by Peiró et al. (2011) in *Austinixa aidae*, thus supporting the above hypothesis.

However, diverging from the results found in this study, Reis et al. (2015) presented results for *Goniopsis cruentata* in which juvenile specimens showed negative allometry and adults positive allometry for the weight/growth relationship. Individual allometries for different locations observed in these crabs indicate that geographic variation (factors such as temperature) may influence the body size determination of Brachyura such as *G. cruentata* because, according to Lira et al. (2015), local conditions influence variations in their biological characteristics. Therefore, geographic variations may be associated with differences in body size, maturity and variation in reproductive characteristics, a behaviour that is present and well defined in other organisms (Olalla-Tárraga et al. 2009).

Geometric morphometry

The combination of morphological methods with morphometric data proved useful for this study, supporting the investigation of the main causes of variation in the shape of the pleon, carapace and chelipeds in this case. Furthermore, studies show that if the complete shape of a structure is considered, the quantity and quality of variables for statistical analysis increases greatly, so geometric morphometry analysis has become an important tool for multidimensional statistical comparison of morphological characters (Bichain et al. 2007).

The variation found in the carapace region of *G. cruentata* reveals a greater sexual dimorphism because no ontogenetic dimorphism was found in same-sex individuals. The sexual dimorphism consisted of the lateral displacement of landmarks 3 and 9, which caused a widening of the females' posterolateral region. This widening resulted in an angular decrease in the region with a smaller displacement of landmarks 1 and 2. It is known that the wider carapace shape in female specimens emerges as a reproductive advantage because females need to develop strategies in this region to accommodate the gonads, and these differences may also reflect an adaptation for internal growth (e.g. gonad and muscle) (López-Greco et al. 2004). Thus, a synoptic study with other physiological and functional methodologies can help to better understand the species' life history.

Because of the lack of variation in the cephalothorax region's shape during the ontogenetic trajectory, we can infer that this region suffers a greater influence of sexual aspects, in which more enlarged females have greater reproductive success (the existence of hierarchy, for example). According to Reis et al. (2015), *G. cruentata* has a life history that prioritizes reproduction over survival, and rapid growth provides selective advantages for both sexes, with fast-growing individuals reaching sexual maturity with larger bodies.

Several authors have already observed differences in cephalothorax shape between sexes in crustaceans (Trevisan et al. 2012), reporting that this distinction is most obvious in the posterior apex of the cephalothorax, specifically in the lateral region, where the posterior region is wider in females than in males. In addition, Marochi et al. (2018) showed, for example, that there is sexual dimorphism of *Aratus pisonii* in carapace shape during the juvenile phase (before the pubertal moult).

In the abdominal region, only the females showed morphological variation (displacement of landmarks 2, 3, and 4 and their respective homologues) involving widening in the sixth, fifth and fourth abdominal segments. This widening caused a reduction at the end of the telson (displacement at landmark 5) and is closely related to egg storage capacity, well discussed by scholars, in which allometric and morphological growth in the female pleon is associated with directional sexual selection arising from an increase in fat reserves and an enlargement of the area to accommodate offspring or eggs (Hartnoll 1978). In both sexes, directional selec-

tion is likely related to increased reproductive success (Kodric-Brown et al. 2006).

The relationship between one-dimensional measurement such as size (represented by the centroid size) and measurement of the entire shape of the pleon (represented by landmarks) allows growth to be studied as an integral approach, thus making it possible to identify the degree of shape change during growth in the pleon region for females. In addition to reflecting internal growth (e.g. gonad and muscle) (Williner and Collins 2013), these changes are linked to the specimens' characteristics because larger females produce a greater number of eggs per clutch for *G. cruentata* (Cobo and Fransozo 1998).

For the male specimens, no morphological variations between developmental stages were found, implying that morphological characteristics are not related to ontogenetic development and that this region does not have a biological need that justifies a reproductive effort for variation in the pleon shape. Similar results were found in freshwater crabs by Silva et al. (2018) and Almeida et al. (2021). The lack of noticeable variation in the pleon of males may due to the fact that the structure is used only for the support and protection of the two pleopod pairs responsible for sperm transfer during mating (Hartnoll 1978).

The *G. cruentata* population showed significant sexual dimorphism but no ontogenetic variation in cheliped morphology and laterality. Regardless of the sexual influence, we observed that the morphology found in the *G. cruentata* chelipeds undergoes a greater expression of laterality (principal component 1) than the sexual aspects (principal component 2) and shows no ontogenetic expression in the shape influence. This variation has been recorded for other Brachyura species, and decapods are classified as either homochelous or heterochelous (the larger cheliped can be either the right or the left). For this study, despite the influence of sex on the cheliped morphology, both sexes showed similar laterality. Heterochely was therefore not influenced by sex, thus diverging from the findings of Silva and Paula (2008) in studies with *Pachygrapsus marmoratus* and *Carcinus maenas*, in which they state that differences in the chelipeds can also be influenced by gender.

The morphological variation of chelipeds in *G. cruentata* may be related to the different functions of the right and left chelipeds. The right (slimmer) cheliped can be used for food handling, while the left (more robust) cheliped is a larger and more powerful grinding tool for breaking down exoskeletons of smaller crabs and other food components. This "function division" was pointed out by Schenk and Wainwright (2001), who compared the morphology underlying cheliped force production and intraspecific cheliped dimorphism in six species of brachyuran crabs, finding that in many crab species males and females exhibit dimorphic chelipeds, as in the case of *G. cruentata*.

The ability to break the shell is directly related to the cheliped's closing force, which varies among species depending on size, leverage and dentition proper-

ties, and the type and angle of the muscle fibres closest to the cheliped (Schenk and Wainwright 2001). However, increased size and variation of the cheliped has also been associated with success in sexual and agonistic interactions (Warner 1982).

The shape of the right and left chelipeds was different in males and females and between sexes, a behaviour not yet recorded for an arboreal species that does not exhibit a high degree of heterochely, such as the species *G. cruentata, A. pisonii* and *A. angustipes*. The fixed finger of the slender and longer cheliped propodus found in females may appear as a facilitator in reaching the abdominal chamber and sensory structures in the egg cleaning and larval release process (Almerão et al. 2010). However, to support the hypothesis presented here, studies on the species' behaviour are necessary. According to Warner (1970), species exhibitingslight sexual dimorphism in chelipeds belong to the category in which ritualized agonistic behaviour is also observed in females. Therefore, the morphological expression existing between males and females of *G. cruentata* should not be limited to ritualized agonistic behaviour, directing female morphology solely towards purely reproductive activities, since the cause of sexually dimorphic chelipeds may offer potential advantages that facilitate sex recognition and therefore courtship, as described in crabs with high sexual dimorphism (*Uca*, Crane 1958; *Hemiplax*, Griffin 1968) and low sexual dimorphism (*Goniopsis*, Herma and Schõne 1963; *Pachygrapsus*, Bovbjerg 1960).

Overall, the results presented here provide new information on the development of secondary characters and their consequences on the shape and size of the carapace and the propodus of the chelipeds in males and females of *Goniopsis cruentata*. Therefore, these functions could be considered morphological characters with sex-dependent differential expression (Hartnoll 1978, Barría et al. 2011). These consequences reflect the evolutionary sexual tendency toward reproduction. Furthermore, traditional morphometry and geometric morphometry proved efficient in investigating and recognizing variations between juveniles and adults and males and females in the carapace, pleon and cheliped with refined detail. This information is extremely important for maintaining and conserving invertebrate species, especially those with commercial value, even if their conservation status is not critical.

CONCLUSION

Based on the data analysis, it was possible to observe that *Goniopsis cruentata* shows sexual dimorphism related to size, with males having a larger carapace than females. This result can be explained by the higher aggressiveness of the males and by sexual selection, as observed in other crustaceans.

When the carapace shape variation was analysed, females showed a wider posterior carapace region because they need a larger compartment for egg development and transportation. The cheliped shape showed heterochely in both males and females, although the

males showed a more robust cheliped, indicating once again the sexual selection action. However, laterality exhibited a greater strength of morphological separation within the species.

AUTHORSHIP CONTRIBUTION STATEMENT

Lucas Nunes da Silva: Conceptualization, methodology, data curation, formal analysis, funding acquisition, investigation. Mauro de Melo Junior: supervision, formal analysis, writing—review and editing. Renata Akemi Shinozaki-Mendes: methodology, data curation, writing—review and editing.

ACKNOWLEDGEMENTS

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