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Tropical Zoology

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## Geographical variation in the morphology and sexual dimorphism of *Acanthochelys spixii* (Testudines, Chelidae) in Brazil

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Body size is an important parameter for ecology, reproduction, evolution, and development of animal species, besides understanding their taxonomic relationships and to establishing the relationships between individual size and shape. We evaluated the variation in body size traits, sexual dimorphism in populations of a turtle species *Acanthochelys spixii* across its distribution in Brazil and we described sex ratio in those populations. Description of the size-classes on these populations was also provided. We sampled 86 adults (16 in Minas Gerais (MG) and 70 in Rio Grande do Sul (RS)). The sex ratio was 1:1 in both sampling areas. Females were larger than males, and specimens from RS were larger than those from MG. The posterior-lobe width (PLW) was deemed the most significant sexing variable in a linear discriminant analysis of specimens within each state. Two variables, PLW and maximum carapace width, together distinguished the four groups (males and females in MG and RS) with 77% accuracy. Overall, most body measures were larger for both males and females in the southernmost population, inhabiting higher latitudes and lower temperatures. Regarding sexual size dimorphism, females had larger PLW than males in MG, and all variables were dimorphic in RS, in general females being larger than males. 10  
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**Keywords:** Chelidae; sex ratio; morphometrics; sexual dimorphism; *Acanthochelys spixii*

### Introduction 25

Body size is an important parameter that can affect the ecology, reproduction, evolution, and development of animal species (Preston and Ackerly 2004). Morphometric studies in turtles have contributed to understanding their taxonomic relationships and to establishing the relationships between individual size and shape, life history, and habitat characteristics (Daza and Páez, 2007). Populations of species that inhabit a wide range of environments frequently display divergent morphologies that correlate with differences in ecological parameters (Rivera 2008). 30

In broader spatial scales, body size variation can be explained by environmental temperatures, known as Bergmann's rule (Mayr 1956). It is often cited as an example of adaptive geographic variation (Mayr 1956) in which there is a within-species tendency for increasing body size with increasing latitude or decreasing environmental temperature (Ashton and Feldman 2003). Other sources also could explain these variations, for instance, seasonality (Ashton et al. 2007). Seasonality is associated with body size, but not in a linear way so that smaller body sizes occur at the intermediate gradient of the predictor variable 35

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(Ashton et al. 2007). Flow regimes (Rivera 2008) and anthropic effects such as hunting pressure, which can select for a given size class (Daza and Páez, 2007, Lovich et al. 2010), and interactions with other species, including predation, have also been examined.

Of interest, and also widespread described in many taxa (Shine 1979, Vollrath and Parker 1992, Owens and Hartley 1998, Cox et al. 2007, McPherson and Chenoweth 2012), sexual size dimorphism (SSD) is found in many reptile species (Anderson and Vitt 1990, Cox et al. 2003). These size differences, however, do not follow a general rule (Cox et al. 2007). In lizards and crocodylians, males are larger than females in most groups (Olson et al. 2002, Cox et al. 2007); for turtles and snakes the inverse seem to be the most general (Shine 1994, Cox et al. 2007). There is evidence that the observed patterns of reptile SSD for large male size are due to sexual selection and for large female size are due to fecundity selection (Shine 1994, Cox et al. 2003, 2007) and ecological divergence between sexes due to intraspecific competition (Preest 1994). *Acanthochelys spixii* (Duméril & Bibron, 1835), which belongs to the family Chelidae, is found in South American regions as Uruguay and Argentina (Carreira et al. 2007, Fritz and Havas 2007, Estrades et al. 2008). In Brazil its distribution ranges since central areas passing through southeastern to south region. (Brandão et al. 2002, Ribas and Monteiro Filho 2002, Fritz and Havas 2007, Brasil et al. 2011). The species inhabits slow-moving water bodies, ponds, wetlands, and other areas with abundant submerged vegetation (Quintela and Loebmann 2009). The biology and ecology of *A. spixii* are poorly understood, and most studies have been performed on specimens in captivity (D'amato, 1992, Brandão et al. 2002, Mocelin et al. 2008, Brasil et al. 2011). Among the publications describing the biology and ecology of *A. spixii*, none has described the morphology of the species in detail. Biometric studies are an important tool for differentiating species and subspecies (Lamb and Lovich 1990, Lovich and Lamb 1995, Seidel et al. 1999), measuring geographical variation within species (Iverson 1985, Lubcke and Wilson 2007), analyzing reproductive parameters (Congdon and van Loben Sels 1991, Daza and Páez, 2007), and evaluating the impact of commercial exploitation (Close and Seigel 1997, Gamble and Simons 2004). Thus, a more detailed understanding of geographic variation in morphology is critical to elucidating the systematic relationships and ecological characteristics of this species.

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In the present study, we sampled populations of *A. spixii* in two Brazilian states separated by nearly 2000 km in order answer the following questions: (i) are there differences in body size across populations? (ii) Do populations differ in sex ratio? and (iii) Do populations show sexual dimorphism?

## Materials and methods

### Study area

*Acanthochelys spixii* individuals were captured in two Brazilian states, Rio Grande do Sul (RS) and Minas Gerais (MG) (Figure 1). Sites were chosen based on previous species records and environmental peculiarity in each one of them. In RS, specimens were collected at six locations from 1994 to 2006, but not annually (Table 1). This region is highly homogeneous and typically of low elevation (mean = 4 m), with a geological origin in the Quaternary. The predominant aquatic habitats are puddles and ponds, notably the Lagoa Mirim and Lagoa dos Patos (Krause et al. 1982) (Figure 1). In MG, specimens were collected at Sempre-Vivas National Park (PARNA das Sempre-Vivas) in 2011. The sampling points were located in regions of the upper São Francisco River Basins, all at elevations above 800 m.

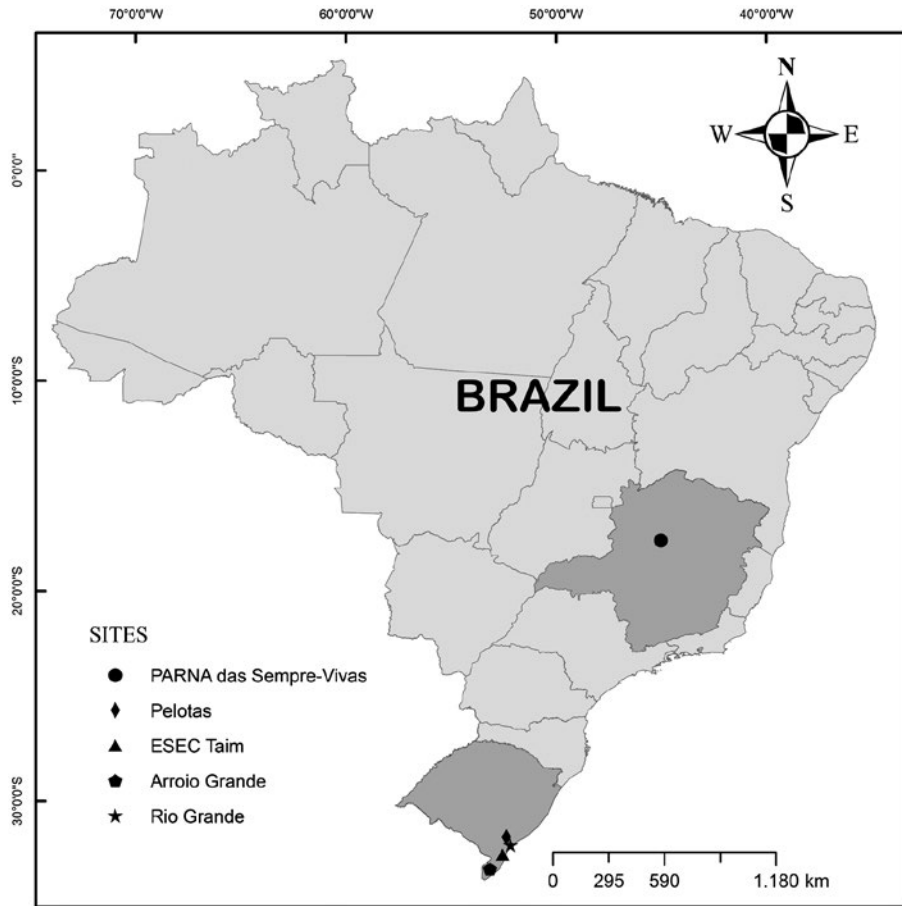


Figure 1. Sampling areas in each state. In Minas Geras (PARNA das Sempre-Vivas) municipality of Diamantina. In Rio Grande do Sul in the municipalities of Pelotas, Arroio Grande, Rio Grande and the Ecological Station Taim (ESEC Taim).

### **Data collection**

Adult *A. spixii* specimens were captured using two types of bait trap: fyke-net traps (FN), with a single opening measuring 30.4 cm in width and 14.7 cm in height, and crab traps (CT), with four openings averaging 40.5 cm around the perimeter (Fagundes et al. 2010). Active searching in the water (ASW) and in the field (ASF) (Bager et al., 2007) and manual capture via snorkeling (SN) (Bager et al., 2010) were also demonstrated (Table 1), but here used only for purpose of morphometric description of the populations. Each individual was weighed (10-g accuracy), and up to 27 additional measurements were taken using a vernier caliper (1-mm or 0.1-mm precision). Figure 2 shows how the measurements were made and their acronyms. Cephalic and mouth width were measured over the tympanic membranes and the maximum width of the lower rhamphotheca, respectively. Males were sexed based on secondary sexual characteristics: tail length (which is longer in males) and plastron concavity. The capture site of each individual was recorded using GPS, and all turtles were marked by notching the marginal scutes (Cagle 1939, Bager et al. 2010) and then returned to their capture sites.

Table 1. Sampling areas with geographic localities, sampling periods, and specimen-capture techniques. FN: Fyke-net traps; SN: manual capture via snorkeling; CT: crab traps; ASF: active searching in the field; ASW: active searching in the water. All coordinates in datum WGS84.

State	Study area	Latitude	Longitude	Sampling period	Capture methods
MG	Diamantina (PARNA das Sempre-Vivas)	17°36'00"	44°60'00"	May 2010	FN/SN
RS	Taim Ecological Station (ESEC Taim)	32°32'51"	52°32'40"	1995, 1998, 2000, 2002, 2003	ASF/CT
	Pelotas	31°43'46"	52°21'51"	2002, 2003, 2004, 2006	ASW
	Arroio Grande	33°14'21"	53°08'12"	2001, 2002	ASW
	Rio Grande	32°06'00"	52°10'00"	1994, 1995	ASW

### Data analysis

20 All the comparative analyses were done based on individuals sampled by the traps and only measurements taken during the first capture were used in the analyses. Juveniles were disregarded due to the small number of individuals captured. Previous analysis did not identify morphometric variation between populations within RS state ( $F_{2,47}$ : 1.15,  $p = 0.28$ ) and for this reason adults were grouped by sex: MG males, MG females, RS males, and RS females. Normality (Shapiro–Wilk test) and homogeneity of variance (Levene test) assumptions were checked. The Kruskal–Wallis test was used to compare different sexes within states (Zar 2010) (MG Male  $\times$  MG Female; RS Male  $\times$  RS Female) and the same sex from different states (MG Male  $\times$  RS Male; MG Female  $\times$  RS Female) in univariate analyses.

30 The original, non-transformed data were used in the statistical analyses, except in the discriminant analysis, in which the value of each variable was divided by the maximum length of the carapace. This approach was used to reduce the effect of size and to improve the allometric shape analyses between males and females (Bager et al. 2010).

35 Sexual dimorphism was analyzed within each region using linear regression (Zar 2010) with carapace length (CL) as the independent variable. Parallelism tests were used to compare the linear-regression results for males and females. When the parallelism test indicated a non-significant difference, we used covariance analyses (with CL as the covariate parameter) to compare the intercepts. In MG, the dependent variables were MCW, CCW, MPL, MVSL, MPW, PLW, MBL, CBL, CPD, CW, MW, and MHS. In RS, the dependent variables were MCW, MPL, MPW, PLW, CPD, and MHS.

40 The linear discriminant analyses (Zuur et al. 2007) of different sexes within states utilized seven variables (PLW, MPW, MHS, MVSL, MCW, MPL, and CPD) in MG and four variables (MCW, MPL, PLW, and MHS) in RS. A linear discriminant analysis was also used to compare the geographic variation in morphology among sexes and states, but only four variables (MCW, PLW, MPL, and MHS) were included in this analysis. The variables were selected to maximize the number of specimens in the analysis.

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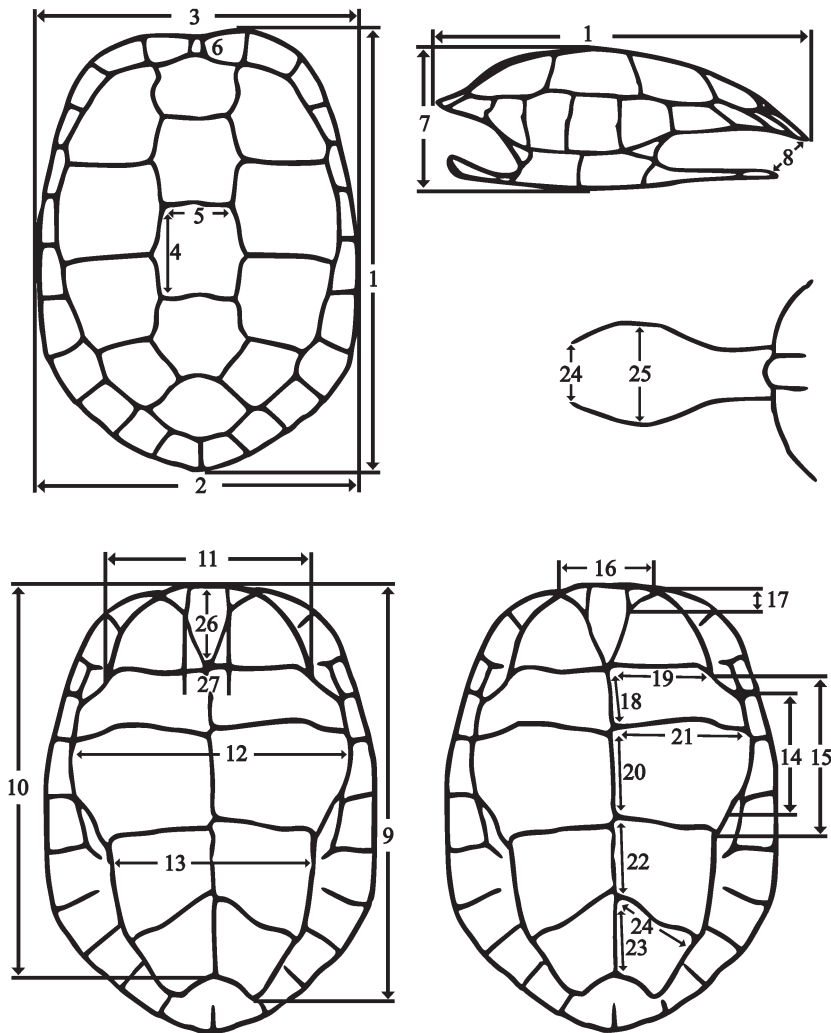


Figure 2. Morphological measurements of *Acanthochelys spixii* (modified from Bager et al., 2010). Key: (1) carapace length (CL); (2) maximum carapace width (MCW); (3) central carapace width (CCW); (4) length of the third central scute (LC3); (5) width of the third central scute (WC3); (6) nuchal scute length (NL); (7) maximum carapace height (MHS); (8) carapace and plastron terminal distance (CPD); (9) maximum plastron length (MPL); (10) mid-ventral suture length (MVSL); (11) anterior lobe width (ALW); (12) maximum plastron width (MPW); (13) posterior lobe width (PLW); (14) central bridge length (CBL); (15) maximum bridge length (MBL); (16) width of left and right gular scutes (WGS); (17) left gular scute length (GSL); (18) left pectoral scute length (PEL); (19) left pectoral scute width (PSW); (20) left abdominal scute length (ASL); (21) left abdominal scute width (ABSW); (22) left anal scute length (ANSL); (23) left anal scute width (ASW); (24) mouth width (MW); (25) cephalic width (CW); (26) intergular scute length (ISL); (27) intergular scute width (ISW).

The sex ratio of males and females was analyzed by Chi-Squared tests (Zar 2010) using the Yates correction and equal expected proportions for both states. We used data from specimens captured only in ESEC Taim for RS state due to sampling method.

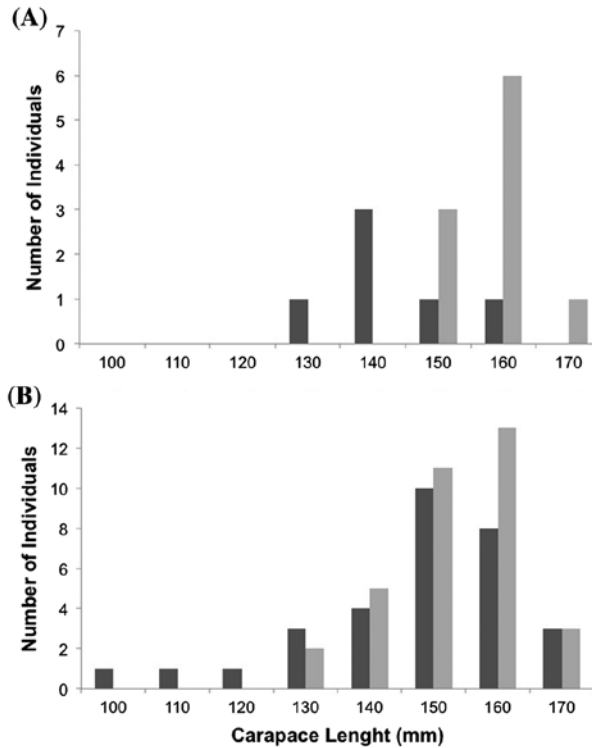


Figure 3. Frequency distributions of carapace length (mm) for male (dark bars) and female (gray bars) *Acanthochelys spixii* in Minas Gerais (A), and in Rio Grande do Sul (B).

SSD were quantified as recommended by Stephens and Wiens (2009):

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$$SSD = (\text{Mean Female CL} / \text{Mean Male CL}) - 1$$

All analyzes were done using BioEstat 5.0 (Ayres et al. 2007). A significance level of  $\alpha = 0.05$  was designated in all tests.

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## Results

In total, 85 individuals were collected: nine females and six males in MG and 38 females and 32 males in RS. Fifteen individuals were found at Sempre-Vivas National Park (Parque Nacional das Sempre-Vivas, in MG state) and the sex ratio (1.5:1) was not significantly different from 1:1 ( $\chi^2 = 0.06$ ;  $p = 0.60$ ). This parameter was also non-significant in ESEC Taim (RS state) (1.18:1,  $\chi^2 = 0.51$ ;  $p = 0.47$ ).

Male CL ranged from 101 to 178 mm, with a modal value of 150 mm, while female CL ranged from 134 to 180 mm, with a modal value of 160 mm (Figure 3). In general, females were larger than males in both regions (Table 2), but CL, MPW, and weight had similar size for females and males in RS, and the same was reported in MG state for the variables LC3, WC3, NCL, PEL, GSL, ISL, and ISW. The only variable for which males exceeded females in both the average value and the extreme values was ISW in MG; however, this difference was not significant.

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Table 2. Descriptive statistics for the 27 variables measured on *Acanthochelys spixii* individuals from Minas Gerais (MG) and Rio Grande do Sul (RS), Brazil. Linear measurements are in millimeters; body weight is in grams. PBS: Kruskal–Wallis analysis between females and males within states; P: Kruskal–Wallis analysis between states for a single sex.

	RS						MG						RS/MG			
	Male			Female			Male			Female			Male	Female		
	n	Min.–Max.	$\bar{X} \pm 1SD$	n	Min.–Max.	$\bar{X} \pm 1SD$	P BS	n	Min.–Max.	$\bar{X} \pm 1SD$	n	Min.–Max.	$\bar{X} \pm 1SD$	P BS	P	
CL	32	101–178	153.2 ± 17.3	35	134–176	159.0 ± 10.4	0.233	6	131–164	148.0 ± 10.6	9	152–180	163.9 ± 7.7	0.009	0.149	0.268
MCW	32	80–125	108.3 ± 10.8	35	101–132	114.8 ± 6.6	0.012	6	85–110	95.0 ± 8.4	9	103–116	107.3 ± 4.0	0.017	0.010	0.002
CCW	–	–	–	–	–	–	–	6	83–109	92.7 ± 8.7	9	94–115	102.6 ± 5.8	0.022	–	–
LC3	–	–	–	–	–	–	–	6	18.7–24.8	22.1 ± 2.8	9	22.5–27	24.5 ± 1.4	0.094	–	–
WC3	–	–	–	–	–	–	–	6	23.5–29.7	25.5 ± 2.6	9	22.3–27.3	24.6 ± 1.5	0.738	–	–
NL	–	–	–	1	–	18.3	–	6	11.6–14.9	13.7 ± 1.5	9	13.4–17.5	15.2 ± 1.3	0.111	–	–
MPL	32	96–168	141.2 ± 15.1	34	128–165	150.4 ± 8.8	0.003	6	119–141	131.4 ± 8.3	9	138–164	148.3 ± 7.5	0.003	0.045	0.416
MVSL	–	–	–	4	143–149	147 ± 2.8	–	6	113.9–131.2	123.7 ± 6.5	9	129.6–152.9	139.0 ± 6.4	0.003	–	0.034
MPW	7	100.1–112.6	104.5 ± 4.8	9	91.2–111.1	104.6 ± 5.8	0.427	6	–	–	–	–	–	–	–	–
ALW	–	–	–	4	89.7–93	90.5 ± 1.5	–	2	64.6–65	64.7 ± 0.3	1	–	73.7	–	–	–
PLW	31	45–83	68.5 ± 8.2	34	63–89	77.2 ± 5.2	<0.001	6	57.5–69	62.0 ± 4.2	9	71–80.6	74.7 ± 3.1	0.001	0.021	0.064
CBL	–	–	–	–	–	–	–	6	25.7–31.3	28.3 ± 2.4	9	31.7–46.4	37.0 ± 4.4	0.001	–	–
MBL	–	–	–	–	–	–	–	6	32.1–37.2	34.9 ± 2.2	9	37.1–49.7	43.0 ± 4.2	0.002	–	–
PEL	–	–	–	4	11–19	13.4 ± 3.8	–	6	10.1–14.7	12.5 ± 1.6	9	9–16.6	13.1 ± 2.6	0.723	–	0.817
PSW	–	–	–	–	–	–	–	6	30.4–37.6	32.5 ± 2.7	9	37.4–41.2	39.3 ± 1.4	0.002	–	–
ASL	–	–	–	–	–	–	–	6	17.3–21.8	19.8 ± 1.7	9	20.7–27.5	25.0 ± 2.0	0.004	–	–
ABSW	–	–	–	–	–	–	–	6	23.9–31.5	27.6 ± 2.5	9	30.8–38	34.8 ± 2.0	0.002	–	–
ANSL	–	–	–	–	–	–	–	6	18.2–22.5	20.2 ± 1.7	9	20–26.1	23.3 ± 2.0	0.015	–	–

(Continued)



Table 2. (Continued).

	RS						MG						RS/MG			
	Male			Female			Male			Female			Male	Female		
	<i>n</i>	Min.- Max.	$\bar{X} \pm 1SD$	<i>n</i>	Min.- Max.	$\bar{X} \pm 1SD$	<i>n</i>	Min.- Max.	$\bar{X} \pm 1SD$	<i>n</i>	Min.- Max.	$\bar{X} \pm 1SD$	<i>P</i>	BS	<i>P</i>	
ASW	-	-	-	-	-	-	6	21.9-25	22.7 ± 1.2	9	26.9-29.6	28.3 ± 0.8	-	0.001	-	
GSL	-	-	-	-	-	-	6	17.1-19.3	18.3 ± 0.8	9	16-24.2	19.9 ± 2.8	-	0.221	-	
WGS	-	-	-	-	-	-	6	50.1-58.8	54.6 ± 3.4	9	55.7-68.1	61.6 ± 4.1	-	0.005	-	
ISL	-	-	-	-	-	-	6	28-34.7	30.9 ± 2.5	9	29.3-37.4	32.3 ± 2.1	-	0.178	-	
ISW	-	-	-	-	-	-	6	17.2-21.3	19.0 ± 1.7	9	16.3-20.9	18.4 ± 1.8	-	0.358	-	
CPD	5	17.4-25.4	20.7 ± 3.5	9	24.8- 29.7	27.1 ± 2.1	6	16.1-23.3	21.3 ± 2.7	9	22.1-34.6	27.3 ± 3.5	0.004	0.004	0.927	0.775
CW	-	-	-	3	28.3-30	29.4 ± 1.0	6	22.6-26	24.8 ± 1.3	9	26-29.6	27.5 ± 1.0	-	0.001	-	0.018
MW	-	-	-	-	-	-	6	17.4-21	19.1 ± 1.4	9	19.2-22.9	20.8 ± 1.3	-	0.030	-	-
MHS	30	42-68	54.4 ± 6.7	33	43-71	61.4 ± 6.8	6	43-55	49.2 ± 4.3	9	52-60	55.6 ± 3.0	0.014	0.014	0.071	0.004
WEIGHT	3	362-372	366.3 ± 5.1	3	540-668	595.3 ± 65.7	6	230-450	316.7 ± 74.5	9	340-530	433 ± 59.8	0.011	0.011	0.120	0.011

The linear regressions for MCW, PLW, CPD, and MHS were significant and had high coefficients of determination, except for MHS in MG females ( $F_{1,8} = 2.410$ ;  $p = 0.157$ ;  $r^2 = 0.14$ ) and CPD in RS males ( $F_{1,4} = 0.443$ ;  $p = 0.555$ ;  $r^2 = 0.36$ ) (Table 3). Because this regression was not significant, analysis of covariance for CPD in RS should be regarded with caution. Overall, females had lower coefficients of determination than males, especially in RS.

The parallelism and covariance analyses to identify sexual dimorphisms in each state showed that PLW was the only dimorphic variable in MG ( $F_{1,13} = 31.01$ ;  $p < 0.001$ ), but all variables were dimorphic in RS. The number of individuals in MG certainly affected this result, and the analyses should be repeated with a larger number of specimens. Overall, SSD was 0.04 in RS and 0.1 in MG.

Linear discriminant analyses between sexes within states identified PLW as the only descriptor variable for sexual dimorphism in this species. The degree of explanation was 93% (Wilks' lambda = 0.3550;  $F_{1,13} = 23.617$ ;  $p < 0.001$ ) in MG and 78.7% (Wilks' lambda = 0.5804;  $F_{1,59} = 42.660$ ;  $p < 0.001$ ) in RS.

The linear discriminant analysis comparing the four groups (sexes  $\times$  areas) was highly significant (Wilks' lambda = 0.2640;  $F_{6,144} = 22.709$ ;  $p < 0.001$ ) (Figure 4), and MCW and PLW were identified as the descriptor variables. The classification matrix had an overall success of 77%. Females from MG were classified with 90% accuracy; the only misclassifications concerned RS females. Males from MG had the worst classification accuracy, 60%, but the small number of individuals ( $n = 6$ ) affected this outcome. Females from RS were classified with 81% accuracy; they were sometimes confused with males from the same state and with females from MG. The model correctly classified 70% of RS males; the rest were confused with females from the same state.

## Discussion

Despite its wide distribution in Brazil, *A. spixii* remains poorly studied. Data on its biology are found predominantly in the gray literature, and there have been several studies on its distribution (D'amato and Morato, 1991, Ghizoni et al. 2011), diet (Brasil et al. 2011), and demographics (Neto et al. 2011). Our results are consistent with previously published descriptions of the morphology and sexual dimorphism of *A. spixii* (D'amato and Morato, 1991, Bujes 2010, Neto et al. 2011). However, they are more comprehensive than previously available descriptions, enabling us to better assess the patterns of geographic variation.

Our data show that female *A. spixii* are significantly larger than males in most traits. Other authors have reported smaller measurements for *A. spixii* than those obtained here. Bujes (2010) reported a mean CL of 136.4 mm for females and 129.4 mm for males, and Neto et al. (2011) gave a mean size of 139.8 mm for females and 128.8 mm for males. The SSD values reported here are the first submitted for this species and demonstrate low dimorphism. The data reported by Neto et al. (2011) also indicate a low SSD (0.08), demonstrating that *A. spixii* follows the pattern of most Chelidae (excluding genera such as *Phrynops*) (Cox et al. 2007, Ceballos et al. 2012) in which females are larger than males. Smaller males could confer an advantage in agile courtship behaviors and mate searching in aquatic species in which male combat is absent, for example, (Berry and Shine 1980). In addition, females could grow larger than males because females gain an advantage in larger size to increase fecundity producing more or larger eggs or reproducing more frequently (Berry and Shine 1980, Stephens and Wiens 2009).

The sex ratios found in the present study confirm the results of Neto et al. (2011) and Bujes (2010), demonstrating a clear pattern of sexual structure throughout the species'

Table 3. Linear regressions, covariance, and parallelism for male and female *Acanthochelys spixii* from Minas Gerais and Rio Grande do Sul States. CL is the independent variable.

State	Sex	n	Regression parameters						Parallelism			Covariance		
			a	b	F	P	r <sup>2</sup>	F	P	F	P	F	P	
MCW	MG	6	-16.78	0.76	43.220	0.004	0.89	4.880	0.050	2.05	0.177			
	Female	9	36.61	0.43	19.410	0.003	0.67							
	RS	31	16.85	0.60	345.313	<0.001	0.92	3.124	0.079	10.11	0.003			
	Female	34	41.34	0.46	37.696	<0.001	0.52							
PLW	MG	6	13.86	0.33	9.280	0.039	0.62	0.099	0.757	31.01	<0.001			
	Female	9	15.44	0.36	32.440	0.001	0.78							
	RS	30	3.83	0.42	134.420	<0.001	0.82	0.305	0.590	39.38	<0.001			
	Female	33	16.36	0.04	30.546	<0.001	0.47							
CPD	MG	6	-10.43	0.21	10.600	0.032	0.66	0.490	0.506	2.87	0.116			
	Female	9	13.42	0.08	0.290	0.606	-0.08							
	RS	5	13.30	0.05	0.443	0.555	0.36	0.101	0.746	30.98	<0.001			
	Female	8	2.51	0.16	12.087	0.010	0.58							
MHS	MG	6	-5.55	0.37	20.440	0.012	0.79	1.401	0.263	1.16	0.306			
	Female	9	24.81	0.19	2.410	0.157	0.14							
	RS	29	4.66	0.33	65.722	<0.001	0.69	9.126	0.004	-	-			
	Female	32	-32.06	0.59	56.428	<0.001	0.63							

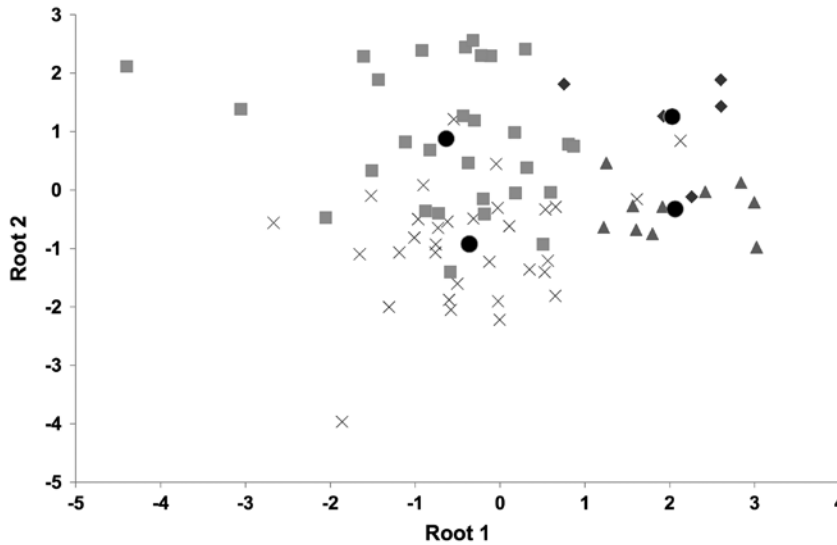


Figure 4. Linear discriminant analysis between males and females of *Acanthochelys spixii* in the Minas Gerais and Rio Grande do Sul states. Triangle: Female MG; x: Female RS; Diamond: Male MG; Square: Male RS and Circle: Centroids.

range. In turtle species with genotypic sex determination, a 1:1 sex ratio is expected to occur (Janzen and Paukstis 1991) and this could be the case, as *A. spixii* is a Chelid species and most Chelid species follow this pattern (Ferreira 2009).

The two populations exhibited differences in size structure, especially for males. The modal class for this sex was 140 mm in MG and 150 mm in RS. No males smaller than 130 mm were captured in MG, preventing us from assessing whether secondary sexual dimorphisms (tail length and concavity) begin to develop at the same size found in RS (CL = 101 mm). Females had larger CPD measurements than males, in contrast to *Hydromedusa tectifera* (A. Bager, unpublished data), which shows higher CPD values for males (most likely resulting from the tail size). A possible explanation is that *A. spixii* is smaller in body size than *H. tectifera*, requiring a larger CPD size for spawning.

The linear regressions for CPD were not significant for MG females or RS males, while those for MHS were not significant for MG females, which had the lowest correlation coefficients among the analyses performed. This result is partly due to the small number of individuals sampled, but other measurements had higher coefficients despite an identical sample size. The parallelism and covariance analyses showed different results between the states. The variables MCW and PLW showed similar growth rates for both sexes in RS (with larger females) and different growth rates for the sexes in MHS. In MG, only PLW showed the larger size of females. This result was confirmed by the discriminant analysis, in which PLW was selected as a descriptor variable.

Comparisons within a given sex between study areas showed that both males and females were larger in RS in some related carapace and plastron measures, but not in others. Males in the two states were equal in size based on CL, CPD, MHS, and weight but differed in MCW and in plastron-related variables (MPL and PLW). Females in the two states were also similar in CL but showed no clear pattern in the variables that differed significantly between states, in contrast to the carapace, plastron, and head of males. Axis

I of the discriminant analysis separated the specimens by state, with MCW as the main group-descriptor variable. The second axis separated the specimens by sex, and PLW was the most significant variable. The discriminant analysis results confirmed that *A. spixii* is monomorphic, and width-related measurements were the descriptor variables.

The morphological differences between individuals may be linked to environmental factors, including air and water temperature and other habitat characteristics (Litzgus and Smith 2010), or to historical factors limiting gene flow between the basins (Clavijo-Baquet et al. 2010). However, the possibility that the morphological differences result from geographical isolation can be ruled out because the populations inhabit different basins instead of contiguous geographic areas (Clavijo-Baquet et al. 2010). Conversely, environmental factors may have affected the morphological differences between the populations studied here because water and air temperature affect the activity and use of space by *A. spixii*. Furthermore, the behavior, breeding season, and feeding ecology of this species vary seasonally (Horta 2008), and the minimum air temperature, water temperature, and mean air temperature are the climatic parameters that best explain the variation in *A. spixii* movement (Horta 2008). This study shows the geographic variation in which individuals from South populations (higher latitudes) are larger than the ones who inhabit lower latitudes for *A. spixii*. Further comparative studies should account for more populations to show how these morphological traits are related to temperature and other environmental conditions for this species in broader areas and quantitatively infer if in fact changes in shape and body size are directly related to environmental conditions.

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No potential conflict of interest was reported by the authors.

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