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Biometric and sexual dimorphism variation of *Hydromedusa tectifera* in Brazil

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Body size has a strong influence on the ecology and evolution of organisms' life history. Turtle species can exhibit variation in body size and shape between populations of conspecifics through usually broad geographical scales. This prediction is timely to be tested in this study with the species *Hydromedusa tectifera*. We aimed to evaluate the variation in body size between and sexual dimorphism within populations of *H. tectifera* in two areas in Brazil. Sampling occurred in Minas Gerais and Rio Grande do Sul states in order to obtain morphometric measures of carapace and plastron of the individuals. We observed sexual dimorphism within populations. In Rio Grande do Sul state, females were larger than males in most of the carapace and plastron measures. In Minas Gerais state, males were larger than females regarding maximum carapace width. Overall, individuals from Rio Grande do Sul state were larger than those from Minas Gerais state. We discuss possible factors that might cause variation in morphology within and between populations of conspecifics. Research on morphology is encouraged to facilitate comparisons among populations in geographically broad areas.

Key words: Body size; geographic variation; South America; turtle.

Body size and related measures influence many aspects of life history and evolution of animals (LaBarbera, 1989; Meiri, 2008). Morphological studies not only analyze shape variations but also try to clarify their causes and effects (Bookstein, 1991). Whether among or within species' populations, the description of morphological variation patterns is essential to highlight evolutionary units in nature (Dos-Reis *et al.*, 2002). The knowledge of this variation plays an important role in the implementation of taxonomic studies (Rohlf & Marcus, 1993), with the investigation of the possible occurrence of new characters or the change in morphology of the current ones.

In freshwater turtle species variation in

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body size and shape between populations of conspecifics is widely recognized, usually in broad geographical scales (Ashton *et al*., 2007; Daza & Páez, 2007; Zuffi *et al*., 2007). Many explanations have already been hypothesized, and the main ones comprise differences in resource availability and quality, population density, environmental conditions (such as temperature and seasonality), and hunting intensity (Rowe, 1994; Ashton *et al*., 2007; Daza & Páez, 2007). In this group, there is a trend for larger body size in higher latitude (i.e. decreased environmental temperature) (Ashton & Feldman, 2003) known as the traditional ecogeographic Bergmann's rule (Mayr, 1956). The Bergmann's rule assumes that larger individuals conserve heat better in cooler than in warmer areas (Ashton *et al*., 2007). Most turtle families that include terrestrial and semi-aquatic species such as the Chelydridae, Emydidae, Kinosternidae and Testudinidae follow this trend (Zuffi *et al*., 1999, Ashton & Feldman, 2003). This pattern is also found in species of the Chelidae family such as *Acanthochelys spixii* in Brazil (Bager *et al*., 2016). However, for many turtle species, incipient knowledge about morphology, population ecology and reproductive biology at local scales is still lacking. Therefore, a comprehensive survey at broader scales for testing this kind of ecological hypothesis become unfeasible.

This is also true for information about sexual size dimorphism (SSD). For instance, Lovich *et al*. (1998) found that males are larger than females in *Glyptemys muhlenbergii* regardless of the location of the populations sampled at varying latitudes. Litzgus & Smith (2010) found that SSD decreased with increasing latitude and was slightly higher inland than in coastal areas, for males and females of *Chrysemys picta*, in North America. Other studies have also evaluated SSD in turtles in terms of geographic variation, such as Lovich *et al*. (1998; 2010), for *Clemmys muhlenbergii* and *Mauremys leprosa*, Zuffi *et al*. (2006) for *Emys orbicularis* and *Emys trinacris* in Italy. For the species *Clemmys guttata*, Rowe *et al*. (2012) studied sexual dimorphism in relation to the size and shape of the carapace and dichromatism in southwest Michigan (USA). Basic knowledge of life history traits are essential and represent the first, necessary, steps for a deeper understanding in order to apply management and conservation practices (Daza & Paéz, 2007).

Hydromedusa tectifera Cope, 1870 is a medium-sized turtle belonging to the family Chelidae with distribution in South--Southern Brazil, Paraguay, Uruguay, Argentina and Bolivia (Fritz & Havas, 2007; van Dijk *et al*., 2014). Knowledge of the ecology and biology of this species is punctual. Clutch size averaged 11.6 eggs per year at the Taim Ecological Station, extreme south of Brazil (Fagundes & Bager, 2007). Population density may reach 218 individuals ha $^{-1}$ and turtle peak of activity is in spring and summer in Argentina (Lescano *et al*., 2008) and Brazil. Its diet is based mainly on leeches, annelids, gastropods, arachnids, insects, and fishes (Lescano *et al*., 2009 , Alcalde *et al*., 2010).

To our knowledge, only Clavijo-Baquet *et al*. (2010) provided evidence about morphological variation among individuals from three different watershed basins across the distribution of *Hydromedusa tectifera*. Adapted to a wide gradient of environmental conditions, species are a good model for testing hypotheses about morphological variation on a broader scale in Brazil. Therefore, we aimed at answering the following questions: 1) Can we observe sexual size dimorphism between males and females within populations of *H. tectifera*? We expected males to be larger than females in accordance with the general pattern of sexual size dimorphism for family Chelidae, mainly in the measurements related to plastron size that are favored during matings. 2) Do different populations of *H. tectifera* display variation in body size and other biometric measurements? We expect individuals from higher latitudes (southern populations) to be larger than individuals of the lower ones in all biometric measures.

MATERIALS AND METHODS

Study area

We sampled turtle populations across two states in Brazil: Minas Gerais and Rio Grande do Sul (hereafter referred to MG and RS), comprising four municipalities, between 1995 and 2011 (Table 1). In MG state sampling occurred in the Lavras municipality, Campo das Vertentes Mesoregion, a transitional area between Cerrado and Atlantic Forest biomes, two Brazilian hotspots of biodiversity (MYERS *et al.*, 2000; MITTERMEIER *et al.*, 2004). The climate is Cwa, with dry winters and rainy summers, and the average annual temperature is 20.4°C (DANTAS *et al.*, 2007). Aquatic environments in this region were composed mainly by swampy areas. In RS state sampling occurred in three close locations of streams and estuarine swamps. The climate in these locations are classified as humid subtropical (hot and wet summers and mild, drier winter) and the average annual temperature varies between 18 to 19°C (MATZENAUER *et al.*, 2011).

Data collection

Turtles were captured in four locations (three in Rio Grande do Sul and one in Minas Gerais state) (Table 1). Each individual was measured for six linear morphometric parameters: carapace length, maximum carapace width, maximum carapace height, maximum plastron length, maximum plastron width and posterior lobe width using a vernier caliper (see BAGER *et al.*, 2016 for morphometric measures details). The sex of adults was established by external examination of secondary sexual characteristics. Males usually have a longer and thicker tail than females and male's plastron is concave. We treated as juveniles those individuals in which sex could not be determined (CL < 145 mm in MG and CL < 119 mm in RS), but were not considered in this study due to low sample size. Capture point of each individual was recorded using GPS, all turtles were marked by notching the marginal scutes (CAGLE, 1939) and then released near the capture spot.

Data analysis

Only measurements taken during the first capture were used in the analyses. Adults were grouped by sex and state of origin: MG males, MG females, RS males and RS females. Morphometric variation within Rio Grande do Sul populations

Table 1: Sampling areas (SA) of *Hydromedusa tectifera* with sampling interval (SI).

State	SA	Latitude (S)	Longitude (W)	SI
Minas Gerais (MG)	Lavras	21°19'45''	44°58'18''	2010/2011
	Arroio Grande	33°14'21''	53°08'12''	2000
Rio Grande do Sul (RS)	ESEC Taim	32°32'51''	52°32'40''	1995/96/98/00/02/06
	Pelotas	31°26'11''	56°22'01''	2001/02/03/06

were computed with an one-way ANOVA to test the hypothesis that morphometrics do not vary with close proximity. Normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene test) assumptions were verified. Descriptive statistics are presented in the text as mean ± standard deviation (range values) of adults for all the variables measured.

In order to detect if sexual dimorphism in biometric measures exists within populations, we applied a one-way analysis of variance (ANOVA) to each population (Zar, 2010). We also fitted linear models in order to test for sexual dimorphism between males and females within each state. Carapace length and sex were used as an independent variable and maximum carapace width, maximum plastron length, maximum plastron width, posterior lobe width and maximum carapace height were used as dependent variables for both states. First, we employed parallelism tests to compare the linear regression slopes for males and females. When this test was non significant, we used covariance analyses (with carapace length as the covariate) to compare the intercepts (Bager *et al*., 2010, 2016).

We computed Linear Discriminant Analysis based on morphometrics of all individuals measured to verify if there was a separation between populations and to identify the variables that most influenced this separation (Zuur *et al.,* 2007). Analyses were done using R (R Development Core Team 2015) and Bioestat (Ayres *et al*., 2007). For all tests, the level of significance was 0.05.

RESULTS

We examined 73 adult turtles, 11 in MG (five males and six females) and 62 in RS (29 males and 33 females). In MG state, males ranged from 181 mm to 244 mm of carapace length and females from 186 mm to 269 mm. In RS state, males ranged from 133 mm to 280 mm of carapace length and females ranged from 137 mm to 298 mm. Morphometric variation based on carapace length was not significant among close locations within Rio Grande do Sul state ($F_{2,49}$ = 1.40, $P = 0.25$) and for this reason, we grouped individuals from RS state to compare to individuals of MG state. For all the morphometric parameters, maximum sizes of males were smaller than those of females within each state (Table 2). Mean size of females from RS state were significantly larger than males regarding the biometric measures: posterior lobe width ($F_{1,60}$ = 12.32, $P = 0.001$) and maximum carapace height ($F_{1,60}$ = 6.44, $P = 0.01$). On the other hand, though in MG state males tended to be on average larger than females, all analyses were non-significant ($P > 0.05$).

Based on linear models, parallelism and covariance tests (Table 3), we also found sexual dimorphism in relative body proportions for some variables within each area. In RS state, females were larger than males in maximum carapace width, maximum plastron length, posterior lobe width and maximum carapace height. This analysis showed that for MG state, males were larger than females only for maximum carapace width. In both states males and females had similar size for all the remaining traits.

Comparing adults among states (Fig.1), the linear discriminant analysis showed that the maximum plastron width (Partial Lambda: 0.77, Can I: 0.81, $p < 0.001$) and posterior lobe width (Partial Lambda: 0.88, Can I: 0.40, $p = 0.04$) were significant at separating groups. The first function explained about 70% of the variation between groups defined *a priori*, and the second function explained 26%. The discriminant function classified groups with a 72% success rate.

DISCUSSION

Our results showed that there is some degree of sexual size dimorphism of *H. tectifera* within states. Maximum carapace height of males was larger than females in MG, but females were larger than males in RS state in most measures. Also, there was a trend of individuals from RS state being larger than MG based on maximum plastron width and posterior lobe width parameters. Despite its wide range of distribution in Brazil, the majority of the studies concerning the basic biology of *H. tectifera* are local and rare. Our study comprises a

Table 2: Descriptive statistics for the morphometric variables measured of *Hydromedusa tectifera* individuals (males and females) from Minas Gerais and Rio Grande do Sul state, Brazil. Linear measurements are in millimeters. Min-Max = minimum and maximum measurements; x̄ = mean; SD = standard deviation; *CL – Carapace length; MCW – Maximum carapace width; MPL – Maximum plastron length; MPW – Maximum plastron width; PLW – Posterior lobe width; MCH – Maximum carapace height.

Variable	Minas Gerais				Rio Grande do Sul			
	Males (N = 5)		Females (N = 6)		Males (N = 29)		Females (N = 33)	
	Min-Max	x̄ ± SD	Min-Max	x̄ ± SD	Min–Max	x̄ ± SD	Min–Max	x̄ ± SD
CL	181.0-244.0	219.6 ± 26.3	186.0-269.0	213.1 ± 33.2	133.0-280.0	208.8 ± 30.2	137.0-298.0	219.8 ± 36.3
MCW	126.0-163.0	148.4 ± 15.8	122.0-176.0	139.1 ± 21.4	107.0-182.0	141.7 ± 18.5	98.0-218.0	151.1 ± 23.9
MPL	153.0-204.0	182.4 ± 21.8	153.0-229.0	178.8 ± 30.4	128.0-257.0	174.2 ± 28.6	110.0-275.0	188.7 ± 33.1
MPW	97.0-140.0	119.0 ± 16.6	91.0-141.0	105.6 ± 19.5	85.0-152.2	118.7 ± 16.0	76.8-176.0	125.6 ± 23.3
PLW	78.9-102.0	92.1 ± 10.7	81.0-121.0	92.1 ± 16.0	67.0-108.0	83.2 ± 9.7	53.5-125.0	96.0 ± 17.0
MCH	56.0-75.0	64.4 ± 8.7	52.0-87.0	63.1 ± 13.0	52.0-82.0	65.8 ± 8.0	43.0-100.0	72.9± 13.0

Table 3: Linear models, covariance, and parallelism for males and females of *Hydromedusa tectifera*. Carapace length is the independent variable for MG and RS states. *MG = Minas Gerais; RS = Rio Grande do Sul; M = male; F = female. F = statistic test and *P* = p value.

Variables	State	Sex	Regression Parameters					Parallelism		Covariance	
			a	b	F	*P*	r^2	F	*P*	F	*P*
MPW	MG	M	23.1	0.43	2.7	0.19	0.30	0.4	0.5	3.2	0.1
		F	-18.0	0.58	66.9	< 0.001	0.97				
	RS	M	14.0	0.50	35.8	< 0.0001	0.84	0.7	0.6	0.5	0.5
		F	3.6	0.56	39.4	< 0.0001	0.82				
MCW	MG	M	16.5	0.60	86.4	< 0.001	0.99	2.0	0.2	35.2	< 0.001
		F	1.7	0.65	8.9	< 0.001	0.99				
	RS	M	20.9	0.58	56.7	< 0.0001	0.86	1.9	0.2	6.2	0.01
		F	12.1	0.63	33.6	< 0.0001	0.91				
PLW	MG	M	-0.3	0.49	14.1	0.03	0.76	0.2	0.7	0.3	0.6
		F	-13.6	0.54	80.47	< 0.01	0.94				
	RS	M	22.1	0.29	117.85	< 0.0001	0.82	0.1	0.9	12.1	< 0.001
		F	28.4	0.30	23.1	< 0.001	0.43				
MPL	MG	M	1.1	1.19	92.5	< 0.001	0.98	1.4	0.3	1.3	0.3
		F	18.8	1.08	64.7	< 0.001	0.99				
	RS	M	-13.6	0.90	79.5	< 0.0001	0.87	0.1	0.8	10.3	< 0.01
		F	-4.3	0.88	67.8	< 0.0001	0.92				
MCH	MG	M	-2.0	0.30	15.6	0.03	0.78	1.9	0.2	0.3	0.6
		F	-17.5	0.37	57.7	< 0.01	0.91				
	RS	M	17.9	0.22	53.3	< 0.0001	0.67	3.0	0.9	0.1	< 0.01
		F	5.1	0.31	96.5	< 0.0001	0.76				

more comprehensive approach from many years of field work that allows us to compare morphological traits among different populations at broad geographical scales.

Other authors also reported similar body sizes for *H. tectifera* through its distribution. For instance, Lescano *et al*. (2008) reported adult male and female carapace length from 100 mm to exceeding 250 mm in a population in Argentina. In the Delta do Jacuí (also RS state), carapace length ranged from 168 mm to 263 mm for females and 170 mm to 224 mm for males (Bujes, 2010), showing that females could attain larger body sizes. The observed sexual size dimorphism, however, with differences in the most important variables separating sexes in both populations has some evolutionary significance. In RS state, females were larger than males considering some well-understood measures of the carapace and plastron sizes. This is not in congruence with the general pattern observed for other Chelidae species and even species of the same genus, for example, *Hydromedusa maximiliani* in Brazil (Novelli & Souza, 2007, Famelli *et al*., 2011). In this species, the variables maximum carapace width, maximum plastron length, posterior lobe width and maximum carapace height were larger in females and these variables might be related

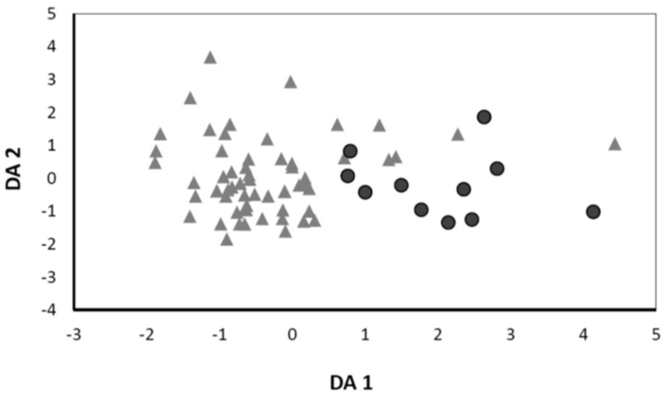

Figure 1: Linear Discriminant Analysis between *Hydromedusa tectifera* individuals from Minas Gerais (black circles) and Rio Grande do Sul (gray triangles) states.

to reproductive output (SOUZA, 2004). Fecundity selection may favor larger females (STEPHENS & WIENS, 2009; CEBALOS *et al.*, 2013) therefore, large females in this species could have increased their reproductive output by producing larger clutches or offspring size. To our knowledge, only FAGUNDES & BAGER (2007) reported information on the reproductive ecology of *H. tectifera* in RS state. Clutch size of this species may reach about 12 eggs on average for a medium-sized turtle. Therefore, a larger body size for females is necessary to carry a larger clutch and/or egg size in this population.

In MG state, in general males were larger than females in most of the biometric measures, but only the maximum carapace width had a significant difference. This fact may be related to the very low sample available for MG. However, carapace width is an important measure of turtle body size and its larger size for males in this population could be due to sexual selection (BERRY & SHINE, 1980). Larger male body size could be favoured when combat between males exist in order to compete for mates with females (STEPHENS & WIENS 2009). Sexual size dimorphism also can arise from factors acting differently on the sexes in the environments that they are restricted (LITZGUS & SMITH, 2010) and natural selection might favour ecological niche divergence (HENDRICK & TEMELES, 1989; SHINE, 1989) resulting in different body sizes (LITZGUS & SMITH, 2010). Males and females, for example, in the genus *Hydromedusa* (SOUZA & ABE, 1998) could partition the resources (food items, microhabitat, space for thermoregulation or foraging) to avoid habitat overlap. How much the sexes feed on different types or quality of resources in different populations can be tested to understand the mechanisms behind the variations in sexual dimorphism in *H. tectifera* across its distribution range.

We also found that individuals from RS state were larger than MG state, mainly females. This is in agreement with a general trend observed for turtles in which body size at lower latitudes and warmer environments tend to be smaller than those of the same species at higher latitudes (ASHTON & FELDMAN, 2003). This is the case for both states in this study. Much has been discussed about the mechanisms by which body size vary among populations of the same species in freshwater turtles (Rowe, 1994; Daza & Páez, 2007; Zuffi *et al*., 2007). This difference among populations could be the result of phenotypic plasticity in response to environmental conditions that are different between locations (Daza & Páez, 2007; Litzgus & Smith, 2010; Zheng *et al*., 2013). For instance, populations in RS state are in areas of marshes, fields, dunes, lakes, streams, and swamps (Gayer *et al*., 1988) ranging from sea level up to 22 m (Matzenauer *et al*., 2011). In MG state the area includes hills and mountains at altitudes around 1000 meters. This might favour a high spatial variability, basically due to relief, which plays a key role in the occurrence of microclimates and conditions (Wrege *et al*., 2009).

The environment may act modifying patterns of individual growth rates that can be dissimilar between populations facing different environments, lastly causing variations in body size. For example, if latitude affects climatic conditions and productivities of water bodies, we could argue that those environments with higher productivity support individuals with larger body sizes (Litzgus & Brooks, 1998; Daza & Paez, 2007). In fact, this could be the case of the RS state populations. All the localities are in an area of high productivity due to the complex environment of lakes connected to the ocean (Niencheski *et al*., 2007). This could lead to individuals that grow faster in a suitable environment (Bury *et al*., 2010) and reach sexual maturity at early ages (and a smaller body size) as upheld by our results. A faster juvenile growth rate also can be explained by an uncertainty on survival at early stages (Congdon *et al*., 1994). If early stages suffer with, for example, high predation pressure, time of exposure during vulnerable stages could be decreased by attaining maturity earlier (Spencer & Janzen, 2010). Once an adult, turtles can continue to grow and attain larger body sizes in these environments, as adult survival probability is commonly high (Congdon *et al*., 1994). To our knowledge, there is no study about nest or hatchling predation of *H. tectifera* in South of Brazil. However, at the same locations we sampled, a sympatric turtle species *Trachemys dorbign*i has an increased predation rate at early stages (Gonçalves *et al*., 2007) suggesting that *H. tectifera* may be also susceptible to this threatning factor.

In summary, this study showed how *H. tectifera* varies in body measurements within at least one of the populations and among them in two different regions in Brazil. The study also highlights the need to understand the ecological process that could help to elucidate body size variation, an important life history trait. Further, studies that are performed with a larger sample size and that take into account growth rate by controlling resource availability could help to explain variation in body traits and elucidate the mechanisms involved in this variation. Research on morphology is encouraged to facilitate comparisons within and among populations in geographically broad areas.

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