REPTILES & AMPHIBIANS

Coracoid Asymmetries in Adult Pondsliders, *Trachemys scripta* (Schoepff 1792), in Spain

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Abstract.—Morphological variation is the result of interplay among multiple intervening factors. Appendicular bone morphology in reptiles has been addressed rarely in the literature and, to our knowledge, the girdles of turtles have never been utilized to address developmental questions of form asymmetry. We quantified left-right symmetry of the coracoids in 42 (10 male and 32 female) Pondsliders, *Trachemys scripta* (Schoepff 1792), an exotic emydid in Spain. We measured form symmetry between the right and left coracoids using geometric morphometric techniques by means of 14 paired landmarks and 66 paired semi-landmarks. Both sexes exhibited fluctuating asymmetry (FA: small and random deviations in the symmetry, but with a variation normally distributed around a zero mean) and directional asymmetry (DA: a greater development on one side, with most asymmetry in the same direction). DA has been described as a product of genotype as well as functional lateralization. The most probable explanation for the detected DA is that it results from a functional imbalance due to limb lateralization, which results from an unequal pull of paired muscles on bones. Low levels of FA, apparently the result of subclinical metabolic osteopathies (presumably attributable to improper diets of most of the animals examined, as they had been kept as pets), are excluded.

Bilaterally symmetric plans, a key feature of the vertebrate body plan, are symmetric under a reflection about the median axis (Stige et al. 2006; Klingenberg 2015). However, these structural and functional symmetries between left and right sides of the body are rarely perfect (Cocilovo et al. 2006; Stige et al. 2006; Hobbs et al. 2018). Asymmetric deviations include fluctuating asymmetry (FA) and directional asymmetry (DA) (Stige et al. 2006; Bravi and Benítez 2013). Within a symmetrical structure, DA occurs when one side develops to a greater extent than the other, with most asymmetry in the same direction (Auffray et al. 1999). FA involves small and random deviations in symmetry, but with a variation normally distributed around a zero mean (Palmer and Strobeck 2001; Tomkins and Kotiaho 2002; Stige et al. 2006). FA is usually thought to originate from random variation in the developmental processes and is therefore considered to be a component of within-individual variation (Klingenberg et al. 2002; Stige et al. 2006). Because it is of non-genetic origin it has been widely used to detect developmental instabilities in individual quality (Auerbach and Ruff 2006; Angelopoulou et al. 2009; Costa et al. 2015), although genetic factors can modulate its expression (Stige et al. 2006; Palmer et al. 2010; Klingenberg 2015).

In turtles, the appendicular skeleton includes the pectoral and pelvic girdles. The pectoral girdle comprises two bones, the scapula, with an acromion process, and the coracoid (alternatively called the procoracoid), all forming a triradiate structure (Nagashima et al. 2013). The scapula is aligned dorsoventrally and attaches to the carapace near the first thoracic vertebra. Ventrolaterally it forms part of the glenoid fossa, effectively the shoulder joint. The acromion process extends medially from each scapula to articulate with the entoplastron via ligaments. The coracoid forms the remainder of the glenoid fossa and extends medially. The majority of the abductor muscles attach to the coracoid processes and the acromial-coracoid ligaments (Barone 1999).

Like most skeletal structures of turtles, the coracoid bones generally are assumed to be bilaterally symmetrical. The left-right symmetry of the coracoid bones corresponds to matching symmetry, which appears in situations where two separate objects exist as mirror images of each other, as opposed to object symmetry, which refers to a single structure that is identical on both sides of a given or selected plane (Rohlf and Bookstein 1990).

Pondsliders, *Trachemys scripta* (Schoepff 1792) (Emydidae), are native to the central and southeastern United States and northern Mexico (Uetz et al. 2024, where they are among the most common turtles (Readel et al. 2008) and where they occupy a wide variety of habitats, including slow-moving rivers, floodplain swamps, marshes, seasonal wet-

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lands, and permanent ponds (Readel et al. 2008; Wyneken et al. 2008). Pondsliders are very popular pets in Spain (Martínez-Silvestre et al. 2015). From 1983 to 1997, 500,000 to 900,000 individuals were imported into Spain (Ministerio de Agricultura Alimentacion y Medio Ambiente 2010), where they have frequently been released into natural habitats.

Geometric morphometrics allows morphological variation to be depicted in a more integrative way than traditional multivariate methods (Claude et al. 2003). As the species has a negative impact on native pond turtle populations and moreover can act as parasite and disease vector, released specimens are captured and legally euthanized (Ministerio de Agricultura Alimentacion y Medio Ambiente 2010). The main object of this study was to use a geometric morphometric approach to quantify for the first time the left-right symmetry of the coracoid in Pondsliders.

Materials and Methods

Specimen collection.—We obtained 42 dead adult Pondsliders (10 males and 32 females) from the Catalonian Reptile and Amphibian Rescue Center (CRARC). Sex was determined visually by size (females are larger), position of the cloaca and thickness and length of the tail (males have enlarged tails), and claws (males have elongated foreclaws) (Martínez-Silvestre et al. 2015; Readel et al. 2008). Corpses used in the study were from adult turtles without detectable abnormalities. Bones were manually obtained after the natural rotting of corpses.

Data collection and geometric morphometric analyses.—Bones were labelled and levelled on a horizontal plane and photographed with a Nikon® D70 digital camera (image resolution 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28–200 mm telephoto lens. The camera was placed parallel to the ground so the focal axis was parallel to the horizontal plane and centered on each specimen. Pictures were digitized using tpsDig version 2.04 software (Rohlf 2015). We identified 14 paired two-dimensional (2D) landmarks (LMs; i.e., discrete and homologous loci in the space characterized by their x- and y-coordinates) and 66 paired semilandmarks (the latter outlining of medial and lateral curves of the bone) (Fig. 1). Semilandmarks were adjusted along the curve of the bone until they were in positions that most closely matched the reference configuration based on minimizing the Procrustes distance with tpsUtil version 1.70 software (Rohlf 2015), creating new aligned coordinates that were analyzed along with LMs (Fig. 2). Landmarks and semilandmarks were digitized twice by the same person on different days to minimize measurement errors. The correlation between the Procrustes and tangent-shape distances was calculated using tpsSmall version 1.33 software (Rohlf 2015).

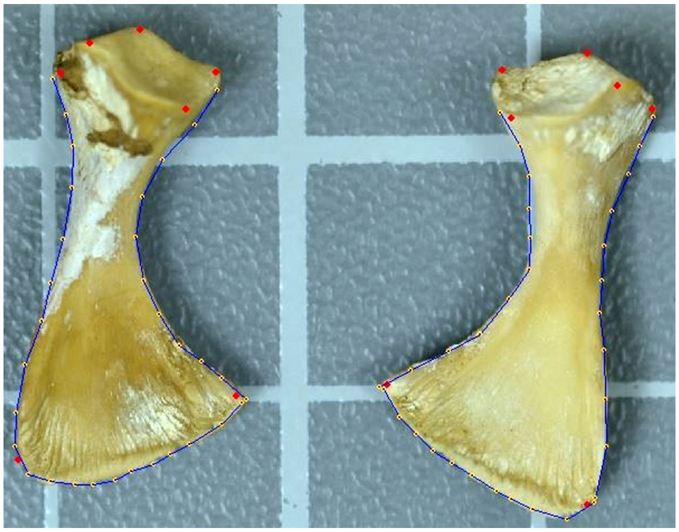

Figure 1. Image illustrating the set of seven landmarks (red symbols) and 33 semi-landmarks (yellow symbols) recorded on each coracoid of Pondsliders (*Trachemys scripta*). Photograph by Pere M. Parés-Casanova.

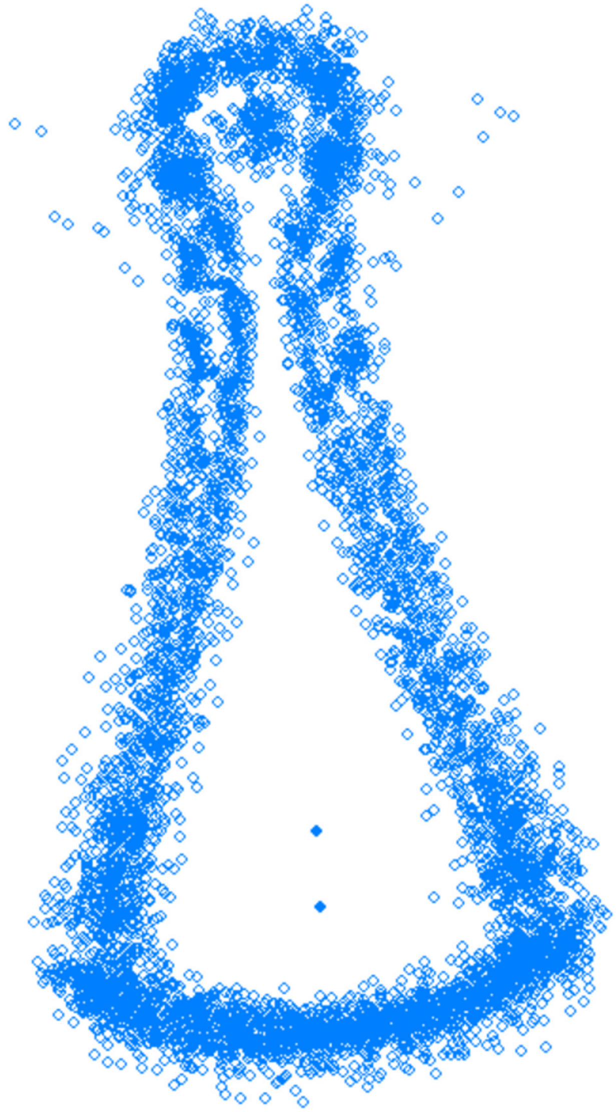

Figure 2. Procrustes superimposition of right (solid symbols) and left (open symbols) coracoids of Pondsliders (*Trachemys scripta*).

Cartesian x-y coordinates were then extracted with a full Procrustes fit in order to remove information about position, orientation, and rotation. The Procrustes procedure allows the treatment of size and shape as two independent components (Rohlf and Bookstein 1990; Bookstein 1991). Size was expressed as values of centroid size (CS: computed as the square root of the summed squared Euclidean distances from each landmark to the specimen centroid) (Bookstein 1991). We then used Procrustes ANOVA to quantify the extent of asymmetric variation (Klingenberg et al. 2002). The coordinates of the superimposed right-left landmarks to assess asymmetry were in relation to individuals (symmetric component of variation), side (DA), their interaction (FA), and measurement error (ME) (Klingenberg and McIntyre 1998). The ANOVA of shape variables tested whether FA was significantly larger than ME. This could be a serious issue because random ME can inflate the extent of variance. Since many statistical analyses are based on the amount of "explained" relative to "residual" variance, inflated variance from ME can result in a loss of statistical power. ME for size was tested with a Wilcoxon *W* paired test with individuals and number of digitizing sessions as factors.

Then we performed a multivariate regression of the Procrustes coordinates as shape variables on the log-transformed CS values to analyze the allometric trends in each sex. Results are reported as a percentage value of the explained total shape variation from the size variation. No allometric effect (10,000 randomization rounds) was evident for sex. Canonical Variate Analysis (CVA) to compare sexes was done using Mahalanobis distance (Md). The Procrustes fit and most of the statistics were executed using MorphoJ software version 1.05 (Klingenberg 2011). For statistical analyses not incorporated into MorphoJ software, we used PAST version 2.17c (Hammer et al. 2001). For all tests, statistical significance was set at 5%.

Results

Variation of the specimens in shape space was perfectly correlated ($r = 0.999998$) with tangent space. This allowed the use of the plane approximation in ulterior statistical analyses and interpretation of results. Wilcoxon reflected similar sizes between replicas ($W = 1828$, $p = 0.849$). ANOVA showed no statistically significant in CS between configuration of right and left coracoids (Table 1). FA (individual × side interaction) exceeded ME more than twofold, so that estimates of FA were not compromised by ME. All other effects were much larger, and the effects of ME were therefore negligible (Table 1). DA (main effect of side) accounted for the bulk of variation (>95% of the total). This left-right asymmetry was larger in males (Md = 52.49; 10,000 randomization rounds) than in females (Md = 47.39) (Fig. 3), but allometry was not significant for males ($p = 0.350$) or females ($p = 0.479$) (Fig. 4).

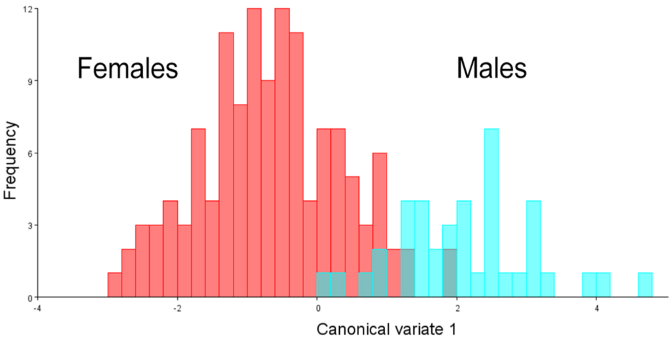

Figure 3. Canonical Variate Analysis for coracoid shape of 42 (10 male and 32 female) Pondsliders (*Trachemys scripta*). The left-right asymmetry was greater in males than in females.

Table 1. Procrustes ANOVA tests performed for shape of the coracoid in 10 male (♂) and 32 female (♀) Pondsliders (*Trachemys scripta*). SS = sum of squares; MS = mean squares; DA = directional asymmetry; FA = fluctuating asymmetry; df = degrees of freedom. FA accounted for significantly more variance than error, exceeding it more than twofold so that the estimates of FA were not compromised. All other effects (DA and Individuals) were much larger, and the effects of measurement error were therefore negligible.

	Effect	SS	MS	df	F	P
♂	Individual	0.15576310	0.00022772	684	1.19	0.013
	Side	0.74030729	0.00974088	76	50.72	< 0.0001
	Individual x side	0.13135762	0.00019204	684	4.38	< 0.0001
	Error	0.06667026	0.00004386	1520		
♀	Individual	0.43006888	0.00018254	2356	1.55	< 0.0001
	Side	2.56736723	0.03378114	76	286.34	< 0.0001
	Individual x side	0.27795175	0.00011797	2356	2.05	< 0.0001
	Error	0.27967711	0.00005749	4864		

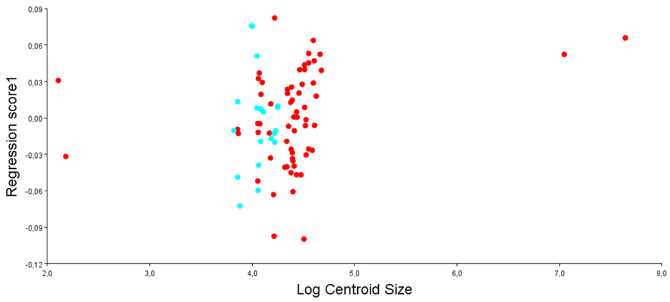

Figure 4. Multivariate regression of the Procrustes coordinates as shape variables on the log-transformed centroid size values to analyze the allometric trends of coracoid shape in 42 (10 male and 32 female) Pondsliders (*Trachemys scripta*). Results were not significant for either males (blue dots) or females (red dots).

Discussion

Our study used 2D geometric morphometric techniques to evaluate asymmetry between the left and right coracoids in adult *T. scripta*. Right coracoids were similar in size to their left counterparts, but we observed a large difference between left and right coracoid shapes, suggesting a presence of matched asymmetry and specifically directional asymmetry.

At this time, biological explanations of this pattern are purely speculative. Laterality results in a preferential asymmetrical use of one side of the body for both sensory input and motor tasks, and it has been shown to exist across a wide range of species (Brown and Magat 2011; Byström et al. 2020). Lateralities have been detected in turtles for social cognition (Sovrano et al. 2018), kinematics (McCall 2014), and righting reflex (Domokos and Varkonyi 2008; Malashichev 2016; Parés-Casanova et al. 2019).

In many species, male-male combat and male-male displays are initiated left side to left side (McCall 2014). In fact, in vertebrates, the right hemisphere appears to be dominant for controlling agonistic behavior and responses to potential threats, so the leftward bias would be stronger in measures of behavior involving aggression and reactivity (e.g., Austin and Rogers 2012). Without fixed or joint connections with other bony structures, compensatory mechanisms for limb lateralization will involve other structures, such as muscles. According to the principle of bone functional adaptation, bone modifies its strength in response to mechanical stress, so a bilateral mechanical asymmetrical loading will leave behind asymmetric skeletal elements. Consequently, unbalanced strength of adjacent muscles would result in an imbalance in the mechanical load of the limbs, and the unilateral preference in muscle load would directly modulate external bone morphology. In other words, lateralized behavior can result in bone asymmetries and, if the magnitude of pectoral girdle shape depends on muscular activity, the lateralized function will affect left-right bone dimensions.

Females had more symmetrical coracoids than males. A possible scenario could involve intense male-male competition for mates, so male girdles would be more asymmetrical than those of females because males engage in increased kinematic activity for fighting. Moreover, turtle girdles in either sex do not become increasingly asymmetrical with age (as a proxy for size), thus reinforcing the functional scenario for explaining DA. However, part of the difficulty in making predictions on this variation stems from the paucity of studies examining growth patterns of bones in turtles.

In addition to a noticeable extent of DA, coracoid bones also exhibit FA. FA has been used as a measure of developmental instability (de Coster et al. 2013), but since the right and left coracoids exhibit low levels of FA, its presence can be ascribed to random environmental factors rather than implicating specific stressors, such as malnutrition, captive conditions, or toxicity. Subclinical metabolic osteopathies (as we can assume improper diets of most animals kept as pets) can also be excluded. Moreover, Lajus et al. (2015) clearly stated that DA inflates FA.

The results of the present study support previous studies on turtle dynamic lateralization. An important future direction would be to study the possible correlation between asymmetries of the girdles and muscular activity by objectively measuring the latter using electromyography, taking also into account any sex-related differences in the asymmetries of girdles.

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In Spain non-native species released in the wild must be captured and legally euthanized, study of corpses for this study required no ethical restriction.

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