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Directional asymmetry in yellow-bellied sliders (*Trachemys scripta scripta*) (Schoepff 1792)

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Abstract. Bilateral symmetry is not uncommon in animal kingdom, and animals can deviate from expected symmetry and manifest some forms of asymmetries. Fluctuating asymmetry is considered a tool for valuating developmental instability, whereas directional asymmetry is inherited. Here we use the method of geometric morphometrics to analyse left/right asymmetries in the plastron of 96 (33 males and 63 females) yellow-bellied sliders (*Trachemys scripta scripta*) with the primary aim to infer and explain morphological asymmetries between sexes in this species. In all individuals analysed we found both fluctuating asymmetry and directional asymmetry for size and shape variation components, and sexual dimorphism for the latter. Fluctuating asymmetry did account a low contribution of the total asymmetric variation, while directional asymmetry assumed the prominent contribution. This may be an adaptive response, which is present in both sexes. We suggest that natural selection for lateralised locomotor efficiency, and not sexual selection (i.e. genetic quality), must be the principal factor for directional asymmetry in this species. The results are consistent with reports from other turtle species using the same technique.

Key words. geometric morphometric, morphological variation, testudines, turtles

Introduction

In geometry, symmetry is defined as invariance under specific transformation (Stige et al., 2006; Klingenberg, 2015). For instance, bilaterally symmetric plans are symmetric under a reflection about the median plane. This symmetry, a key feature of vertebrate body plans, is rarely perfect (Cocilovo et al., 2006; Stige et al., 2006). These deviations can be, among others fluctuating asymmetry (FA) and directional asymmetry (DA) (Stige et al., 2006; Bravi and Benítez, 2013). Within a symmetrical structure, DA happens whenever one character is more developed on ~~in~~ one side of the plane of symmetry than on ~~in~~ the other, while FA is defined as the non-directional deviation from bilateral symmetry (right-left differences) (Palmer and Strobeck, 2001; Tomkins and Kotiaho, 2001; Stige et al., 2006). FA has been widely to detect developmental instability individual quality, although with variable results (Auerbach and Ruff, 2006; Angelopoulou et al., 2009; Costa et al., 2015). FA is usually thought to originate from random variation in the developmental processes, being therefore a component of within-individual variation (Klingenberg et al., 2002; Stige et al., 2006). It is of non-genetic origin, although genetic factors may modulate its expression (Stige et al., 2006; Palmer et al., 2010; Klingenberg, 2015).

Typically, scutes of turtle shells are arranged in longitudinal rows with strict bilateral symmetry and certain quantitative characteristics (Cherepanov, 2014). Plastrons and carapaces have repeated structures at both sides of the median plane, although some asymmetries have been described even under normal conditions by many authors (Davis and Grosse, 2008; Rivera and Claude, 2008; Băncilă et al., 2012; Buică and Cogălniceanu, 2013; Cherepanov, 2014; McCall, 2014; Malashichev, 2016). Some of these publications demonstrate that asymmetry can be maintained by natural selection for functional reasons, although asymmetry could reduce biomechanical performance. Moreover, among turtles, males of some species have evolved exaggerated morphological traits to fight each other to gain mates, while females tend to have bigger

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sizes (Berry and Shine, 1980). If there are different sizes between genders, would be different degrees of asymmetries between genders?

Emydidae is a family of turtles with 12 genera and approximately 43 currently recognised species and includes many common North American species such as the red-eared slider (*Trachemys scripta*) (Stephens and Wiens, 2009). Adult females of some emydid species are up to 158% longer in carapace length than males (Stephens and Wiens, 2009). The plastron contains, as a general pattern (as species variation can appear), of twelve left and right scutes (gular, humeral, pectoral, abdominal, femoral and anal) and one central (intergular) scute. Sliders are ideal candidates for bilateral asymmetry assessment due to the bilateral characteristics of the plastron. The bilateral characteristics and relative flat surface of the plastron allows for easy digital measurements from photographs. Moreover, because they possess a very dorsoventrally flattened body shape and live both in water and on land, they provide an important model for evaluating the effects of morphological design on both hydrodynamic and terrestrial performance in vertebrates. Finally, sliders, being very spread as captive animals, are easy to study in big samples.

The yellow-bellied slider (*Trachemys scrita scripta*) belongs to the family of true pond turtles (Emydidae; subfamily Emydinae) (Readel, Warner, Holberton, and Phillips, 2008) (Martínez-Silvestre, Hidalgo-Vila, Pérez-Santigosa, and Díaz-Paniagua, 2015). It is native to the southeastern United States and is the most common turtle species in its range (Readel et al., 2008). It occupies in a wide variety of habitats, including slow-moving rivers, floodplain swamps, marshes, seasonal wetlands, and permanent ponds. *T. scripta* is popular as pet (Martínez-Silvestre et al., 2015). From 1983 to 1997 500,000 to 900,000 specimens were introduced to Spain where many escaped or were released into the wild; the species seems to have established stable populations in various parts of Spain (Ministerio de Agricultura Alimentacion y Medio Ambiente, 2010). Moreover, as the species has negative impacts on native pond turtle population fitness and can act as parasite and disease vectors, non-captive specimens are captured and legally euthanised (Ministerio de Agricultura Alimentacion y Medio Ambiente, 2010). The species is currently included in the European law of invasive species so some control measures have been adopted, among which ~~is~~ includes the capture and sacrifice of animals. These animals are then available for scientific study.

We analyse symmetric and asymmetric components of left-right asymmetries in the plastron of the yellow-bellied slider turtle (*Trachemys scripta scripta*) by means of geometric morphometric techniques, with the aim to detect FA and/or DA to assess and describe differences between sexes, and to determine if asymmetry is correlated with size. Such a relationship (in the correct direction) could provide support for our hypothesis that locomotor performance can impose strong selective forces acting to constrain asymmetry. This study is part of a larger project to study asymmetries in land and water turtles.

Materials and methods

Specimen collection.—A sample of 33 males (92.8-193.6 mm) and 63 females (95.2-256.5 mm) *T. s. scripta* were obtained from the CRARC, which had been collected as pets. Clear cases of plastron pathologies, such as fractures or deformities, which caused gross bony deformations *intra vitam*, were not detected. Sex was determined visually by: 1) general size (females are larger), 2) position of cloaca and thickness and length of tail (males have enlarged tails), 3) claws (males have elongated foreclaws) (Readel et al., 2008) (Martínez-Silvestre et al., 2015). All specimens used in the study were adults without any detectable abnormalities (such as injuries or unusual additional scutes or plates). The authors did not consider necessary apply the ethical official agreement because this study was done on animals euthanised according to the law and so not due to our research sampling.

Data collection and geometric morphometric analyses.—Turtles were labelled and levelled on a horizontal plane, and then photographed in their ventral view. Image capture was performed with a Nikon® D70 digital camera (image resolution of 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 plane so the focal axis of the camera was parallel to the horizontal plane of reference and centred on the plastron. A scale was included in the images to standardise each specimen's size (mm). Pictures were posteriorly digitised using tpsDig version 2.04 (Rohlf, 2015). In total, 17 two-dimensional (2D) landmarks (LMs, homologous anatomical points) were used on the ventral side of the plastron (Figure 1). Ten were bilateral and seven were midline landmarks. These landmarks correspond to the intersections between bony plates or epidermal scutes, and thus are type 1 or 2 according to Bookstein's nomenclature (Bookstein, 1991). Landmarks were digitised twice by the same

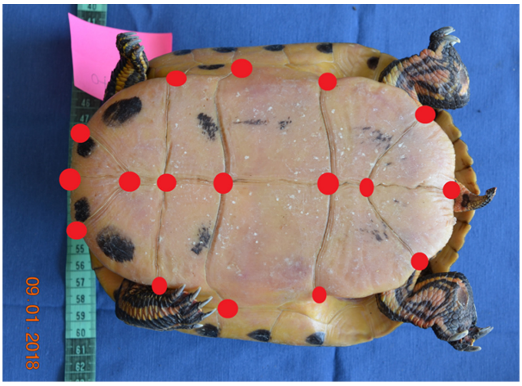

Figure 1. Landmarks digitised on the ventral side of the plastron. In total, 17 two-dimensional (2D) landmarks were used on the ventral side of plastron. Ten of them were bilateral and seven were midline landmarks.

person (JA) on two different days in the same order, to assess measurement error.

Cartesian *x-y* coordinates were then extracted with a full Procrustes fit, a procedure that removes information about position, orientation and rotation and standardise each specimen to unit centroid size (CS -computed as the square root of the summed squared Euclidean distances from each landmark to the specimen centroid, and providing the estimation of the size of the structure under study-) (Bookstein, 1991). Due to the symmetry of the structure, reflection was removed including the original and the mirror images of all configurations in the analysis and superimposing all of them simultaneously; all information on the asymmetry of the studied structures were used to observe eventual phenomena of FA and DA (Klingenberg et al., 2002). We used Procrustes ANOVA (analysis of variance), as assessed for study on symmetry, to quantify the amount of symmetric variation and asymmetry; results are reported as sum of squares (SS) and means squares (MS) that are dimensionless (Klingenberg et al., 2002). Using a Procrustes ANOVA, we partitioned variance in size or shape among individuals (i.e., averaged replicas representing the 'true' sample variance) and residual component (i.e., variation among replicas). Procrustes SS were computed from both the symmetric and asymmetry components. Additionally, to compare the individual-reflection interactions to measurement error (the latter estimated from the total variation of the entire landmark configuration) (Bravi and Benítez, 2013), it was performed a MANOVA test for both symmetric and asymmetry components. Finally, a regression of Procrustes coordinates on CS (log-transformed) was done for each sex separately to study allometry.

Shape variation was evaluated by means of a PCA (Principal Component Analysis), which was performed from the covariance matrix of symmetric and asymmetry components of shape variation. Symmetric component is the average of left and right sides and represents the shape variation component, whereas the asymmetry component represents the individual left-right differences. Differences between sexes were then evaluated with a CVA (Canonical Variate Analysis). All analyses were done using MorphoJ version 1.05 (Rohlf, 2015).

Results

The ANOVA tests whether individual variation is significantly larger than error. Measurement error resulted bigger than FA (MS value for FA, compared to MS value for individuals, Table 1). This is potentially a very serious issue because random measurement error can inflate the amount of variance and, since many statistical analyses are based on the amount of "explained" relative to "residual" variance, can result in loss of statistical power. On the other hand, systematic bias can affect statistical analyses by biasing the results, i.e. variation due to bias is incorporated in the analysis and treated as biologically meaningful variation.

Procrustes ANOVA indicated that variation between individuals was significant for size (p<.0001), as well

Table 1. Procrustes ANOVA test performed for both centroid size and shape; DA = directional asymmetry; FA = fluctuating asymmetry; df = degrees of freedom. Individual differences accounted for significantly more variance than error (MS value for FA, compared to MS value for individuals).

Centroid Size					
Effect	SS	MS	df	F	P
Individual	52633906	554041.116	95	4.55	<.0001
Error	11677939.5	121645.204	96		

Shape					
Effect	SS	MS	df	F	P
Individual	0.19193060	0.00013469	1425	10.43	<.0001
DA	0.00710281	0.00047352	15	36.66	<.0001
FA	0.01840375	1.291E-05	1425	0.28	1
Error	0.13132726	4.56E-05	2880		

as for shape (p<.0001), the latter result confirmed by a MANOVA test for symmetric component of variation (Pillai trace=9.32, p<.0001). Additionally, both DA and FA emerged as highly significant and were confirmed by a MANOVA test (for DA Pillai trace=0.87, p<.0001; for FA Pillai trace=8.56, p<.0001).

We then used PCA to assess and describe this pattern of individual variation and asymmetry. PCA for the symmetric component of variation (DA) showed that the first three PCs explained 52.3% of the total shape variation, with the all other PC which account for no more than 9.7% of variation (see Figure 2 and Table 2 for details); LMs located on pectoral, abdominal and femoral scutes contributed mostly to the whole shape variation. On the reverse PCA for the asymmetric component of shape variation (FA) showed that the first PC contributed alone for 65.6% of the total variation and each other PCs contributed no more than 9.7% to the total shape variation (Table 2).

Morphological variability between sexes was assessed and displayed with CVA, which showed significant differences in the symmetric component (p=0.0004) but not in the asymmetric component (p=0.547). Regression of shape of size for the symmetric component was significant for males (p=0.0007, 5.606 % of shape variation explained by size variation) and for females (p<.0001, 5.424 % of shape variation explained by size variation).

Discussion

In this study, authors have applied the general method for geometric morphometric analysis of symmetric shape variation and asymmetry in *T. s. scripta*). The method used allowed the decomposition of the total shape variation into components of symmetric variation (i.e. differences among sexes) from components of asymmetry (multiple components might occur according to the symmetry of the object). The use of landmarks to study bilaterally symmetric animals has been questioned by many authors (Bravi and Benítez, 2013), since they increase the degrees of freedom without adding much additional information (Dryden and Mardia, 1998). Here we used the entire body (the entire plastron), which considers the variation in form holistically. This approach allows a more realistic view of the variation in shape in a deformation network.

In spite of considerable anthropic intervention on the species, FA did account for a low contribution to the total asymmetric variation. The prominent role of phenotypic asymmetry was on DA. This observation

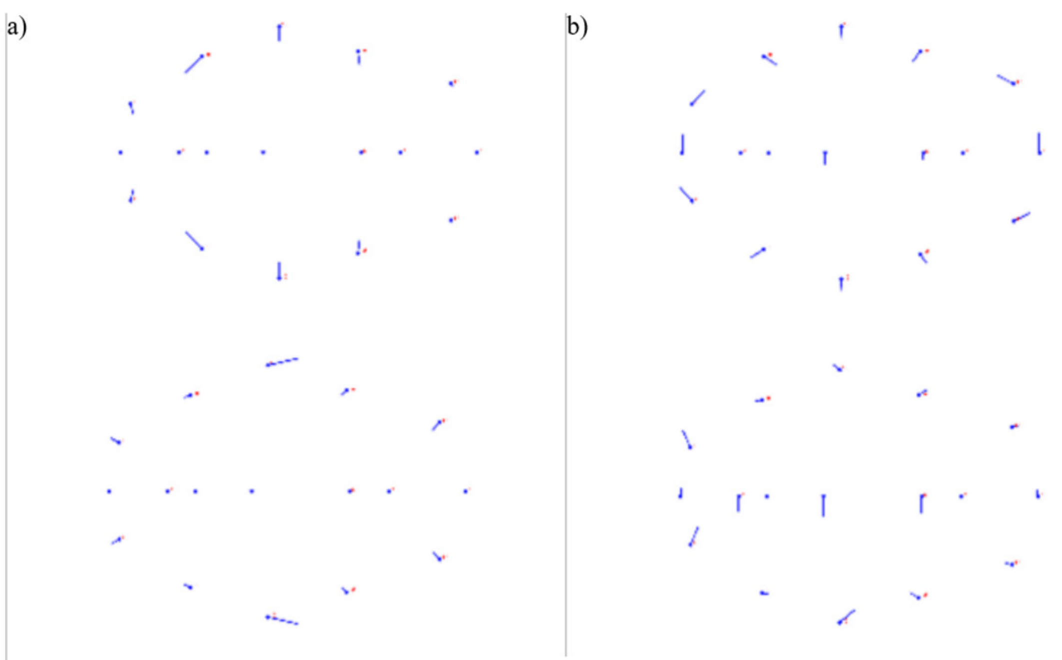

Figure 2. PCA shape deformations: a) for symmetric and b) for asymmetry components of variation.

Table 2. Principal component analysis of shape variation, for both symmetric and asymmetry components for 15 Principal Components (PC). Values reported are the eigenvalues and percentage which each PC (Principal Component) accounts for.

	Symmetric	Component		Asymmetric	Component	
PC	Eigenvalue	Variance	Cumulative %	Eigenvalue	Variance	Cumulative %
1.	0.0003465	21.339	21.339	0.00005028	30.479	30.479
2.	0.00027256	16.785	38.124	0.00003648	22.112	52.591
3.	0.00023140	14.250	52.374	0.00002157	13.075	65.665
4.	0.00015732	9.688	62.062	0.00001592	9.649	75.314
5.	0.00012594	7.756	69.818	0.00000834	5.058	80.372
6.	0.00009311	5.734	75.552	0.00000723	4.383	84.755
7.	0.00008671	5.340	80.892	0.00000557	3.375	88.130
8.	0.00007950	4.896	85.788	0.00000410	2.486	90.616
9.	0.00005423	3.339	89.127	0.00000319	1.931	92.547
10.	0.00005160	3.178	92.305	0.00000269	1.633	94.180
11.	0.00004282	2.637	94.942	0.00000246	1.489	95.669
12.	0.00003283	2.022	96.964	0.00000214	1.300	96.968
13.	0.00002363	1.455	98.419	0.00000194	1.173	98.141
14.	0.00001601	0.986	99.405	0.00000174	1.055	99.196
15.	0.00000966	0.595	100	0.00000133	0.804	100

could be assumed to be an adaptive response, which is present in both sexes. Over half of the symmetric shape component variation ~~of~~ (DA) is associated with the first three PCs, with middle variation between them indicating that there is no external pressure determining shape change (medium sexual dimorphism or allometry) (Bravi and Benítez, 2013). On the whole DA displayed a clear shape variation pattern, indicating that a side was consistently different from the other side. DA has been studied in several organisms including multiple groups of reptile (McCall, 2014).

Evidence of asynchronous locomotion has been reported in many animals (McGreevy, Landrieu, and Malou, 2007) (Malashichev, 2016). Many species present a preferential use of thoracic and/or pelvic appendages for locomotion, so a shape asymmetry could be explained by this preferential use. This finding would be consistent with the fact than bigger animals would be more “side-preferent” because of their higher need to be “faster”. In fact, lateralities have been demonstrated in many other turtle species, aquatic and terrestrial (Băncilă et al., 2012), (McCall, 2014) (Malashichev, 2016) (Young, Vest, Rivera, Espinoza, and Blob, 2017).

To conclude, from our study emerged the presence of both DA and FA in yellow-bellied slider turtle plastrons, but only the DA values appeared statistically important. These results on the whole indicate the presence of some functional asymmetries in the shell structure, apparently not sufficient to infer developmental stability, but instead they should act to counteract stressors and maximise fitness for, in this case, a lateralised locomotor function.

Plastron asymmetries must be explored further through future studies, as well as kinematic models in living individuals. As studied tortoises were kept as pets raised in captivity, with limited space for locomotion, perhaps results would be different among wild animals. Anyway, from our results emerge other promising areas of research in testudines.

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References

Angelopoulou, M.V., Vlachou, V., Halazonetis, D.J. (2009): Fluctuating molar asymmetry in relation to environmental radioactivity. Archives of Oral Biology **54**(7): 666–670. https://doi.org/10.1016/j.archoralbio.2009.04.008

Auerbach, B.M., Ruff, C.B. (2006): Limb bone bilateral asymmetry: Variability and commonality among modern humans. Journal of Human Evolution **50**(2): 203–218. https://doi.org/10.1016/j.jhevol.2005.09.004

Băncilă, R.I., Plăiaşu, R., Tudor, M., Samoilă, C., Cogălniceanu, D. (2012): Fluctuating Asymmetry in the Eurasian Spur-Thighed Tortoise, *Testudo graeca ibera* Linneaus, 1758 (Testudines: Testudinidae). Chelonian Conservation and Biology **11**(2): 234–239.

Berry, J.F., Shine, R. (1980): Sexual size dimorphism and sexual selection in turtles (order Testudines). Oecologia **44**(2): 185–191. https://doi.org/10.1007/BF00572678

Bookstein, F.L. (1991): *Morphometric Tools for Landmark Data: Geometry and Biology*. (Cambridge University Press, Ed.), *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge. https://doi.org/10.1002/sim.4780120711

Bravi, R., Benítez, H.A. (2013): Left-right asymmetries and shape analysis on *Ceroglossus chilensis* (Coleoptera: Carabidae). Acta Oecologica **52**: 57–62. https://doi.org/10.1016/j.actao.2013.07.007

Buică, G., Cogălniceanu, D. (2013): Using digital images in the study of fluctuating asymmetry in the spur-thighed tortoise *Testudo graeca*. Turkish Journal of Zoology **37**(6): 723–729. https://doi.org/10.3906/zoo-1302-19

Cherepanov, G.O. (2014): Patterns of scute development in turtle shell: Symmetry and asymmetry. Paleontological Journal **48**(12), 1275–1283. https://doi.org/10.1134/S0031030114120028

Cocilovo, J.A., Varela, H.H., Quevedo, S. (2006): La asimetría bilateral y la inestabilidad del desarrollo. Revista Argentina de Antropologia Biologica **8**(1): 121–144.

Costa, M., Mateus, R.P., Moura, M.O. (2015): Constant fluctuating asymmetry but not directional asymmetry along the geographic distribution of *Drosophila antonietae* (Diptera, Drosophilidae). Revista Brasileira de Entomologia **59**(4): 337–342. https://doi.org/10.1016/j.rbe.2015.09.004

Davis, A.K., Grosse, A.M. (2008): Measuring Fluctuating Asymmetry in Plastron Scutes of Yellow-Bellied Sliders: The Importance of Gender, Size and Body Location. The American Midland Naturalist **159**(2): 340–348. https://doi.org/10.2307/20491338

Dryden, I.L., Mardia, K.V. (1998): *Statistical Shape Analysis*. Chichester: Wiley.

Klingenberg, C.P. (2015): Analyzing Fluctuating Asymmetry with Geometric Morphometrics: Concepts, Methods, and Applications. Symmetry **7**: 843–934. https://doi.org/10.3390/sym7020843

Klingenberg, C.P., Barluenga, M., Meyer, A. (2002): Shape analysis of symmetric structures: Quantifying variation among individuals and asymmetry. Evolution **56**(10): 1909–1920. https://doi.org/10.1111/j.0014-3820.2002.tb00117.x

Malashichev, Y. (2016): Asymmetry of righting reflexes in sea turtles and its behavioral correlates. Physiology and Behavior **157**(157): 1–8. https://doi.org/10.1016/j.physbeh.2016.01.001

Martínez-Silvestre, A., Hidalgo-Vila, J., Pérez-Santigosa, N., Díaz-Paniagua, C. (2015): Galápago de Florida – *Trachemys scripta* (Schoepff, 1792). In A. Salvador, A., Marco (Ed.), *Enciclopedia Virtual de los Vertebrados Españoles* (pp. 1–41). Madrid: Museo Nacional de Ciencias Naturales. Retrieved from http://www.vertebradosibericos.org/

McCall, W.C. (2014): *Evaluating bilateral asymmetry of the adult Western Pond Turtle (Emys marmorata)*. California State University, Chico.

McGreevy, P.D., Landrieu, J.-P., Malou, P.F.J. (2007): A note on motor laterality in plains zebras (*Equus burchellii*) and impalas (*Aepyceros melampus*). Laterality **12**(5): 449–457. https://doi.org/10.1080/13576500701275881

Ministerio de Agricultura Alimentacion y Medio Ambiente. (2010): *Catálogo de especies exóticas invasoras*.

Palmer, A.R., Strobeck, C. (2001): Fluctuating Asymmetry Analyses Revisited. In Oxford University (Ed.), *Developmental Instability (DI): Causes and Consequences*. Oxford: Michal Polak, Publisher.

Palmer, M., Linde, M., Morales-Nin, B. (2010): Disentangling fluctuating asymmetry from otolith shape. Marine Ecology Progress Series **399**: 261–272. https://doi.org/10.3354/meps08347

Readel, A.M., Warner, J.K., Holberton, R.L., Phillips, C.A. (2008): Maturational changes in male slider turtles (*Trachemys scripta*) from Illinois. Herpetological Conservation and Biology **3**(2): 170–175. https://doi.org/10.1017/S0898030614000141

Rivera, G., Claude, J. (2008): Environmental media and shape asymmetry: a case study on turtle shells. Biological Journal of the Linnean Society **94**: 483–489.

Rohlf, F.J. (2015): The tps series of software. Hystrix **26**(1): 9–12. https://doi.org/doi:http://dx.doi.org/10.4404/hystrix-26.1-11264

Stephens, P.R., Wiens, J.J. (2009): Evolution of sexual size dimorphisms in Emydid turtles: ecological dimorphism, Rensch's rule, and sympatric divergence. Evolution **63**(910–925). https://doi.org/10.1111/j.1558-5646.2008.00597.x

Stige, L.C., David, B., Alibert, P. (2006): On hidden heterogeneity in directional asymmetry - Can systematic bias be avoided? Journal of Evolutionary Biology **19**(2): 492–499. https://doi.org/10.1111/j.1420-9101.2005.01011.x

Tomkins, J.L., Kotiaho, J.S. (2001): Fluctuating Asymmetry. In *Encyclopedia of Life Sciences* (pp. 1–5). https://doi.org/10.1055/s-0031-1275301

Young, V.K.H., Vest, K.G., Rivera, A.R.V., Espinoza, N.R., Blob, R.W. (2017): One foot out the door: Limb function during swimming in terrestrial versus aquatic turtles. Biology Letters **13**(1). https://doi.org/10.1098/rsbl.2016.0732

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