FULL ARTICLE

ISSN Online 1678-4456

Skull asymmetries in wild boar (*Sus scrofa* LINNAEUS, 1758)

Assimetrias do crânio de javalis (Sus scrofa *LINNAEUS, 1758*)

Pere M. Parés-Casanova[1]

[1] University of Lleida, Departament of Animal Science, Lleida – Catalonia, Spain

ABSTRACT

Organisms can develop different kinds of asymmetry when deviations from expected perfect symmetry occur. Among others are fluctuating asymmetry (FA) and directional asymmetry (DA). FA represents small random differences between corresponding parts on the left and right sides of an individual in bilaterally paired structures. It is thought that FA reflects an organism's ability to cope with genetic and environmental stress during growth. DA occurs whenever one side on the plane of symmetry develops more than the other side, and has a genetic component. In this research, we examined the expression of morphological symmetry in 38 skulls of different age groups of wild boar (*Sus scrofa*), on their ventral aspect, using two-dimensional coordinates of 27 landmarks. Analyses showed the presence of significant FA and DA in the entire sample, detecting also distinctive differences between age groups. The obtained results show that the shape differences in different age groups could reasonably be a consequence of a response to environmental factors for FA and a masticatory lateralization for DA.

Keywords: Environmental stress. Geometric morphometric techniques. Mastication. Lateralization. Ontogeny. Symmetry.

RESUMO

Os organismos podem desenvolver diferentes tipos de assimetria quando ocorrem desvios da pefeita simetria esperada. Entre os diversos tipos de assimetria existentes duas merecem especial destaque: a flutuante (AF) e a directional (AD). A AF é representada por pequenas diferenças casuais entre as partes correspondents das laterais direita e esquerda de um indivíduo em estruturas pareadas bilateralmente; acredita-se que elas reflitam a habilidade de um organismo adaptar-se a fatores estressantes genéticos e ambientais observados durante o seu crescimentos. A AD ocorre quando um lado da assimetria plana desenvolve-se mais do que o outro e há um componente genético. No presente trabalho foi analisada a expresssão da simetria morfológica de 38 crânios de diferentes grupos etários de javalis (*Sus scrofa*), nos seus aspectos ventrais, com o emprego de duas coordenadas dimensionais de 27 pontos anatômicos homólogos. As análises efetuadas revelaram a existência de valores significantes de AF e AD em toda a amostra trabalhada, detectando inclusive diferenças entre os grupos etários. Os resultados obtidos demonstraram que as diferentes formas observadas nos diferentes grupos etários podem ser consequência de uma resposta a fatores ambientais para a AF e a lateralização da mastigação para a AD.

Palavras chave: Stress ambiental. Técnicas geométricas e morfométricas. Mastigação. Lateralização. Ontogenia. Simetrial.

Correspondence to:
Parés-Casanova, P.M
University of Lleida, Departament of Animal Science
Av. Alcalde Rovira Roure, 191, 25198 Lleida – Catalonia, Spain
e-mail: peremiquelp@ca.udl.cat

Submited: September 29, 2018
Approved: February 02, 2019

How to cite: Parés-Casanova PM. Skull asymmetries in wild boar (*Sus scrofa* LINNAEUS, 1758). Braz J Vet Res Anim Sci. 2019;56(1):e150704. https://doi.org/10.11606/issn.1678-4456.bjvras.2019.150704.

Introduction

Bilateral symmetry, a key feature of vertebrate body plans, is rarely perfect, and mild asymmetry can be found in normal growth and development as a typical adaptation of organisms to their environments. Live organisms can develop different kinds of asymmetry, among others, including fluctuating asymmetry (FA) and directional asymmetry (DA). FA represents small random differences between corresponding parts on the left and right sides of an individual, and because of its characteristics, it is usually considered to be a measure of developmental noise (Graham et al., 2010; Palmer, 1994; Palmer & Strobeck, 2001). DA occurs whenever one side of the plane of symmetry develops more than the other side, and has a genetic component (Valen, 1962) (Palmer & Strobeck, 2001). FA is not normally an adaptive trait, where symmetry is ideal. DA can be distinguished by showing significantly biased measurements towards traits being larger on either the left or right sides.

Several investigations devoted to the study of skull asymmetry have shown the potential use of these analyses in the detection of environmental stress. In the present study, left-right ventral asymmetry in wild boar (*Sus scrofa*) skulls were analyzed using geometric morphometric techniques, with the aim of detecting and quantifying asymmetry and assessing possible differences based on age.

Materials and Methods

Specimen collection

Thirty-eight skulls of wild boar were obtained from a vulture feeding point located in Catalonia (NE Spain). Skulls belonged to hunted animals of different ages. Individuals corresponded to different age groups, according to molar teeth eruption: only M^1 fully erupted (n = 2); M^2 fully erupted (n = 18); and M^3 fully erupted (complete molar series, n = 18). Their sex was unknown.

Data collection and geometric morphometric analyses

Skulls were labelled and levelled dorsally on a horizontal plane, and then the ventral view was photographed. Image capture was performed with a Nikon® D70 digital camera (image resolution of 2240 × 1488 pixels) equipped with a Nikon AF Nikkor® 28-200 mm telephoto lens. The camera was placed on a tripod parallel to the ground plane so that the focal axis of the camera was parallel to the horizontal plane of reference and centered on the ventral aspect of each skull. A scale was included in the images to standardize each specimen size (in mm). Skulls were digitised using tpsDig version 2.16 (Rohlf, 2010). In total, 27 two-dimensional (2D) landmarks (LMs, homologous anatomical points) were used on the ventral aspect of the cranium (Figure 1). Twenty-three were bilateral and four (1, 2, 3, and 27) were midline LMs. All of these LMs are thought to encompass elements of the whole skull (both viscerocranium as splanchnocranium). As differences in FA among samples are normally very small, DA and measurement error can make up a sizable portion of the between-side and within-individual variance (Palmer & Strobeck, 2001). LMs were digitized twice (using the same image) by the same person on two different days, in the same order.

In order to compare procrustes to tangent space distances between individuals, the Generalized procrustes analysis superimposition (equivalent to generalized least squares) procedure of Rohlf (2010) was performed on each data set

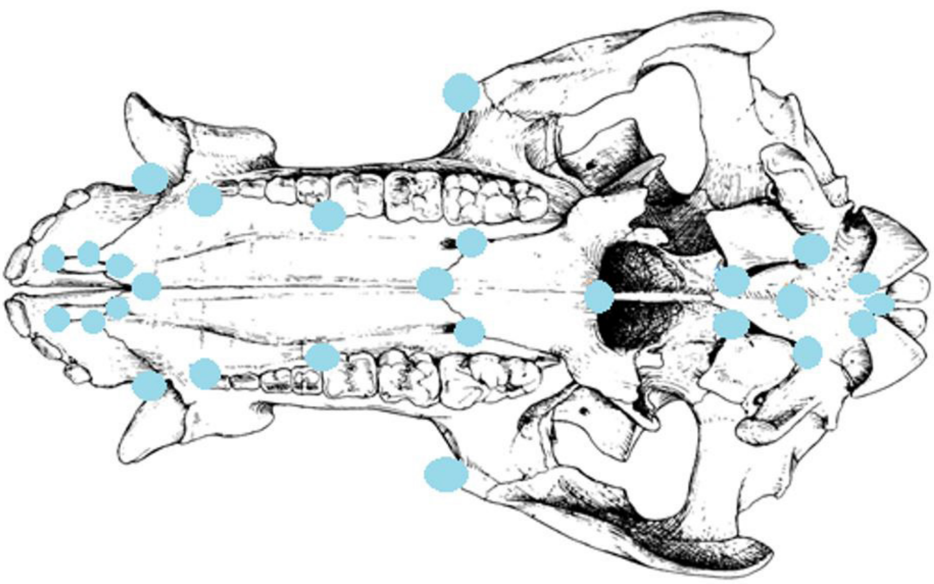

Figure 1 – Used landmarks for geometric morphometric analysis of the wild boar skull (ventral aspect). Four landmarks were on the mid-sagittal plane. The rest were bilaterally equivalent. Source: author.

using TpsSmall version 1.20 (Rohlf, 2015). The approximation of shape space by tangent space presented a high correlation (1.000). This high degree of approximation of shapes in the sample (i.e., shape space) by the reference shape (i.e., tangent space) allowed accurate capture of the nature and extent of shape deformations in subsequent statistical analyses.

Shape asymmetry

Cartesian *x-y* coordinates were then extracted with a full procrustes fit (Dryden & Mardia, 1998), a procedure that removes information about position, orientation, and rotation and standardizes each specimen to unit centroid size (CS). CS is a measure computed as the square root of the summed squared Euclidean distances from each LM to the specimen centroid, and provides an estimation of the size of the structure. Due to the symmetry of the structure, reflection was removed, including the original and mirror images of all configurations in the analysis, which were superimposed simultaneously (Klingenberg, 2015), and all information on the asymmetry of the studied structure was used to observe the eventual phenomena of FA and DA. To analyze shape asymmetry, we used a two-way, mixed-model analysis of variance (ANOVA) with repeated measures, as it is usually used in studies on symmetry (Klingenberg et al., 1998). Shape asymmetry can be viewed as the distance of the actual shape from its closest symmetrical variant. FA and DA, as assessed using LM-based methodology, are different from traditional methods of asymmetry analysis (Palmer & Strobeck, 2001), but measure the same biological phenomena as in traditional morphometrics (Palmer & Strobeck, 2001). However, it was not expected absolute numbers with respect to particular LMs, as asymmetry is a measure of the distance between complete LM configurations (procrustes distance). The results are reported as the sums of squares and mean squares, which are dimensionless. In these analyses, the main factors were individuals and sides. The side × individuals interaction assessing the extent of FA was evaluated by the F statistic, using the remainder mean square over the mean square due to measurement error. Additionally, to avoid the assumption of having isotropic (equal and independent) variation in all LMs, a multivariate analysis of variance (MANOVA) was performed for both symmetric and asymmetric components.

The shape variation in the whole dataset was assessed by performing a principal component analysis (PCA), taking into account both symmetric and asymmetric components of variation; the symmetric component is the average of the left and right sides and represents the shape variation component, whereas the asymmetry component represents the individual left-right differences. Differences between the three age groups were assessed with a canonical variate analysis (CVA), a multivariate statistical test that allows finding of shape characteristics that best distinguish between several groups of individuals. The results are reported as the Mahalanobis distances, a multivariate measure of distance relative to the within-sample variation.

Size asymmetry

Additionally, CS (Bookstein, 1991) was used as a basic characteristic describing size differences between age groups. Differences in CS between age groups were analysed with the Kruskal-Wallis test.

All analyses were performed using MorphoJ version 1.05 (Klingenberg, 2011) and PAST version 3.01 (Hammer et al., 2001). Statistical treatments were computed with 10,000 permutation runs.

Results

The results of the measurement error assessment are presented in Table 1 and show that the variance as a result of repeated measurement was smaller in comparison with the FA variance, thus indicating that precision was unlikely to constrain the results of subsequent statistical analyses in the present study. Size, which was different between age groups (H_c=6.818, p=0.0090), was progressively larger with increasing age (Figure 2), but shape differences were only present between the

Table 1 – Analysis of variance (ANOVA) of skull size and shape for *Sus scrofa* (n = 38), on the basis of the position of all 27 landmarks

		SS	MS	Df	*F*	*p* (param.)
Shape	Individual[a]	0.03447429	0.0000510730	675	12.75	< 0.001***
	Side[b]	0.00059789	0.0000239155	25	5.97	< 0.001***
	Ind x Side[c]	0.00270334	0.0000040050	675	1.21	0.002**
	Error	0.00464736	0.0000033195	1400		
Size	Individual	2487718.211383	92137.711533	27	4.26	< 0.001***
	Error	605705.936808	21632.354886	28		

SS: Sum of Squares; MS: Mean of Squares Df: Degrees of freedom. [a] Individual effect represents the variation between individuals in the symmetric component of shape. [b] Side, systematic difference between the original and mirrored copy of each individual, Directional Asymmetry. [c] Ind x Side quantifies Fluctuating Asymmetry. Error, the residual variation due to measurement error. Levels of significance: ** for $p \leq 0.01$, *** for $p \leq 0.001$

M^3 group and the others ($p<0.05$). ANOVA indicated that the variation between individuals was significant based on size (Table 1) as well as shape. Additionally, both DA and FA emerged as highly significant and the significance was confirmed by MANOVA. For the symmetric component, Pillai trace = 19.31, $p < 0.0001$.

PCA for the symmetric component of variation showed that the first three PCs explained 68.0% of the total shape variation, with all of the other PCs accounting for no more than 9% of the variation (Table 2); all LMs contributed quite equally to the whole shape variation. The reverse PCA for the asymmetric component of shape variation (FA) showed that the first three PCs contributed 64.0% to the total variation (Table 2). Morphological variability between age groups was assessed and displayed with CVA, which showed that age groups appeared well differentiated for both symmetric and asymmetric components (Figures 3 and 4).

Significant differences (in all cases, $p < 0.05$) appeared between the three age groups analyzed in both the symmetric component (Mahalanobis distances: 1M *vs.* 2M=15.993; 1M *vs.* 3M = 15.072; 2M *vs.* 3M=15.099) and the asymmetry component (Mahalanobis distances: 1M *vs.* 2M=14.671; 1M *vs.* 3M=12.417; 2M *vs.* 3M=6.781) of variation. Only the former was positively correlated with age ($p = 0.0098$).

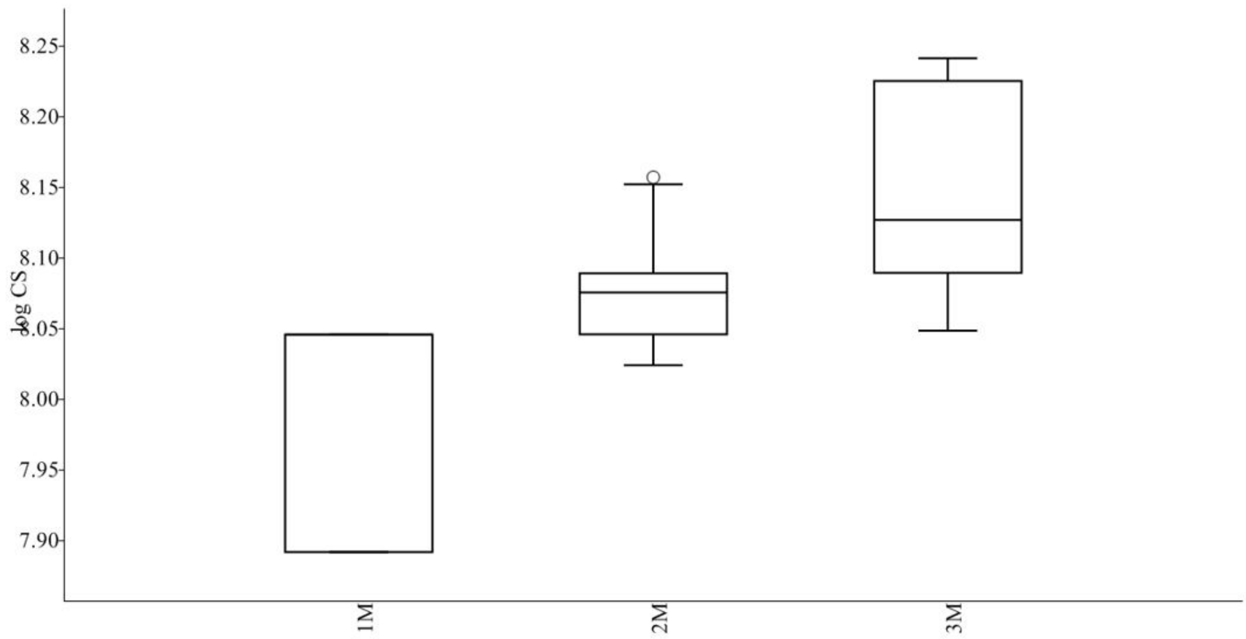

Figure 2 – Box plots of log of centroid size (CS) for the age groups considered according to molar (M) eruption:1M, M^1 fully erupted (n = 2); 2M, M^2 fully erupted (n = 18); and 3M, M^3 fully erupted (complete molar series, n = 18). The bottom and top of the box represent the first and third quartiles, respectively, and the band inside the box is the second quartile (the median). Lines extending vertically from the boxes (*whiskers*) indicate variability outside the upper and lower quartiles. An outlier is only present in the second group. Size, which was different between age groups ($H_c = 6.818$, $p = 0.0090$), was progressively larger with increasing age

Table 2 – Principal component analysis (PCA) of shape variation, for both symmetric and asymmetry components

PC	Symmetric			Asymmetric		
	Eigenvalues	% variance	Cumulative %	Eigenvalues	% variance	Cumulative %
1	0.00023825	37.318	37.318	0.00001594	31.839	31.839
2	0.00011379	17.825	55.143	0.00000976	19.493	51.332
3	0.00008236	12.900	68.043	0.00000635	12.685	64.017
4	0.00005255	8.231	76.275	0.00000448	8.947	72.964
5	0.00003597	5.634	81.908	0.00000288	5.752	78.716
6	0.00002620	4.105	86.013	0.00000257	5.143	83.859
7	0.00002053	3.216	89.229	0.00000188	3.762	87.622
8	0.00001948	3.051	92.280	0.00000153	3.059	90.681
9	0.00001020	1.597	93.877	0.00000092	1.836	92.517
10	0.00000881	1.380	95.258	0.00000082	1.643	94.160
11	0.00000755	1.183	96.441	0.00000062	1.241	95.401
12	0.00000665	1.042	97.483	0.00000051	1.011	96.413

The values reported are the eigenvalues and percentages for which each principal component accounts

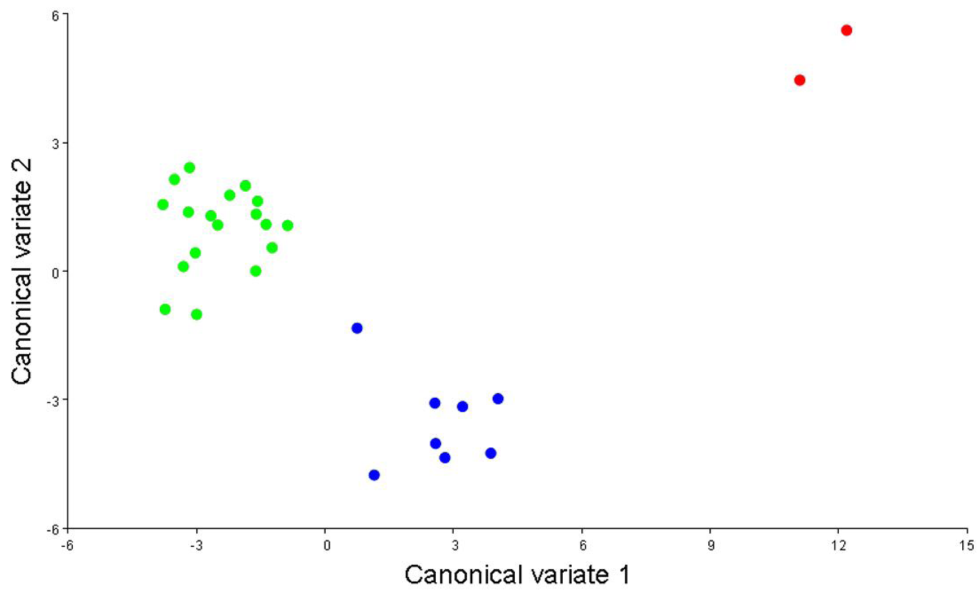

Figure 3 – Scatter plot of canonical variate analysis showing differences between age groups, for the symmetric component of variation
Groups appeared well differentiated.

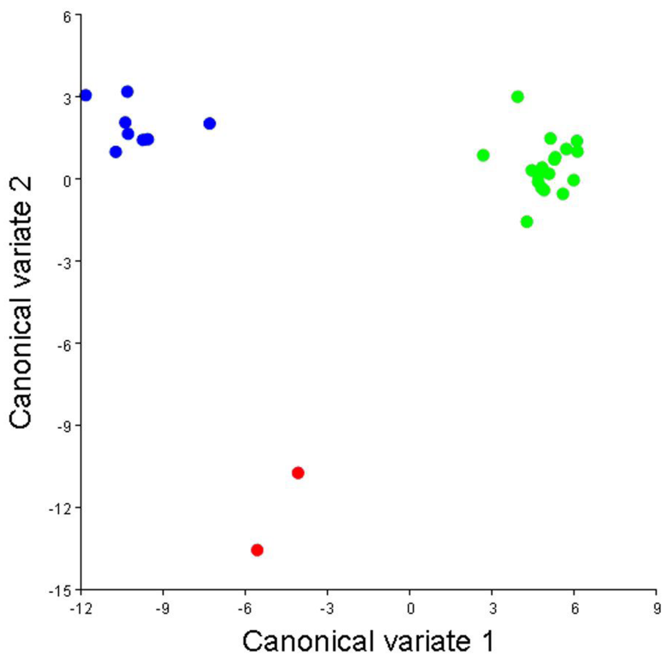

Figure 4 – Scatter plot of canonical variate analysis showing differences between age groups, for the asymmetric component of variation
Groups appeared well differentiated

Discussion

Within a symmetrical structure, fluctuating asymmetry (FA) is defined as the non-directional deviation from bilateral symmetry, while directional asymmetry (DA) happens whenever one character has developed more on one side of the plane or planes of symmetry than on the other. The results showed the presence of both FA and DA. The geometric morphometric method used also allowed decomposition of the total shape variation into symmetric (i.e., differences among individuals) from asymmetric (i.e., multiple components occurring according to the symmetry of the object). The results obtained in the preliminary analyses indicated that the measurement error is low relative to the magnitude of FA. Consequently, for this research, in which the size of the sample was limited by factors other than design, we considered that the relationship between number of individuals and repetitions used in this work was appropriate to get reliable FA estimates. Using this approach, purely symmetric variation for the first three PCs spanned 68.0% of the total variation. This was similar to the variation described by individual patterns of asymmetry, which accounted for 64.0% of the total morphological variation in all three age groups, and both components allowed the differentiation of individuals according to their age. Thus, the data demonstrated age-dependent asymmetry, both individually and per age group.

Since both sides of the skull are produced by the same genome, the degree of symmetry must reveal the individual's ability to canalise development in the face of stress. It was believed that, in the samples examined, ventral craniofacial FA could just reflect responses to stress factors, which are different for each of the considered age groups. These were subtle stress factors, as no skull deformities were observed and similar results have been obtained for other domestic species. DA could have a functional basis, due to masticatory lateralization, well described in this species for mandibles (Parés-Casanova, 2014a) and in other domestic mammals (Parés-Casanova, 2014b; Parés-Casanova & Bravi, 2014), and also for other traits (Leśniak, 2018; Parés-Casanova & Kucherova, 2013; White et al., 1974; Wilson et al., 2009). In fact, the masticatory apparatus is dominated by the masseter muscle which has its origin on the skull. Necessarily, fibers that attach to different surfaces of an aponeurosis must have different orientations, raising the possibility that differential contraction of fibers could change the direction of muscle pull.

Finally, the results of the present paper showed also that the shape differences obtained from different age groups could reasonably be simply a consequence of different responses to the same local environmental factors. However, this supposition must be investigated in further research.

Conclusion

In studied wild boar skulls on their ventral aspect, both fluctuant and directional asymmetries appeared highly significant and age-dependent. FA is tauthor declares that hought to reflect the environmental pressures experienced throughout development, with greater pressures resulting in higher levels of asymmetry, while DA would reflect a masticatory lateralization.

Conflict of interest

The author declares he has no conflicts of interest.

Ethics Statement

The author declares that each contribution to this article has been acknowledged and source of information from other peoples' published or unpublished works have been cited referenced. I certify that I am solely responsible for text of the article and work included in the article along with any incomplete reference.

REFERENCES

Bookstein FL. Morphometric tools for landmark data: geometry and biology. New York: Cambridge; 1991. Chapter 1, Introduction; p. 435.

Dryden IL, Mardia KV. Statistical shape analysis. Chichester: Wiley; 1998.

Graham JH, Raz S, Hel-Or H, Nevo E. Fluctuating asymmetry: methods, theory, and applications. Symmetry. 2010;2(2):466-540. http://dx.doi.org/10.3390/sym2020466.

Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electronica. 2001;4(1):1-9.

Klingenberg CP. MorphoJ: An integrated software package for geometric morphometrics. Mol Ecol Resour. 2011;11(2):353-7. http://dx.doi.org/10.1111/j.1755-0998.2010.02924.x. PMid:21429143.

Klingenberg CP. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry. 2015;7(2):843-934. http://dx.doi.org/10.3390/sym7020843.

Klingenberg CP, McIntyre GS, Zaklan SD. Left-right asymmetry of fly wings and the evolution of body axes. Proc Biol Sci. 1998;265(1402):1255-9. http://dx.doi.org/10.1098/rspb.1998.0427. PMid:9699316.

Leśniak K. Directional asymmetry of facial and limb traits in horses and ponies. Vet J. 2018;232(1):46-51. PMid:29428084. http://dx.doi.org/10.1016/j.tvjl.2017.12.001.

Palmer AR. Fluctuating asymetry analysis: a primer. In: Markow T, editor. Developmental instability: its origins and evolutionary implications. Dordrecht: Kluwer; 1994. p. 335-364. vol. 93.

Palmer AR, Strobeck C. Fluctuating asymmetry analyses revisited. In: Polak M, editors. Developmental instability (DI): causes and consequences. Oxford: Oxford University Press; 2001.

Parés-Casanova PM. Existence of mandibular directional asymmetry in the European wild boar (Sus scrofa Linnaeus, 1758). J Morphol Sci. 2014a;31(4):1-5. http://dx.doi.org/10.4322/jms.064613

Parés-Casanova PM. Size asymmetries in equine upper molar series. ECORFAN Journal. 2014b;5(13):2055-69.

Parés-Casanova PM, Bravi R. Directional and fluctuating asymmetries in domestic sheep skulls. J Zool Biosci Res. 2014;2(3):11-7.

Parés-Casanova PM, Kucherova I. Horn antisymmetry in a local goat population. Int J Res Agric Food Sci. 2013;1(2):12-7.

Rohlf FJ. Digitalized landmarks and outlines [Internet]. New York: Department of Ecology and Evolution, State

University of New York at Stony Brook; 2010 [cited 2019 Sept 11]. Available from: http://life.bio.sunysb.edu/morph/

Rohlf FJ [Internet]. TpsSmall v. 1.33. 2015 [cited 2019 Sept 11]. Available from: http://life.bio.sunysb.edu/morph/

Valen LV. A study of fluctuating asymmetry. Evolution. 1962;16(2):125-42. http://dx.doi.org/10.1111/j.1558-5646.1962.tb03206.x.

White AA, Panjabi MM, Hardy RJ. Analysis of mechanical symmetry in rabbit long bones. Acta Orthop Scand. 1974;45(3):328-36. http://dx.doi.org/10.3109/17453677408989153. PMid:4406999.

Wilson GH, McDonald K, O'Connell MJ. Skeletal forelimb measurements and hoof spread in relation to asymmetry in the bilateral forelimb of horses. Equine Vet J. 2009;41(3):238-41. http://dx.doi.org/10.2746/042516409X395561. PMid:19469228.

Financial support: Author declares this study has been exempt of financial support.

Authors Contributions: The author conceived and designed the analysis, collected the data and performed the analysis, and wrote the paper.