Sphenoid bone is more asymmetrical than palatine bone among small ruminants

Pere M. Parés-Casanova and Xènia Domènech-Domènech

Department of Animal Science, ETSEA, University of Lleida, Lleida, Spain

ABSTRACT
A sample comprised by 53 dry modern skulls of adult small ruminants (sheep n = 36 and goat n = 17) from a comparative collection, absent of bony pathologies, was studied on their basal craniofacial aspect. A total of 26 points (2 sagittal landmarks and 24 semilandmarks) and 32 points (4 landmarks and 28 semilandmarks) were chosen on the sphenoid bone and palatine bone respectively and analysed by means of geometric morphometric techniques. The interaction of individuals and sides (fluctuating asymmetry) showed a highly significant difference for both bones, as well as side effect (directional asymmetry), being levels of detected fluctuating asymmetry higher in sphenoid (25.4%) than in palate (12.8%). Asymmetric component differentiated sheep and goats. Detected basicranial asymmetry can be viewed as a common finding among small ruminants.

Introduction
The palatine bone (os palatinum) is a paired structure between the maxilla, sphenoid and pterygoid bones. It is composed of a horizontal plate (lamina horizontalis, which is flattened dorsoventrally and forms part of the hard palate), a perpendicular plate (which forms the dorsal and lateral walls of the nasopharyngeal meatus) and the choanae (Barone 1999). The horizontal plate takes part in the constitution of the bony vault of the palate, caudally to the palatine process of the maxilla (Barone 1999).

The sphenoid bone is located at the central skull base and is commonly considered as a complex bone in the animal skeleton (Barone 1999). It forms the rostral part of the base of the neurocranium and is composed of two segments, the pre-sphenoid (os præsphenoidale), rostrally, and the basisphenoid (os basisphenoidale), caudally (Barone 1999). The sphenoid bone joins the orbit and facial region with the cranial convexity and the skull base.

Developmental instability arises from genetic or environmental stressors that disturb the normal developmental pathways of continuous characters, producing developmental noise (Graham et al. 1993). This instability results from the difference between left and right in ideal bilaterally symmetrical organisms or parts of them, which we expect to be zero, and it provides a measure of how well an individual can buffer its development against internal and external environmental stress during ontogeny (Klingenberg and McIntyre 1998). Developmental instability in phenotypic traits is commonly measured as fluctuating asymmetry (FA) (Van Valen 1962). There are many publications that assume FA as a good estimator of developmental stability, e.g. the organism’s ability to buffer minor developmental accidents (Alibert et al. 1994). Both genomic and environmental changes can increase FA which represents a possible deterioration in developmental homeostasis apparent in adult morphology (de Coster et al. 2013; Ducos and Tabugo 2014).

The literature lacks articles on morphology relating to the palate and sphenoid bones among domestic mammals. A recent article by the authors compared the sphenoid bone between sheep and goats, but did not consider possible asymmetries (Parés-Casanova and Domènech-Domènech 2021). Using geometric morphometrics, we aim to investigate the morphological variability between palatine and sphenoid bones in domestic sheep (Ovis aries) and goats (Capra hircus). Specific questions to be addressed by this study are:

(1) Which is the level of asymmetry for palatine and sphenoid bones?
(2) Can we differentiate palatine and sphenoid bones according to asymmetries in both species?
(3) Is directional or fluctuating asymmetry predominant on these bones?

Materials and methods
A sample of skulls from domestic small ruminants was studied. The material was held in the Laboratory of Veterinary Anatomy, Department of Animal Science, University of Lleida, in Catalonia (Spain) but any of the skulls presenting craniofacial trauma were previously excluded. As the bones are separated by cartilage but ossifies with age, senile individuals were not sampled due to the difficulty to delimitate clearly sutures. Final sample was of 53 dry modern skulls of adult small ruminants: sheep (n = 36) and goat (n = 17). No permits were required for the described study, which complied with all relevant sanitary regulations and needed no in vivo manipulation.
**Imaging**

Each skull on its basal aspect was photographed on high resolution in standardized ventral view with a digital camera. Images were captured with a Nikon® D70 digital camera (image resolution of 2240 × 1488 pixels) equipped with a Nikon AF Nikkor® 28–200 mm telephoto lens. Scale was given for each photograph by placing a ruler. The images were stored in JPG format. For each skull, sphenoid and palate were pictured separately as it is required to level the skull differently in order to obtain a good parallel view of each bone.

**Landmark selection**

To capture the form, we used a combination of sagittal landmarks and paired semilandmarks for the contours. A total set of 26 points (2 landmarks and 24 semilandmarks) were chosen to analyse the sphenoid bone, and a set of 32 points (4 landmarks and 28 semilandmarks) were chosen to analyse the palate bone. The x and y coordinates of these landmarks and semi-landmarks were digitized using TpsDig 2.04 v. 1.40 (Rohlf 2015). The file to determine the sliding direction of the semilandmarks was created in TpsUtil v. 1.70 (Rohlf 2015). Since levels of error can account for a large fraction of between-side variance and alter the results, digitalizations were made twice in order to assess this error.

**Digitation and shape analysis**

A Generalized Procrustes Analysis (GPA) approach eliminates the scale, and the translational, and rotational differences of the coordinate data of the landmarks among subjects (Webster and Sheets 2010). The coordinate data of each specimen are usually scaled by its centroid size (CS, the square root of the sum of squared distance between each landmark and the plastron centroid) (Bookstein1991). The CS and GPA-scaled coordinates represent surrogates of size and shape, respectively (Webster and Sheets 2010) (Figures 1 and 2).

To detect the components of variances and deviations, a Procrustes ANOVA was then used. In this analysis, the *individuals* effect denoted the individual variations of shape and size of each turtle, the *individuals mean square* was the measure of total phenotypic variation and it is random, the main effect of *sides* indicated the variation between sides and considered as the measure of DA, and the *individuals x sides* was
a mixed effect indicating FA (Klingenberg and McIntyre 1998). Finally, measurement error represents the variation due to measurement error in taking landmarks of the same individual in separate sessions (Klingenberg and McIntyre 1998). In Procrustes ANOVA there are more degrees of freedom than in conventional ANOVA because the squared deviations are summed over all the landmark coordinates (instead of a single sum of squares in conventional ANOVA). Therefore, the number of degrees of freedom is that for ordinary ANOVA multiplied by the shape dimension, which is, for our two-dimensional coordinate data, twice the number of landmarks minus four (the number of coordinates minus two dimensions for translation and one each for scaling and rotation) (Klingenberg and McIntyre 1998).

When studying morphological variation, estimation of the allometric effect (defined as the dependence of shape on size) is a necessary step. We investigated by performing a regression of asymmetric component on CS (independent variable). CS was transformed to its logarithm to increase the fit of the model and number of randomization rounds was 10,000. Finally, a Canonical Variate Analysis (CVA) was used to ordinate specimens to maximize separation of species with respect to their within-group variances.

Morphometric and statistical analyses were conducted using the MorphoJ v. 1.06c (Klingenberg 2011) using object symmetry (the plane of symmetry passing through the landmark configuration) and PAST v. 2.17c (Hammer et al. 2001) packages. Confidence level was established at 95%.
Results

In the Procrustes ANOVA, where three factors (individual, side and interaction individual*side) were analysed, error was very low, less than 7.1% (Table 1). The interaction of individuals and sides (FA) showed a highly significant difference (p < 0.0001) for both bones, as well as side factor (DA). Levels of detected FA was clearly higher in sphenoid (25.4%) than in palate (12.8%) but being similar for DA (39.8% and 42.5% respectively) (Table 1). Similar results appear if we consider both species separately.

Multivariate regression of asymmetric component onto log CS showed that the allometric effect was not significant (p = 0.456 and p = 0.766 for palatine and sphenoid bones, respectively, based on a permutation 10,000 rounds) and that size accounted only for 0.889% and 0.367% respectively. On CVA, the asymmetric shape differences in each species on the shape space were scattered on the first two canonical variate axes (CV1 and CV2) and species did not overlap for both bones (p < 0.05) (Figures 3 and 4).

Discussion

The sphenoid bone of the skull base is an unpaired bone with many foramina and grooves that allow nerves and blood vessels to pass through or along it (Barone 1999). Essentially, the sphenoid serves as a key conduit for the passage of nerves and blood vessels of the head and neck. Major foramina and fissures include the foramen orbitotundum and foramen ovale, and important cranial nerves such as ophthalmic, maxillary, oculomotor and abducens have their pass there (Sisson and Grossman 1985), so it seems surprising the significative presence of FA on it. Sphenoid FA was moreover higher than FA detected in palate, this bone supporting important vital functions, notably feeding and breathing (Kimmel et al. 2009). The presphenoid and the basisphenoid fuse late in life. Thus during ossification processes it would be easier to appear asymmetry in the adult, which in turn would be expressed as FA. Palatine asymmetry can be explained by lateralized masticatory function, which has been described in sheep and goat (Parés-Casanova and Bravi 2014; Parés-Casanova 2017). Asymmetry of the sphenoid bone has been found in human skulls (Kim et al. 2003) as well of the palate (Moreira et al. 2008).

So, in conclusion, the detected lack of accurate correspondence between two sides must be considered as normal on the basal craniofacial skeleton in small ruminants.

Supporting information

The contents of all supporting data are the sole responsibility of the authors. Queries or any other issues regarding errors are requested to be addressed to first author.

Table 1. Results of procrustes ANOVA for palatine and sphenoid bones, both for size (expressed as centroid size) as shape.

| Effect     | 1/Palatine bone | 2/Sphenoid bone |
|------------|-----------------|-----------------|
|            | Effect          | Effect          |
| Individual | Individual      | Individual      |
|            | 309.0425        | 216.6308        |
| Error      | 14.70735        | 1.346449        |
|            | 0.277497        | 0.025405        |
|            | 52              | 52              |
|            | 21.42           | 163.98          |
|            | <.0001          | <.0001          |
| Shape      | Individual      | Individual      |
|            | 0.993374        | 0.296675        |
|            | 0.023102        | 0.006560        |
|            | 0.362417        | 0.216841        |
|            | 0.344287        | 0.134567        |
|            | 1560            | 1248            |
|            | 3.02            | 1.37            |
|            | <.0001          | <.0001          |
|            | 1560            | 24              |
|            | 2.15            | 1.57            |
|            | <.0001          | 0.0388          |
|            | 1560            | 1248            |
|            | 3.28            | 3.28            |
|            | <.0001          | <.0001          |

Note: There were significative levels of ‘side’ (directional asymmetry) and ‘side*individual’ (fluctuating asymmetry) effects for both bones. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless).

Figure 3. Canonical variate analysis for palatine bone. Asymmetries appear as deformation grids. There were significative differences between Ovis and Capra.
Disclosure statement
No potential conflict of interest was reported by the author(s).

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Figure 4. Canonical variate analysis for sphenoid bone. Asymmetries appear as deformation grids. There were significant differences between Ovis and Capra.