The Relationship Between Cephalic Scales and Bones in Lizards: A Preliminary Microtomographic Survey on Three Lacertid Species

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ABSTRACT

In the last two decades, there has been a great deal of interest in the morphology and anatomy of the lizard skull in an ecological and evolutionary perspective. However, the relationship between variations in many key anatomical features remains largely unknown. Using microtomography and geometric morphometrics, we examined the relationship between bones and scales associated with the parietal foramen in the three lizards species most common in the Italian peninsula: *Podarcis muralis*, *P. sicula*, and *Lacerta bilineata*. The imprints of the scales are clearly recognizable on the outer bone surface, and this may suggest a structural interaction between these elements. The temporal osteoderms are visible in the larger males and in the larger females of *L. bilineata*, but they are absent in the smaller specimens of *L. bilineata* and in all *Podarcis* specimens. Two parallel rows of pterygoid teeth are present in all the specimens of *L. bilineata* and are absent in the smaller male of *L. bilineata* and in both *Podarcis* species. Cheek osteoderms occurred only in the largest specimens of our sample (i.e., large *L. bilineata*), being possibly related to hyperostotic processes and densitometric thresholds more than to phylogeny. Minor differences may be also associated with the form of the parietal foramen. In absolute terms the parietal foramen tends to be largest in *L. bilineata* but in relation to skull length the foramen tends to be larger in *P. muralis*. In this latter species the foramen is also more elongated. In all three species the fronto-parietal suture occupies a similar location relatively to the scale spatial organization. A shared allometric pattern shows that the main vault enlargement can be localised at the areas anterior to the fronto-parietal suture, providing further information on the possible morphogenetic dynamics associated with the interaction between scales and bones around this structure. Anat Rec, 293:183–194, 2010. © 2009 Wiley-Liss, Inc.

Key words: lacertids; cranial anatomy; tomography; fronto-parietal suture; *Podarcis*; *Lacerta*

INTRODUCTION

The lizard family Lacertidae comprises about 280 species distributed throughout Eurasia and Africa (Arnold et al., 2007). The fossil history of the family extends back at least to the Lower Eocene, about 45–50 My ago (Böhme and Weitschat, 1998, 2002; Borsuk-Bialynicka et al., 1999; Evans, 2003; Hipsley et al., 2009). Despite the large amount of investigation, the phylogenetic relationships within this family are still not completely understood (Arnold, 1973; Oliverio et al., 1998, 2000; Harris et al., 2005; Arnold et al., 2007).

In the last two decades, there has been a great deal of interest in interspecific and intraspecific variation in the morphology and anatomy of lizard skull (bones, scales, osteoderms) to obtain new insights into lizard evolution (e.g., Arnold, 1989; Barahona and Barbadillo, 1998; Metzger, 2002; Arnold et al., 2007). An anatomical report on the ossification of lacertid skull shows that the bones of the snout region (premaxilla, maxilla and nasal bones) ossify before those of the skull roof, and the posterior region of the frontal areas ossifies before the anterior region of the parietals (Barahona and Barbadillo, 1998). An equivalent of similar antero-posterior growth gradient, associated with a differential allometric rate, has been described in several lacertids, as well as in other lizard taxa, in both the skull (Monteiro and Abe, 1997) and the cephalic scales (Bruner et al., 2005; Bruner and Costantini, 2007). The fronto-parietal suture, which is positioned under the fronto-parietal scales, has been hypothesized to be a relevant pace-maker of the head growth and development (Bruner and Costantini, 2007). The degree of interdigitation at the fronto-parietal suture restricts dorsoventral bone movements, modulating the cranial kinesis (Barahona and Barbadillo, 1998; Metzger, 2002). In addition to anatomical constraints, diet and mating both have a role in shaping the head morphology (including both bones and cephalic scales), mostly through the stress generated by muscles (jaw adductors and nuchal attachments) involved in feeding, coupling, or fighting (Moss and Young, 1960; Buckland-Wright, 1978; Bruner et al., 2005; Huyghe et al., 2009). The muscular system may exert structural pressures and tensions on the surrounding anatomical elements; however, bones may represent a morphogenetic pace-maker in many cephalic regions by means of modulator structures, such as the fronto-parietal suture.

It is widely accepted that morphological differences between species reflect differences in diet, ecology and patterns of bone growth (Moreno and Carrascal, 1993; Niehoff et al., 2004; Herrel et al., 2008; but see Schwenk, 2000, 2002). The way such morphological differences have evolved may provide useful information on morphogenetic patterns and selective pressures acting on them. Morphogenesis is a polyphasic and multifactorial process in which a genetic program is decoded through the interaction with environmental factors and expressed within a given biomechanical context (Cheverud, 1996; Klingenberg, 2002). In this framework, functional morphometrics largely deals with the biomechanical environment, trying to identify, describe, and understand the structural relationships between the elements of the anatomical system (Moss and Young, 1960). Under this scenario, applications of geometric modeling to study the morphology and the biomechanical dynamics of skull proved useful to quantify differences between species, providing helpful information on the structural organization of the skull (Hallgrimsson et al., 2007). Lacertids are one of the most diverse groups of lizards in terms of dietary specialization, and although they have been well described in the literature (e.g., Arnold, 1989; Barahona and Barbadillo, 1998; Herrel et al., 2004, 2008; Evans, 2008), the degree to which the mechanics of their skulls differs among species remains unexamined.

In this study, we carried out an exploratory computed microtomography (μCT) survey together with morphometric analyses to supply information on cranial anatomy and morphology and on the relationships between scales and bones in the three lacertid species: the common wall lizard *Podarcis muralis* (Laurenti, 1768), the Italian wall lizard *Podarcis sicula* (Rafinesque-Schmaltz, 1810) and the western green lizard *Lacerta bilineata* (Daudin, 1802). All the three species represent the most common lizard species in the Italian peninsula, although they can be found elsewhere in Europe (Arnold and Burton, 1978; Corti and Lo Cascio, 1999; Sindaco et al., 2006; see for a general description of anatomy Barahona and Barbadillo, 1998; Arnold et al., 2007). They belong to the tribe Lacertini (around 108 species). The molecular differences suggest that this tribe diversified around 12–16 My ago, producing three main evolutionary units (Arnold et al., 2007): the *Lacerta-Timon* clade of large-bodied lizards probably dispersed earliest, followed by *Algyroides* and then *Podarcis*. This study is aimed at providing information on specific anatomical cranial traits in these Mediterranean lizards, and on the variation of some neurocranial structures related to the cranial morphogenetic dynamics.

MATERIALS AND METHODS
Sampling and μCT Analysis

The sample includes specimens of *P. muralis*, *P. sicula*, and *L. bilineata* collected as prey remains from nest-boxes of Eurasian kestrels *Falco tinnunculus* (Costantini et al., 2005, 2007). Prey remains were used only when the preservation was sufficient enough to investigate head morphology and recognize the species. All the individuals were recovered around Rome, between 1999 and 2005. Of each species, we included in this study three males and three females chosen from a larger sample (Bruner et al., 2005; Bruner and Costantini, 2007) in order to have a head size gradient (i.e., from small to large) for each sex and for each species, respectively.

The specimens were scanned using a high-resolution X-ray microtomography (μCT-80, Scanco Medical, Switzerland), at 45 kV X-ray tube voltage, 88 μA, 0.35 degree rotation step and ~800 ns exposure time per individual shadow projection. The voxel size was $36 \times 36 \times 36$ μm, with an average exposure time of 120 min per scan. CT data analysis, automatic and semiautomatic segmentation, surface rendering, and traditional metrics, were performed using Mimics 11.11 (Materialise, Belgium).

Anatomy and Morphometrics

The microtomographic sections and the computed surface reconstructions were used to investigate anatomical differences between the three species. Basic differences between the densitometric components in these three groups were found and are discussed accordingly.
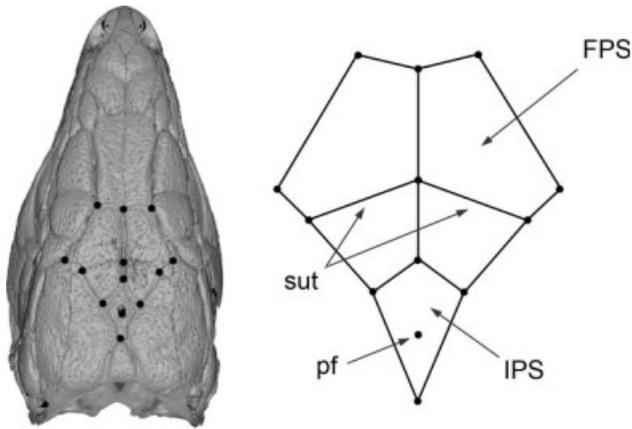
Fig. 1. Configuration with twelve 2D landmarks describing the geometry of the fronto-parietal (FPS) and interparietal (IPS) scales, the posterior border of the parietal foramen (pf), and the position of the fronto-parietal suture (sut).

Taking into account the specific morphology of the interparietal area in the western green lizard and the possible relationship with the underlying pineal system (Bruner and Costantini, 2009), the dimensions (length and width) of the parietal foramen were measured and compared. The average between the length and width of the foramen was used as index of size. The ratio between the length and width was used to estimate the general shape of the foramen. The head length was measured as the midsagittal interlandmark distance between the anteriormost point of the muzzle to the posterior margin of the occipital scale. The linear relationships between these diameters have been quantified by using a reduced major axis approach. Allometry has been tested through permutations and 95% confidence intervals for slopes after log-log transformation on the pooled sample.

The upper vault morphology was analyzed using a 2D bilateral configuration including 13 landmarks, describing the principal boundaries of the scales, the position of the fronto-parietal suture, and the parietal foramen (Fig. 1). The suture divides the fronto-parietal scale into anterior and posterior district. The species-specific average configurations were compared after Procrustes superimposition, which operates a normalization of the coordinates through translation to a common centroid, scaling to unitary centroid size, and rotation as to minimize the least-square differences between corresponding landmarks (Bookstein, 1991). The whole sample has been investigated according to the principles of geometric morphometrics (Zeldtich et al., 2004). After Procrustes superimposition, the covariance matrix has been analyzed by multivariate ordination techniques (PCA, Principal Component Analysis; CVA Canonical Variates Analysis). The correlation between shape and size (allometry) has been computed by multivariate regression of the shape variables (Procrustes coordinates projected into the tangent space, see Bookstein, 1991) onto the centroid size. Taking into consideration the exploratory and preliminary nature of this survey and the limited number of specimens, correlation between size and shape has been analyzed pooling the data from the three species, most of all to verify whether or not the position of the fronto-parietal

suture changes with size. Differences among these three groups are largely based on common allometric variation, although with some minor size-free traits (Bruner and Costantini, 2009).

Results from a preliminary 3D analysis using the same configuration (i.e., including also the z-coordinate for the superior-inferior axis, associated with the vertical position of the landmarks) did not show marked differences from those obtained using a 2D approach. This is probably because of the fronto-parietal vault is almost completely flat in lacertids. Hence, the 2D approach was then used, to decrease the number of variables (from three per landmark to two per landmark) without loosing much information. Geometric morphometrics was computed on symmetrised bilateral configurations using MorphoJ 1.00 (Klingenberg, 2008). Basic statistics was computed with PAST 1.91 (Hammer et al., 2001).

RESULTS

General Anatomy

All three species have a similar skull shape (slightly flatter and sharpened in both *Podarcis* species). *Lacerta bilineata* possesses much more extensive osteoderms than either species of *Podarcis* and has a relatively larger postorbital area. In *L. bilineata*, palatine bones are closer to each other than in either *Podarcis*. Nares tend to be more rounded and relatively larger in *L. bilineata*.

At the optimal bone-density energy, the scales are not visible. Nevertheless, the traces of the borders of each scale are imprinted on the bone surface, appearing as grooves, and can be easily detected after 3D rendering (Figs. 1, 2). All the skull volumes show similar and comparable density ranges and values, except for the statolithic masses or otoliths in the inner ear, which display a very high attenuation coefficient, producing white overflow in some specific areas (Fig. 3). The upper vault bones are pneumatized, with an intermediate layer including large air cells (Fig. 3).

A series of four separate supraocular osteoderms are present in all the specimens (Fig. 4). Their degree of fusion is variable, being higher in larger specimens and lower in smaller specimens of each species, respectively. A row of unfused supraciliary osteoderms are also visible in *L. bilineata*, but not in either *Podarcis* species. The temporal osteoderms underlying the temporal scales on the side of the head (herein *cheek osteoderms*) are well visible, while appearing fenestrated in the two larger males and in the two larger females of *L. bilineata*. In this same area, the masseteric osteoderm is also visible. In contrast, the temporal osteoderms are absent in the two smaller specimens of *L. bilineata* and in all *Podarcis* specimens. Two parallel rows of pterygoid teeth are present in all the specimens of *L. bilineata* and are absent in the smaller male of *L. bilineata* and in both *Podarcis* species (Fig. 5). The exact number of pterygoid teeth is not easily determinable because they tend to fuse with each other. However, using the cuspids as a landmark, they vary from around 6 teeth in smaller specimens to around 25 teeth in larger specimens. The size of pterygoid teeth slightly differs, but they seem to have a similar orientation. Generally, in every specimen the fronto-parietal suture shows a complex outline with a variable but evident degree of interdigitation.
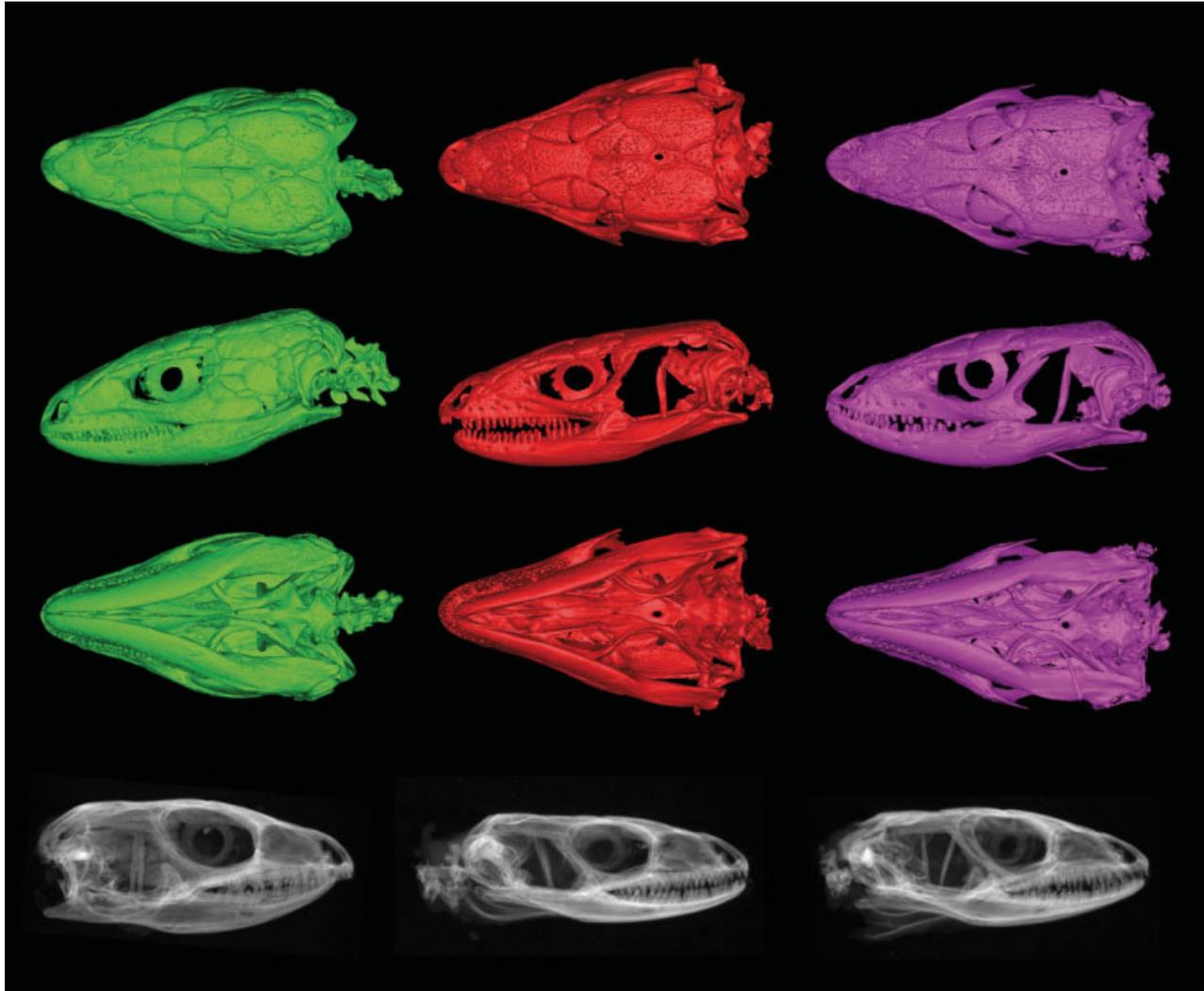
Fig. 2. The 3D models by surface rendering show the upper, left lateral, and lower views for males from *L. bilineata* (green), *P. muralis* (red), *P. sicula* (purple). Scout views (below) show the same species through projection of standard deviation gray values or the raw data. Not at scale. The specimens were CT-scanned with a voxel size of 36 μm, and analyzed by digital reconstructions.

Parietal Foramen

Figure 6 synthesizes the mean and standard deviation for the head length, and length and width of the parietal foramen. In absolute terms, the parietal foramen tends to be largest in *L. bilineata*, but in relation to skull length the foramen tends to be largest in *P. muralis* (Fig. 6). In *P. muralis*, the foramen is also more elongated. The small sample sizes per species prevent meaningful statistics, but it seems that there are differences between species in the relationship between parietal foramen morphology (size and shape) and skull length.

Considering the pooled sample, the length and width of the parietal foramen are correlated ($r = 0.87$; $P < 0.0001$; Fig. 7). The major axis coefficient between length and width is 0.75. After log-log transformation the hypothesis of isometry cannot be rejected (95% confidence interval for slope: 0.85–1.29; mean $= 1.03$). Hence, the general proportions between length and width of the fo-

ramen are constant, independently by the size of the foramen itself. *P. muralis* shows, however, negative residuals along this pattern, remarking the antero-posterior stretched shape of the foramen as suggested by the univariate analysis (Fig. 7). There is also a correlation between the head length and the foramen size ($r = 0.76$; $P = 0.0004$). In this case, the log-log regression cannot also reject the hypothesis of isometry (95% confidence interval for slope: 0.61–1.24; mean $= 0.87$). Therefore, we can hypothesize that the dimension of the parietal foramen does not change in relation to changes in head size. However, note that the correlation between the head length and the foramen size is not very robust and the data are definitely dispersed compared to the correlation between length and width of the foramen.

The relationship between parietal foramen and head length suggests that *P. muralis* differs from the remaining two species. In individuals with skulls around 13-mm long, *P. muralis* has a much smaller parietal foramen
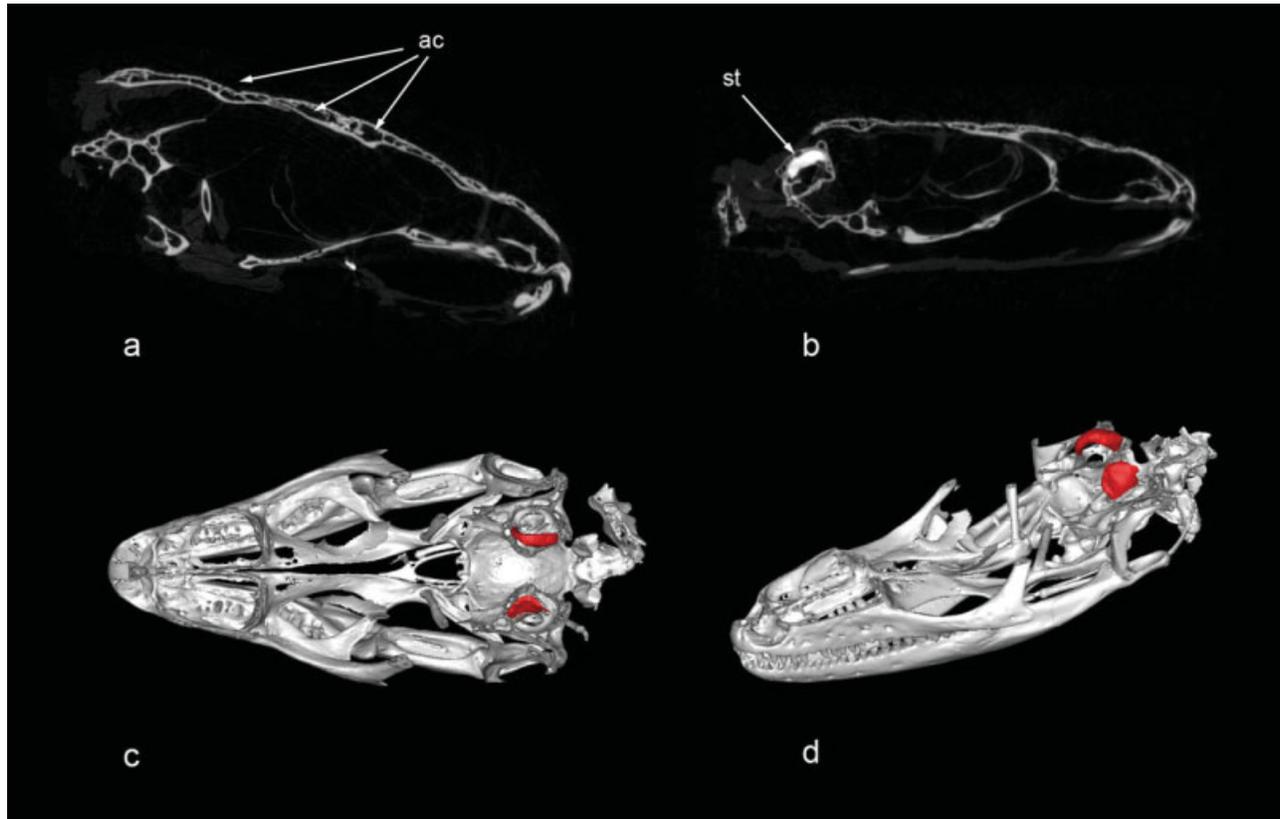
Fig. 3. The midsagittal CT section shows the air cells (ac) between the inner and outer bone layers of the vault **(a)**. The statolithic masses (st) are evidenced as the only high-density component of the skull **(b)**. The 3D rendering **(c, d)** shows the volumes of the statolithic masses after transversal section of the skull.

compared to *P. sicula*. However, in individuals with larger skulls (17 mm), the parietal foramen of *P. muralis* is similar to that of *P. sicula* and of the small *L. bilineata* specimens. The ratio between the length and width of the foramen does not correlate with the head length.

Geometric Morphometrics

Despite the small sample size, a permutation test after Procrustes superimposition suggests significant differences between *P. muralis* and the other two species ($P = 0.01$) and, even indicative, between *P. sicula* and *L. bilineata* ($P = 0.11$) (Fig. 8). The fronto-parietal scale is longer and narrower in *L. bilineata*, wider and shorter in *P. muralis*. The interparietal scale is narrower in *P. sicula*, and wider in *L. bilineata*. *Lacerta bilineata* shows also a marked constriction at the fronto-parietal suture. The parietal foramen is more anterior in *P. muralis* and more posterior in *P. sicula*, relatively to the whole configuration and particularly to the boundary of the interparietal scale. The morphospace from the PCA shows a steep and discontinuous decrease in the variance explained after the fifth component (cumulating 88% of the total variance; Fig. 9). The first principal component (32%) is associated with a relative contract on and narrowing of the fronto-parietal scale, and an anterior contraction of the interparietal scale. The parietal foramen is shifted forward, and there is the development of a definite lateral process at the lateral border of the fronto-parietal suture.

The second component (22%) is associated with widening of the anterior portion of the fronto-parietal scale, lengthening of the posterior area, and reduction of the interparietal scale. These two components together separate *P. muralis* from the other two species.

Following Canonical Variates Analysis, the Mahalanobis distances are significant for all the comparisons, while the Procrustes distances are significant only for the differences between *P. muralis* and the other two groups (Fig. 10).

The first variate separates, progressively, *L. bilineata*, *P. muralis*, and *P. sicula* because of a widening at the frontoparietal suture and a narrowing of the interparietal scale. This discrimination axis is mainly correlated with the fourth principal component, which explains 10% of the whole variance. The second variate separates *L. bilineata* and *P. sicula* from *P. muralis*, because of a larger anterior portion of the frontoparietal scale, smaller posterior portion of the frontoparietal, and a more posteriorly located parietal foramen in the latter. This axis correlates with both the first two principal components.

A multiple regression of the centroid size on the first five components of shape is also significant ($r^2_{adj} = 0.81$; $P < 0.0001$), showing that the allometric vector is not related with a specific component, but is distributed across the morphospace, involving PC1, PC2, PC4, and PC5. The centroid size shows a significant or indicative correlation with all these axes ($0.03 < P < 0.08$). A regression of the whole shape variation onto the centroid
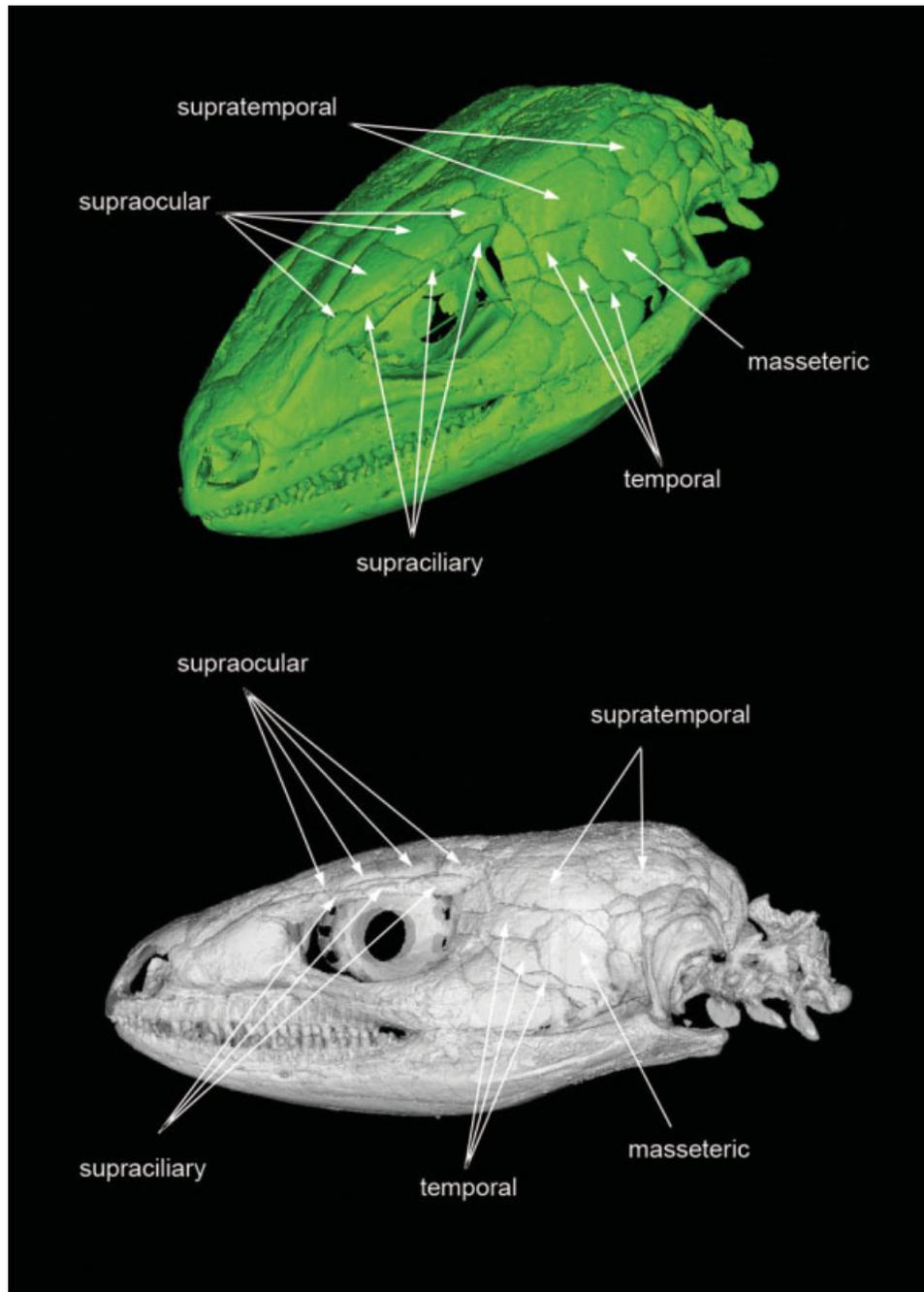
Fig. 4. 3D model (opaque and transparent) of the head structures in *L. bilineata*, showing the main osteoderms.

size is also significant (permutation test, $P = 0.008$; partial least square analysis, $r = 0.84$), mainly loaded onto the first two principal components, and is associated with a longitudinal stretching of the anterior part of the fronto-parietal suture, a reduction of the posterior part, and a widening of the interparietal scale (Fig. 11). The same pattern in the opposite direction shows geometrical areas of compression in the anterior part of the fronto-parietal scale and at the anterior border of the interparietal scale, suggesting that the allometric geometrical di-

lation at the fronto-parietal areas is associated with the anterior portion and not with the posterior one.

DISCUSSION
Cranial Morphology in Lizards

The cranial system in lizards involves a complex morphogenetic process because of the complex architecture of cranial bones (Barahona and Barbadillo, 1998), fine-tuned kinematic relationships (Metzger, 2002), and
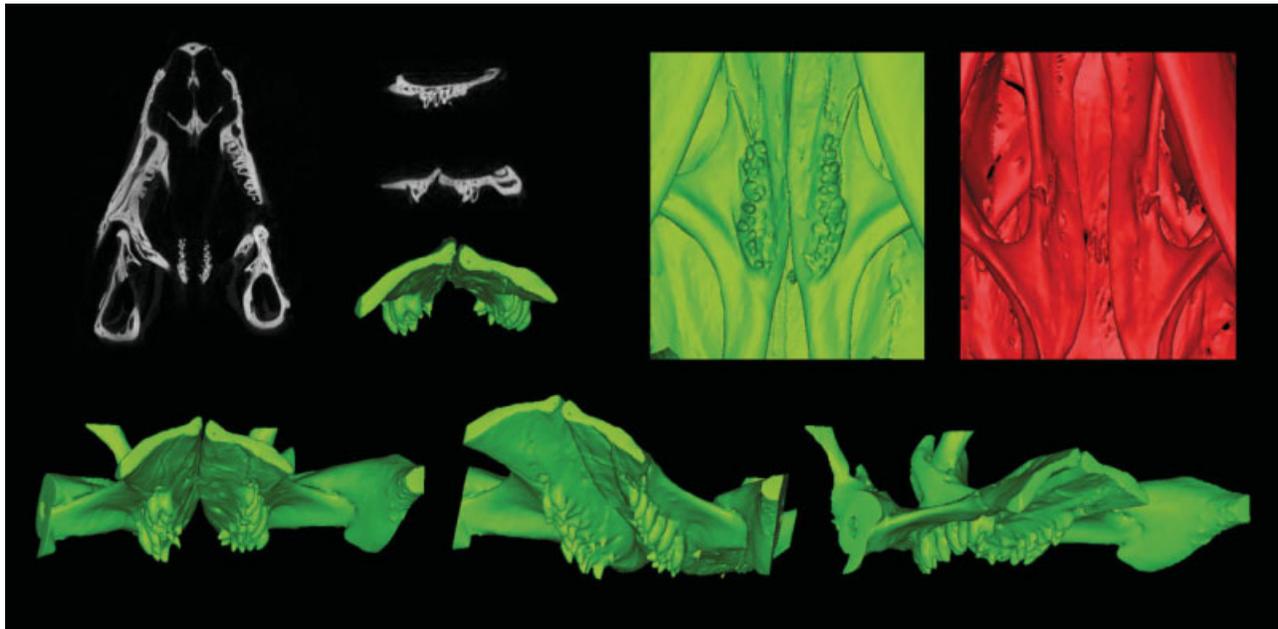
Fig. 5. The CT sections show the pterygoid teeth in *L. bilineata*, in transversal, sagittal, and coronal section. The 3D reconstructions show the pterygoid structures with different perspectives in *L. bilineata* (in green) and *P. muralis* (upper right frame, in red). Pterygoid teeth are lacking in the all *Podarcis* specimens.
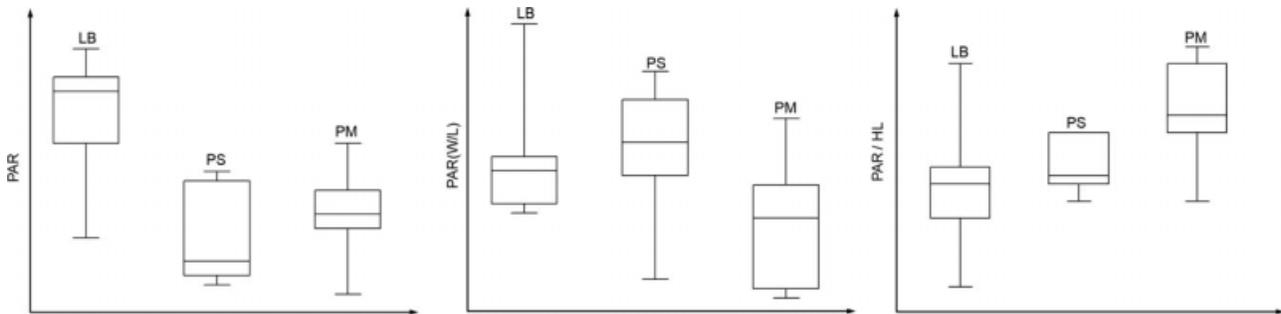

Fig. 6. Nonparametric distributions (median, interquartile, and range) for parietal foramen size (PAR: width + length divided by two), parietal foramen shape [PAR(W/L): ratio between width and length], and adjusted parietal foramen size (PAR/HL: parietal foramen size divided by head length) for *L. bilineata* (LB), *P. sicula* (PS), and *P. muralis* (PM).

possibly an interaction between bones and keratin scales (Bruner and Costantini, 2007). This study is aimed at introducing some relevant characters of the lacertid skull by means of microtomographic sections and digital reconstructions, focusing on elements of the fronto-parietal junction.

The bone parts show a rather heterogeneous densitometric composition, with the exception of otolithic masses, which are the only separated high-density components. A similar situation can be seen in other reptiles, even if with marked differences in the relative size of the otolithic volumes (Maisano and Rieppel, 2007). The otolithic masses are crystalline structures composed of both organic and inorganic material and localised in vestibular macular organs, where they mainly work as static organs (Carlström, 1963). Although the formation and function of otolothis are similar across Vertebrates, striking differences were found for their occurrence and crystallographic properties (Carlström, 1963). The inorganic phase contains calcium carbonate ($CaCO_3$) in its aragonite and calcite forms, but aragonite predominates in reptiles with an estimated ratio of 3 to 1 (Carlström, 1963; see also Piscopo et al. 2004 for a case study of *P. sicula*). A crystallographic analysis of a diverse group of Lepidosauria species (*Anguis fragilis, Varanus flavus, Natrix natrix and Vipera berus*) showed that otholitis are often similar in size and shape across different species (Carlström, 1963).

Scales are not recorded at the current densitometric range, but their imprints are clearly recognizable on the outer bone surface. Such traces suggest a tight structural contact between bones and dermal elements, probably through fibrous connections. The impressions of scales are imprinted into the vault bones, suggesting osteolithic processes, which are generally associated with pressures during the osteogenesis. However, in this
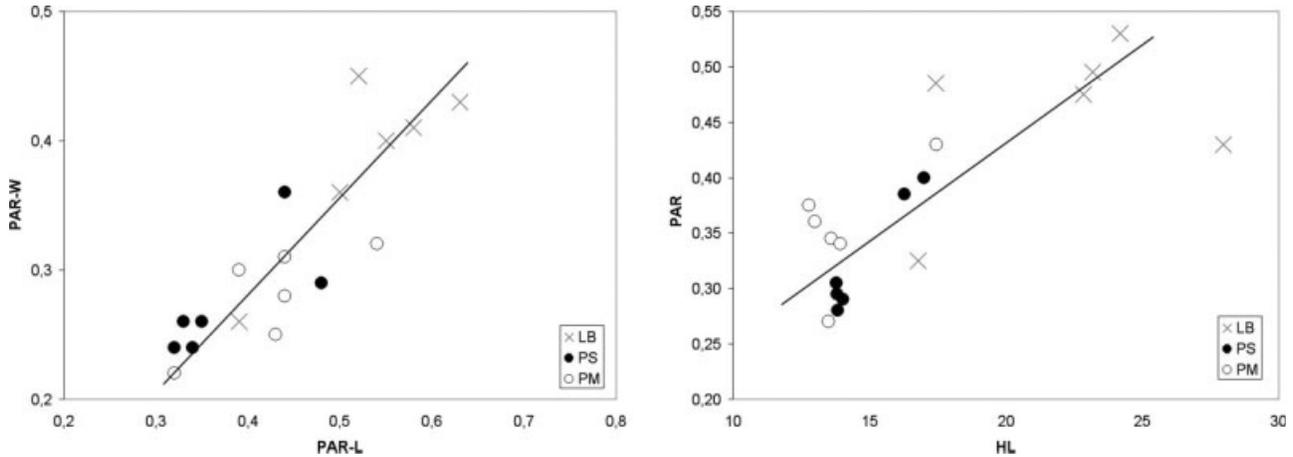
Fig. 7. Bivariate regression (reduced major axis) between parietal foramen length (PAR-L) and width (PAR-W), and between head length and parietal foramen size (PAR). LB: *L. bilineata*; PS: *P. sicula*; PM: *P. muralis*.

case it can be hypothesized that tensile strains may regulate the physical interactions between scales and bones. Bones are displaced by morphogenetic modeling, inducing a certain stretching on the dermal elements. Such interactions may generate constraints, restricting the changes in size (growth) and shape (development) during the morphogenesis. It is clear that this relationship is far from understood and it may represent a fruitful field of investigation in the future, most of all performing detailed ontogenetic studies. The tomographic analysis also shows a good level of pneumatisation in the vault bones. Taking into account the scarce functional information on the formation of air cells in the skull of vertebrates, this trait should be also considered carefully when investigating the lizard cranial system.

Two more traits warranting attention are osteoderms (in this case cheek osteoderms) and pterygoid teeth. Osteoderms are dermal sclerifications (Moss, 1969) that develop as a result of epithelial-mesenchymal interactions just below the pigment layer of the dermis (Krauss, 1906). Pterygoid teeth are small and conical structures attached at the surface of pterygoid bones, where they form a short row (Mahler and Kearney, 2006; Reilly et al., 2007). These two traits may be interpreted as hyperostotic traits that occur as a final result of a high level of bone deposition relatively to the rate of bone absorption. Hyperostotic characters (called "epigenetic" in anatomy and osteology) may result from pathological/subpathological conditions (e.g., excess of ossification through variation of the osteocytes/osteoclasts activity) or simply from prolongation/acceleration of ontogenetic pathways, both at intraspecific and interspecific level. The presence of cheek osteoderms and pterygoid teeth only in the largest specimens of our sample (adult green lizards) may be interpreted through two different hypotheses: (1) an allometric extension of growth and development patterns shared by lacertids, resulting in the expression of these traits only in the largest individuals; (2) a species-specific character of *L. bilineata*, expressed only in adults. In both cases, it can be a non-adaptive consequence, or a selected adaptation associated with specific functions. The first hypothesis seems
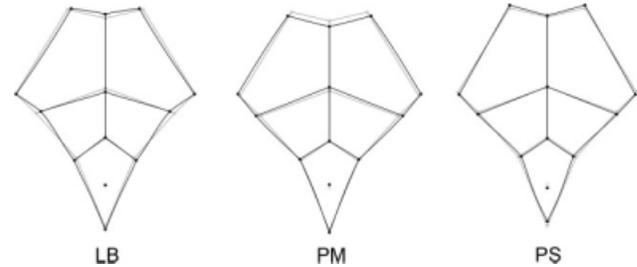

Fig. 8. Mean configuration from the three species (bold links) superimposed on the grand-mean (thin links); LB: *L. bilineata*; PM: *P. muralis*; PS: *P. sicula*.

to be more plausible for osteoderms because they have been found in adults but not in juveniles in a number of lizard species, suggesting that their degree of ossification is related with the developmental phase (Barahona and Barbadillo, 1998; Bever et al., 2005; Arnold et al., 2007). As regards pterygoid teeth, they could prove useful for feeding. On the other hand, for cheek osteoderms the adaptation is less obvious, even if it can be related with a structural protection of the head. Of course, some caution should be taken in interpreting these traits because of limits of the densitometric approach. For example, as regards osteoderms, the development of bone supports can be more or less discrete, through thresholds that could not be detected with the current tomographic parameters.

The Variation of the Fronto-Parietal Structures

The present analysis provides a geometric comparison of the fronto-parietal scale morphology relatively to the position of the fronto-parietal suture. This area is an important source of variation for head bones (Barahona and Barbadillo, 1998) and the head scales (Bruner et al., 2005; Bruner and Costantini, 2007) of lacertids. The fronto-parietal geometry differs between the three species, being most distinct in *P. muralis*. Differences between the average shapes are mainly related with a
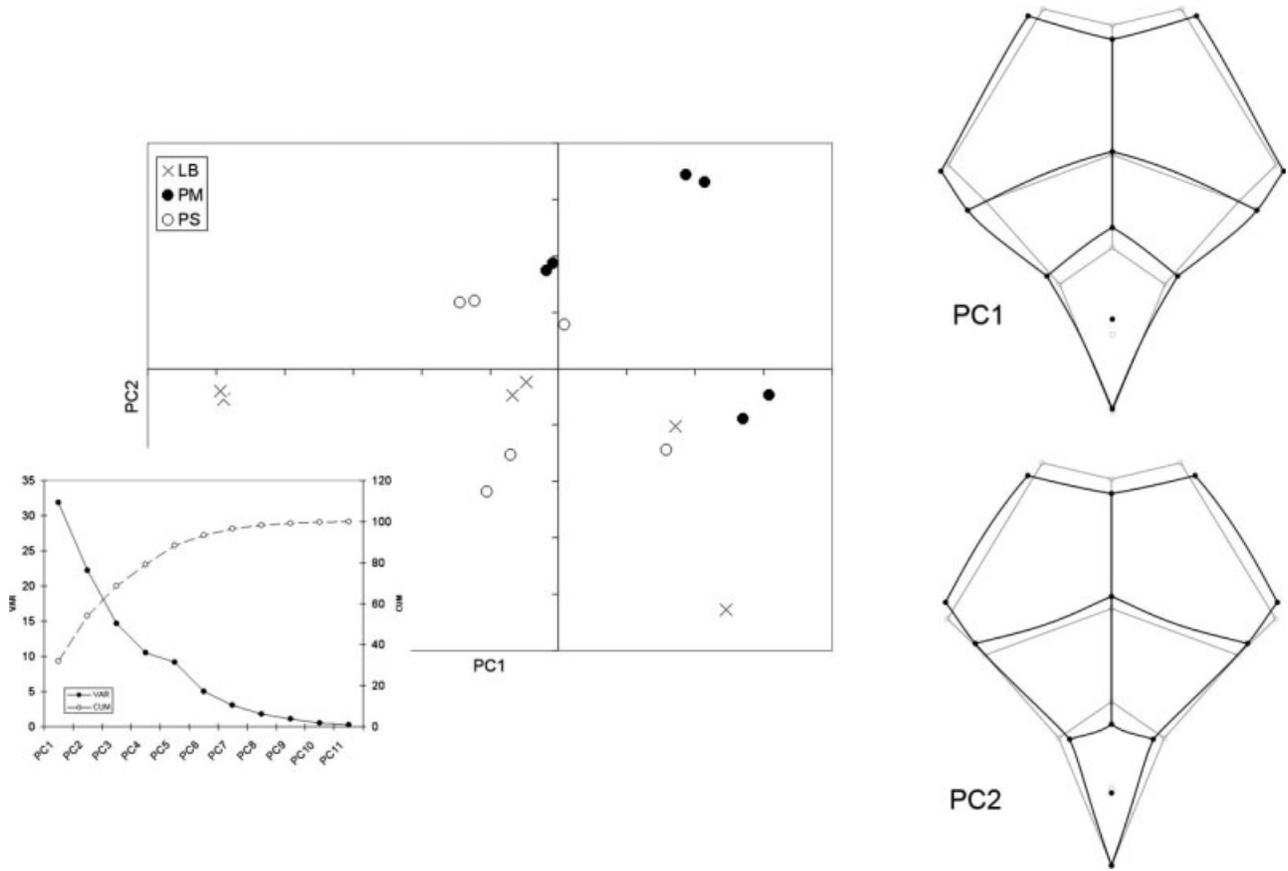
Fig. 9. First and second principal components of shape variation (LB: *L. bilineata*; PM: *P. muralis*; PS: *P. sicula*). The wireframes show the shape changes along the two axes (gray links: negative values; bold link: positive values). The scree plot shows the individual (VAR) and cumulative (CUM) variance for each principal component.

lateral extension of scales, while the intraparietal suture shows no evident variation in its relative position. Hence, it seems that in all three species the suture occupies a similar location relatively to the superficial dermal (scales) spatial organization. We can state that in all three species both the anterior and posterior parts of the fronto-parietal scale (as divided by the suture) have a similar proportion. Even the first principal component, representing the main pattern of covariation between the elements, does not involve any important changes in the relative position of the suture. Apart from rough morphological differences or similarities among groups, we should assume that the covariation patterns observed in a given taxonomical sample is the direct result of the functional matrix generated by the levels of integration between the different anatomical components. Geometric morphometrics allows the recognition and quantification of those morphological vectors associated with spatial organization within a given anatomical model. Accordingly, the resulting patterns can be useful to provide hypotheses on the structural relationships between the morphological elements. Although the vault becomes larger, there is a change in the proportions of the anterior and posterior parts of the fronto-parietal scale: the first increases in length and the second decreases in width. The fronto-parietal scale covers the fronto-parie-

tal suture, which is considered a relevant biomechanical structure for the kinetic organization of the lacertid skull (Frazzetta, 1962). In the species examined in this study, the level of interdigitation at this suture could limit kinetic movements of the skull. However, this suture should be also carefully studied for its possible role as a morphogenetic pace-maker of the head shape during ontogeny, and more detailed studies are needed in this direction. In any case, this preliminary analysis suggests that as the head size increases, the anterior component of the fronto-parietal area undergoes a relative antero-posterior expansion, whereas the posterior one becomes relatively smaller. The expansion of the anterior portion can be interpreted according to three different processes: (1) an active growth of the frontal bone, which presses and displaces the neighboring elements; (2) a passive expansion of the frontal bone, following the separation of neighboring elements because of endocranial growth; (3) an active bone deposition at the anterior edge of the fronto-parietal suture, determining the enlargement and forward displacement of the frontal bone. All these three processes have relevant roles in the cranial morphogenesis (Enlow, 1990). To test these three hypotheses, we will need information on the histological activity at the fronto-parietal suture, and on the dynamics of brain growth through ontogenetic studies.
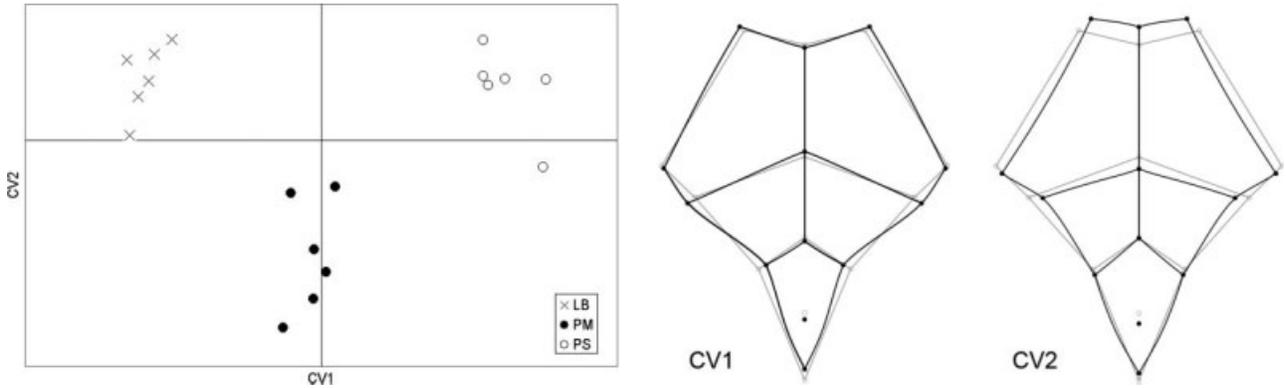
Fig. 10. First and second canonical variates (LB: *L. bilineata*; PM: *P. muralis*; PS: *P. sicula*). The wireframes show the shape changes along the two axes (gray links: negative values; bold link: positive values).
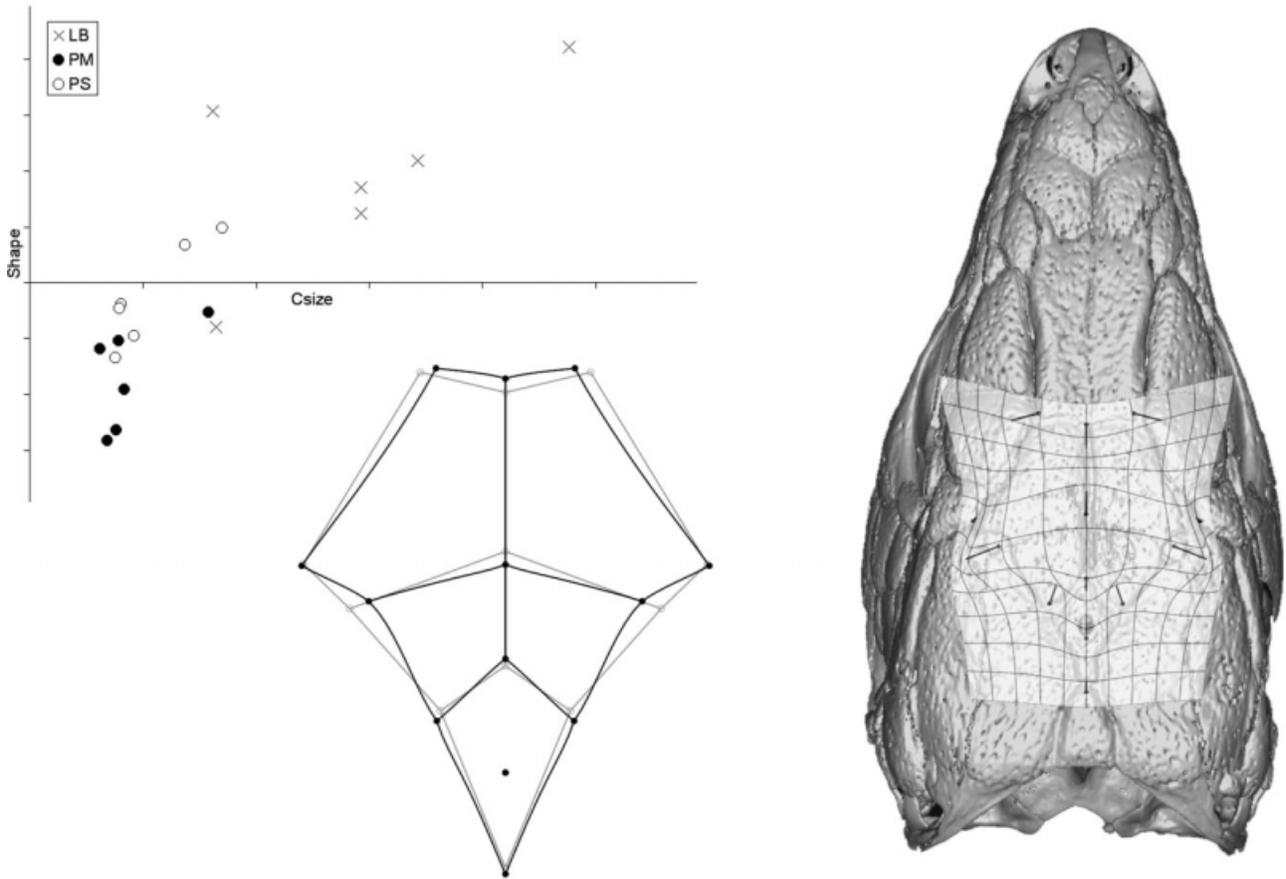

Fig. 11. Centroid size plotted onto the allometric vector, as linear combination of shape changes associated with size variation. (LB: *L. bilineata*; PM: *P. muralis*; PS: *P. sicula*). The wireframe shows the shape variation along the allometric pattern, from smaller (gray links) to larger (bold links) specimens. The deformations grid shows the opposite pattern (from larger to smaller specimens) directly onto a lizard head.

A further element associated with the fronto-parietal structures (in particular with the interparietal scale) is the parietal foramen, projecting into the parietal eye (Eakin and Westfall, 1960). The current analysis suggests that the proportion between length and width of the foramen is generally constant, independently by the foramen size, and that the foramen size does not change in relation to changes in head size. Hence, differences between species may be due to species-specific traits more than to allometric variations. *Lacerta bilineata*

probably shows at the same time specific interparietal scale morphology and long pineal extensions (Bruner and Costantini, 2009). On the other hand, according to the present data, it seems that *P. muralis* may display a different morphology of the parietal foramen when compared with the other two species, having a relatively larger (compared with the head length) and more elongated passage. However, because of the limited sample size in terms of statistical significance and biological representation, the present results should be intended as explorative and descriptive, and larger samples are needed to confirm these quantitative trends.

Biomechanics of Fronto-Parietal Region

The role and function of fronto-parietal region in the development and evolution of reptiles have been of a wide interest among functional morphologists (e.g., Barahona and Barbadillo, 1998; Metzger, 2002; Evans, 2008). This region experiences relatively high mechanical stresses, resulting from the bite force and the muscle forces acting upon the skull (Rayfield, 2005; Moazen et al., 2009). Considering the contiguity of the brain in this region, the role and function of the fronto-parietal suture and the parietal foramen become even more significant. It is widely accepted that the level of interdigitation of fronto-parietal suture may play a pivotal role in the relative movement between the frontal and parietal and this varies between Squamata. The species studied here (*L. bilineata, P. muralis* and *P. sicula*) show a high degree of interdigitation across this suture, and such interdigitation should constrain micro-movements across this joint (see also Frazzetta, 1962; Barahona and Barbadillo, 1998), which can play an important role in releasing the strain generated during biting in this region to protect the braincase (e.g., De Vree and Gans, 1987; Moazen et al., 2009).

Structural differences in the skull roof and fronto-parietal region can affect the local stress distribution around the parietal foramen during biting (Moazen et al., unpublished data). This suggests that there may be a morphogenetic response or adaptation in terms of size and location of the parietal foramen in the skull roof. The differences in size or shape of the parietal foramen described in the present study among these species may be therefore related to the biomechanical framework characterizing the biting performance of each species. This hypothesis requires further investigation.

Allometric changes in the antero-posterior vault proportions described in this study should be also interpreted in terms of biomechanical functions. Such changes are supposed to influence the biomechanical distribution of biting forces, characterizing the different figures recorded for small and large species (Herrel et al., 2004). Mechanical properties of the integument vary in different regions and can be modulated by the internal pressure exerted by the underlying structures (e.g., Wainwright et al., 1978; Hebrank, 1980; Gemballa and Bartsch, 2002; Rivera et al., 2005). To our knowledge, there is no data on the mechanical properties of fronto-parietal scales in lizards, but larger fronto-parietal scales could serve as protective shell for the underlying fronto-parietal suture. Gemballa and Bartsch (2002) suggested that ganoid scales in fishes may be able to damp torsion together with the stratum compactum and internal body pressure. Considering the significance of the fronto-parietal region in the mechanics of the *Lacertids* and *Podarcis* (and, in general, of squamates), fronto-parietal scales may play a role in damping the strain generated during biting or other activities in this region. The study of the relationships between biomechanical performance and fronto-parietal morphology could be intended as a promising topic for future investigations on lizard functional anatomy.

CONCLUSIONS

In this article, we explore through microtomographic reconstructions the cephalic anatomy in three lacertid lizards. Apart from considerations on possible epigenetic traits like pterygoid teeth and specific osteoderms, the results presented here supply information on the spatial relationships between the dermal elements of the upper vault and the underlying bone structures. Our results suggest that the main vault enlargement can be localized at the areas anterior to the fronto-parietal suture, providing further information on the possible morphogenetic dynamics associated with the interaction between scales and bones around this structure.

Because of the limited sample size, this survey should be interpreted as a descriptive study. Further analyses should be aimed at increasing the sample size, both in terms of individuals and species. Further geometrical analysis will need to be provided to take into considerations the relationships between cranial shape, bite parameters, and muscle arrangements. Biomechanical models and histological assessments will further help to move a step ahead within this issue, providing evidence on the biological nature of such morphological variations.

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