Head morphological variation in *Podarcis muralis* and *Podarcis sicula*: a landmark-based approach

Emiliano Bruner, David Costantini*

Abstract. The geometry of the cephalic scales in lizards is easily represented by using landmark based approaches. The cephalic scales may prove useful structures in the investigation of the evolution and morphogenesis of lizards because of their biomechanical relationships with the underlying bones and muscular anatomy. In the present paper the head morphology in *Podarcis muralis* and *Podarcis sicula* is compared by using geometric morphometrics and Euclidean distance matrix analysis. The head shape in these two species is largely influenced by a shared allometric pattern, with *P. sicula* displaying a reduced range of variation. This pattern is probably influenced by the cranial morphogenesis at the fronto-parietal suture, and by the parieto-occipital musculature involved in diet and social behaviours. Minor species-specific differences are evidenced, and should be further investigated.

The dermic scales of reptiles may prove useful as tool in the study of the intra- and inter-species morphological variations (Bruner et al., 2005; Kaliontzopoulou et al., 2007). Many species of lacertids possess well shaped cephalic scales whose geometric properties make them suitable markers for phylogenetic, biogeographic or taxonomic investigations (e.g. Arnold, 1989). Furthermore, the tight relationship between the dermal, muscular, and skeletal systems makes the study of the scales useful in the investigation of growth patterns and morphogenesis.

The diversification of the genus *Podarcis* probably started in the Miocene, with *P. muralis* and *P. waglieriana* separating at about 11 Myrs, from a common group that separated at 15-17 Mys from the *P. sicula* lineage (Oliverio et al., 1998, 2000). In the present work we investigate the causes underlying the morphological variation of the cephalic scales in two small-sized lacertids, namely *Podarcis muralis* (Laurenti, 1768) and *Podarcis sicula* (Rafinesque-Schmaltz, 1810). Such approach allows the description of the patterns of structural covariation which is necessary in order to draw hypotheses on morphogenesis and functional anatomy, representing the first step to delineate the general anatomical model, and the basic phenotypic organisation.

The bilateral configuration used to analyse the geometrical head variation is based on 14 bidimensional landmarks, localised at the boundaries of the frontal, frontoparietal, interparietal, parietal, and occipital scales (fig. 1a). Landmarks were sampled from photographs taken in dorsal view, using tpsDig 1.20 (Rohlf, 1998a). The sample numbers 66 *P. muralis* and 66 *P. sicula*, collected in the surrounding areas of Roma between 2000 and 2005, from 6 different areas. When occurring in the same regions, *P. muralis* is prevalently found in wet and wooded areas, while *P. sicula* prefers open and sunny areas (Capula et al., 1993). The sample includes both specimens photographed in the field and prey remains of Eurasian kestrels (*Falco tinnunculus*). Only adult specimens were considered. Although sex dimorphism can be relevant in lizards, a large percentage of individuals in the current sample cannot be properly *a priori* sexed, because of their incomplete status of preservation. Taking into account that a subjective *a posteriori* sex determination is not recommended to provide an actual analysis of the sexual dimorphism, and considering the main target of the present paper (a descriptive analysis of the main structural patterns in these two species), sexes are pooled.

Coordinates were superimposed using the Procrustes registration (Bookstein, 1991). Shape differences between the two species were tested by using the Integrated Morphology Package (Sheets, 2004). Pairwise comparisons between average configurations were performed by using Morpheus et al. (Slice, 2000). Procrustes superimposition was also used to perform a multivariate ordination analysis according to the principles of geometric morphometrics (Zelditch et al., 2004). A Principal Component Analysis of the shape variables was computed with tpsRelw 1.18 (Rohlf, 1998b). Centroid size and allometric vectors were computed using tpsRegr 1.20 (Rohlf, 1998c), to compare the patterns of static allometry. Size and shape correlation was tested by

---

Ornis italica, Piazza Crati 15, 00199 Roma, Italy
*Corresponding author; e-mail:
david.costantini@uniroma1.it

Amphibia-Reptilia 28 (2007): 566-573

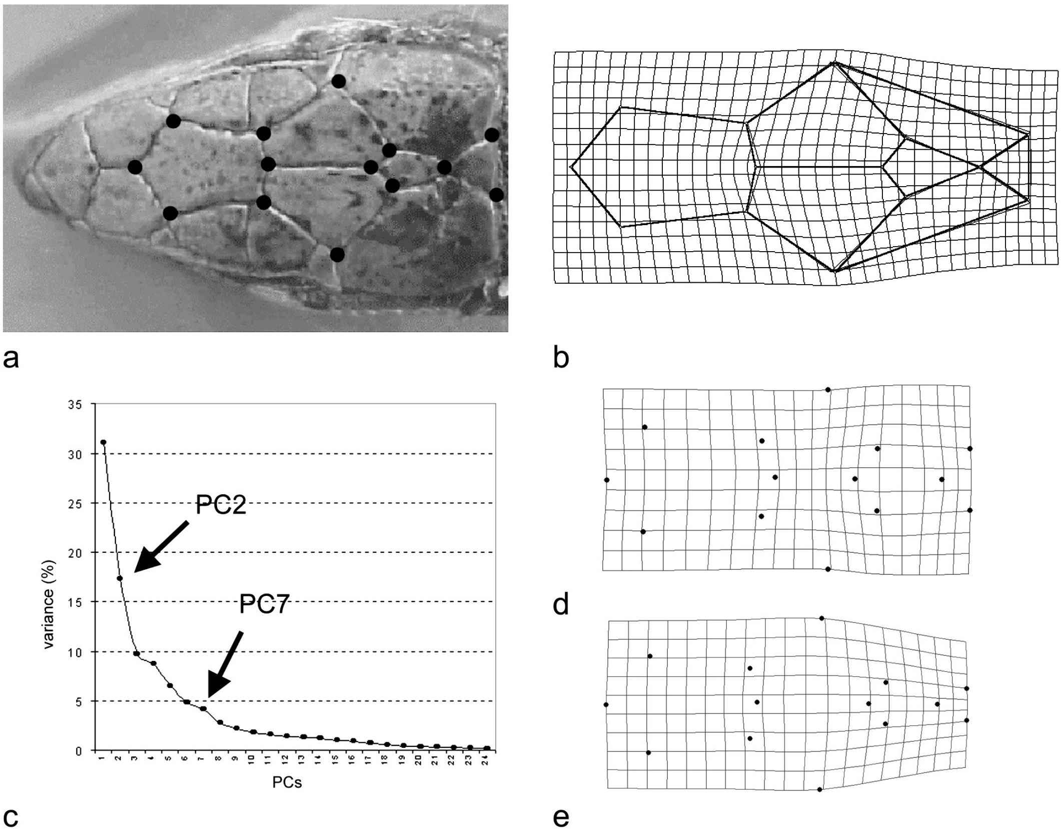

Figure 1. Fourteen two-dimensional landmarks were sampled on the lizard's head using dorsal photographs (a). The average configuration from *Podarcis sicula* (bold links) is superimposed to the average configuration from *Podarcis muralis* (thin links) (b). Spatial differences are visualised using thin-plate spline interpolation grids (magnified ×3). The morphospace computed using the shape variables shows two major axis of variation, and scree after the seventh components (c). The first component (31% of the total variance) is largely size related, accounting for the whole allometric variation (d), while the second (17%) separates *P. sicula* because of occipital and interparietal reduction.

using tpsPLS 1.11 (Rohlf, 2002). APS 2.41 (Penin, 2001) was used to test the contribution of each principal component to the allometric vector through multivariate regression onto centroid size. APS was also used to compute a discriminant analysis between the two species. In the regression and discrimination analyses, only the right hemiconfiguration was used, to exclude redundancies and the correlated problems of matrix algebra associated with the symmetrical geometry. Stand6b (Sheets, 2004) was used to standardise the shape variation of the two species at the average pooled centroid size, according to their species-specific allometric patterns. Univariate and bivariate statistics were computed by using PAST 1.59 (Hammer et al., 2001) and Statistica 5.1 (StatSoft Inc.).

The two species were also analysed by using the Euclidean distance matrix analysis (EDMA; Richstmeier et al., 1992), by visualising the actual differences through Form Difference Maps (Bruner et al., 2005). Form Difference Maps are based on interpolating functions computed at each landmark using the average value of all the ratios describing the variation of the distances from that point to all the others. Maps therefore describe the degree of increasing/decreasing distance between the set of landmarks. They are very effective in showing compression/dilation surface in absolute terms, thus according to the actual form differences. EDMA was performed using WinEdma (Cole, 2002), and maps were computed using Surfer 7.0 (Golden Software, Inc.).

The differences in shape between *P. muralis* and *P. sicula* were significant after Procrustes registration (Goodall test: $F_{14,1820} = 4.18$; $P < 0.001$; permutation test: 1000 permutations, $P < 0.001$). The differences between the mean configurations concern (in *P. sicula*) the reduction of the occipital scale, a minor backward stretching of the interparietal scale, and enlargement of the anterior half of the frontoparietal structures with relative shortening of the frontal scale (fig. 1b). The centroid size is not normally distributed in the pooled sample (Shapiro-Wilk: $P < 0.002$). *P. sicula* displays greater average size (Mann-Whitney U test; $P = 0.02$) but reduced variation (F test: $P = 0.01$; Levene test: $P = 0.003$).

The PCA shows a morphospace largely characterised by the first two components (i.e., a "planar" morphospace; fig. 1c), with a scree plateau after the seventh component. We thus used the first two axes to describe the main variations, and the first seven to analyse the allometric and discriminant vectors. PC1 and PC2 explain 31% and 17% of the total variance, respectively, and together they account for 48% of the morphological variability. Along the PC1 axis, the two groups display the same average value (Mann-Whitney U test; $P = 0.80$) but with a lower variation in *P. sicula* (F-test: $P = 0.01$; Levene test: $P = 0.004$). Along this component the occipital scale shortens, the interparietal enlarges anteriorly, the frontoparietal displays longitudinal compression, and the frontal scale lengthens showing a relative widening of the anterior breadth (fig. 1d). The second component is associated with occipital and interparietal contraction (fig. 1e). Considering the distribution of the two species on this axis, *P. sicula* shows higher average value for this pattern (Mann-Whitney U test; $P = 0.03$) but no differences in the variance (F-test: $P = 0.55$; Levene test: $P = 0.31$).

The shape variables are size-related (Wilk's lambda: $P < 0.001$; Goodall: $F_{14,1820} = 23.40$, $P < 0.001$; permutation test: 1000 permutations $P < 0.001$; $R = 0.70$; fig. 2a). Allometric changes involve occipital stretching, interparietal compression, frontoparietal longitudinal enlargement, and frontal shortening (fig. 2b). A multivariate regression on centroid size using the first seven principal components is significant, but entirely loaded onto PC1 (normalised multivariate correlation coefficient = 0.97; $R^2 = 0.44$, $P < 0.001$). The other components show no relevant contribution to the allometric vector. Along the entire allometric vector, *P. sicula* shows a reduced variation (Levene: $P < 0.05$). The analysis of covariance fails to account for the differences in the overall shape between the two species when the centroid size is taken into account as a covariate ($P = 0.36$). The differences between the two species using only the PC1 values, on the other hand, are significant when corrected by using the centroid size as a covariate ($F_{1,129} = 4.72$; $P = 0.03$). According to the centroid size vs. PC1 major axis regression, similar slopes but different intercepts are identified in the two species using the 95% confidence intervals.

The allometric vectors computed independently for each group show some minor differences (fig. 2c, 2d). In *P. muralis* there is a wider range of allometric variation, the frontal shortening is mostly related to the anterior lateral landmark (leading to a more lance-shaped morphology), the interparietal reduction is loaded onto all the three landmarks, and the occipital stretching is directed backward. In *P. sicula* the allometric range is more limited, the frontal shortening is associated with a more generalised longitudinal reduction, the interparietal compression is more marked at the anterior edge, and the occipital stretching includes a certain narrowing.

Considering the same slopes but different intercepts for these two trajectories, the individuals have been scaled allometrically to a common average pooled centroid size, but according to their species-specific allometric vector. Differences between the two scaled groups are still significant (Hotelling $F_{18,113} = 7.74$; $P < 0.001$; Goodall $F_{14,1828} = 4.94$; $P < 0.001$; permutation $n = 400$; $P = 0.0025$). The shape differences between the two scaled means involve in *P. sicula* the longitudinal frontal shortening, fronto-parietal bending and widening, and occipital compression (fig. 2e). These non-allometric differences are also supported by a discriminant analysis between the two groups performed on the first 7 components ($R^2 = 0.20$, $F_{7,124} = 4.18$, $P < 0.001$), showing in *P. sicula* shorter and more squared frontal scales, larger fronto-parietal anterior areas, anterior re-

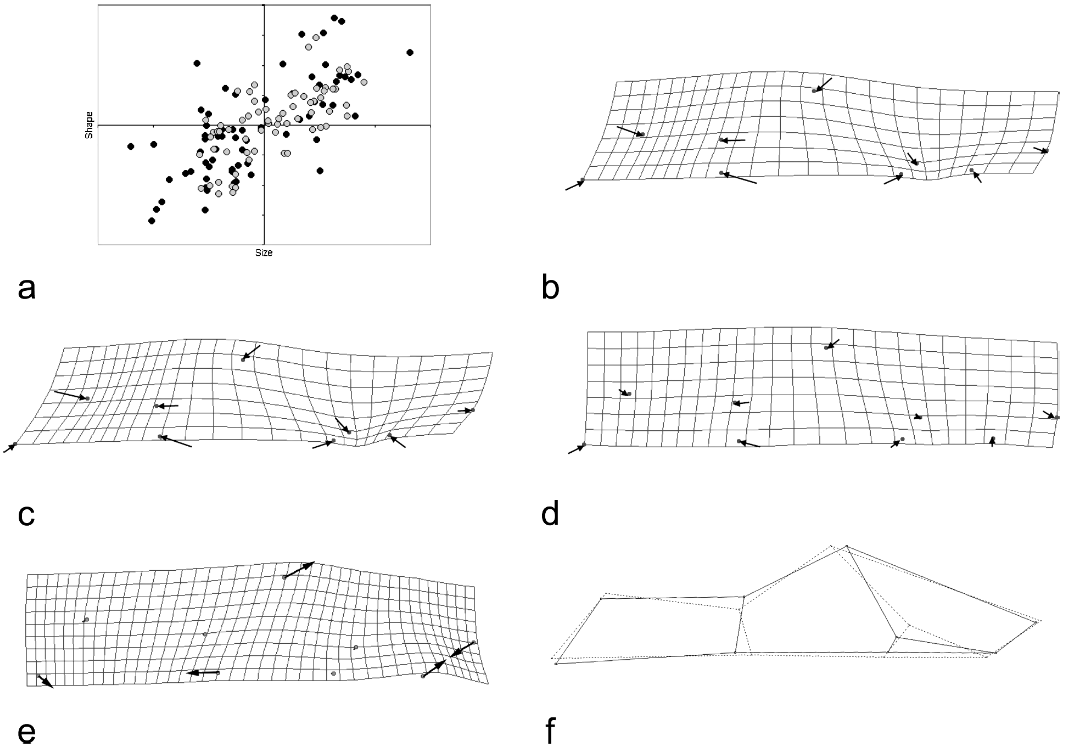

Figure 2. The correlation between the size and shape vectors (a) after partial least-square analysis pooling *P. muralis* (black dots) and *P. sicula* (grey dots) shows a shared allometric component (b). However, the species-specific allometric vectors in *P. muralis* (c) and *P. sicula* (d) display some differences in both magnitude and direction of the vectors. The main non-allometric differences can be visualised comparing the mean shapes after species-specific allometric standardisation (e) and the discrimination vector (f), both from *P. muralis* to *P. sicula*. The analyses and the deformation grids are computed using the right (unilateral) hemi-configuration.

duction of the interparietal scale, and reduced occipital surface (fig. 2f).

The Euclidean distance matrix analysis shows significant differences between the two species (bootstrap T: $P < 0.001$), with *P. sicula* displaying only minor absolute differences in the interlandmarks lengths (mean ratio = 1.07; median: 1.07; SD: 0.03; range: 0.98-1.12). Considering the interlandmark distances which on average are above or below one standard deviation from the mean *P. sicula*/*P. muralis* ratio, *P. sicula* is characterised by a smaller frontal length and occipital width, and larger anterior development of the frontoparietal area. The interparietal scale is more narrow and posteriorly elongated (fig. 3a). The Form Difference Map efficiently synthesises these differences, stressing the frontal relative shortening, occipital reduction, interparietal posterior stretching, and frontoparietal enlargement (fig. 3b). It is worth noting that the mean values are always larger than one (that is, the mean diameters are always larger in *P. sicula*), so differences are related to a larger and smaller degree of lengthening, and no actual shortening takes place.

Although the morphological ranges of variation largely overlap, *P. muralis* and *P. sicula* show differences both in size and shape. *P. sicula* has a larger average size, but *P. muralis* is more variable, at least in the present sample. The reduced variability in *P. sicula* compared to *P. muralis* can be interpreted in two ways: sampling bias or biological signals. In the first case, the use of kestrel prey remains and differential predation may have biased the sample, even if the data available on the kestrel diet preferences

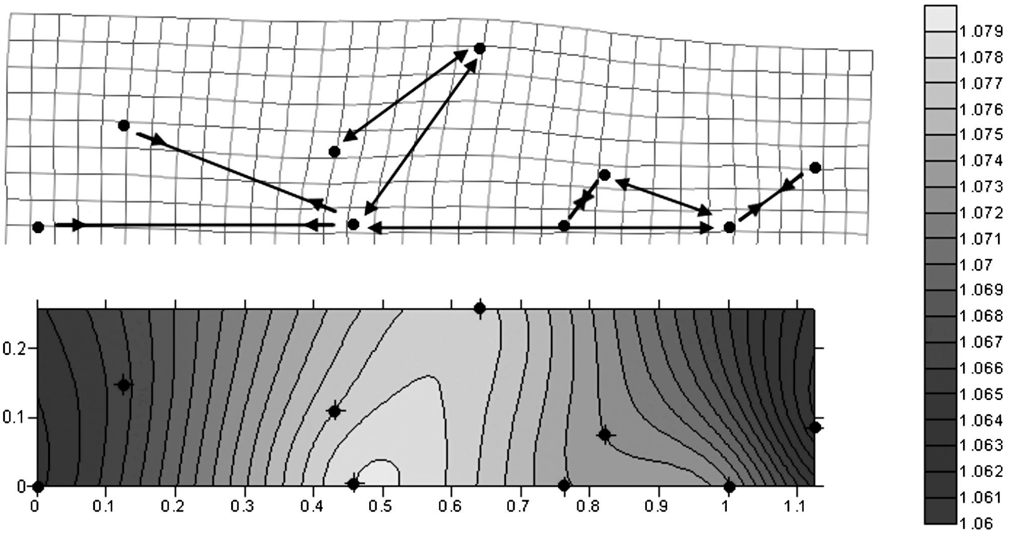

Figure 3. EDMA comparison between *P. muralis* and *P. sicula*; a) the arrows show the most increasing (divergent arrows) and decreasing (convergent arrows) diameters (half configuration) onto the *P. muralis* vs. *P. sicula* deformation grid; b) the Form Difference Map shows the interpolated values (half configuration) using the average ratio for each landmark (dark: shortening; light: lengthening). The scale bar refers to the ratio value (1 means no differences).

of the two species of *Podarcis* do not corroborate this hypothesis (Costantini et al., 2005). If the data is to be interpreted otherwise, on the other hand, the reduced variability might be related to a biological (evolutionary or physiological) signal, which requires further investigation. The fact that *P. muralis* has been hypothesised to display a high degree of phenotypic plasticity because of its variable morphology and molecular homogeneity should be noted (Oliverio et al., 2000), and the hypothesis of actual different degrees of variation between the two species thus finds further evidence.

The mean shape differences are rather small, represented in *P. sicula* by the enlargement of the anterior half of the fronto-parietal scales (involving relative shortening of the frontal area), narrowing of the interparietal scale, and reduction of the occipital scale. The first axis of variation is represented by a shared allometric component, involving frontal shortening, longitudinal stretching of the fronto-parietal scales, compression of the interparietal area, and backward stretching of the occipital structures. The second axis is influenced by species-specific differences associated (in *P. sicula*) with a contraction of the occipital and interparietal areas.

If we visualise the allometric pattern through the vectorial displacement, two blocks can be recognised: a group of anterior landmarks converging towards the centre of the frontal scale, and a group of posterior landmarks involving the compression of the posterior surfaces (fig. 4a). If we visualise the opposite pattern (i.e., from larger to smaller individuals) beyond the actual range of variation, a plane of structural compression crossing the frontoparietal scale is evidenced (fig. 4b). That region covers the fronto-parietal suture of the skull exactly, separating the frontal from the parietal bones (see Barahona and Barbadillo, 1998). As suggested also in the case of *L. bilineata* (Bruner et al., 2005), this suture is clearly a very significant source of morphogenetic variation in the lizard's head morphology along the ontogenetic patterns, and possibly within the evolutionary radiation of this group. The fronto-parietal suture is supposed to be a paedomorphic trait in lizards, whose ontogenetic change extends further than is usual in the primitive lizard taxa (Arnold, 1989). In fact, most advanced lacertids

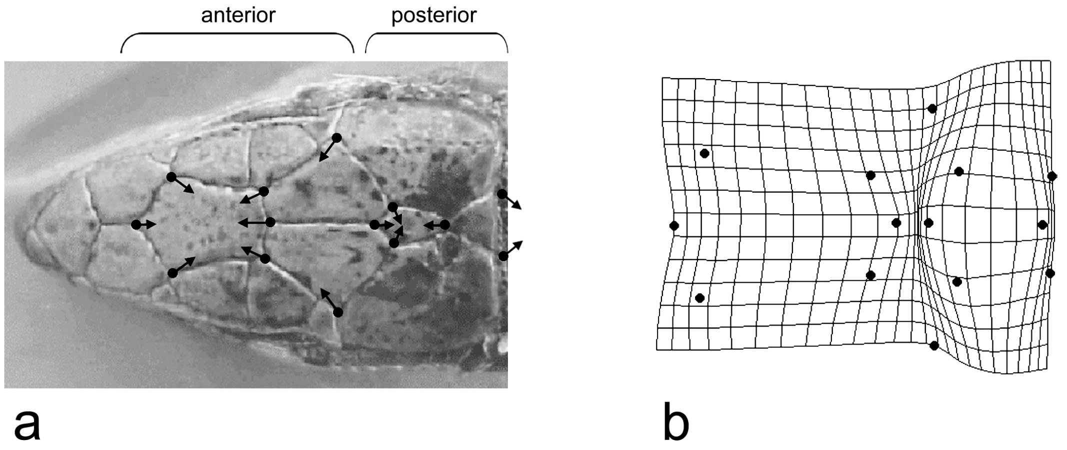

Figure 4. The allometric pattern is visualised through vector displacement onto the head of *P. muralis* (a), showing the anterior and posterior blocks of landmarks. The opposite pattern, visualised through thin-plate spline deformation and exaggerated beyond the variation of the current adult sample, shows a creasing area localised above the fronto-parietal suture (b). This area is supposed to be relevant in the head morphogenesis, as a major source of growth and development.

show a simple fronto-parietal suture similar to states often found in juveniles of more primitive forms. The number and degree of development of the fronto-pairetal interdigitations are also involved in the skull's kinetic. Their increase is known to cause the loss of movement in the mesokinetic axis, a condition which is widespread in lacertid lizards (Arnold, 1993; Barahona and Barbadillo, 1998). It seems therefore that the morphogenetic processes at the fronto-parietal boundary are one major determinant of the allometric variation, possibly related to secondary structural changes such as a relative frontal shortening (anteriorly) and anterior compression of the interparietal area (posteriorly). On the other hand, we hypothesise that the posterior areas (interparietal and occipital regions) are influenced by a second major allometric component, represented by the biomechanical relationship with the associated muscular system. This structural network is characterised by the development of the parietal muscles, the masticatory functions, and the nuchal muscles found in the furthest posterior surfaces (Guibè, 1970; Haas, 1973). A positive allometry for the parietal structures has also been evidenced in large teiids (Monteiro and Abe, 1997), and in the ossification of the lacertid skull in general (Barahona and Barbadillo, 1998). Through the functional interface of the muscular system, diet composition and prey capture are known to be the major factors underlying the lizard skull morphology because of the relationship between mouth size and jaw adduction (Verwaijen et al., 2002; Stayton, 2005). However, both species of *Podarcis* feed mostly on insects, and their diet largely overlaps in terms of prey types (Capula et al., 1993). We recently hypothesised also the possible role of behavioural components related to intra-specific male to male and male to female interactions, jaw biting force, and parieto-occipital musculature in shaping skull morphology (Bruner et al., 2005).

Minor differences between the species-specific allometric trajectories can be nevertheless evidenced. The species-specific allometric vectors suggest differences in the way the frontal scale shortens, and in the changes at the interparietal and occipital areas. Assuming different intercepts but non-significant differences in the slope of the allometric vectors (as supported by the present analysis), the comparison between allometrically-scaled means of the two species stresses the anterior enlargement of the fronto-parietal scale and occipital reduction in *P. sicula*. The discrimination analysis adds also in this species the anterior narrowing of the interparietal scale. Interestingly, all these informations are well synthesised by the form difference maps, accounting for the absolute metric changes.

In the present analysis the different approaches involved suggest that the head morphology in *P. muralis* and *P. sicula* is largely characterised by a common allometric pattern, in which the bone growth at the frontoparietal suture and the development of the occipito-parietal muscular system mould the dermal structures, probably representing the most significant structural determinants of the cephalic scale morphology. Furthermore, *P. muralis* shows a larger degree of variation, and some traits (associated in *P. sicula* with occipital reduction and anterior enlargement of the frontoparietal areas) may be species-specific. These differences will deserve further attention. Clearly the present survey should be integrated with future studies on sexual dimorphism in these two species, that may reveal more subtle and detailed structural trajectories accounting for the intra-specific variation. Furthermore, taking into account the degree of morphological variation in these taxa and the geographical localisation of the current sample, it will be interesting to test possible inter-populational differences. Both species display an ample degree of variability, which has lead to the discussion of a possible subspecific taxonomy, of local endemisms, and of the limited existing knowledge of the genetic boundaries between populations (Corti and Lo Cascio, 1999; Sindaco et al., 2006). Nevertheless, considering the functional relationships between cranial structures and the evolutionary niche in vertebrates, the study of the cephalic scales in lizards should be regarded as a useful case study in the investigation of the network between ontogenetic, ecological, and behavioural components, relying upon the anatomy of elements with a clear geometrical organisation.

Acknowledgements. We are grateful to the scientific association *Ornis italica* for supporting this work, and to ENEL and ACEA for providing the logistic support for the monitoring of the nest-boxes on their utility lines.

References

Arnold, E.N. (1989): Towards a phylogeny and biogeography of the Lacertidae: relationships within an old-world family of lizards derived from morphology. Bull. Brit. Mus. (Nat. Hist.) **55**: 209-257.

Arnold, E.N. (1993): Phylogeny and Lacertidae. In: Lacertids of the Mediterranean region. A biological approach, p. 1-16. Valakos, E.D., Böhme, W., Pérez-Mellado, V., Maragou, P., Eds, Athens, Hellenic Zoological Society.

Barahona, F., Barbadillo, L.J. (1998): Inter- and intraspecific variation in the post-natal skull of some lacertid lizards. J. Zool. **245**: 393-405.

Bookstein, F.L. (1991): Morphometrics tools for landmarks data: geometry and biology. Cambridge, Cambridge University Press.

Bruner, E., Costantini, D., Fanfani, A., Dell'Omo, G. (2005): Morphological variation and sexual dimorphism of the cephalic scales in *Lacerta bilineata*. Acta Zool. **86**: 245-254.

Capula, M., Luiselli, L., Rugiero, L. (1993): Comparative ecology in sympatric *Podarcis muralis* and *P. sicula* (Reptilia: Lacertidae) from the historical centre of Rome: what about competition and niche segregation in an urban habitat? Boll. Zool. **60**: 287-291.

Cole, T.M. III (2002): WinEDMA: Software for Euclidean distance matrix analysis. Version 1.0.1 beta. Kansas City, University of Missouri, Kansas City School of Medicine.

Corti, C., Lo Cascio, P. (1999): I Lacertidi italiani. Palermo, L'Epos.

Costantini, D., Casagrande, S., Di Lieto, G., Fanfani, A., Dell'Omo, G. (2005): Consistent differences in feeding habits between neighbouring breeding kestrels. Behaviour **142**: 1409-1421.

Guibé, J. (1970): La musculature. In: Traité de zoologie, anatomie, systématique, biologie. Reptiles, caractères généraux et anatomie, Tome XIV, fascicule II, p. 144-193. Grassé, P.-D., Ed., Paris, Masson et C^{ie}, Éditeurs, Libraires de l'Académie de Médecine.

Haas, G. (1973): Muscles of the jaws and associated structures in the Rhynchocephalia and Squamata. In: Biology of the Reptilia, Vol. 4, p. 285-490. Gans, C., Parsons, T.S., Eds, London and New York, Academic Press.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001): PAST: Paleontological statistics software package for education and data analyses. Palaeontologia electronica **4**: 1-9.

Kaliontzopoulou, A., Carretero, M.A., Llorente, G.A. (2007): Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* lizards. J. Morphol. **268**: 152-165.

Monteiro, L.R., Abe, A.S. (1997): Allometry and morphological integration in the skull of *Tupinambis merianae* (Lacertilia: Teiidae). Amphibia-Reptilia **18**: 397-405.

Oliverio, M., Bologna, M.A., Monciotti, A., Annesi, F., Mariottini, P. (1998): Molecular phylogenetics of the Italian *Podarcis* lizards (Reptilia, Lacertidae). Ital. J. Zool. **65**: 315-324.

Oliverio, M., Bologna, M.A., Mariottini, P. (2000): Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). J. Biog. **27**: 1403-1420.

Penin, X. (2000): Applied Procrustes Software 2.3.

Richtsmeier, J.T., Deleon, V.B., Lele, S.R. (2002): The promise of geometric morphometrics. Yearbook Phys. Anthrop. **45**: 63-91.

Rohlf, F.J. (1998a): tpsDig 1.20. Ecology and Evolution, SUNY at Stony Brook, NY.

Rohlf, F.J. (1998b): tpsRelw 1.18. Ecology and Evolution, SUNY at Stony Brook, NY.

Rohlf, F.J. (1998c): tpsRegr 1.20. Ecology and Evolution, SUNY at Stony Brook, NY.

Rohlf, F.J. (2002): tpsPLS 1.11. Ecology and Evolution, SUNY at Stony Brook, NY.

Sheets, H.D. (2004): Integrated Morphometrics Package; Dept. of Physics, Canisius College, Buffalo, NY 14208. Available at: http://www.canisius.edu/∼sheets/morphsoft.html.

Sindaco, R., Doria, G., Mazzetti, E., Bernini, F. (2006): Atlas of Italian Amphibians and Reptiles. Firenze, Edizioni Polistampa.

Slice, D. (2000): Morpheus et al. Ecology and Evolution, SUNY at Stony Brook, NY.

Stayton, C.T. (2005): Morphological evolution of the lizard skull: a geometric morphometrics survey. J. Morph. **263**: 47-59.

Verwaijen, D., Van Damme, R., Herrel, A. (2002): Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. Funct. Ecol. **16**: 842-850.

Zelditch, M.L., Swidersky, D.L., Sheets, H.D., Fink, W.L. (2004): Geometric morphometrics for biologists. San Diego, Elsevier.

Received: October 24, 2006. Accepted: March 22, 2007.