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Patterns of cranial ontogeny in lacertid lizards: morphological and allometric disparity

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Abstract

We explored the ontogenetic dynamics of the morphological and allometric disparity in the cranium shapes of twelve lacertid lizard species. The analysed species (*Darevskia praticola*, *Dinarolacerta mosorensis*, *Iberolacerta horvathi*, *Lacerta agilis*, *L. trilineata*, *L. viridis*, *Podarcis erhardii*, *P. melisellensis*, *P. muralis*, *P. sicula*, *P. taurica* and *Zootoca vivipara*) can be classified into different ecomorphs: terrestrial lizards that inhabit vegetated habitats (habitats with lush or sparse vegetation), saxicolous and shrub-climbing lizards. We observed that there was an overall increase in the morphological disparity (MD) during the ontogeny of the lacertid lizards. The ventral cranium, which is involved in the mechanics of jaw movement and feeding, showed higher levels of MD, an ontogenetic shift in the morphospace planes and more variable allometric patterns than more conserved dorsal crania. With respect to ecology, the allometric trajectories of the shrub-climbing species tended to cluster together, whereas the allometric trajectories of the saxicolous species were highly dispersed. Our results indicate that the ontogenetic patterns of morphological and allometric disparity in the lacertid lizards are modified by ecology and functional constraints and that the identical mechanisms that lead to intraspecific morphological variation also produce morphological divergence at higher taxonomic levels.

Introduction

It is a truism in evolutionary biology that an understanding of how ontogeny, phylogeny and ecology mediate morphological diversification is crucial for understanding the process of evolution and that, on morphological grounds, evolution is almost entirely related to changes in size and shape. During ontogeny, the organism changes shape because of changes in the timing or relative growth of its anatomy. The size-related shape changes during developmental time are defined as ontogenetic allometry (Huxley & Tessier, 1936; Klingenberg, 1998; Gayon, 2000). The change in the ontogenetic allometric relations is regarded as one of the main forces that drive morphological evolution (Gould, 1966; Klingenberg, 1998; Frankino *et al.*, 2005; Wilson & Sanchez-Villagra, 2010, 2011). Therefore, a relationship between the changes in ontogenetic trajectories and morphological disparity (MD) is expected. 'Morphological disparity', a term primarily used to describe variations among body plans (Runnegar, 1987), is commonly used in a broader manner to denote a measure of morphological diversity (Gould, 1989, 1991; Foote, 1997; Gavrilets, 1999; Zelditch *et al.*, 2003b). Complementary to MD, the concept of allometric disparity was proposed as a measure of the diversity of ontogenetic trajectories (Gerber *et al.*, 2008; Wilson & Sanchez-Villagra, 2010).

Many recent studies have focused on the phenomenon of evolving ontogenetic trajectories (Solignac *et al.*, 1990; Klingenberg & Froese, 1991; Klingenberg & Spence, 1993; Mitteroecker *et al.*, 2005; Wilson & Sanchez-Villagra, 2010, 2011; Piras *et al.*, 2011). In general, allometric scaling relationships are considered to be phylogenetically stable (West *et al.*, 1997; Gould, 2002), that is, the directions of the ontogenetic trajectories in

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more closely related taxa should be conserved (Weston, 2003; Frédérich & Sheets, 2010; Wilson & Sanchez-Villagra, 2010). However, recent studies of various vertebrate taxa showed that the ontogenetic allometry could be considerably variable (Klingenberg & Froese, 1991; Zelditch *et al.*, 2003b; Kaliontzopoulou *et al.*, 2008; Adams & Nistri, 2010; Klingenberg, 2010; Frédérich & Vandewalle, 2011; Wilson & Sanchez-Villagra, 2011), even on the intraspecific level (Solignac *et al.*, 1990; Kaliontzopoulou *et al.*, 2010; Rodriguez-Mendoza *et al.*, 2011). Additionally, the allometric diversity tends to be increased between different ecomorphs (Zelditch *et al.*, 2003b; Gerber *et al.*, 2007; Frédérich & Vandewalle, 2011).

To explore the relationship between ontogenetic allometry and MD at higher taxonomic levels, we analysed the variation in cranium shape in a group of diverse lacertid lizard species, representing multiple genera. The lacertid lizard cranium is a good model system for the study of ontogeny and morphological and allometric disparity because (i) lacertids share common cranial morphology (Arnold, 1973, 1989; Arnold *et al.*, 2007) and (ii) the morphology of the cranium exhibits important information related to refuge use and the jaw movement mechanics involved in feeding, competitive, reproductive and antipredatory behaviour (e.g. Herrel *et al.*, 1996, 1999, 2001). Therefore, even small differences in the cranium morphology may have important biomechanical and ecological implications (Herrel *et al.*, 1999, 2001, 2007; Verwaijen *et al.*, 2002; Lailvaux *et al.*, 2004).

The selected sample consists of 12 species representing different phylogenetic lineages within the family Lacertidae: *Darevskia praticola* (Eversmann 1834), *Dinarolacerta mosorensis* (Kolombatović 1886), *Iberolacerta horvathi* (Méhely 1904), *Lacerta agilis* (Linnaeus 1758), *Lacerta trilineata* (Bedriaga 1886), *Lacerta viridis* (Laurenti 1768), *Podarcis erhardii* (Bedriaga 1882), *Podarcis melisellensis* (Braun 1877), *Podarcis muralis* (Laurenti 1768), *Podarcis sicula* (Rafinesque 1810), *Podarcis taurica* (Pallas 1814) and *Zootoca vivipara* (Von Jacquin 1787). Although intensively studied, the phylogenetic relationships between and within genera are uncertain with numerous cases of hidden diversity and paraphyly. Even after numerous reassessments with different molecular markers included, the polytomy of the main clades remained unresolved (Fu, 1998, 2000; Harris *et al.*, 1998; Arnold *et al.*, 2007; Mayer & Pavličev, 2007; Pavličev & Mayer, 2009; Kapli *et al.*, 2011). The position of the genera *Dinarolacerta*, *Lacerta* and *Podarcis* has been particularly problematic (Godinho *et al.*, 2005; Harris *et al.*, 2005; Podnar *et al.*, 2005, 2007; Poulakakis *et al.*, 2005; Pavličev & Mayer, 2009). The four alternative cladograms of the six studied genera based on morphology and different DNA markers are given in Figure S1.

Regardless of their taxonomic position, the analysed species have different habitat preferences and belong to different ecomorphotypes (Arnold, 1987, 1998). Morphologically, the saxicolous species (such as *D. mosorensis* and *I. horvathi*) tend to be dorsoventrally flattened with a pronounced flat cranium morphotype, whereas the terrestrial and shrub-climbing species (the *D. praticola*, *Z. vivipara*, *Lacerta* spp. and most of the *Podarcis* species) tend to have laterally compressed, cylindrical bodies with deep, robust crania (Arnold, 1973, 1987, 1989; Vanhooydonck & Van Damme, 1999).

Our study had three main aims: (1) to explore the MD of cranium shape across the ontogenetic stages; (2) to explore whether the patterns of MD could be explained by changes in the ontogenetic trajectories; and (3) to explore the species-specific ontogenetic trajectories of cranium shape changes and to test whether a similar pattern of ontogenetic shape changes is present across the different species within genera, between genera and across different ecomorphs.

Materials and methods

Samples

A total of 670 specimens (237 neonatal juveniles, 218 adult females and 215 adult males) representing 6 genera and 12 lacertid species that inhabit the Balkan Peninsula were used in this study. The analysed specimens were from the Herpetological Collections of the Institute for Biological Research, 'Siniša Stanković', Belgrade; the Herpetological Collections of the Natural History Museum of Montenegro, Podgorica; the Science and Research Centre of Koper, Slovenia; and the State Institute for Nature Protection of Croatia, Zagreb (Table S1). By choosing neonates as the starting point and adults as the final point of the ontogenetic trajectory, we captured the shape changes during the complete post-natal morphological development. According to the available data, the sexual dimorphism in lizards is not pronounced at the early juvenile stage (Cooper & Vitt, 1989; Andrews & Stamps, 1994; Watkins, 1996). The level of sexual dimorphism in the cranium shape at the older juvenile and subadult stages was determined for the *Podarcis* spp. lizards (Kaliontzopoulou *et al.*, 2008). Because the preliminary exploratory analyses in our study showed that neonates are morphologically uniform, we decided to unite the neonates as a group within each species. For the adults, the sexual maturity of the specimens was determined through an analysis of previously described reproductive characters (Bejaković *et al.*, 1996; Ljubisavljević *et al.*, 2010b).

According to Arnold (1998), the analysed species can be grouped into three different ecomorphotypes: (i) terrestrial lizards that inhabit vegetated habitats (*D. praticola*, *L. agilis*, *Z. vivipara*, *P. melisellensis*, *P. sicula* and *P. taurica*); (ii) shrub-climbing or semi-arboreal species (*L. trilineata* and *L. viridis*); and (iii) saxicolous species that prefer vertical habitats, such as rocks, boulders and walls (*D. mosorensis*, *I. horvathi*, *P. erhardii* and *P. muralis*). According to a detailed ecological survey of the Balkan Peninsula lacertids (Arnold, 1987) and our fieldwork observations, we divided the terrestrial lacertids into two distinct groups. The first group consisted of the ground-dwelling lizards, which prefer lush vegetation and overgrown terrain in humid (*D. praticola* and *Z. vivipara*) or moderately dry conditions (*L. agilis*). The members of the second group prefer fairly dry, open terrains (dry meadows, steppes, agricultural land and open, rocky terrains) with low vegetation and scarce bushes (*P. melisellensis*, *P. sicula* and *P. taurica*). An overview of sample sizes, sampling locations and habitat preferences is provided in Table 1.

Cranium preparations and landmarks

Following a procedure provided by Dingerkus & Uhler (1977), the crania of all specimens were cleared with trypsin and KOH, stained with Alizarin Red S and preserved in glycerol. Images of the neonate crania were obtained with a Nikon SMZ800 stereoscopic zoom microscope (Nikon Instruments, Europe, B.V.) with a Moticam 2000 digital camera (resolution 2.0 MP, Motic Group Co., Ltd. Xiamen, China). Images of the adult crania were obtained with a Sony DSC-F828 digital camera (resolution 8.0 MP; Sony Corp., Tokyo, Japan). Each cranium was submerged in glycerol with the parietal (dorsal cranium) or palate (ventral cranium) side positioned parallel to the photographic plane in the centre of the optical field. The distance to the stereomicroscope or camera lens was consistent to minimize and equalize the distortion and image aberration because of parallax (Mullin & Taylor, 2002). The 25 two-dimensional landmarks on the dorsal cranium and 34 landmarks on the ventral cranium were digitized (by A.U.) using TpsDig software (Rohlf, 2005). The main criteria for the choice of these landmark configurations were the presence of the landmarks in all of the specimens, their reliability in providing an adequate summary of the cranial morphology and a concordance with previous studies of cranial size and shape variation in lacertid lizards (Ljubisavljević *et al.*, 2010a, 2011; Urošević *et al.*, 2012) (Fig. 1; Table S2).

Statistical analyses

A generalized Procrustes analysis (GPA) was applied to obtain a matrix of Procrustes coordinates, from which the differences introduced by position, scale and orientation were removed (Rohlf & Slice, 1990; Bookstein, 1996; Dryden & Mardia, 1998). To explore the variation in cranial shape, we used the averages of the original

Table 1 The sample sizes, locations and habitat preferences. The neonatal specimens were hatched in the laboratory. The gravid females from natural populations were transferred to the laboratory to lay eggs. The eggs were incubated in the laboratory and initially used for studies of reproductive biology (Bejaković *et al.*, 1996; Ljubisavljević *et al.*, 2007, 2008, 2010b, 2012; Tomašević-Kolarov *et al.*, 2010).

Species	Neonate	♀	♂	Locality	Hab.
D. praticola	25	23	23	Serbia: Vršac Mountains	ter 1
D. mosorensis	16	20	20	Montenegro: Mt. Lovćen	sax
I. horvathi	15	10	9	Slovenia: Mangart Saddle, Predel, Kluže, Tabor Rock	sax
L. agilis	25	18	20	Serbia: Kovin, Mt. Zlatibor; FYR Macedonia: Mt. Šara, Mt. Korab, Dešat	ter 1
L. trilineata	13	5	8	Montenegro: Kotor, Lake Skadar, Mt. Rumija, Lastva Grbaljska, Sutorman, Orljevo; FYR Macedonia: Mariovo	sarb
L. viridis	25	19	12	Serbia: Deliblato Sands	sarb
P. erhardii	16	28	29	Serbia: Pčinja	sax
P. melisellensis	19	19	18	Montenegro: Lake Skadar	ter 2
P. muralis	25	26	26	Serbia: Belgrade	sax
P. sicula	26	11	19	Croatia: Zagreb; Krk island	ter 2
P. taurica	17	26	24	Serbia: Deliblato Sands	ter 2
Z. vivipara	15	13	7	Serbia: Mt. Stara Planina	ter 1

Habitat code: ter 1 = terrestrial, overgrown habitats, lush vegetation; ter 2 = terrestrial, low, sparse vegetation, dry habitats; sarb = shrub-climbing (semi-arboreal); sax = saxicolous.

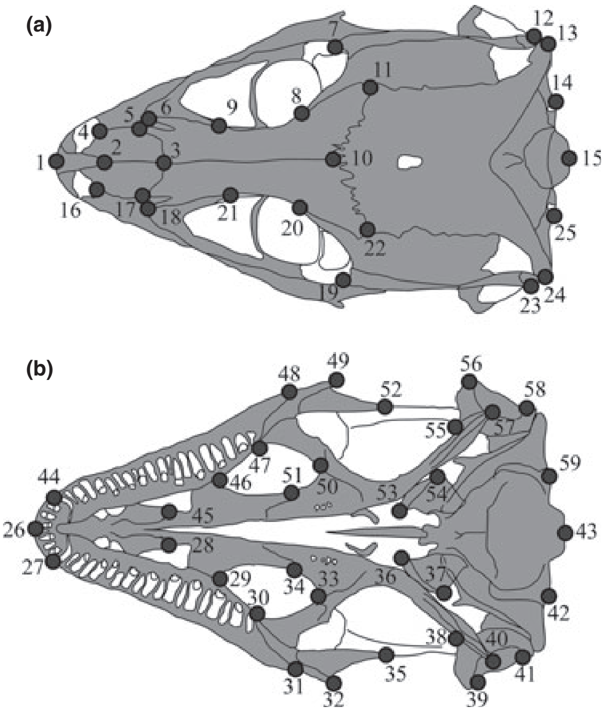

Fig. 1 The 52 symmetric and 7 median landmarks digitized on the (a) dorsal and (b) ventral side of the cranium.

and mirrored configurations of each specimen, which constitute the symmetric component of shape variation (Klingenberg *et al.*, 2002). The shape variables (the symmetric component of shape variation) were obtained using MorphoJ software (Klingenberg, 2011). We used centroid size (CS) as a measure of size, which was calculated as the square root of the summed squared distances of each landmark from the centroid of the form. Centroid size is uncorrelated with shape in the absence of allometry (Bookstein, 1991).

Differences in size and shape and the test for phylogenetic signal

Because the significant sexual dimorphism in cranium size and shape characterizes the lacertid lizards at the adult stage (Herrel *et al.*, 1999, 2001; Verwaijen *et al.*, 2002; Ljubisavljević *et al.*, 2010a, 2011), we performed all analyses of shape variation on adult females and males separately. The differences in the sizes between species were tested by a one-way ANOVA on the CS for neonates, females and males separately. To test for variation in the cranium shape, we performed a MANOVA on the shape variables and species as a factor for each group (neonates, females and males) separately.

We conducted a principal component analysis (PCA) of the covariance matrix of shape variables for each group separately to explore the variation in cranium shape among the 12 species and to visualize the morphospaces. The PCA was performed using MorphoJ software (Klingenberg, 2011).

To test for the phylogenetic signal, we used four alternative trees given in Figure S1, which were all published phylogenies trimmed only to include the six genera studied (see Fig. S1 for detailed description). We applied the criteria of squared-change parsimony to reconstruct the values of the internal nodes of the four phylogenies from the shape averages of the terminal taxa and tested for phylogenetic signal using a permutation approach to simulate the null hypothesis of the complete absence of phylogenetic structure by randomly reassigning shape configurations to the terminal nodes of the phylogeny (see Klingenberg & Gidaszewski, 2010 and references therein). We also tested the effect of phylogenetic uncertainty for our set of four trees following a procedure described by Klingenberg *et al.* (2012).

Morphological disparity

Morphological disparity can be quantified as the empirical distribution of taxa in morphospace (Foote, 1992). The MD was calculated by the following equation:

$$\mathrm{MD} = \Sigma d_{\mathrm{i}}^2/(N-1)$$

where d_{i} is the Procrustes distance of the mean shape of species i from the overall centroid of shape (i.e. the grand mean shape of all species) and $N =$ the total number of all species (Foote, 1993; Zelditch *et al.*, 2003b). MD was calculated for the neonatal juveniles and adult females and males separately. The disparity levels were also calculated for genera represented by more than one species (*Lacerta* spp. and *Podarcis* spp.) and for each of the four ecomorphs. All of the calculations were made with the DisparityBox program, IMP software (Sheets, 2000).

We tested the similarities between the morphological spaces of the dorsal and ventral cranium occupied by the neonates, females and males, using the PCA-based method developed by Mezey & Houle (2003), to further illustrate the pattern of disparity on a family level. The morphospace of each group is represented as a plane defined by the initial two PC axes. The null hypothesis states that the observed angle between the planes of two groups is within the range of angles observed within one or both of the groups individually. To determine the within-group angles, PCAs were performed for each group independently, and the ranges of angles that could be generated within a group were obtained by resampling (900 bootstraps). The observed between-group angle was then compared with the within-group angles. If the between-group angle exceeded the 95% range of the within-group angles for both groups generated by the bootstrap procedure, then we can assert that the observed angle between the planes is significant and that the patterns of variation shift during ontogeny. The confidence interval of the between-group angle was obtained by resampling (300 bootstraps). The angles between the planes were calculated by Space-Angle, IMP software (Sheets, 2000).

Ontogenetic allometry

The analyses of ontogenetic allometry were performed using a multivariate regression of the shape variables on the CS. The null hypothesis states that size and shape are independent. The statistical significance and visualization of allometric shape changes were obtained with the MorphoJ software (Klingenberg, 2011). The regression rates and correlation coefficients (R^2) were obtained by the Regress6k program, IMP software (Sheets, 2000).

The divergence in the ontogenetic allometric trajectories of the different species and ecomorphs was analysed using a multivariate analysis of covariance (MANCOVA) with the species or ecomorph (habitat code) as the factors and the CS as a covariate. A significant species × CS or habitat × CS interaction would indicate that the species- or ecomorph-specific allometric slopes significantly diverge. We also tested the differences in static allometric slopes between neonates and adults of different species with age and species as the factors and the CS as a covariate. The significant species × age × CS interaction would indicate that age-specific allometric slopes are also species-specific and significantly diverge.

The divergence between the species-specific allometric slopes was further explored by comparing the angles between the vectors of allometric coefficients. This angle was calculated as the arccosine of the signed inner products between the normalized regression vectors. The between-group angle was then compared to the upper 95% confidence interval of the within-group angle ranges obtained by bootstrapping (900 runs). If the between-group angle exceeds the 95% confidence interval of the two within-group angles, the difference is judged to be statistically significant at the 5% level (Zelditch *et al.*, 2003a, b, 2004). Pairwise comparisons of allometric vectors were made using the VecCompare program, IMP software (Sheets, 2000).

Allometric disparity

To visualize the allometric disparity, we summarized the interspecific dissimilarity between the ontogenetic allometry trajectories (presented as a pairwise comparison of the ontogenetic trajectory angles between species) with scatter plots calculated using nonmetric multidimensional scaling (NMDS) on the previously calculated angle matrices (Frédérich & Vandewalle, 2011). An NMDS analysis was performed with the PAST statistical package (Hammer *et al.*, 2001). To visualize the optimal separation of the allometric trajectories based on habitat preference, we conducted a canonical variate analysis (CVA) on the NMDS scores for all three axes based on the *a priori* assignment of the habitat preference groups (Wilson & Sanchez-Villagra, 2010).

The standard statistical analyses (ANOVA, MANOVA, MANCOVA and CVA) were performed with the Statistica software package (Statistica for Windows; StatSoft, Inc., Tulsa, OK).

Results

Statistically significant differences were observed for the cranium size between the species for neonates, females and males (ANOVA, $P < 0.0001$ for all comparisons, Table S3). The species also significantly differed in cranium shape (MANOVA, $P < 0.0001$ in all comparisons, Table S4).

A PCA of the dorsal cranium shape variables showed that *Z. vivipara*, *I. horvathi* and *D. praticola* were gradually separated from *L. trilineata*, *L. viridis* and *P. taurica* at the neonatal stage. The shape changes were related to a general narrowing of the cranium, an elongation of the mid-face, a posterior movement of the fronto-parietal suture and a reduction in the cranium base (Fig. 2). *Lacerta agilis* and *L. viridis* were also characterized with the widest crania, an elongated mid-face and posteriorly moved fronto-parietal suture, whereas *P. muralis* and *P. erhardii* had narrower crania, a shorter mid-face and anterior fronto-parietal suture (Fig. 2).

The general pattern of shape variation appeared to be preserved throughout ontogeny; the pattern of the dorsal cranium shape variation in females and males corresponded to the pattern in neonates. The main difference was that the shrub-climbing species were completely separate from the rest of the species at the adult stage (Fig. 2).

Podarcis erhardii, *P. melisellensis* and *P. taurica* clustered together at the neonate stage for the ventral cranium. These species had narrow crania, reduced occipitals and enlarged jaw adductor muscle chambers compared to *I. horvathi* and *L. agilis* that possessed wide crania, enlarged occipitals and small jaw adductor muscle chambers. *Darevskia praticola* and *Dinarolacerta mosorensis* had shorter and narrower crania, enlarged occipitals and smaller jaw adductor muscle chambers compared to *L. trilineata* and *L. viridis* that possessed elongated crania, a wide jugal region, reduced occipitals and enlarged jaw adductor muscle chambers (Fig. 3). For the ventral cranium of the females, the PC1 described a gradient from *L. trilineata* with an elongated cranium, a wide jugal region, a reduced cranial base and an enlarged jaw adductor muscle chamber to *I. horvathi, D. mosorensis* and *Z. vivipara* with short crania, narrow jugal regions, enlarged cranial bases and reduced jaw adductor muscle chambers. The PC2 described a gradient from *P. muralis* and *P. sicula* with narrow crania, enlarged cranial bases and reduced jaw adductor muscle chambers to *L. agilis* with a wide cranium, reduced cranial base and enlarged jaw adductor muscle chambers. *L. trilineata* and *L. viridis* were well separated along the PC1 (Fig. 3). The pattern of the ventral cranium shape variation in males was similar to the pattern observed in females. The shrub-climbing species were well separated from the other species (Fig. 3).

We found no evidence for phylogenetic signal in the shape of dorsal and ventral crania for any of the four alternative trees ($P > 0.05$; see Table S5). Because the range of P-values obtained by permutation test for all four alternative trees was nonsignificant, we could rule out phylogenetic uncertainty as an explanation for the nonsignificant results.

Morphological disparity

Comparisons of the morphological disparities for all 12 species showed that males had a significantly higher disparity of the dorsal cranium than neonates. For the ventral cranium, both females and males had a statistically higher MD than the neonates. The sexual dimorphism in the MD was also statistically significant with males being more disparate than females (Fig. 4a).

The MD of the *Podarcis* species was significantly lower than the disparity level calculated for all 12 species (for both the dorsal and ventral crania). The disparity level

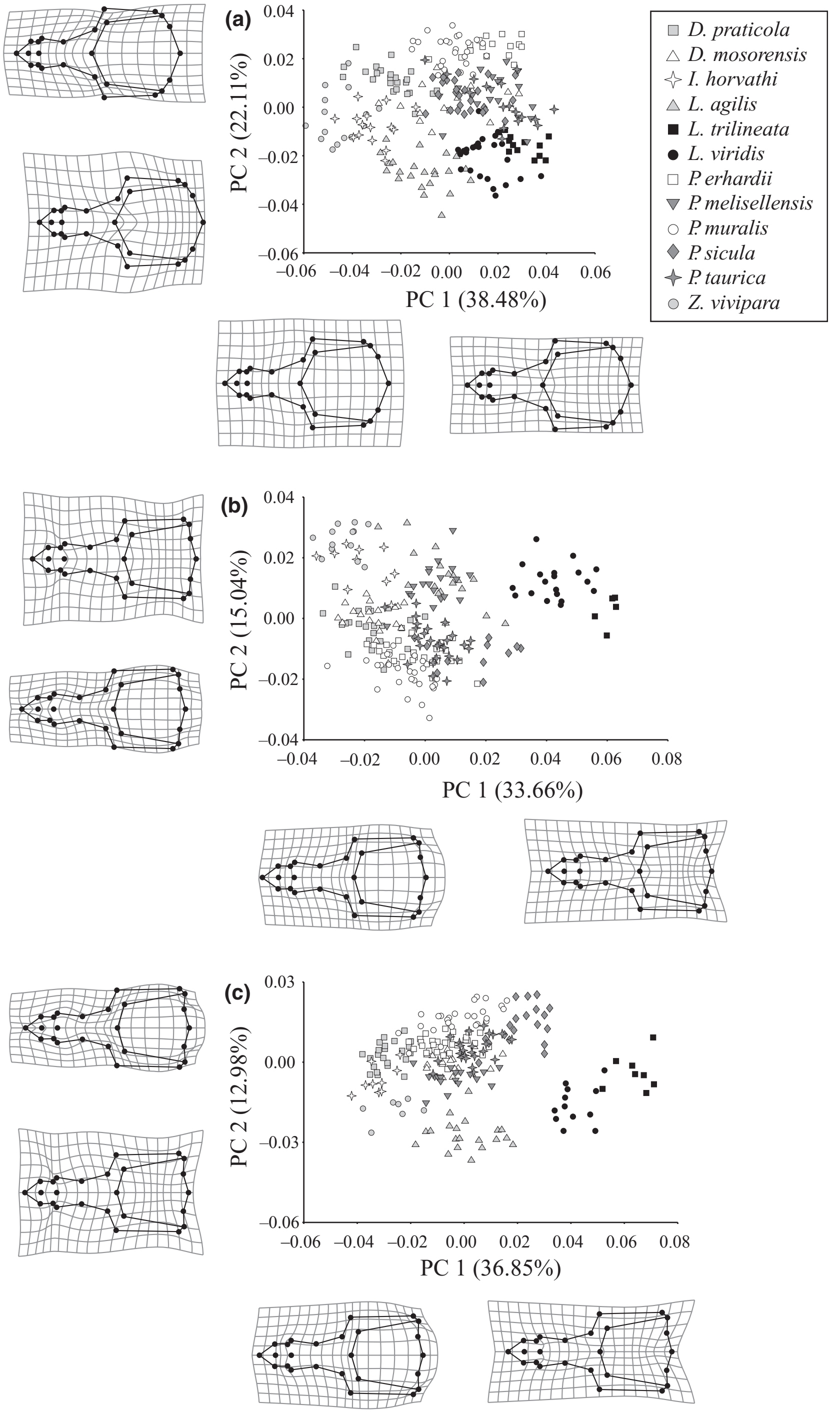

Fig. 2 The morphospaces of the dorsal cranium of the (a) neonates, (b) females and (c) males obtained by the principal component analysis (PCA). The deformation grids illustrate the shape changes associated with the first two PC axes. The line between landmarks 13, 11, 10, 22 and 24 defines the parietal bone. The habitat preferences are represented by colour code: empty symbols = sax, light grey fill = ter1, dark grey fill = ter2 and black symbols = sarb.

of the *Lacerta* species for the dorsal cranium was not significantly different from the dorsal cranium disparity level calculated for the 12 analysed species, except for the lower disparity level of the neonates. The disparity level of the ventral cranium of the *Lacerta* species was significantly smaller than the disparity level of the ventral cranium calculated for the 12 analysed species (Fig. 4a).

The shrub-climbing (sarb) and terrestrial species that inhabit terrains with low vegetation (ter 2) had low disparity levels. The terrestrial species that inhabit densely vegetated habitats (ter 1) had a higher level of disparity

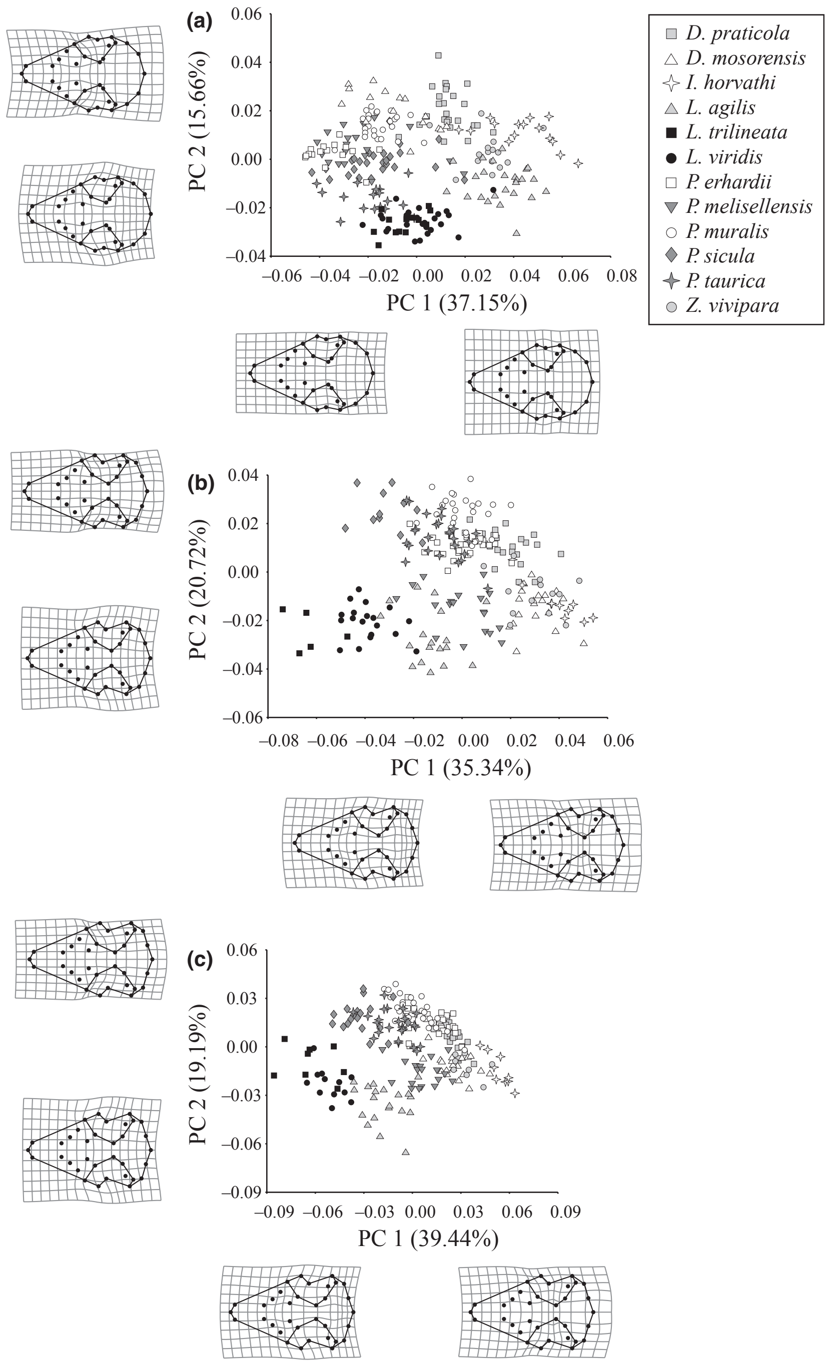

Fig. 3 The morphospaces of the ventral cranium of the (a) neonates, (b) females and (c) males obtained by the principal component analysis (PCA). The deformation grids illustrate the shape changes associated with the first two PC axes. The lines between landmarks 31, 32, 33, 35, 36, 37, 39 and 40 as well as 48, 49, 50, 52, 53, 54, 56 and 57 define the jaw adductor muscle chambers. The habitat preferences are represented by colour code: empty symbols = sax, light grey fill = ter1, dark grey fill = ter2 and black symbols = sarb.

than the previous two groups, and in all three habitat groups, the disparity level increased from the neonatal to the adult stage. However, the level of MD decreased in females for the saxicolous (sax) group (Fig. 4b).

The difference between the plane angles was statistically significant for the ventral cranium between the neonates and adult females and males. No differences were observed for the dorsal cranium (Table 2).

Ontogenetic allometry

The species × CS interaction was significant for females and males and for both the dorsal and ventral cranium sides, which indicated that the ontogenetic trajectories of the females and males were species-specific (Table 3).

The habitat × CS interaction was also significant in both sexes. This result indicated that the ontogenetic

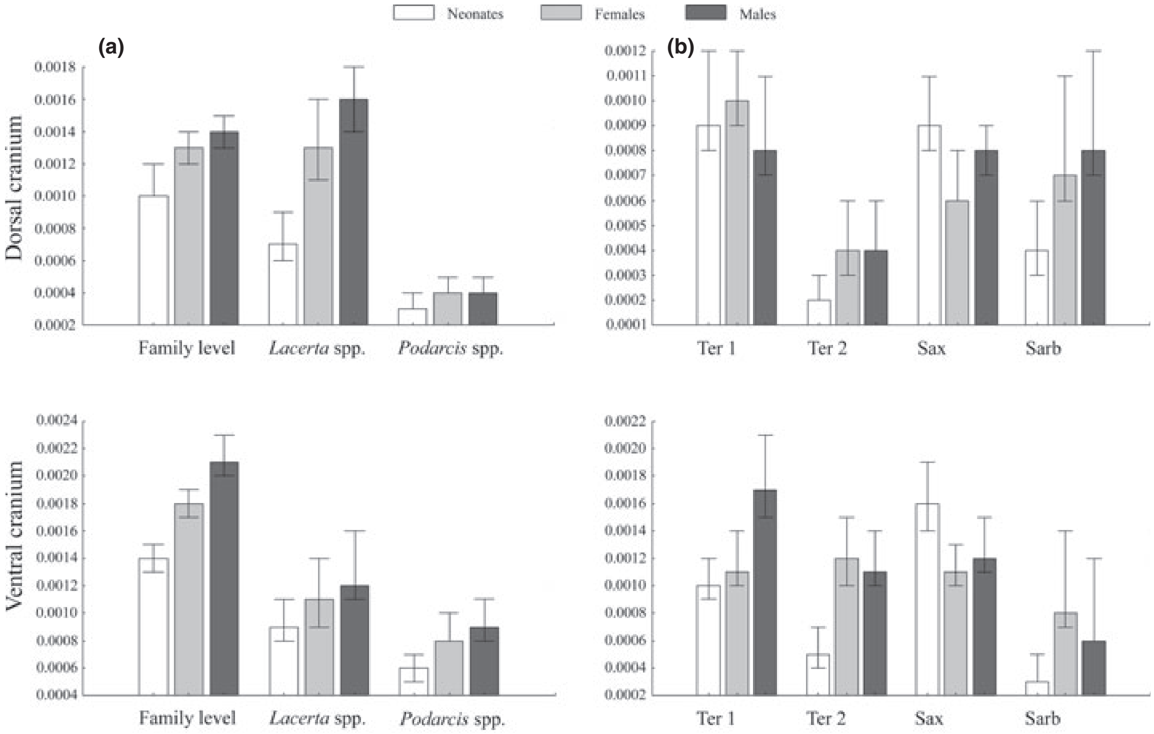

Fig. 4 The magnitude of the morphological disparity (MD) of the neonates, adult females and adult males, provided as means and 95% confidence intervals for the dorsal and ventral crania of all studied species of lacertid lizards. The MD is calculated (a) for all 12 species and for the *Lacerta* and *Podarcis* species separately and (b) for each ecomorph. The 95% confidence intervals were obtained by a bootstrap procedure (N = 900).

Table 2 Comparison of plane angles between neonates, females and males for dorsal and ventral crania for all 12 species

View	Group 1	Group 2	Between groups	Within group 1	Within group 2
Dorsal	Neonates	Females	51.09°	17.01°	81.41°
	Neonates	Males	75.14°	17.17°	73.63°
	Females	Males	40.87°	78.51°	76.07°
Ventral	Neonates	Females	**39.77°**	30.45°	18.54°
	Neonates	Males	**40.22°**	29.09°	19.31°
	Females	Males	20.26°	18.31°	20.22°

Numbers given in boldface indicate statistical significance of between-group angles after repeated bootstrapping test (N = 300).

trajectories of both sexes significantly differed in relation to the habitat (Table 4).

The species × age × CS interaction was also significant for both cranium sides, which indicated that the static allometry was different in neonates and adults and that these differences were species-specific (Table 5).

The results of the multivariate regression of the shape variables on cranium size revealed that the ontogenetic shape changes were significantly correlated with the changes in size during growth. Allometry explained the high per cent of shape changes during ontogeny: from 75.61% in *P. taurica* females to 92.59% in *L. trilineata* males for the dorsal cranium and from 72.43% in *P. taurica* females to 91.87% in *L. trilineata* males for the ventral cranium (Table 6).

Although the MANCOVA indicated that the species significantly diverged in the pattern of ontogenetic allometries, the visual inspection of the thin-plate spline deformation grids indicated that all species had a similar trend of ontogenetic shape changes from the neonates to adult females and males. The pattern of ontogenetic shape changes was universal for the dorsal cranium: an elongation of the rostral region and mid-face, a shortening and narrowing of the parietal bone, a reduction in the orbits, a narrowing of the cranium in the region of the post-frontal and post-orbital bones, a posterior movement of the squamosal and supratemporal processes and a reduction in the occipital bones (Fig. 5).

The ontogenetic shape changes for the ventral cranium were also similar for all species. These changes involved a reduction in the cranium base, a widening and shortening of the basipterygoid processes, a shortening and posterior and medial shift of the quadrates, a narrowing of the cranium in the jugal region, a shortening of the tips of the jugal bones and an expansion

Table 3 The changes in ontogenetic allometry among the species as tested by a multivariate analysis of covariance (MANCOVA).

Sex	Cranium side	Effect	Wilks' λ	*F*	Effect d.f.	Error d.f.	*P*-value
♀	Dorsal	Species	0.0036	12	253	4103.18	0.0000
		CS	0.0188	930	23	409.00	0.0000
		Species × CS	0.0025	13	253	4103.18	0.0000
	Ventral	Species	0.0008	12	352	4203.57	0.0000
		CS	0.0144	855	32	400.00	0.0000
		Species × CS	0.0005	13	352	4203.57	0.0000
♂	Dorsal	Species	0.0032	13	253	4073.30	0.0000
		CS	0.0196	882	23	406.00	0.0000
		Species × CS	0.0021	14	253	4073.30	0.0000
	Ventral	Species	0.0005	13	352	4172.30	0.0000
		CS	0.0136	898	32	397.00	0.0000
		Species × CS	0.0003	14	352	4172.30	0.0000

d.f., degrees of freedom; CS, centroid size.
The significant species × CS interaction indicates that the allometric slopes diverge among the species.

Table 4 The effect of habitat preference on ontogenetic allometry as tested by a multivariate analysis of covariance (MANCOVA).

Sex	Cranium side	Effect	Wilks' λ	*F*	Effect d.f.	Error d.f.	*P*-value
♀	Dorsal	Hab	0.1559	16	69	1270.53	0.0000
		CS	0.0219	826	23	425.00	0.0000
		Hab × CS	0.1094	20	69	1270.53	0.0000
	Ventral	Hab	0.1490	12	96	1246.20	0.0000
		CS	0.0219	580	32	416.00	0.0000
		Hab × CS	0.1031	15	96	1246.20	0.0000
♂	Dorsal	Hab	0.1340	18	69	1261.57	0.0000
		CS	0.0206	872	23	422.00	0.0000
		Hab × CS	0.0753	25	69	1261.57	0.0000
	Ventral	Hab	0.0954	15	96	1237.22	0.0000
		CS	0.0183	691	32	413.00	0.0000
		Hab × CS	0.0542	21	96	1237.22	0.0000

d.f., degrees of freedom; CS, centroid size.
The significant Hab × CS interaction indicates that the allometric slopes differ with habitat preference.

Table 5 The differences in static allometry between neonates and adults among the species as tested by a multivariate analysis of covariance (MANCOVA).

Cranium side	Effect	Wilks' λ	*F*	Effect d.f.	Error d.f.	*P*-value
Dorsal	Species	0.2913	1	506	6217.00	0.0000
	Age	0.6644	6	46	577.00	0.0000
	CS	0.6583	7	46	577.00	0.0000
	Species × age × CS	0.3136	1	506	6217.00	0.0000
Ventral	Species	0.1187	2	704	6103.22	0.0000
	Age	0.6706	4	64	559.00	0.0000
	CS	0.6215	5	64	559.00	0.0000
	Species × age × CS	0.1189	2	704	6103.22	0.0000

d.f., degrees of freedom; CS, centroid size.
The significant Species × Age × CS interaction indicates that the static allometric slopes differ between neonates and adults and among the species.

of the jaw adductor muscle chambers (Fig. 6). The shape changes of both cranium sides, which were slightly more pronounced in males, were species-specific and followed a size-related pattern. These changes were the most pronounced in the *L. trilineata* and *L. viridis*, which are the largest species, and the least pronounced in *D. praticola*, *I. horvathi* and *Z. vivipara*, which are the smallest of the species studied.

Table 6 Rates of ontogenetic shape changes (i.e. lengths of ontogenetic trajectories in units of Procrustes distance), regression coefficients (R^2), per cent of allometric shape changes and statistical significance of allometry obtained by multivariate regression of shape variables (Procrustes distances) on centroid size (CS).

		Dorsal				Ventral			
		Rate	R^2	Shape changes %	*P*-value	Rate	R^2	Shape changes %	*P*-value
D. praticola	♀	0.1190	0.9857	87.02%	0.0001	0.1043	0.9824	80.78%	0.0001
	♂	0.1199	0.9890	86.71%	0.0001	0.1175	0.9898	85.09%	0.0001
D. mosorensis	♀	0.0796	0.9816	77.17%	0.0001	0.0774	0.9835	74.24%	0.0001
	♂	0.0886	0.9880	83.24%	0.0001	0.0873	0.9835	80.42%	0.0001
I. horvathi	♀	0.1016	0.9728	82.74%	0.0001	0.1039	0.9818	80.79%	0.0001
	♂	0.1096	0.9851	86.14%	0.0001	0.1068	0.9880	83.69%	0.0001
L. agilis	♀	0.1025	0.9837	81.21%	0.0001	0.1136	0.9882	80.10%	0.0001
	♂	0.1068	0.9920	84.32%	0.0001	0.1279	0.9959	85.99%	0.0001
L. trilineata	♀	0.1138	0.9971	92.27%	0.0001	0.1134	0.9951	90.42%	0.0001
	♂	0.1160	0.9937	92.59%	0.0001	0.1183	0.9968	91.87%	0.0001
L. viridis	♀	0.1123	0.9817	86.39%	0.0001	0.1044	0.9836	84.55%	0.0001
	♂	0.1073	0.9915	88.84%	0.0001	0.1081	0.9888	88.93%	0.0001
P. erhardii	♀	0.1144	0.9793	81.31%	0.0001	0.1122	0.9775	82.12%	0.0001
	♂	0.1084	0.9796	83.22%	0.0001	0.1214	0.9864	85.19%	0.0001
P. melisellensis	♀	0.0904	0.9764	78.96%	0.0001	0.0902	0.9499	73.68%	0.0001
	♂	0.0906	0.9794	81.39%	0.0001	0.0934	0.9656	78.34%	0.0001
P. muralis	♀	0.1098	0.9847	83.35%	0.0001	0.1137	0.9841	84.37%	0.0001
	♂	0.1115	0.9895	87.39%	0.0001	0.1243	0.9903	89.07%	0.0001
P. sicula	♀	0.1187	0.9843	82.52%	0.0001	0.1256	0.9785	81.71%	0.0001
	♂	0.1094	0.9911	87.58%	0.0001	0.1220	0.9876	87.34%	0.0001
P. taurica	♀	0.0956	0.9574	75.61%	0.0001	0.0973	0.9471	72.43%	0.0001
	♂	0.0940	0.9721	80.32%	0.0001	0.1020	0.9756	82.59%	0.0001
Z. vivipara	♀	0.1110	0.9895	83.66%	0.0001	0.0858	0.9733	73.28%	0.0001
	♂	0.1199	0.9923	85.13%	0.0001	0.0989	0.9816	78.40%	0.0001

The pairwise comparison of ontogenetic trajectories (represented as multivariate regression vectors) indicated that the patterns of ontogenetic allometry were species- and sex-specific. The average between-species angle was 24.4° (a range of 12.5–35.7°) for the female dorsal cranium, 22.4° (a range of 12.1–33.5°) for the male dorsal cranium, 27.4° (a range of 13.4–43.5°) for the female ventral cranium and 24.3° (range 11.2–40.7°) for the male ventral cranium. The vector matrices for the dorsal and ventral crania are provided as Table S6. The angle matrices were used in the NMDS analysis to represent the lacertid allometric space. The topologies of the NMDS plots were similar for each cranium side in females and males but greatly differed between the dorsal and ventral crania (Fig. 7). For the dorsal cranium, the gradual positioning of the species in allometric space was concordant to the increase in species size from *I. horvathi* and *D. praticola* to *L. trilineata* (in which *D. mosorensis* had a similar allometric vector to the larger species). The species of the genus *Podarcis* tended to cluster together, and *D. praticola, I. horvathi* and *Z. vivipara* were also closely grouped. The shrub-climbing *L. trilineata* and *L. viridis* diverged from the rest of the species with *D. mosorensis* and *L. agilis* diverging in an identical direction in females and grouping approximately intermediately between the shrub-climbing lacertas and the remaining species in males. For the ventral cranium, most of the species were closely clustered, whereas *D. mosorensis, P. erhardii* and *P. melisellensis* diverged along the first axis. The most evident difference between the allometric spaces of the dorsal and ventral crania was that the gradient from the small to large species present along the first axis for the dorsal cranium was disrupted for the ventral cranium. The parallelisms between habitat preference and cranium allometry were evident for the shrub-climbing and terrestrial species. The saxicolous group appeared to be the most dispersed group in the allometric space.

The most obvious separation of ontogenetic allometries was observed in the dorsal cranium of females, in which the CV1 accounted for 97.16% of the variance (Fig. 8). The large shrub-climbing lacertas were well separated from all other species along the CV1. The terrestrial species that prefer densely vegetated habitats clustered closely, as did the terrestrial species that prefer low vegetation; these two groups were well separated along the first and second axes. However, the

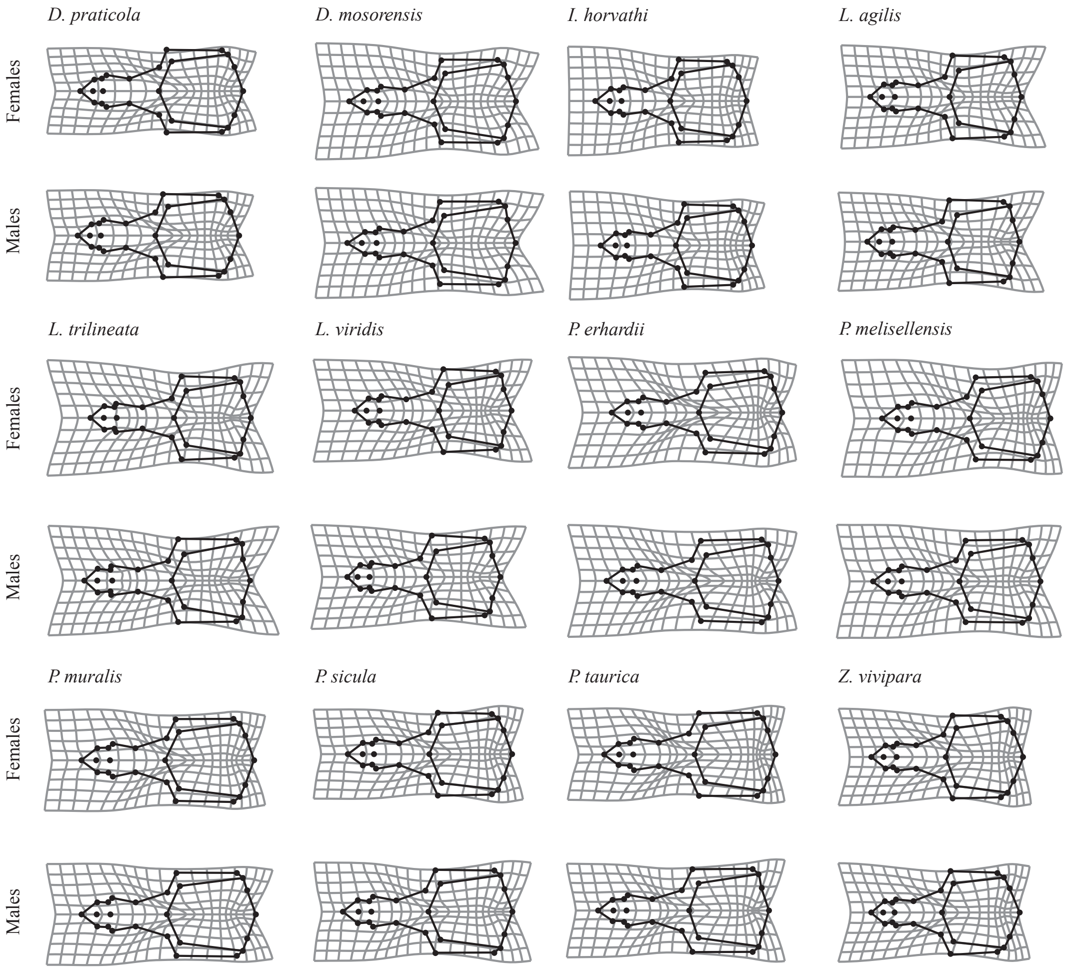

Fig. 5 The ontogenetic shape changes of the dorsal cranium illustrated by thin-plate spline grids. The represented shape changes are from neonates to adult females and males, presented in the original ratios. The line between landmarks 13, 11, 10, 22 and 24 defines the parietal bone.

saxicolous group was divided along the CV2. The saxicolous *Podarcis* spp. clustered together with the terrestrial *Podarcis* spp., whereas *I. horvathi* and *D. mosorensis* clustered with the terrestrial species that prefer densely vegetated habitats. In males, the *Podarcis* separated along the CV1 (65.35%), whereas the shrub-climbing group was closer to the terrestrial species that prefer densely vegetated habitats along the CV1 and separated along the CV2 (31.55%). The *D. mosorensis* was close to the shrub-climbing group, whereas *I. horvathi* was grouped inside the densely vegetated terrestrial group.

The habitat groups were more disrupted for the ventral cranium. The female *Podarcis* spp. clustered together along the CV1 (70.25%), but *P. taurica* was separated from this cluster along the CV2 (28.04%) and was located close to *D. mosorensis.* The terrestrial species that prefer densely vegetated habitats and the *I. horvathi* cluster was present and separated from the *Podarcis* cluster along the CV1. The shrub-climbing group was close to the *Podarcis* spp. cluster. The male *Podarcis* spp. group was separated from the rest of the species along the CV1 (79.04%). The *D. mosorensis* and *I. horvathi* clustered with *Z. vivipara* and *D. praticola*, whereas *L. agilis* clustered with the shrub-climbing group, which was well separated along the CV2 (20.95%).

Discussion

The ontogenetic aspects of MD

The inspection of the morphospaces and the comparison of plane angles between the neonates, females and males of 12 lacertid lizard species showed that the general pattern of cranium shape disparity was present in the analysed groups. The general shape gradient progresses from the smaller species with shorter and

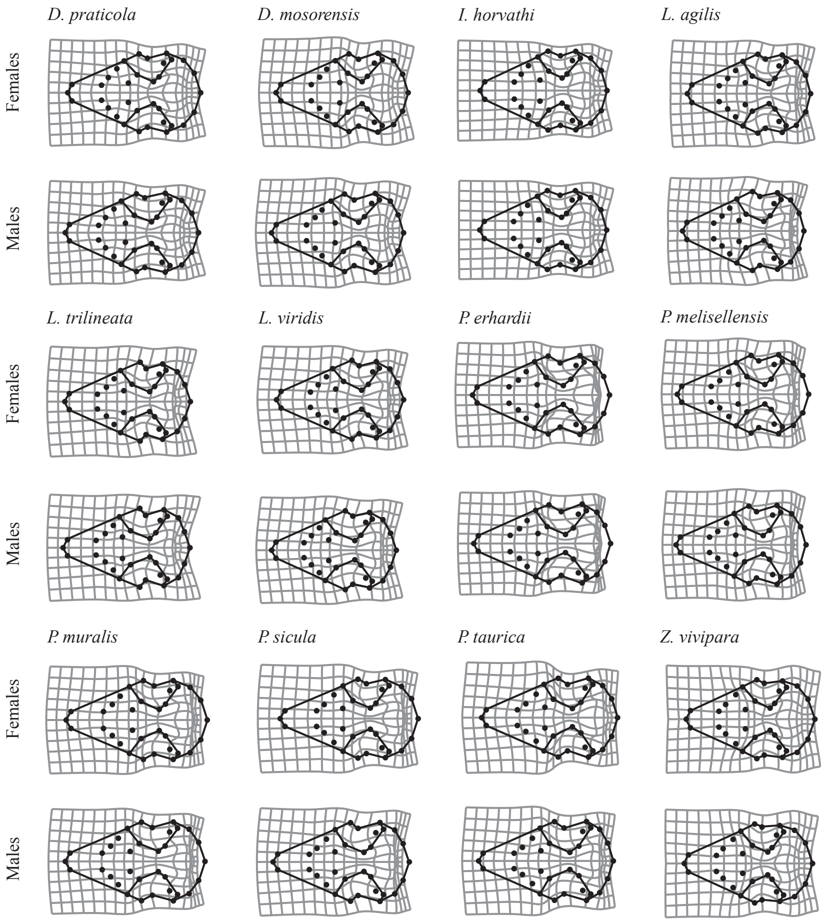

Fig. 6 The ontogenetic shape changes of the ventral cranium illustrated by thin-plate spline grids. The represented changes are from the neonates to adult females and males, presented in the original ratios. The lines between landmarks 31, 32, 33, 35, 36, 37, 39 and 40 as well as 48, 49, 50, 52, 53, 54, 56 and 57 define the jaw adductor muscle chambers.

wider crania to the larger species with elongated crania, reduced occipital bones and overgrown dermal skeletal elements, particularly in the posterior regions. The shape changes were less pronounced in the neonate stage with a more 'compact' clustering of the species in the morphospace compared to the adult stage. Regarding sex differences, males were slightly more dispersed in the morphospace than females. Some differences between the dorsal and ventral cranium sides were also recorded. The shape disparity for the dorsal cranium (which is more related to the growth of the brain and main sensory organs) appears to be more conserved, whereas the ventral cranium (more directly related to foraging) shows a significant increase in disparity from the neonates to adult females and males. For the ventral cranium, there is also a shift in the morphospace planes during the course of ontogeny. Different components of complex morphological structures, such as the cranium, could have different patterns of developmental morphospace partitioning (Eble, 1998, 2003). In lizards, the ventral cranium is more susceptible to the different selective pressures related to diet and sexual selection (Herrel *et al.*, 2007; Ljubisavljević *et al.*, 2010a, 2011; Urošević *et al.*, 2012). Consequently, in comparison with the dorsal cranium, the disparity of the lacertid lizard ventral cranium could be driven by different functional and ecological constraints (see Zelditch *et al.*, 2003b; Frédérich & Vandewalle, 2011; Urošević *et al.*, 2012).

The lack of phylogenetic signal in the variation in cranium shape for all of the alternative generic-level phylogenies prevents us from estimating the effect of the phylogenetic constraints on the pattern of MD of the analysed lacertid species. Nevertheless, the comparisons of the MD within the two genera *Lacerta* and *Podarcis* (which were represented by a number of species in this study) indicate that phylogenetic constraints could affect MD on lower taxonomic levels because the low level of MD characterizes the genus *Podarcis* for which the monophyletic origin has been obviously supported (Arnold, 1973, 1989; Arnold *et al.*, 2007; Pavličev & Mayer, 2009). However, lacking well-supported phylogenetic trees, which would include all of the species present in our study, disables us from testing the effect of phylogeny on the subgeneric-level MD.

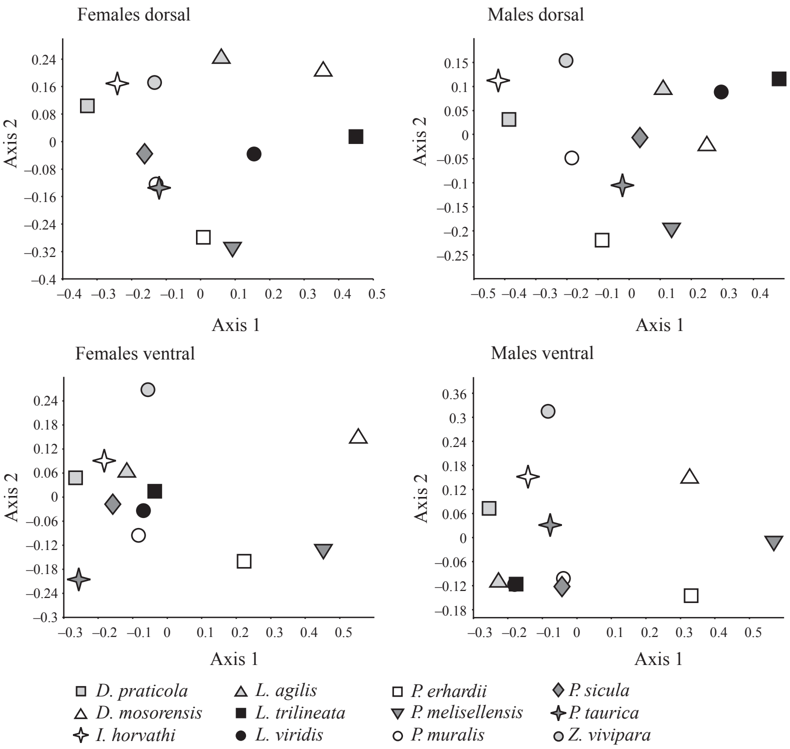

Fig. 7 The allometric spaces of the 12 species of lacertid lizards represented by the nonmetric multidimensional scaling (NMDS) plots on the matrix of pairwise angles among the allometric vectors for the female dorsal cranium, female ventral cranium, male dorsal cranium and male ventral cranium. The habitat preferences are represented by colour code: empty symbols = sax, light grey fill = ter1, dark grey fill = ter2 and black symbols = sarb.

However, a higher level of adult disparity can be positively correlated with the higher level of ecological and functional diversity of a particular developmental stage (Frédérich & Vandewalle, 2011). Our analyses showed that different ecological groups have similar patterns of shape disparity from the neonatal to adult stage with the exception of the saxicolous lizards, which showed a tendency of decreasing shape disparity throughout ontogeny. In saxicolous lizards, the ontogenetic paths apparently converged from the more disparate juveniles to the less disparate adults. The saxicolous group in our study is phylogenetically heterogeneous (see Arnold *et al.*, 2007; Pavličev & Mayer, 2009), and the saxicolous lacertid lizards are noted for a shared morphology constrained by vertical habitat dwelling and seeking shelter in crevices (Arnold, 1973; Kaliontzopoulou *et al.*, 2010). The observed pattern of MD of the saxicolous lacertid lizards could be achieved by different allometric paths (Zelditch *et al.*, 2003b; Gerber *et al.*, 2007; Adams & Nistri, 2010).

Ontogenetic allometry

Our study shows that allometry explained a high percentage of the cranium shape variation in the analysed lacertid lizard species. The ontogenetic shape changes correspond to the ontogenetic and static allometric patterns previously described for lacertid lizards (Bruner *et al.*, 2005; Bruner & Constantini, 2007, 2009; Kaliontzopoulou *et al.*, 2008; Ljubisavljević *et al.*, 2010a, 2011; Piras *et al.*, 2011; Urošević *et al.*, 2012). The general anterior–posterior gradient is present, reflected by an elongation and narrowing of the cranium, growth of the dermal skeletal elements, an enlargement of the jaw adductor muscle chambers, a posterior shift of the fronto-parietal suture and reduction in the cranial base. The changes are the most pronounced in the largest species (*L. agilis*, *L. trilineata*, *L. viridis* and *Podarcis sicula*). These changes also correspond with the general pattern of the ontogenetic shape changes in tetrapods (Emerson & Bramble, 1993). The important ontogenetic shape changes are related to the biomechanically important traits of the cranium – the fronto-parietal suture, which is an important mesokinetic articulation and stress-amortization point (Moazen *et al.*, 2008, 2009), and jaw adductor muscle chamber (Herrel *et al.*, 1999, 2007).

The ranges of the calculated between-species angles indicate a significant level of allometric diversity within the lacertids. Rodents have a comparable range of allometric trajectories (Wilson & Sanchez-Villagra, 2010). Bony fishes tend to have more variable allometric trajectories, ranging from highly conserved (Klingenberg & Ekau, 1996) to highly dispersed (Zelditch *et al.*, 2003b; Frédérich & Vandewalle, 2011), even on the intraspecific level (Rodriguez-Mendoza *et al.*, 2011).

The distribution of the allometries in allometric space differs for the dorsal and ventral crania. However, for

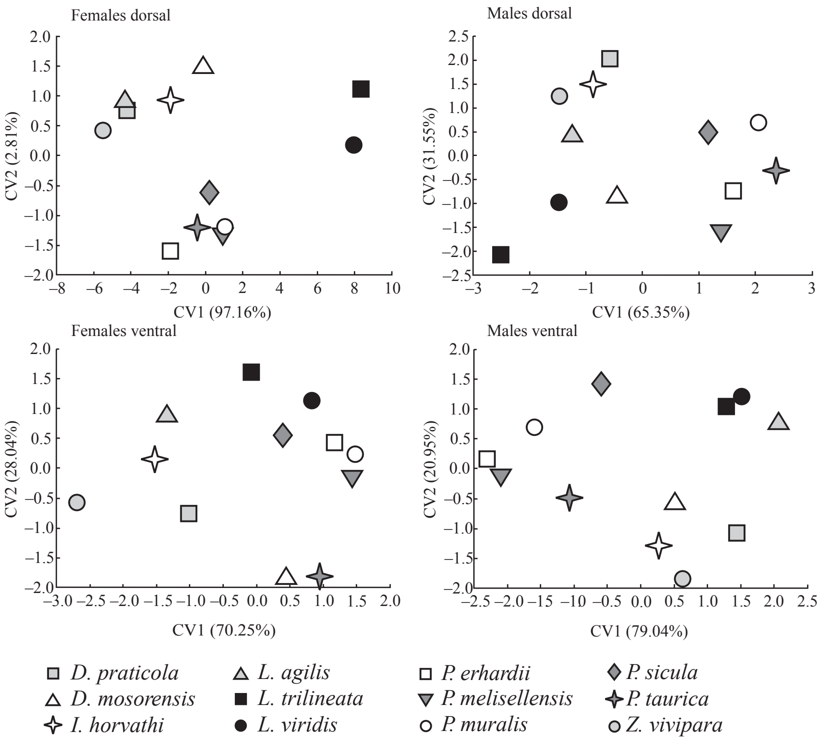

Fig. 8 The canonical variate analysis (CVA) of the allometric trajectories of the 12 species of lacertid lizards for the female dorsal cranium, female ventral cranium, male dorsal cranium and male ventral cranium using the predefined habitat preference (ecomorph) group. The habitat preferences are represented by colour code: empty symbols = sax, light grey fill = ter1, dark grey fill = ter2 and black symbols = sarb.

both cranium sides, the shrub-climbing species tend to cluster together, whereas the allometries of the saxicolous species are the most dispersed. The allometries of the saxicolous *Podarcis* spp. lizards clustered closely with the allometries of the terrestrial *Podarcis* spp. lizards. The allometric vectors of the *Podarcis* species tended to cluster together in the allometric space, which indicates that the allometric trajectories within this well-supported monophyletic group could possibly be phylogenetically constrained, as was proposed by Piras *et al.* (2011).

We can also conclude that the effect of habitat preference is more obvious for the dorsal cranium, particularly in females, whereas the allometric patterns of the ventral cranium are further modified by different factors. Because the structures of the ventral cranium are directly involved in the mechanics of jaw movement, different functional requirements (related to different types of diet, territorial and mating behaviours, such as male territorial combats and grabbing females during copulation) could also influence ontogenetic trajectories of the ventral cranium (Verwaijen *et al.*, 2002; McBrayer & Anderson, 2007; Ljubisavljević *et al.*, 2010a, 2011; Urošević *et al.*, 2012).

In sum, our study observed that the pattern of the lacertid lizard dorsal cranium shape disparity is conserved throughout ontogeny. There is a morphological divergence of the ventral cranium, and there is a significant shift in the morphospace planes with adult morphospaces being more disparate than neonatal morphospaces. Differences in shape disparity between adult and neonatal morphospaces could be explained by different static allometries of neonates and adults. Different patterns of the ventral cranium MD could be explained by different functional and ecological constraints. In general, studies of ontogenetic shape changes could identify the underlying mechanisms and processes that lead to the divergence between populations and between lineages. The observed pattern of allometric variability could be related to differences in habitat preference, but the effect of functional constraints should also be taken into account. These patterns of MD and allometric shape changes indicate that the identical mechanisms that produce intraspecific morphological variation (Kaliontzopoulou *et al.*, 2010) also produce morphological variation among lacertid lizard species (this study).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Four alternative phylogenetic trees of six lacertid lizards genera studied, trimmed from published phylogenies: (a) phylogeny based on the 64 morphological characters, from Arnold *et al.* (2007); (b) phylogeny based on the 620 bp of the mitochondrial DNA sequences (cyt b and 12S rRNA gene sequences) from Arnold *et al.* (2007); (c) phylogeny based on the 1600 bp of the nuclear DNA sequences (rag1 and c-mos gene sequences) from Pavličev & Mayer (2009) and (d) consensus phylogeny based on the 5727 bp of the four mitochondrial and two nuclear DNA sequences (cyt b, 12S rRNA, 16s rRNA, CO1, rag1 and c-mos gene sequences) from Kapli *et al.* (2011).

Table S1 The origin of samples (collection) and collection of serial numbers.

Table S2 The number and descriptions of dorsal and ventral cranial landmarks.

Table S3 The one-way analysis of variance (ANOVA) between the species for the dorsal and ventral cranium size of the neonates, females and males.

Table S4 Multivariate analysis of variance (MANOVA) between species for the dorsal and ventral crania shape of neonates, females and males.

Table S5 Test for phylogenetic signal and phylogenetic uncertainty in the symmetric component of the cranium shape. Permutation tests for each phylogenetic tree were done with 10 000 bootstrap series. If the range of *P*-values for resampled trees includes significant as well as non-significant ones, the phylogenetic uncertainty has a marked effect on the results of phylogenetic tests.

Table S6 The results of the pairwise comparison of ontogenetic trajectories. Upper section is for females; lower section is for males.

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