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Multilevel assessment of the Lacertid lizard cranial modularity

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Abstract

Different factors and processes that produce phenotypic variation at the individual, population, or interspecific level can influence or alter the covariance structure among morphological traits. Therefore, studies of the patterns of integration and modularity at multiple levels—static, ontogenetic, and evolutionary, can provide invaluable data on underlying factors and processes that structured morphological variation, directed, or constrained evolutionary changes. Our dataset, consisting of cranium shape data for 14 lizard species from the family Lacertidae, with substantial samples of hatchlings and adults along with their inferred evolutionary relationships, enabled us to assess modularity and morphological integration at all three levels. Five, not mutually exclusive modularity hypotheses of lizard cranium, were tested, and the effects of allometry on intensity and the pattern of integration and modularity were estimated. We used geometric morphometrics to extract symmetric and asymmetric, as well as allometric and nonallometric, components of shape variation. At the static level, firm confirmation of cranial modularity was found for hypotheses which separate anterior and posterior functional compartments of the skull. At the ontogenetic level, two alternative hypotheses (the "anteroposterior" and "neurodermatocranial" hypotheses) of ventral cranial modularity were confirmed. At the evolutionary level, the "neurodermatocranial" hypothesis was confirmed for the ventral cranium, which is in accordance with the pattern observed at the ontogenetic level. The observed pattern of static modularity could be driven by functional demands and can be regarded as adaptive. Ontogenetic modularity and evolutionary modularity show the same developmental origin, indicating conservatism of modularity patterns driven by developmental constraints.

KEYWORDS
evolution, Lacertidae, modularity, morphological integration, ontogeny, skull

1 | INTRODUCTION

Despite increasing interest in the subject of morphological integration—the phenomenon initially defined by Olson and Miller (1958) as covariation of morphological traits as a result of developmental or functional interactions—information about many aspects of integration and modularity is still missing. Morphological modules, units of tightly intercorrelated traits but relatively independent from other such units, can be regarded as building blocks within an organism allowing selection to act on the modules without altering other characteristics of the organism. Covariation among traits can arise through shared development or the actions of pleiotropic loci and reflect functional relationships (Klingenberg, 2008). Modules can be shaped by selective pressures (Cheverud, 1996; Sanger, Mahler, Abzhanov, & Losos, 2011; Wagner & Altenberg, 1996), but evolutionary changes can also be largely constrained or directed by integration and modularity (Klingenberg & Marugán-Lobón, 2013; Lande, 1980; Olson & Miller, 1958; Wagner, 1996). One of the main tasks of

research is to explore patterns of integration and modularity and reveal processes that underlie a specific pattern of covariation among traits and produce modules (Wagner, Pavlicev, & Cheverud, 2007).

The methods of geometric morphometrics, especially ones separating asymmetric (intraindividual) and symmetric (interindividual) components of shape variation, were crucial in facilitating quantitative analyses of morphological modularity/integration at different levels, particularly developmental and functional aspects of integration and modularity (Klingenberg, 2014). The covariation pattern of the asymmetric component of shape variation reflects the developmental aspect of modularity (Klingenberg, Barluenga, & Meyer, 2002; Klingenberg & McIntyre, 1998; Klingenberg & Zaklan, 2000). The reasoning here lay in the fact that traits which have common development will share the same pattern of fluctuating asymmetry (FA), the differences between the left and right sides being due to random imprecisions in developmental processes (Klingenberg, 2015; Klingenberg, Mebus, & Auffray, 2003).

The covariation pattern of symmetric (interindividual) variation in shape reflects functional integration. Functional integration arises when particular traits are associated with performing a certain function (Cheverud, 1996; Winther, 2001). According to the "matching hypothesis," functional and developmental patterns of modularity/integration should match, due to the adaptive evolution of developmental pathways (Breuker, Debat, & Klingenberg, 2006; Cheverud, 1984; Wagner & Altenberg, 1996). Morphological variation and patterns of integration and modularity can be studied at three levels: static, ontogenetic, and evolutionary (Klingenberg, 2014) (Figure 1). At the static level, the pattern of morphological integration is estimated from variation among individuals that belong to the same population or species and same ontogenetic stage. This is the most frequently studied level (Cheverud, 1982; Ivanović & Kalezić, 2010; Jojić, Blagojević, & Vujošević, 2011; Jojić, Blagojević, & Vujošević, 2012; Klingenberg & Zaklan, 2000). However, covariation patterns change over the course of ontogeny, and selection can act on any ontogenetic stage, leading to evolutionary changes. Studying ontogenetic integration (covariation patterns across ontogenetic stages in the same population or species) offers an insight into the dynamics of changes and timing when selection may have acted on development (Ackermann, 2005). Finally, studying integration across species and taxa can reveal preservation of genetic and developmental integration patterns and disclose evolutionary modules (Klingenberg, 2008; Sanger et al., 2011). By studying modularity and morphological integration at multiple levels, we can gain a better understanding of the process of evolution itself and discover links between static, ontogenetic, and evolutionary integration (Drake & Klingenberg, 2010; Klingenberg & Zaklan, 2000; Mitteroecker, Gunz, & Bookstein, 2005; Monteiro, Bonato, & dos Reis, 2005; Monteiro & Nogueira, 2010).

From the first pioneering study of modularity and integration (Cheverud, 1982), the cranial skeleton, as a complex morphological structure with heterogeneous development and multiple functions, has been frequently used as a model-system for studies of
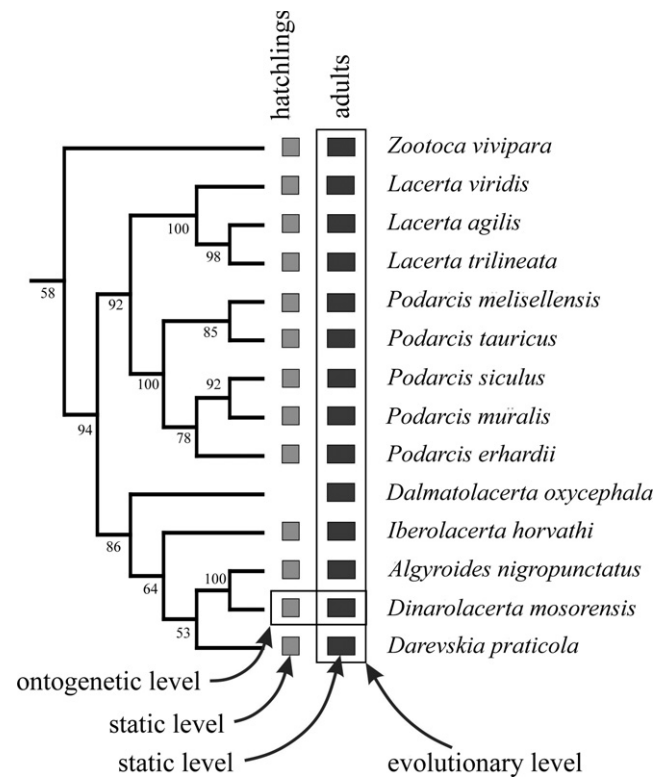
FIGURE 1 Levels at which hypotheses were tested with phylogeny of 14 lacertid species as studied by Pyron et al. (2013). The horizontal rectangle represents the ontogenetic level, while the vertical rectangle represents the evolutionary level. The small light-gray rectangles represent samples of hatchlings of each species, while the dark-gray rectangles stand for samples of adults for each species

modularity and integration. However, studies of cranial morphological integration have been done mostly on mammals (Álvarez, Perez, & Verzi, 2015; Cheverud, 1982; Goswami, 2006a,b; Jojić et al., 2011, 2012; Porto, De Oliveira, Shirai, De Conto, & Marroig, 2009), with other taxa being sporadically represented (Ivanović & Kalezić, 2010; Klingenberg & Marugán-Lobón, 2013; Monteiro & Abe, 1997; Sanger et al., 2011). In mammals, two modules can be recognized: (i) the braincase (the neurocranial module); (ii) the facial module (Bookstein et al., 2003; Cardini & Polly, 2013; Cheverud, 1996; Goswami, 2006a,b).

The pattern of covariation within the cranial skeleton or head structures in lizards has been explored in several studies. The hypothesis proposed by Monteiro and Abe (1997) recognizes dermatocranial parts (rostrum, midface, and dermatocranial parts of the braincase), which are functionally related; and endochondral elements, which share the same development and are also functionally related (Monteiro & Abe, 1997). Later studies tested a range of alternative developmental and functional hypotheses with the overall conclusion that cranial modularity is not evolutionarily conserved, but rather reflects functional demands of specific skull morphologies (Sanger et al., 2011). It was also pointed out that the anterior and posterior parts of the head are relatively independent modules, and

that this modularity pattern can arise due to relatively late ossification and prolonged growth of the posterior dorsal parts of the head leading to increased plasticity of this region (Lazić, Carretero, Crnobrnja-Isailović, & Kaliontzopoulou, 2015). On the other hand, the anterior-posterior pattern of modularity observed on the dorsal and ventral cranial structures can be interpreted as functional, as the a priori functional hypotheses were confirmed at the static level.

Despite numerous studies on morphometric variation in lacertid lizards (Bruner & Constantini, 2007; Herrel, Spithoven, Van Damme, & De Vree, 1999; Hipsley, Miles, & Müller, 2014; Hipsley & Müller, 2017; Kaliontzopoulou, Carretero, & Llorente, 2008; Lazić, Carretero, Crnobrnja-Isailović, & Kaliontzopoulou, 2016; Lazić et al., 2015; Ljubisavljević, Urošević, Aleksić, & Ivanović, 2010; Urošević, Ljubisavljević, & Ivanović, 2013), a phylogenetically based comparative approach is greatly hampered by the fact that the phylogeny of the lacertids is still, after multiple efforts, largely unresolved. Recently, Pyron, Burbrink, and Wiens (2013) offered a seemingly resolved phylogeny of a clade included in the "megaphylogeny" of the squamates which contained thousands of species and multiple genes. In the context of the Lacertini tribe, it was suggested that supermatrix phylogenies should be treated with caution when integrating intergeneric relationships into ecological studies (Mendes, Harris, Carranza, & Salvi, 2016). However, despite possible shortcomings, it is still a reasonable choice for topology in evolutionary studies on lacertids, which include a fairly large number of species (Figure 1).

Consisting of information about size and shape of the ventral and dorsal cranium for a large sample of hatchlings and adults of 14 species of lacertid lizards, our dataset enabled us to explore morphological integration and modularity at different levels and to compare patterns across levels. Because allometry is a known factor contributing to integration (Hallgrímsson et al., 2006; Klingenberg, 2009; Zelditch & Fink, 1995), we explored modularity and integration taking allometric effects into account. Specifically, we employed methods of geometric morphometrics in order to:

1. Explore patterns of integration at the static, ontogenetic, and evolutionary levels (Figure 1);
2. Compare patterns of variation and covariation across levels; and
3. Estimate the effects of allometry on morphological integration of the lacertid lizard cranium.

In studying the modularity and integration of dorsal cranium structures, we tested five alternative hypotheses (Figure 2):

1. The "*Anolis* skull shape hypothesis" suggests two independent modules of the dorsal rostrum and cranium and was derived from studying morphological diversification of the skull of *Anolis* lizards (Sanger et al., 2011).
2. The "mammalian morphometric hypothesis" (Sanger et al., 2011), which reflects cellular origins of the dorsal cranium and suggests facial and braincase (neurocranial) modules, was derived from extensive studies of mammalian skull modularity (Goswami, 2006a,b). However, it should be stressed that precise landmark

homologies between the mammalian and the squamate skull are fairly unclear.

3. The "tripartite hypothesis" was based on division of the dorsal cranium into three separate functional modules, viz., the rostrum, the orbital region, and the braincase (Sanger et al., 2011).
4. The "neurodermatocranial hypothesis" is a developmentally based hypothesis which according to their embryonic origin separates the ventral cranium into two distinct modules comprising elements of the neurocranium and the dermatocranium.
5. The "anteroposterior hypothesis" is a functionally based hypothesis which divides the ventral cranium into anterior (the jaw and the palate) and the posterior (the braincase and the jaw adductor muscle chambers) regions (Ljubisavljević et al., 2010; Urošević, Ljubisavljević, Jelić, & Ivanović, 2012; Urošević et al., 2013) (Figure 2).

At the static level, these hypotheses were tested on both the symmetric and the asymmetric components of shape variation in order to detect developmental modularity (Klingenberg, 2014; Klingenberg et al., 2003).

2 | MATERIAL AND METHODS

2.1 | Studied sample

In this study, we used a total of 760 specimens (239 hatchlings, 261 adult females, and 260 adult males) representing 14 species and eight genera of lacertids (a complete list of species with their common and scientific names and authorities is provided in Appendix 1). As we lacked a sample of neonates for the species *D. oxycephala*, analyses of ontogenetic modularity and integration were conducted on 13 species. The specimens, which were previously collected for studies of reproductive biology and other morphological studies (Ljubisavljević, Polović, Urošević, & Ivanović, 2011; Ljubisavljević et al., 2010; Urošević et al., 2012, 2013), were taken from the herpetological collections of the Institute for Biological Research, "Siniša Stanković," Belgrade and herpetological collections of the Natural History Museum of Montenegro, Podgorica. Samples of *I. horvathi* and *P. siculus* were previously donated by the Science and Research Centre of Koper, Slovenia and by the State Institute for Nature Protection of Croatia, Zagreb, respectively (Appendix 1).

2.2 | Cranium preparation and landmarks

The crania of all specimens were cleared using the enzyme trypsin and potassium hydroxide (Dingerkus & Uhler, 1977), except in the case of the crania of *D. oxycephala*, which were cleared by dermestid beetles. All skeletons were stained with Alizarin Red S and preserved in glycerol (see Appendix 1 for collection numbers). Crania of hatchlings were photographed using 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). Adult crania were photographed
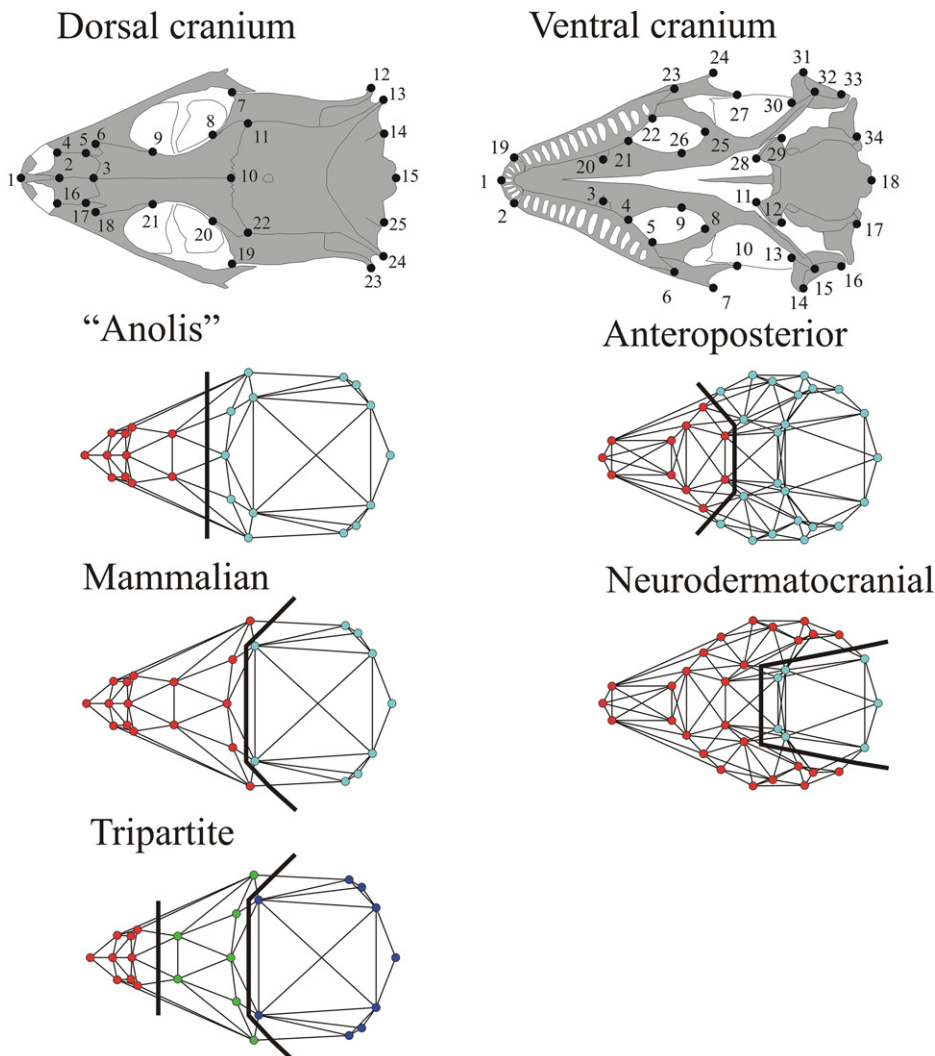
FIGURE 2 Landmark constellations for the dorsal and ventral crania and the five modularity hypotheses tested. Adjacency graphs are represented by thin lines, while thick lines separate the hypothesized modules

using a Sony DSC-F828 digital camera (resolution 8.0 MP; Sony Corp., Tokyo, Japan). Each cranium was submerged in glycerol and positioned in the center of the optical field in order to minimize the effects of distortion and parallax (Mullin & Taylor, 2002); the parietal (dorsal cranium) and palatal (ventral cranium) sides were positioned parallel to the photographic plane, while the distance from the crania to the stereo-microscope or camera lens was kept constant. The sets of landmarks for the dorsal (25) and ventral (34) cranium (Figure 2) were digitized by the same person using Tps Dig2 software (Rohlf, 2013). These sets of landmarks were chosen using as criteria the presence of the landmarks in all specimens and reliability in providing an adequate summary of cranial morphology, and they were successfully used in previous studies of lacertid cranium morphology (Ljubisavljević et al., 2010, 2011; Urošević et al., 2012, 2013).

2.3 | Shape variables

Shape variables for the dorsal and ventral cranium were extracted using generalized Procrustes superimposition. The symmetric and the

asymmetric components of variation were obtained for both structures (Klingenberg, 2015). The symmetric component of variation was calculated as an average of the landmark configuration and its mirror reflection (the averages of the left and right sides were calculated for bilaterally symmetric landmarks). The asymmetric component of variation represents differences in shape between a structure with symmetry and its mirror image, that is, it reveals differences in shape between the left and right sides (Klingenberg, 2015; Klingenberg et al., 2002).

2.4 | Statistical analyses

We accounted for sexual dimorphism by doing analyses with sexes pooled within, as we were interested in morphological differences at the species-specific level. To test for the statistical significance of fluctuating asymmetry (FA) and estimate the level of measurement error, we employed Procrustes ANOVA with the main effect of individuals representing interindividual variation, the main effect of side representing directional asymmetry, the individual × side interaction representing the measure of FA, and the residual term representing

the measurement error (Klingenberg & McIntyre, 1998). For this analysis, the sample of *P. muralis*, which comprises around 10% of the total sample, was digitized twice.

In order to explore shape variation within and between species in our sample, for adult specimens (with sexes pooled within), we employed PCA analysis based on the covariance matrix of shape variables of the dorsal and ventral crania. To test for a phylogenetic signal, we used the permutation approach. It simulates the null hypothesis of the absence of phylogenetic structure by randomly reassigning shape configurations to the terminal nodes of the phylogeny (Klingenberg & Gidaszewski, 2010). Statistical significance of the phylogenetic signal was assessed via the permutation test (10,000 rounds). Phylogenetically independent contrasts were calculated as weighted differences in values for sister nodes (Felsenstein, 1985; Rohlf, 2001). For this analysis, we used the phylogenetic tree published by Pyron et al. (2013), trimmed to the 14 investigated species (Figure 1).

We reconstructed morphological evolution of the lacertid cranium by applying the criteria of squared-change parsimony, which reconstructs internal nodes from the shape averages of terminal taxa (Maddison, 1991) and scales branch lengths according to the estimated divergence time. Using the criteria of square-change parsimony described above, PC scores were mapped onto the phylogeny in order to visualize evolutionary shape changes in the dorsal and ventral cranium (Klingenberg & Gidaszewski, 2010; Maddison, 1991).

To account for patterns of static allometry, we did a pooled within-species multivariate regression of shape on the log-transformed centroid size (log CS) (Monteiro, 1999). As preliminary analyses showed that females and males share common static allometric slopes, we performed the multivariate regression on pooled sexes.

In order to assess the common ontogenetic allometry, we did a pooled within-species multivariate regression of shape variables of hatchling and adult individuals on the log-transformed centroid size (log CS). The statistical significance of allometric shape changes was obtained via the permutation test.

Evolutionary allometry was estimated via multivariate regression of phylogenetically independent contrasts of shape onto phylogenetically independent contrasts of size (Figueirido, Serrano-Alarcón, Slater, & Palmqvist, 2010; Perez, Klaczko, Rocatti, & dos Reis, 2011). To obtain the nonallometric component of shape variation (corrected for evolutionary allometry), the regression residuals from independent contrast regression were used as shape variables.

2.5 | Patterns of modularity and integration

To test the hypotheses of modularity, we applied contingency analyses (partitions) and calculated the *RV* coefficient as a measure of the strength of association between the sets of landmarks (Escoufier, 1973; Klingenberg, 2009). The *RV* coefficient can range from 0 (if there is no association between the modules) to 1 (if there is perfect covariance) (Escoufier, 1973). In order to evaluate the strength of covariation between modules, the *RV* coefficient of the landmark partition specified by each hypothesis was compared to the *RV*

coefficients of all possible alternative partitions. If the proportion of partitions with *RV* coefficient smaller than or equal to the value of the hypothesis was less than 0.05 (95% confidence interval), the modularity hypothesis was confirmed (Klingenberg, 2009). Each hypothesis was tested with a set of 10,000 random spatially contiguous partitions. Although the use of *RV* coefficients was recently criticized as being sensitive to differences in sample size and variable count (Adams, 2016), the use of alternative approach on dataset by Jojić et al. (2012) yielded the same result regarding modularity. Further, we did our analyses on a very large sample with high number of variables and gained differences in RV coefficient at different levels which could not be attributed solely to sample size or variable count. We conclude that, although one should be aware of the *RV* coefficient shortcomings, especially when comparing the results from various datasets, using this approach at different levels on the same dataset yields biologically meaningful results.

For defining spatially contiguous sets of landmarks, we used adjacency graphs based on Delaunay triangulation, with landmarks that are directly connected by skeletal tissue, but also, especially in the case of the ventral cranium, paying attention to important functional connections by tendons or muscles (Herrel et al., 1999; Klingenberg, 2009; Ljubisavljević et al., 2010).

The modularity hypotheses (Figure 2) were tested on different levels: static, ontogenetic, and evolutionary (Figure 1). At the static level, symmetric and asymmetric components of shape variation were analyzed, reflecting functional, and developmental covariation, respectively.

Static level—The common pattern of static modularity for adults was examined by testing modularity hypotheses for both the dorsal and the ventral cranium, and symmetric and asymmetric shape variables pooled within species and sex shape. The test of hypotheses was repeated on allometry-corrected symmetric and asymmetric shape variables.

Ontogenetic level—For ontogenetic integration/modularity, alternative modularity hypotheses were tested on a mixed adult and hatchling sample pooled within species (available for 13 species). To assess modularity patterns independent from ontogenetic allometry, ontogenetic modularity was also tested for the nonallometric component of shape variation.

Evolutionary level—The patterns of evolutionary modularity were examined using a phylogenetically based approach (Klingenberg & Marugán-Lobón, 2013). To assess evolutionary modularity independent from size-related evolutionary shape changes (i.e., evolutionary allometry), we also tested modularity hypotheses on regression residuals from the regression of independent contrasts (Klingenberg & Marugán-Lobón, 2013).

The pooled within species (for hatchlings) and species and sex (for adults) matrices of the symmetric and asymmetric components of shape variation were compared using the matrix correlation test. To assess statistical significance of the matrix correlation, a permutation test with 10,000 iterations (permuting landmarks) was conducted.

The variances of the eigenvalues scaled to the mean eigenvalue can be used as a measure of the overall level of shape integration

(Hallgrímsson et al., 2006; Wagner, 1990; Young, 2006). We calculated scaled variance of eigenvalues (SVE) for the covariance matrices pooled within species (for hatchlings) and species and sex (for adults) for the total shape variation as well as for the nonallometric component. All shape analyses were conducted using the MorphoJ software (Klingenberg, 2011).

3 | RESULTS

The FA was statistically significant, for both the dorsal and the ventral cranium, for hatchlings and adults alike (Supporting Information Table S1). The mean squares of FA and interindividual variation exceeded the digitizing error threefold or more, and therefore, the asymmetric component of shape variation can be used as a valid variable for testing hypotheses of developmental modularity/integration.

A statistically significant phylogenetic signal in skull shape was found for both the dorsal ($p = 0.026$) and the ventral ($p = 0.003$) cranium. The constructed phylomorphospaces (PCA plots with the phylogeny superimposed) demonstrated a gradient along PC 1 from *Z. vivipara*, *I. horvathi*, and *D. praticola*—which have wider crania with a shorter rostrum, enlarged orbits, and expanded occipital region—to *L. trilineata*, *L. viridis*, and *P. siculus*—which have narrower crania with an elongated rostrum and relatively smaller orbital and occipital regions. The obtained PC 2 describes a gradient from *Lacerta* spp. and *Z. vivipara*—with a wide rostrum and mid-cranium, frontoparietal suture shifted posteriorly, and reduced occipital region—to *A. nigropunctatus* and *D. oxycephala*—with a narrow rostrum and mid-cranium, fronto-parietal suture shifted anteriorly, and an enlarged occipital region (Figure 3). The obtained PC 3 describes a gradient from *L. agilis*, *Z. vivipara*, and *D. mosorensis*—with a shorter and wider cranium, enlarged orbits, and reduced occipital region—to *D. oxycephala*—with an elongated and narrower cranium with smaller orbits and an enlarged occipital region (Figure 3). For the ventral cranium, there is a gradient along PC 1 from *L. trilineata*, *L. viridis*, and *P. siculus*—with enlarged jaw adductor muscle chambers, quadrates shifted posteriorly, and a relatively smaller cranium base—to *I. horvathi*, *Z. vivipara*, *D. oxycephala*, and *D. mosorensis*—with relatively reduced jaw adductor muscle chambers, quadrates shifted anteriorly, and an enlarged cranium base. Along PC 2, there is a gradient from *D. oxycephala*—with a very narrow and elongated cranium—to *L. agilis*, *L. viridis*, *P. melisellensis*, and *Z. vivipara*—with a short and wide cranium (Figure 4). The obtained PC 3 described a gradient from *A. nigropunctatus* and *L. trilineata*—with slightly elongated and narrower cranium, longer quadrates, and an elongated cranium base—to *P. melisellensis*—with a slightly shortened and wider cranium, short quadrates, and a shortened cranium base (Figure 4).

Static allometry explained a relatively large percentage of shape variation for the dorsal (19.82%) and ventral (28.88%) cranium and was statistically significant ($p < 0.0001$) (Supporting Information Figure S1). For the asymmetric component of shape variation, allometry explained a relatively small percentage of shape variation for the

dorsal (0.77%, $p < 0.0001$) and ventral (0.26%, $p = 0.1472$) cranium. Ontogenetic allometry explained a large percentage of total shape variation for the dorsal (74.84%) and ventral (73.47%) cranium and was statistically significant in both comparisons ($p < 0.0001$) (Supporting Information Figure S2). Evolutionary allometry also explained a large percentage of variation for the dorsal (48.47%) and ventral (33.13%) cranium and was statistically significant ($p < 0.0001$ and $p = 0.0004$, respectively, Supporting Information Figure S3). As the evolutionary allometry proved to be significant, we did a phylomorphospace reconstruction based on nonallometric data. Residuals corrected for evolutionary allometry were obtained by applying the regression vectors from the evolutionary allometry (independent contrast regression analysis) to the mean shape of species, which removes the effect of evolutionary allometric scaling from the differences among species. These residuals were then used to explore the nonallometric dataset. However, there were only minor differences between allometry-corrected and the original phylomorphospaces, so the corrected phylomorphospaces are provided as supplementary figures (Supporting Information Figures S4 and S5).

3.1 | Patterns of static modularity

Of the five tested hypotheses, the "*Anolis*" hypothesis was supported for hatchlings and adults (Table 1). For adults, relatively independent variation between the rostrum and cranium was detected for both the allometric and nonallometric components of shape variation. Such modularity was detected in variation of the asymmetric component, indicating developmental integration of these two parts of the skull (Table 1). The "anteroposterior" hypothesis was supported for adults only (Table 1).

3.2 | Ontogenetic modularity

At the ontogenetic level, the "anteroposterior" hypothesis was confirmed. After correction for allometry, modularity was confirmed only for the "neurodermatocranial" hypothesis (Table 1).

3.3 | Evolutionary modularity

The covariations between hypothesized modules at the evolutionary level were generally high, indicating low modularity and correlated evolutionary changes in lizard cranium shape. Only the "neurodermatocranial" hypothesis was supported when the allometry-included dataset was used (Table 1), indicating that allometry contributes to overall integration of the cranium at the evolutionary level.

The matrix correlations between covariance matrices of the symmetric and asymmetric components of shape variation were high and statistically significant (Table 2).

The overall integration of the cranium (SVE values) was similar in all cases, but hatchlings tended to have slightly higher values than adults. Allometry contributes significantly to overall integration of the cranium, as SVE values were in all cases slightly lower for the nonallometric component of variation in cranium shape (Table 3).
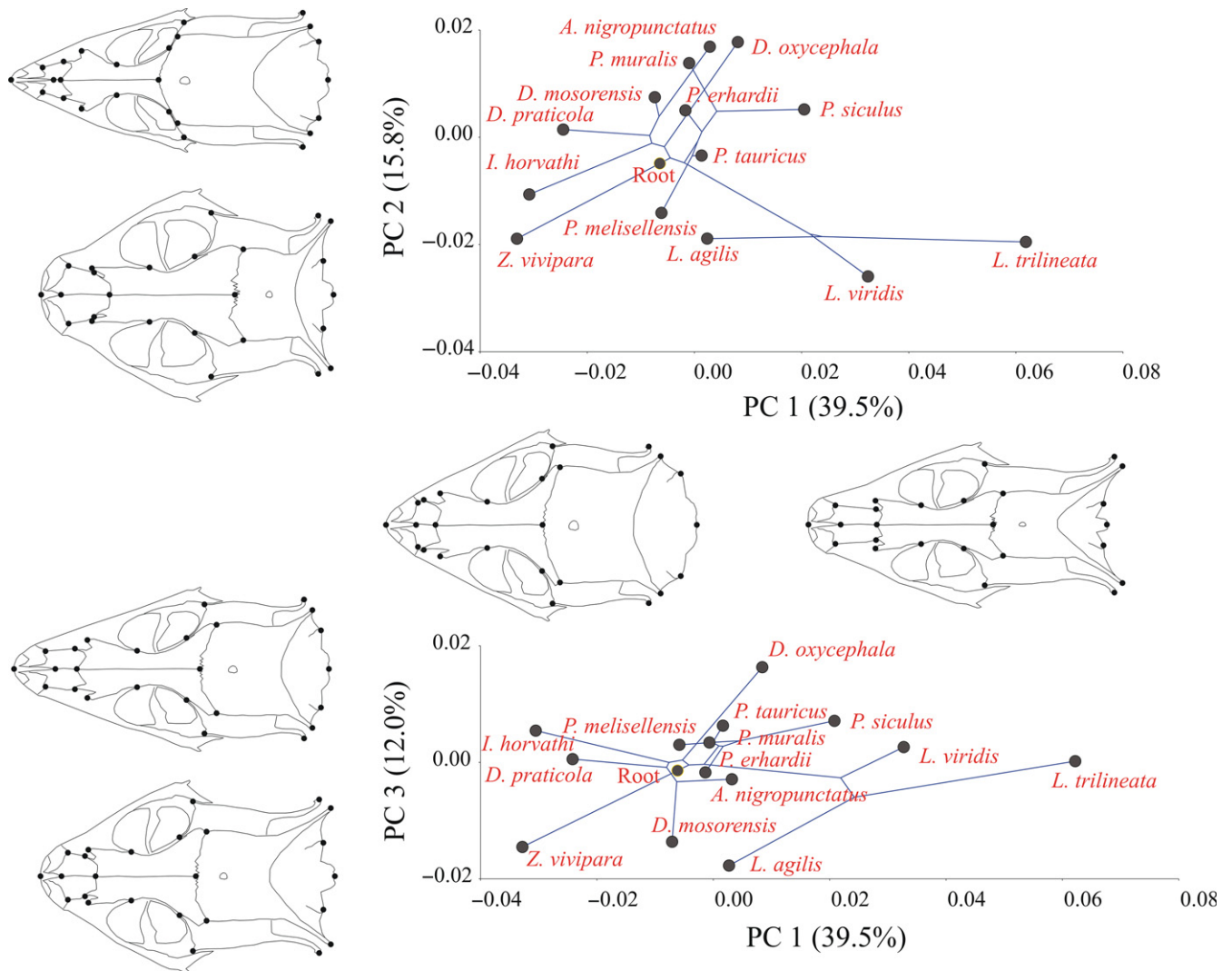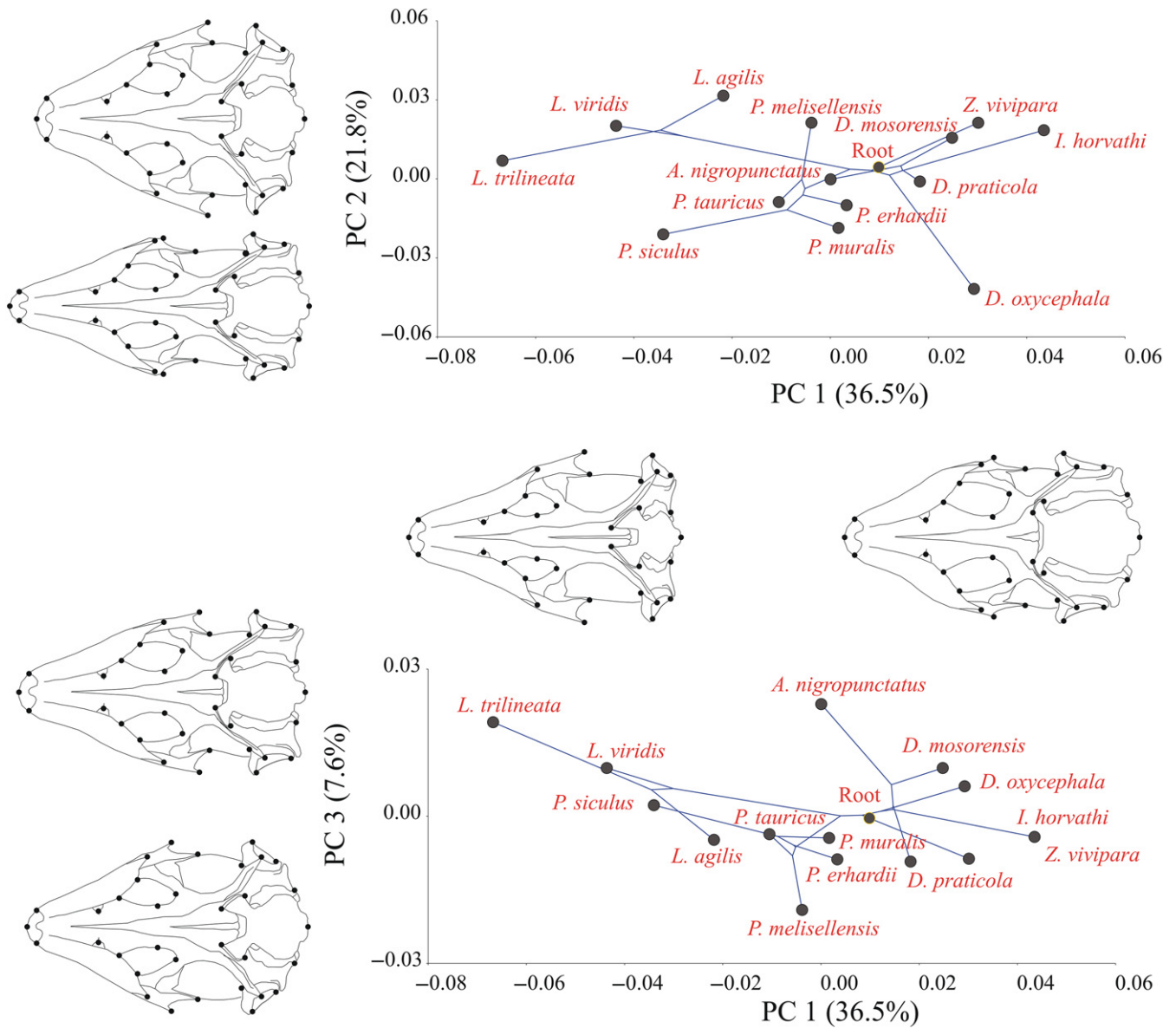
FIGURE 3 Phylomorphospaces of dorsal cranium shape for the first three PC components. The shape changes corresponding to maximal PC scores are presented along each axis

4 | DISCUSSION

The main shape gradients of the studied lacertid species correspond with what has been previously described, namely, a shift from the generally smaller terrestrial or saxicolous species with short and wide crania having an enlarged cranium base, to the generally larger terrestrial or semiarboreal species with elongated narrow crania, a reduced cranium base, and overgrown dermal skeletal elements (Urošević et al., 2013). Unlike the results of previous research (Urošević et al., 2012, 2013; but see Hipsley & Müller, 2017), there was a significant phylogenetic signal for shape of both the dorsal and the ventral cranium. Phylomorphospaces show some cases of morphological overlap, convergence, and homoplasy among the taxa, which are generally expected for Palaearctic lacertids (Urošević et al., 2013) and for the family as a whole (Hipsley & Müller, 2017; Hipsley et al., 2014). Allometry was significant at all three analyzed levels, and static and ontogenetic and evolutionary allometry share a common pattern, similar to the patterns previously described for lacertids (Bruner

& Constantini, 2007, 2009; Bruner, Constantini, Fanfani, & Dell'Omo, 2005; Hipsley & Müller, 2017; Kaliontzopoulou et al., 2008; Ljubisavljević et al., 2010, 2011; Piras et al., 2011; Urošević et al., 2012, 2013). Previous studies have shown that lizard cranial modularity is influenced by epigenetic and developmental processes (Monteiro & Abe, 1997). For the genus *Anolis*, it was found that the pattern of cranial modularity is not conserved across the group, thereby reflecting functional demands and unique skull shapes (Sanger et al., 2011).

Our multilevel study of lacertid cranial modularity reveals a somewhat different pattern of covariation at the static compared to the ontogenetic and evolutionary levels. At the static level (for adults), the covariation pattern of the dorsal cranium corresponded to the "*Anolis*" hypothesis, which separates the anterior (rostrum) and the posterior cranium. This hypothesis was confirmed for the symmetric and asymmetric components of shape variation, indicating a functional and/or developmental basis of covariation between the rostrum and the posterior part of the skull in lacertid lizards.

FIGURE 4 Phylomorphospaces of ventral cranium shape for the first three PC components. The shape changes corresponding to maximal PC scores are presented along each axis

Interestingly, the "*Anolis*" hypothesis was based on the extreme diversity of *Anolis* lizards, driven by complex functional demands (Johnson, Revell, & Losos, 2010; Losos, 2009; Sanger et al., 2011). Compared to *Anolis*, the lacertid cranium is generally uniform across species and genera (Arnold, Arribas, & Carranza, 2007), but some divergence of lacertid cranium shape is also driven by complex functional and ecomorphological demands, including ones related to shelter use, feeding, reproductive activity, and antipredatory behavior (Herrel, Van Damme, & De Vree, 1996; Herrel, Van Damme, Vanhooydonck, & De Vree, 2001; Herrel et al., 1999). It has been documented that even minor shape divergences can have important biomechanical and ecological connotations (Herrel et al., 1999, 2001; Urošević et al., 2012, 2013; Verwaijen, Van Damme, & Herrel, 2002), which might explain similarities in the pattern of covariation and could be common for lizards in general. The anteroposterior

hypothesis, which divides the ventral cranium into functional modules (corresponding to the "*Anolis*" hypothesis for the dorsal cranium), was also supported at the static level. According to our results, anteroposterior modularity of the ventral cranium is driven by size-related shape changes, as these modules could not be detected for nonallometric data. Previous allometric analyses (Ljubisavljević et al., 2010; Urošević et al., 2012, 2013) showed that there is positive allometric growth of the anterior component coupled with negative allometric growth of the braincase and posterior parts of the cranium, and that this growth pattern can be reflected through the observed modularity pattern.

As effects of size influence all parts of the structure jointly, allometry is expected to be a strong integrating factor and counteract the existing patterns of modularity (Klingenberg, 2009). That was confirmed in empirical studies on skull shape in various taxa such as

TABLE 1 RV coefficients calculated at the static, ontogenetic, and evolutionary levels of testing five cranial modularity hypotheses. The asymmetric component of shape variation is indicated as/Asymm. Nonallometric shape data are indicated as/Nall. Numbers given in boldface with an asterisk indicate proportions of partitions with RV coefficients less than the a priori hypothesis (<0.05)

| Level | Dataset | Anolis | Mammalian | Hypothesis | | |
				Tripartite	Anteroposterior	Neurodermatocranial
Static						
Functional	Adults	**0.177***	0.206	0.159	**0.327***	0.305
	Adults/Nall	**0.202***	0.262	0.193	0.304	0.255
Developmental	Adults/Asymm	0.103	0.112	0.080	0.267	0.138
	Adults/Asymm/Nall	**0.102***	0.114	0.080	/	/
Functional	Hatchlings	0.360	0.388	0.349	0.460	0.351
	Hatchlings/Nall	**0.259***	0.314	0.267	0.426	0.338
Developmental	Hatchlings/Asymm	0.185	0.196	0.141	0.165	0.118
	Hatchlings/Asymm/Nall	0.1753	0.184	0.131	/	/
Ontogenetic						
	Hatchlings + adults	0.835	0.896	0.850	**0.872***	0.903
	Hatchlings + adults/Nall	0.370	0.405	0.336	0.409	**0.240***
Evolutionary						
	Indep. contrasts	0.743	0.801	0.690	0.784	**0.580***
	Indep.contrasts/Nall	0.595	0.764	0.589	0.728	0.663

TABLE 2 Matrix correlation (MC) between covariance matrices of symmetric and asymmetric components of shape variation for dorsal and ventral crania of hatchlings and adults. For the landmark permutation test, data are significant at $p < 0.05$

| | Dorsal | | Ventral | |
	MC	p	MC	p
Hatchlings	0.608	0.0159	0.643	0.0001
Adults	0.775	0.0001	0.668	0.0001

TABLE 3 Scaled variance of eigenvalues (SVE) for dorsal and ventral crania of adults and hatchlings. Nonallometric data are indicated as Nall

	Dorsal	Ventral
Hatchlings	0.000518	0.000706
Hatchlings/Nall	0.000460	0.000679
Adult	0.000509	0.000610
Adult/Nall	0.000486	0.000554

mammals (Hallgrímsson et al., 2006) and birds (Klingenberg & Marugán-Lobón, 2013), but not in newts (Ivanović & Kalezić, 2010). Our results indicate that in lacertid lizards, the overall static integration (SVE) decreased after correction for allometry (the nonallometric component of shape variation), which is in agreement with the classic formulation of allometry as an integrating factor (Klingenberg, 2009, 2014).

At the static level, the overall shape integration (SVE) shows that the hatchling cranium is more integrated and less modular than the adult cranium. There is no common trend in the ontogeny of modularity and integration among taxa: in fishes, integration decreases during early and increases during later ontogeny (Fischer-Rousseau, Cloutier, & Zelditch, 2009) in tailed amphibians, integration tends to increase during ontogeny (Ivanović, Kalezić, & Aleksić, 2005) in rodents, on the other hand, it decreases slightly (Willmore, Leamy, & Hallgrímsson, 2006). For the studied lacertid species, it was previously shown that morphological disparity increases during ontogeny (Urošević et al., 2013). The shift from a more integrated cranium at the hatchling stage to the modular adult cranium could be a mechanism that increases morphological diversity among different genera and ecomorphs.

In ontogenetic series with substantial size increase due to growth, the overall integration should be mainly of ontogenetic origin (Klingenberg, 2014). In our data, functional modularity (anteroposterior modularity of the ventral cranium) at ontogenetic level was detected, which coincides with the modularity of the ventral cranium observed at the static level. This could be expected, as variation at the static level can partially result from variation in ontogenetic trajectories (Pélabon et al., 2013). However, the pattern of variation of the nonallometric component of shape variation reveals dermatoneurocranial modularity, which separates the ventral cranium into modules according to the early development and embryonic origin of neurocranial and dermatocranial elements. As allometry of the lacertid cranium has been proven to be influenced by functional constraints (Ljubisavljević et al., 2010; Urošević et al., 2012, 2013), then allometry itself could be important for masking the developmental

modularity (neurodermatocranial) pattern with the functional (antero-posterior) pattern, rather than being just a factor which increases the global shape integration (Hallgrímsson et al., 2006; Klingenberg, 2009; Klingenberg & Marugán-Lobón, 2013).

At the evolutionary level, the only hypothesis that was confirmed was the developmental hypothesis, which was also found at the ontogenetic level for nonallometric data. These results indicate that developmental integration is evolutionarily conservative and ontogenetically constrained. In cases when static and evolutionary integrations correspond, the often favored evolutionary scenario is that of neutral evolution by drift (Drake & Klingenberg, 2010; Klingenberg, Duttke, Whelan, & Kim, 2012; Monteiro et al., 2005). In our data, the lack of concordance between static and evolutionary patterns indicates that the functional covariation patterns which are observed at the static level are likely adaptive and could arise through selection.

Overall, the patterns of modularity and morphological integration in the lacertid cranium are complex and vary across different levels. Static modularity seems to be functionally influenced, while both ontogenetic modularity and evolutionary modularity show a developmental pattern. Allometry is an important integrating factor, but it could also be a constraint that directs and shapes the covariation pattern in lizards. The matching patterns of cranial modularity for the symmetric and asymmetric components of shape variation at the static level are in line with the "matching hypothesis" (Breuker et al., 2006; Cheverud, 1984; Wagner & Altenberg, 1996) and suggest that there is strong functional/developmental interaction during postnatal ontogeny, which is partially masked by allometry. Further studies, which would include more morphologically diversified phylogenetic lineages and more precise, three-dimensional capturing of skull shape, could provide additional information about the complex patterns of morphological evolution of the lacertid cranium.

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SUPPORTING INFORMATION

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APPENDIX 1

The origin of samples and collection numbers. *N*: number of individuals. Ha – Hatchlings.

Species	N	Locality	Collection numbers
Dalmatian algyroides *Algyroides nigropunctatus* (Duméril and Bibron, 1839) Collection: NHMM	Ha: 2 ♀: 23 ♂: 24	Montenegro: Lake Skadar; FYR Macedonia: Prespansko lake	Ha: A1-3, A7-1; ♀: L244–L252, L254–L256, L261, L262, L272, L287, L305, L306, L310, L311, L315, L317, L318; ♂: L220, L240–L242, L258–L260, L275, L280, L281, L285, L286, L288–L292, L295, L296, L298–L302
Sharp-snouted rock lizard *Dalmatolacerta oxycephala* (Duméril and Bibron, 1839) Collection: IBISS	Ha:/ ♀: 20 ♂: 21	Montenegro: Lake Skadar	Ha:/; ♀: 10O1, 16O1, 17O1, 25O1, 27O1–29O1, 32O1, 36O1, 59O1, 60O1, 62O1, 66O1, 79O1, 80O1, 83O1, 92O1, 95O1, 105O1, 116O1; ♂: 40O1–42O1, 44O1, 47O1–49O1, 51O1, 55O1–57O1, 70O1, 74O1–76O1, 81O1, 82O1, 89O1, 110O1, 111O1, 115O1
Meadow lizard *Darevskia praticola* (Eversmann, 1834) Collection: IBISS	Ha: 25 ♀: 23 ♂: 23	Serbia: Vršac Mountains	Ha: P2-2, P4-4-P4-6, P5-3, P5-5, P5-6, P7-2-P7-4, P8-2, P8-5-P8-7, P9-2, P9-6, P10-1, P10-5, P12-1, P12-6, P13-1, P13-5, P15-1, P15-5, P15-6; ♀: G22131-G22153; ♂: G22154-G22163, G22192-G22204
Mosor rock lizard *Dinarolacerta mosorensis* (Kolombatović, 1886) Collection: NHMM	Ha: 16 ♀: 20 ♂: 20	Montenegro: Mt. Lovćen	Ha: MOS 3d, MOS 5b-d, MOS 6b, MOS 6c, MOS 7b, MOS 9b, MOS 9c, MOS 10a, MOS 10c, MOS 11d, MOS 12c, MOS 12d, MOS 14b, MOS 15a, MOS 15c; ♀: L20, L21, L26, L27, L32, L33, L37, L38, L41, L46-L49, L53, L64, L66, L89, LM8, LM9, LM18; ♂: L23-L25, L28, L31, L36, L45, L36, L45, L50, L51, LM3-LM6, LM11-LM14, LM17, LM19, LM22
Horvath's rock lizard *Iberolacerta horvathi* (Méhely, 1904) Collection: IBISS	Ha: 15 ♀: 10 ♂: 9	Slovenia: Mangart Saddle, Predel, Kluže, Tabor Rock	Ha: Ih 1,1, Ih 1,2, Ih 2,1-2,4, Ih 3,1, Ih 3,3, Ih3,4, Ih 4,1, Ih 9,1, Ih 11,1, Ih 11,2, Ih 12,1, Ih 12,3, Ih 13-2; ♀: 2F, 4F-10F, 13F; ♂: 1M-10M
Sand lizard *Lacerta agilis* (Linnaeus, 1758) Collection: IBISS	Ha: 25 ♀: 18 ♂: 20	Serbia: Kovin, Mt. Zlatibor; FYR Macedonia: Mt. Šara, Mt. Korab, Dešat	Ha: Ag 1-1-Ag 1-8, Ag 2-1-Ag 2-12, Ag 3-1, Ag 3-3, Ag 3-6, Ag 3-8, Ag 3-11, Ag 3-12; ♀: G4751, G4758, G4764, G4765, G4768, G4772, G4775, G4779, G4786, G22560-G22568; ♂: G4752, G4754, G4756, G4767, G4769-G4771, G4774, G4777, G4780, G4782-G4785, G22569-G22574
Balkan green lizard *Lacerta trilineata* (Bedriaga, 1886) Collection: IBISS	Ha: 13 ♀: 5 ♂: 8	Montenegro: Kotor, Lake Skadar, Mt. Rumija, Lastvagrbaljska, Sutorman, Orljevo; FYR Macedonia: Mariovo	Ha: G22608–G22620; ♀: G22621, G22622, G22631–G22633; ♂: G22623–G22630
Green lizard *Lacerta viridis* (Laurenti, 1768) Collection: IBISS	Ha: 25 ♀: 19 ♂: 12	Serbia: Deliblato Sands	Ha: V1-4, V2-1, V2-5, V4-7, V6-2, V6-8, V8-4, V8-5, V9-1, V9-2, V10-1, V10-5, V10-6, V11-4, V13-1, V13-3, V13-6, V14-1, V14-3, V14-4, V16-1, V16-5, V16-8, V19-9, V19-10; ♀:G22280-G22298 ♂: G22299-G22310
Erhard's wall lizard *Podarcis erhardii* (Bedriaga, 1882) Collection: IBISS	Ha: 16 ♀: 28 ♂: 29	Serbia: Pčinja	Ha: E2-3, E6-1, E6-3, E6-4, E7-1-E7-3, E12-2, E13-2-E13-4, E17-1-E17-3, E19-1, E19-2; ♀: G22100–G22127, G22129; ♂:G22130, G22164–G22191
Dalmatian wall lizard *Podarcis melisellensis* (Braun, 1877) Collection: IBISS	Ha: 19 ♀: 19 ♂: 18	Montenegro: Lake Skadar	Ha: Mel1or 2ml3-1, Mel2ml1-1, Mel2ml1-2, Mel3or4ml3-1, Mel3or4ml3-2, Mel5ml1-1, Mel5ml3-2, Mel5ml3-4, Mel6ml3-2, Mel6ml3-3, Mel6ml3-3*, Mel8ml1-2, Mel8ml2-3, Mel9ml1-2, Mel9ml1-3, Mel15ml1-1-Mel15ml1-4, Mel15ml1-4*; ♀:G10147–G10150, G10152, G10153, G10155–G10157, G10159, G10162, G10164, G10165, G10189, G10190, G10192, G10193, G10199, G10200; ♂:G10170, G10171, G10173, G10174, G10176, G10201, G10203–G10207, G10209, G10211–G10214, G10216, G10233

(Continues)

APPENDIX 1 (Continued)

Species	N	Locality	Collection numbers
Common wall lizard *Podarcis muralis* (Laurenti, 1768) Collection: IBISS	Ha: 25 ♀: 26 ♂: 26	Serbia: Belgrade	Ha: M1-3, M1-4, M2-2, M2-4, M6-1, M6-4, M7-1, M7-2, M8-1, M8-2, M9-1, M9-2, M9-4, M12-1, M12-3, M15-2, M16-1, M16-2, M18-2, M18-5, M18-6, M20-1, M20-2, M22-2, M22-1; ♀: G22029, G22032-G22035, G22037, G22038, G22043, G22045, G22046, G22050-G22065; ♂:G22002-G22007, G22009-G22028
Italian wall lizard *Podarcis siculus* (Rafinesque, 1810) Collection: IBISS	Ha: 26 ♀: 11 ♂: 19	Croatia: Zagreb; Krk island	Ha: AK60, PS1-PS13, PS19-PS21, PS25, PS26, PS35, PS37, PS38-1, PS38-2, PS41, PS57, PS75; ♀:PS1, PS3/1, PS3.1, PS7/1, PS7/2, PS8/1, PS8/4, PS11, PS13, PS14/2, PS?; ♂:PS2-PS8, PS30-PS41
Balkan wall lizard *Podarcis tauricus* (Pallas, 1814) Collection: IBISS	Ha: 17 ♀: 26 ♂: 24	Serbia: Deliblato Sands	Ha: T2-1, T2-2, T5(8), T5(9), T6-1, T6-2, T6(12), T7-1, T7-13, T8-2, T9-1, T9-2, T5(10), T10-1, T10-2, T12-1, T12-2; ♀:G21808-G21833; ♂:G21877-G21900
Viviparous lizard *Zootoca vivipara* (von Jacquin, 1787) Collection: IBISS	Ha: 15 ♀: 13 ♂: 7	Serbia: Mt. Stara Planina	Ha: ZV50-1-ZV50-7, ZV64-1-ZV64-4, ZV64-6, ZV64-7, ZV70-3, ZV70-9; ♀:003DJ, 004DJ, 008DJ-011DJ, 49DJ, 63DJ2, 70DJ3, G21804, G21806, G22066, G22805; ♂:005DJ, 006DJ, 012DJ, 013DJ, 60DJ, 74DJ, 78DJ

Collection code: IBISS – Herpetological Collections of the Institute for Biological Research, "SinišaStanković", Belgrade; NHMM – Herpetological Collections of Natural History Museum of Montenegro, Podgorica. Asterisk denotes different individuals with duplicate collection numbers.