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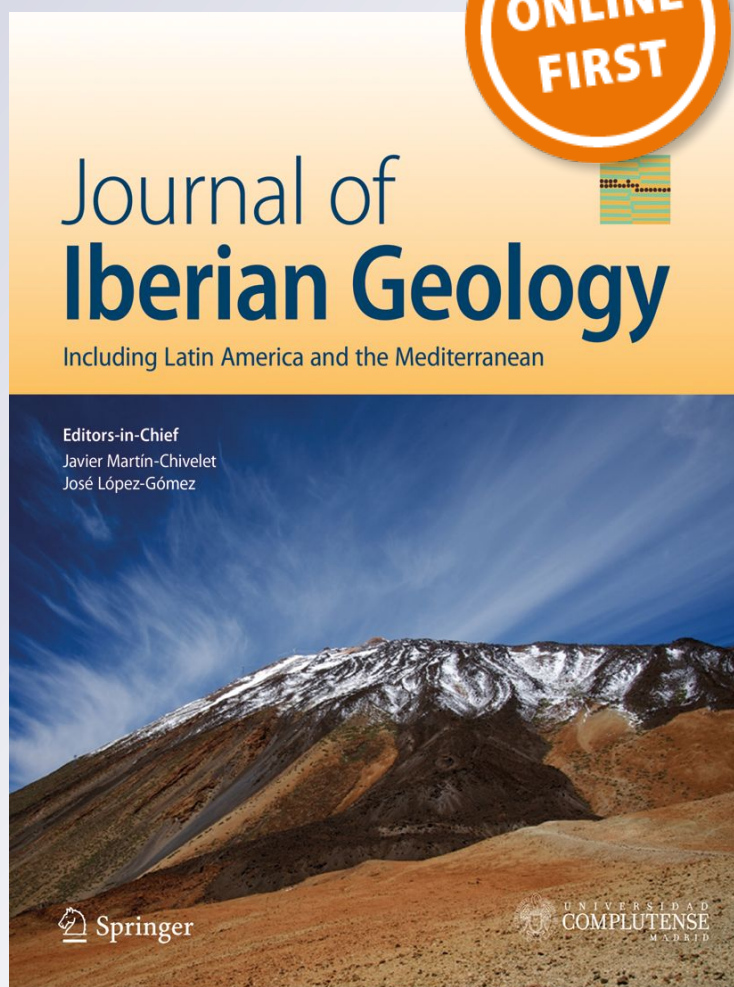
Celeste M. Pérez-Ben & Raúl O. Gómez

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Morphological integration and evolution of the skull roof in temnospondyl amphibians

Celeste M. Pérez-Ben[1] · Raúl O. Gómez[1,2]

Abstract

Morphological integration refers to the phenotypic interdependence of two or more traits and is estimated by the degree of covariation or correlation among traits at different levels, such as at the intraspecific and evolutionary scales. Intraspecific integration of morphological traits results from the interaction among traits at the genetic, developmental, and functional levels and it has been proposed that it channels morphological evolution by modulating variability. In this work, we test whether the intraspecific integration might have channeled the morphological evolution of the skull roof in a major tetrapod radiation, that of extinct temnospondyl amphibians. To do this, we quantified the patterns of intraspecific integration of different species and explored their relationships with the evolutionary patterns of integration and disparity of three clades of temnospondyls using geometric morphometrics. We recovered that, at the intraspecific level, the integration patterns of the total shape of the skull roof are conserved across the clade and over geological time, but that the integration among individual bones varies in every species considered. We did not find a correlation between the patterns of integration among individual bones at the intraspecific and evolutionary levels, nor between the strength of intraspecific integration of each bone and their respective disparity. These results suggest that the intraspecific integration might have not affected significantly the morphological evolution of the skull roof in temnospondyls over geological time. Thus, it seems that the morphological evolution of this skeletal part might have been driven more by selective pressures than by shared developmental constraints inherited from the temnospondyl ancestor.

Keywords Morphological integration · Disparity · Morphological evolution · Temnospondyli

Resumen

La integración morfológica refiere a la interdependencia fenotípica de dos o más rasgos y es estimada por el grado de covariación o correlación de dichos rasgos a diferentes niveles, como el intraespecífico y el evolutivo. La integración intraespecífica de rasgos morfológicos resulta de la interacción de caracteres a nivel genético, del desarrollo y funcional y ha sido propuesta como canalizadora de la evolución morfológica al modular la variabilidad. En este trabajo, ponemos a prueba si la integración intraespecífica pudo haber canalizado la evolución morfológica del techo craneano en una de las grandes radiaciones de tetrápodos, la de los anfibios temnospóndilos extintos. Para ello, cuantificamos los patrones de integración intraespecífica de diferentes especies y exploramos su relación con los patrones evolutivos de integración y disparidad en tres clados de temnospóndilos usando morfometría geométrica. Obtuvimos que, a nivel intraespecífico, los patrones de integración de la forma total del techo craneano son conservados dentro del clado y a través del tiempo geológico, pero que la integración entre huesos individuales varía en todas las especies consideradas. No encontramos una correlación entre los patrones de integración entre los huesos individuales a nivel intraespecífico y evolutivo, ni entre la magnitud de la integración intraespecífica de cada hueso y su respectiva disparidad. Estos resultados sugieren que la integración intraespecífica pudo no haber afectado de manera significativa la evolución morfológica del techo craneano de los temnospóndilos a través del tiempo geológico. Por lo tanto, la evolución morfológica de esta parte del esqueleto

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habría sido dirigida en mayor medida por presiones selectivas que por restricciones del desarrollo heredadas del ancestro temnospóndilo.

Palabras clave Integración morfológica · disparidad · evolución morfológica · Temnospondyli

1 Introduction

The different parts of organisms are coordinated in their size and shape because they develop, function, and evolve jointly (Klingenberg 2013). This phenotypic interdependence of two or more traits is called morphological integration and is estimated by the degree of covariation or correlation among these traits at different levels, such as at the intraspecific and evolutionary scales (Klingenberg 2008). Intraspecific integration refers to the covariation of traits in a given developmental stage of a single species, whereas evolutionary integration deals with the covariation of traits among different species.

At the intraspecific level, the covariation of characters at the morphological level results from the interaction among traits at the genetic, developmental, and functional levels (Goswami et al. 2014). In particular, some modules at the morphological level might reflect morphogenetic components consisting of spatially delimited cell populations that are internally coordinated in response to epigenetic interactions and are almost autonomous with respect to patterns of formation and differentiation (Hall 2003; Zelditch et al. 2008). At the evolutionary level, traits covary when they are inherited or selected jointly (Monteiro and Nogueira 2009). Taking this into account, the study of morphological integration allows shedding light on the mechanisms that work at other levels, bridging different facets of evolutionary biology (Goswami et al. 2015). Because of this, morphological integration has become a prominent concept in evolutionary biology in the last two decades (Klingenberg 2014) and many recent works have characterized large-scale patterns of integration and addressed their relationship with changes in environment (e.g., Badyaev et al. 2005), function (e.g., Young and Hallgrímsson 2005), and development (e.g., Kelly and Sears 2011; Goswami et al. 2015). Most of these works have focused on living species, usually model organisms, but a few studies on integration in extinct taxa have been carried out in the last years (e.g., Bell et al. 2011; Maxwell and Dececchi 2013; Goswami et al. 2015), as the quantitative methods used to calculate the integration patterns can be also applied to fossils.

It has been proposed that intraspecific integration channels morphological evolution by modulating variability (Willmore et al. 2007; Klingenberg 2010). A strong integration among a set of traits, as that expected within a module, might limit its range of variation or slow down its evolutionary rate, since variation in one of these highly integrated

traits might entail disadvantageous variation in the other or the module as a whole and, thus, would be negatively selected (Wagner and Altenberg 1996). In this scenario, a strong integration at the intraspecific level might result in relatively low morphological disparity within a given clade, being the disparity a measure of the morphological divergence among taxa. Conversely, the same covariation structure might likewise increase variation or accelerate evolutionary rates due to positive selection of a single trait that, in turn, might indirectly favor change in the other integrated traits, facilitating transformation of functional structures and leading to a faster displacement in the morphospace (Goswami and Polly 2010a). Therefore, it becomes evident that the study of morphological integration is essential to understand morphological evolution and, to do this, it is necessary to understand the relationships between patterns of integration at different scales in relation to morphological disparity (Klingenberg and Marugán-Lobón 2013).

In this work we explore these relationships in a major tetrapod radiation, that of extinct temnospondyl amphibians. Remarkably, studies on morphological integration in tetrapods have almost exclusively focused on extant mammals (e.g., Ackermann and Cheverud 2000; Young and Badyaev 2006; Zelditch et al. 2009), whereas other groups, such as amphibians, remain largely understudied. In this context, the present work adds novel information to our general knowledge on the subject by dealing with a non-mammalian group of extinct taxa. In particular, we studied by geometric morphometric methods the patterns of intraspecific and evolutionary integration of the skull roof of different temnospondyl species and clades, respectively, aiming to:

1. Explore whether these patterns are conserved or vary in the group. We tested variation in: (a) the patterns and total integration of the overall shape of the skull roof and (b) the patterns of integration among bones (i.e., inter-bone integration). By overall shape we refer to the total landmark configuration used to describe the skull roof (see below), whereas the individual bones are represented by sub-sets of landmarks and, thus, the inter-bone integration refers to the relationship among these sub-sets. We focused on the study of bones as individualized units because they constitute morphogenetic units (Monteiro et al. 2005).

2. Test whether the intraspecific integration might have channeled the morphological evolution among different clades of temnospondyls. To do this, we first evalu-

ated whether the degree of intraspecific integration within individual bones (i.e. intra-bone integration) of the different species correlates with the disparity of the bones in the respective clades these species belong to. We tested two extreme hypotheses: (1) high intra-bone integration constrains variation, resulting in low disparity (i.e., constraint hypothesis), versus (2) high intra-bone integration facilitates variation, leading to high disparity (i.e., facilitation hypothesis). Secondly, we tested whether the patterns of inter-bone integration at the evolutionary level follow the patterns recovered at the intraspecific level.

2 Materials and methods

Morphological integration has been typically studied by "traditional morphometrics" based on interlandmark distances (e.g., Bell et al. 2011). However, geometric morphometric approaches have recently started to be developed and applied to address this issue (e.g., Monteiro and Nogueira 2009). For this study, we decided to use geometric morphometric techniques because they allow characterizing the patterns of morphological variation in detail, while keeping the anatomic context of the studied structures (Klingenberg 2015). All the analyses in this work were carried out in R 3.3.0 (R Core Team 2016).

2.1 Intraspecific level

We studied different aspects regarding the integration of the skull roof at the intraspecific level in the temnospondyl species *Apateon pedestris* (n = 15), *Micromelerpeton credneri* (n = 19), *Archegosaurus decheni* (n = 11), and *Mastodonsaurus giganteus* (n = 12). These taxa were selected because they are representative of different lineages across the temnospondyl radiation and we had access to a relatively high number of well-preserved individuals for each of them. In addition, the seymouriamorph *Discosauriscus austriacus* was used as outgroup for comparisons. The specimens used are listed in the Online Resource 1.

We restricted the analyses to the frontals, parietals, postparietals, and tabulars, which are all dermal bones of the skull roof, because they are usually the best preserved cranial elements and are relatively flat, which allowed us to compare two-dimensionally preserved species (i.e., *Micromelerpeton*, *Apateon*, and *Discosauriscus*) with those preserved in three dimensions (i.e., *Mastodonsaurus* and *Archegosaurus*). The lateral skull bones were not taken into account because they tend to preserve poorly and not in their natural position in bidimensional specimens.

We photographed or drew each specimen using a microscope with camera lucida. On each image, we digitized 12 landmarks on the skull roof (Fig. 1) using TPSDIG2 2.26 (Rohlf 2016). Landmarks were selected based on the trade-off between capturing the skull shape in detail and maximizing the number of specimens over which landmarks could be digitized confidently. Landmarks were digitized in the best-preserved cranial half.

2.1.1 Matrix repeatability and total cranial integration

For each species separately, the landmark configurations of the individuals were superimposed by a Generalized Procrustes Analysis (GPA; Rohlf and Slice 1990; Bookstein 1991) using the R package geomorph (Adams and Otárola-Castillo 2013). Then, we calculated the correlation matrix of the Procrustes coordinates using congruence coefficients (Burt 1948) as the measure of correlation. We chose the congruence coefficient over the similar canonic correlation coefficient because the former seems to be more robust when sample sizes are small (Goswami and Polly 2010b). This correlation matrix represents the integration pattern of the total shape (Goswami and Polly 2010b).

In order to account for the statistical robustness of the correlation patterns of each species, we calculated the repeatability of each correlation matrix by autocorrelation (Goswami and Polly 2010b). To do this, the dataset for each species was resampled 10,000 times with replacement and the correlation matrix was calculated in each
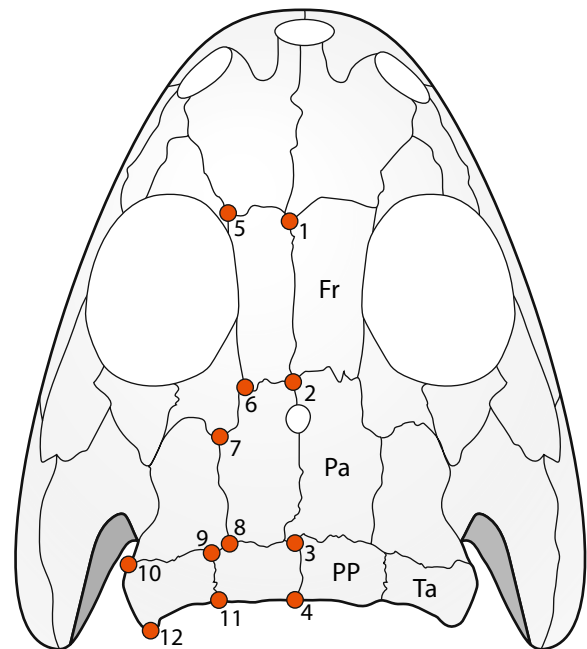

Fig. 1 Landmarks of the skull roof used in the geometric morphometric analyses. *Fr* frontal, *P* parietal, *PP* postparietal, *Ta* tabular

iteration. The new correlation matrices were compared with the respective original one by correlation matrix analysis. The average of the matrix correlation of the 10,000 repetitions was taken as an estimator of the repeatability of the correlation matrices (Goswami et al. 2012).

We compared the patterns of morphological integration of the total shape (i.e., the correlation matrices) between each pair of species by matrix correlation analyses (Goswami 2006). Given that the sampling error makes that the theoretical range (i.e., $+1$, -1) could not be reached, we corrected this error, as suggested by Cheverud (1996), by dividing the matrix correlations obtained by the maximum correlation reachable, calculated as the square root of the product of the repeatabilities of the matrices being compared (Goswami and Polly 2010b). Statistical significance of the analyses was estimated by Mantel test. The Mantel test consists of a permutation test that evaluates whether the similarity between two matrices is significantly higher than the expected between two random matrices of the same size (Goswami and Polly 2010b).

We quantified the total integration of the skull roof as the relative standard deviation of eigenvalues (RSDE) of the correlation matrices (Pavlicev et al. 2009). The dispersion of eigenvalues is useful to summarize the degree of integration or modularity of a dataset because the principal components (i.e., eigenvectors) reflect the covariation among variables: a strongly integrated system will have most of its variance explained by few principal components; thus, the dispersion of the eigenvalues will be high because only few axis will have high eigenvalues, whereas the rest will have very low ones (Goswami et al. 2012).

To calculate whether the RSDE is significantly different between each pair of species, we resampled 10,000 times the individuals of each species with replacement and calculated the RSDE in each iteration. The probability of one species to have a higher RSDE is the number of times that this occurs in the resampling procedure divided by the number of iterations (Young and Hallgrímsson 2005).

2.1.2 Intra-bone integration and integration patterns among bones

We calculated the integration of each dermal bone separately by partitioning the total set of landmarks (i.e., each partition represents a single bone; Online Resource 2). Those landmarks situated in the suture between two bones were included in the partitions of both elements. For each partition, we calculated the correlation matrix of the Procrustes coordinates and the RSDE in the same way as for the total configuration of landmarks. For each species, we tested whether there are significant differences between each pair of bones as before for the differences among species.

In order to evaluate the integration patterns among bones, we followed the protocol of Monteiro et al. (2005) modified by Zelditch et al. (2012), suitable to study the correlation among complex traits (Zelditch et al. 2008) such as cranial bones. For each species and each bone (i.e., partition) separately, we calculated the Euclidean distance between each pair of individuals, obtaining a distance matrix for each bone in each species. By doing this, the correlations are estimated for shapes instead of individual landmarks (Zelditch et al. 2008). Then, for each species, we calculated the matrix correlation between each pair of distance matrices, obtaining what Monteiro et al. (2005) called "integration matrix". The correlation between distance matrices indicate whether the variation in the shape of one partition (i.e., in this case, a bone) is related to the variation of the other partition. When both partitions show the same pattern of variation among individuals, their respective distance matrices are strongly associated and, thus, the matrix correlation is statistically significant. Conversely, matrix correlations close to zero indicate that both partitions differ in their variation structure (Zelditch et al. 2008). We calculated the matrix correlation for a pair of bones as a partial correlation according to the matrices of the other bones and estimated the statistical significance by Mantel test (Monteiro and Nogueira 2009).

2.2 Evolutionary level

We studied three temnospondyl clades that include the four temnospondyl species used in the intraspecific analyses: (1) Dissorophoidea (includes, *Apateon* and *Micromelerpeton*), (2) Capitosauria (includes *Mastodonsaurus*), and (3) Eryopidae + non-Stereospondyli Stereospondylomorpha (includes *Archegosaurus*). We considered eryopids together with the non-Stereospondyli stereospondylomorphs in order to have a larger number of species. Although they constitute a paraphyletic group respect to Stereospondyli, we did this because they formed a monophyletic group before the Triassic radiation of stereospondyls according to recent phylogenetic hypotheses (e.g., Schoch 2013).

The phylogenetic hypotheses used for the clades were obtained from the literature. We considered the topology used by Pérez-Ben et al. (2018) for dissorophoids (Online Resource 3). For capitosaurs, we followed the tree obtained by Schoch (2008) (Online Resource 4). For eryopids and non-Stereospondyli stereospondylomorphs, we used the topology recovered by Schoch and Witzmann (2009a) with *Sclerocephalus bavaricus* and *S. jogischneideri* added by hand following another work of the same authors (Schoch and Witzmann 2009b; Online Resource 5).

We used images of the skull roof in dorsal view consisting of photographs of fossil specimens studied by first hand and photographs, interpretative drawings, or reconstructions

from the literature (Online Resource 1). We only included interpretative drawings and reconstructions whose accuracy could be checked, except for some species of the clades (2) and (3) for which we did not have access to the fossil material by first hand nor by photographs. In this latter case, we only considered species represented by well-preserved skulls according to Schoch and Milner (2000, 2014). We decided to include these species, in spite of the lower confidence in their reconstructions, in order to have a sample of taxa large enough to evaluate the integration patterns. On the images, we digitized the same set of landmarks used in the intraspecific analyses.

2.2.1 Skull shapes in the morphospace

We constructed a morphospace to summarize the diversity in skull shape in the sample of temnospondyls used in this work and provide a visual morphological framework for the discussion of the integration analyses. To do this, we superimposed the landmark configurations of the total set of species by GPA and performed a principal component analysis (PCA). We visualized the shape variations in the morphospace by deformations grids depicting deformations from the total species mean shape to: (1) the shapes corresponding to extreme values on the PC1 and PC2; (2) the mean shapes of the three temnospondyl clades considered; and (3) the mean shapes of the temnospondyl species used in the intraspecific analyses.

2.2.2 Integration patterns among bones

We calculated the patterns of integration between bones in the same way as in the intraspecific analysis, but using species of a given clade instead of individuals of a single species. The matrix of phylogenetic distances was included in the partial correlation analyses to take into account the phylogenetic structure, following Monteiro and Nogueira (2009). To calculate the phylogenetic distances, given that the topologies used herein lack branch lengths because they are the result of cladistic analyses, we set all branch lengths to one, which corresponds to a speciational model of evolution (Garland et al. 1992).

2.2.3 Disparity

To measure the disparity of each dermal bone in each clade, we followed the protocol of Goswami and Polly (2010a). We calculated the partial Procrustes distance (i.e., the squared root of the sum of the squared Euclidean distances between homologous landmarks of the species and the consensus configuration) for each partition and species. The disparity of each partition in a clade was defined as the sum of the partial Procrustes distances of every species of the respective clade. Given that the disparity of the different bones cannot be compared because the partitions have different number of landmarks, comparisons were made by a randomization test. This test consisted in comparing the disparity observed for each bone to the distribution of disparity of random configurations with the same number of landmarks of the given partition obtained from the total set of landmarks. We considered that a bone has a significantly high disparity when it was higher than 95% of the disparity values generated from the random configurations. Conversely, we considered that a bone has a significantly low disparity when it was lower than 95%. For each partition, 10,000 random configurations were generated and a GPA carried out and the disparity calculated as described before for each iteration.

3 Results

3.1 Intraspecific integration

Matrix repeatability is highest for *Discosauriscus* and *Micromelerpeton*, the two best sampled species (Table 1). The total integration of the skull roof, calculated as RSDE, do not show significant differences for any pair of species (i.e., we obtained $p > 0.05$ in every pair-comparison), except for *Archegosaurus* and the seymouramorph *Discosauriscus* (Table 2).

We recovered a significant correlation (i.e., $p < 0.05$ of Mantel test) between the correlation matrices of every pair of species. This means that the patterns of integration of the total shape do not differ significantly among species (Table 2). The highest matrix correlation, with and without correction for sampling error, is between *Archegosaurus* and *Apateon* and the lowest, between *Discosauriscus* and *Mastodonsaurus.*

Table 1 Sample size, matrix repeatability, and total integration (measured as the relative standard deviation of eigenvalues of correlation matrices; RSDE) of species

	Discosauriscus	*Apateon*	*Micromelerpeton*	*Archegosaurus*	*Mastodonsaurus*
Sample size	21	15	19	11	15
Matrix repeatability	0.94	0.93	0.94	0.9	0.92
RSDE	0.26	0.32	0.29	0.33	0.32

Table 2 Lower triangle: pair-wise matrix correlation between species

	Discosauriscus	Apateon	Micromelerpeton	Archegosaurus	Mastodonsaurus
Discosauriscus		0.1	0.22	**0.02**	0.12
Apateon	0.71 (0.76)		0.75	0.3	0.63
Micromelerpeton	0.78 (0.83)	0.75 (0.8)		0.08	0.7
Archegosaurus	0.73 (0.8)	0.8 (0.87)	0.77 (0.84)		0.4
Mastodonsaurus	0.7 (0.75)	0.76 (0.82)	0.77 (0.83)	0.75 (0.82)	

Values obtained after correcting for sampling error are between parentheses. All correlations with $p < 0.05$. Upper triangle: pair-wise comparison of the relative standard deviation of eigenvalues (RSDE) of correlation matrices between species. The p value of RSDE-column > RSDE-row is reported. In bold, $p < 0.05$

Table 3 Intra-bone integration measured as the relative standard deviation of eigenvalues (RSDE) of correlation matrices of the subsets of landmarks representing individual bones

	Frontal	Parietal	Postparietal	Tabular
Discosauriscus Fr > P, PP PP > P T > P	0.52	0.29	0.48	0.49
Micromelerpeton Fr > Ta PP > Pa, Ta	0.5	0.35	0.46	0.37
Apateon PP > Fr, Pa, Ta	0.43	0.35	0.5	0.42
Archegosaurus Fr > Pa, Ta	0.6	0.43	0.45	0.38
Mastodonsaurus Fr > Ta PP > Pa, Ta	0.55	0.45	0.59	0.39

Statistically significant differences ($p < 0.05$) between pairs of bones are reported below taxon names

Fr frontal, *P* parietal, *PP* postparietal, *Ta* tabular

The results of the intra-bone integration and the significant differences between pairs of bones are shown in Table 3. Postparietals and/or frontals are the only bones that show a higher intra-bone integration respect to other bones in the species analyzed. The patterns of integration among bones (i.e., partial correlations) are shown in Fig. 2, where only significant correlations are reported ($p < 0.05$). As observed in the figure, the patterns differ in every species.

3.2 Skull shapes in the morphospace

PC1 and PC2 account for the 69.5% of the variance (PC1, 54.4%; PC2, 15.1%). The ordination of taxa in the morphospace and the deformation grids listed above are shown in Fig. 3. Positive values of PC1 indicate shorter and wider bones of the skull roof than the consensus configuration. Positive values of PC2 also indicate shorter and wider parietals, but, in contrast to PC1, longer frontals, postparietals, and tabulars.

Dissorophoids are characterized by positive values on PC1, having skulls markedly shorter than the other two clades, and are widespread over PC2. In contrast, Capitosauria and Eryopidae + Stem-Stereospondyli are distinguishable over PC2, with capitosaurs having positive values. The four temnospondyl species considered in the intraspecific analyses are wide apart in the reduced morphospace PC1–PC2.

3.3 Disparity and evolutionary integration

The results of disparity are shown in Fig. 2 and the values reported in the Online Resource 6. We did not recover a low disparity for any bone in any clade considered. Remarkably, the postparietal is the only element showing a high disparity in every clade. The patterns of integration among bones are shown in Fig. 2, where only significant correlations are reported ($p < 0.05$). As shown in the figure, the patterns differ among clades.

4 Discussion

4.1 Intraspecific integration

It is noteworthy that the analyses were carried out using relatively small samples, with the poorest sampled species (i.e., *Archegosaurus*) represented by only 11 specimens. This issue should be taken into account, even in spite of the high matrix repeatability values recovered, when evaluating the confidence of the results of this study.

The total integration of the skull roof does not differ statistically among the temnospondyls studied and the matrix correlations are high (i.e., higher than 0.7) in the comparisons of every pair of species. This points to a conservation of the integration patterns of the total shape of the skull roof in the clade and through geological time, as the sampled species include both Paleozoic taxa (i.e., *Apateon*, *Micromelerpeton*, and *Archegosaurus*) and the Triassic *Mastodonsaurus*. These results are consistent with comparative studies in living species that document that correlation matrices of traits show a limited divergence among closely related taxa (Ackermann and Cheverud 2000; Maxwell and Dececchi 2013). On the other hand, the fact that no differences were obtained between temnospondyls and *Discosauriscus*
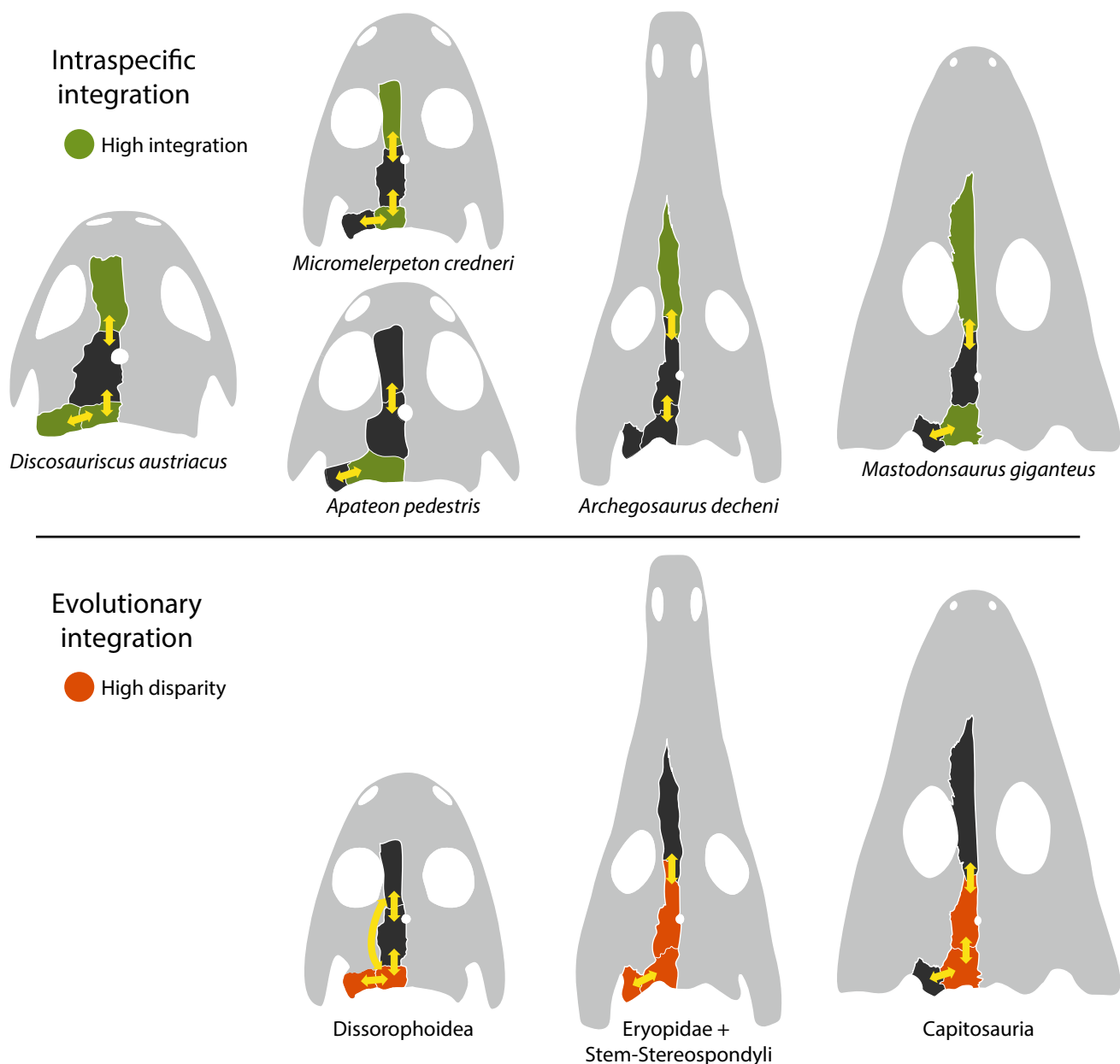
Fig. 2 Patterns of integration among bones at the intraspecific and evolutionary levels, intra-bone integration, and disparity. Statistically significant integration between a pair of bones (i.e., partial correla- tion) is indicated with arrows. High intra-bone integration and high disparity highlighted. Taxa are aligned with their respective clades. Numerical results are reported in the Online Resource 6

(except for the total integration of the latter and *Archego- saurus*, which is in one end of the temnospondyl range) indicates that the patterns of integration recovered are not exclusive of Temnospondyli.

Conversely, the analysis of the integration among bones based on the partial correlations does reveal differences among taxa. These patterns vary in every species considered, even between *Apateon* and *Micromelerpeton*, two species closely related (Fröbisch and Schoch 2009). The differences obtained in the results from the correlation matrix and those

from partial correlations (i.e., conservation versus no con- servation of patterns, respectively) might be due to the dif- ferent types of characters used to perform the analyses. The correlation matrix and the analyses based on it (i.e., total integration and matrix correlation) consider each landmark as an individual character and, thus, they evaluate integra- tion among landmarks (Goswami and Polly 2010b), whereas in the method used herein to study the correlation among
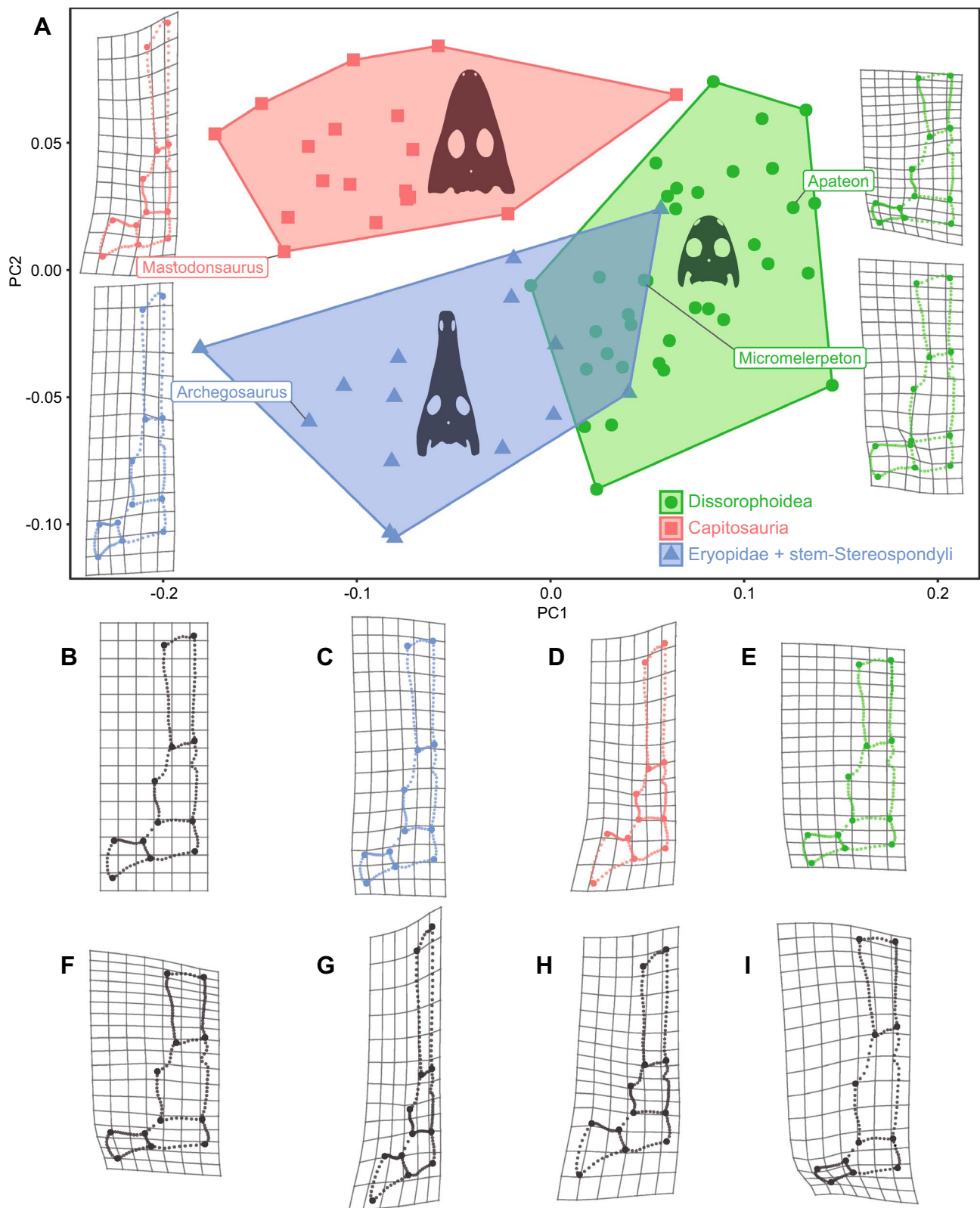
Fig. 3 **a** Ordination of taxa in the first and second PCs of the principal component analysis with clades indicated by symbols and colors. The grids show the deformation between the consensus configuration and the mean shapes of the species used at the intraspecific analyses; **b** consensus configuration; **c–i** grids showing the deformation between the consensus configuration and the mean shapes of **c** Eryopidae + stem-Stereospondyli, **d** Capitosauria, and **e** Dissorophoidea, and the maximum and minimum values of PC1 and PC2 (**f**, **g** and **h**, **i**, respectively)

bones characters are the subsets of landmarks that represent the different bones (Monteiro et al. 2005; Zelditch et al. 2012).

Regarding the intra-bone integration, tabulars and parietals show the lowest integration in every temnospondyl species. This is interesting because, whereas parietals are the first dermal bones to ossify in the ontogeny of *Apateon caducus* and *A. pedestris* (the only temnospondyls for which early ossification sequences are known; Schoch 1992), tabulars are the last to form. Therefore, the intra-bone integration does not seem to be related to the time of ossification. Furthermore, the strength of this type of integration is not related to the relative position of the bones in the skull: parietals are completely surrounded by other bones, whereas tabulars only articulate with other elements in their anterior and medial margins. Taking into account that this integration pattern is present in species of different temnospondyl clades, geological ages, and habitats but it is not recovered in *Discosauriscus*, it seems that stronger developmental constraints on frontals and postparietals than on parietals and tabulars were inherited from the temnospondyl ancestor.

4.2 Evolutionary integration

The three clades of temnospondyls here analyzed differ in their patterns of evolutionary integration among cranial bones. Furthermore, we did not found a correlation between the evolutionary pattern of a clade and the intraspecific pattern of the species of that clade. These results suggest that the coordinated evolution of the bones of the skull roof might have been driven more by selective pressures than by shared developmental constraints inherited from the last common ancestor. This is consistent with the high variation of the integration patterns among bones recovered here at the intraspecific level and with empirical studies in which the observed evolutionary integration is more frequently attributed to common selection than to shared developmental patterns (Monteiro et al. 2005).

4.3 Disparity and intra-bone integration

According to the constraint hypothesis, bones with high intra-bone integration at the intraspecific level will show a low morphological disparity. Conversely, the facilitation hypothesis predicts that bones with low intra-bone integration will be associated with a high disparity. We recovered that the bones with high disparity are either highly or poorly integrated at the intraspecific level. In other words, the integration of a bone is not related consistently to its disparity in any of the clades studied (Fig. 2). Therefore, it remains unclear whether high intra-bone integration facilitates or limits morphological disparity. The lack of evidence supporting one hypothesis over the other might be due to the alternation of facilitation and constraint throughout temnospondyl evolution or to the actual lack of relationship between integration and disparity. In any case, if the instrumental problem of small sample sizes is put aside, it seems that intra-bone integration did not affect significantly the morphological evolution of the skull roof in temnospondyls over geological time, mirroring previous results for Carnivora and Primates (Goswami and Polly 2010a). Additional work on morphological integration in Permo-Triassic temnospondyls is needed in order to further testing these hypotheses in the group.

5 Conclusions

This work represents the first study on integration in temnospondyls and, more generally, in Paleozoic tetrapods. Integration studies are challenging in extinct tetrapods because the fossil preservation limits greatly the sample size, as only well preserved specimens are suitable for the analyses. In spite of this reduction in statistical power, addressing the integration patterns of fossil taxa is essential to understand how integration evolves over millions of years. In this regard, the integration data presented here coming from this major radiation of amphibians constitutes a necessary addition to that derived mainly from mammals for testing if patterns vary across tetrapod lineages and over deep geological time.

References

Ackermann, R. R., & Cheverud, J. M. (2000). Phenotypic covariance structure in tamarins (genus Saguinus): A comparison of variation patterns using matrix correlation and common principal component analysis. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 111*(4), 489–501.

Adams, D. C., & Otárola-Castillo, E. (2013). geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution, 4*(4), 393–399.

Badyaev, A. V., Foresman, K. R., & Young, R. L. (2005). Evolution of morphological integration: Developmental accommodation of stress-induced variation. *The American Naturalist, 166*(3), 382–395.

Bell, E., Andres, B., & Goswami, A. (2011). Integration and dissociation of limb elements in flying vertebrates: A comparison of pterosaurs, birds and bats. *Journal of Evolutionary Biology, 24*(12), 2586–2599.

Bookstein, F. (1991). *Morphometric tools for landmark data: Geometry and biology*. Cambridge: Cambridge University Press.

Burt, C. (1948). Factor analysis and canonical correlations. *British Journal of Statistical Psychology, 1*(2), 95–106.

Cheverud, J. M. (1996). Developmental integration and the evolution of pleiotropy. *American Zoologist, 36*(1), 44–50.

Fröbisch, N. B., & Schoch, R. R. (2009). Testing the impact of miniaturization on phylogeny: Paleozoic dissorophoid amphibians. *Systematic Biology, 58*(3), 312–327.

Garland, T., Jr., Harvey, P. H., & Ives, A. R. (1992). Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology, 41*(1), 18–32.

Goswami, A. (2006). Cranial modularity shifts during mammalian evolution. *The American Naturalist, 168*(2), 270–280.

Goswami, A., Binder, W. J., Meachen, J., & O'Keefe, F. R. (2015). The fossil record of phenotypic integration and modularity: A deep-time perspective on developmental and evolutionary dynamics. *Proceedings of the National Academy of Sciences, 112,* 4891–4896.

Goswami, A., & Polly, P. D. (2010a). The influence of modularity on cranial morphological disparity in Carnivora and Primates (Mammalia). *PLoS One, 5*(3), e9517.

Goswami, A., & Polly, P. D. (2010b). Methods for studying morphological integration and modularity. *The Paleontological Society Papers, 16,* 213–243.

Goswami, A., Polly, P. D., Mock, O. B., & Sánchez-Villagra, M. R. (2012). Shape, variance and integration during craniogenesis: Contrasting marsupial and placental mammals. *Journal of Evolutionary Biology, 25*(5), 862–872.

Goswami, A., Smaers, J. B., Soligo, C., & Polly, P. D. (2014). The macroevolutionary consequences of phenotypic integration: From development to deep time. *Philosophical Transactions of the Royal Society B, 369*(1649), 20130254.

Hall, B. K. (2003). Unlocking the black box between genotype and phenotype: Cell condensations as morphogenetic (modular) units. *Biology and Philosophy, 18*(2), 219–247.

Kelly, E. M., & Sears, K. E. (2011). Reduced integration in marsupial limbs and the implications for mammalian evolution. *Biological Journal of the Linnean Society, 102,* 22–36.

Klingenberg, C. P. (2008). Morphological integration and developmental modularity. *Annual Review of Ecology Evolution and Systematics, 39,* 115–132.

Klingenberg, C. P. (2010). Evolution and development of shape: Integrating quantitative approaches. *Nature Reviews Genetics, 11*(9), 623.

Klingenberg, C. P. (2013). Cranial integration and modularity: Insights into evolution and development from morphometric data. *Hystrix, the Italian Journal of Mammalogy, 24*(1), 43–58.

Klingenberg, C. P. (2014). Studying morphological integration and modularity at multiple levels: Concepts and analysis. *Philosophical Transactions of the Royal Society B, 369*(1649), 20130249.

Klingenberg, C. P. (2015). Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. *Symmetry, 7*(2), 843–934.

Klingenberg, C. P., & Marugán-Lobón, J. (2013). Evolutionary covariation in geometric morphometric data: Analyzing integration, modularity, and allometry in a phylogenetic context. *Systematic Biology, 62*(4), 591–610.

Maxwell, E. E., & Dececchi, T. A. (2013). Ontogenetic and stratigraphic influence on observed phenotypic integration in the limb skeleton of a fossil tetrapod. *Paleobiology, 39*(1), 123–134.

Monteiro, L. R., Bonato, V., & Dos Reis, S. F. (2005). Evolutionary integration and morphological diversification in complex morphological structures: Mandible shape divergence in spiny rats (Rodentia, Echimyidae). *Evolution and Development, 7*(5), 429–439.

Monteiro, L. R., & Nogueira, M. R. (2009). Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. *Evolution: International Journal of Organic Evolution, 64*(3), 724–744.

Pavlicev, M., Cheverud, J. M., & Wagner, G. P. (2009). Measuring morphological integration using eigenvalue variance. *Evolutionary Biology, 36*(1), 157–170.

Pérez-Ben, C. M., Schoch, R. R., & Báez, A. M. (2018). Miniaturization and morphological evolution in Paleozoic relatives of living amphibians: A quantitative approach. *Paleobiology, 44*(1), 58–75.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/. Accessed Mar 2016.

Rohlf, F. J. (2016). *TPSDIG2 2.26*. Stony Brook: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J., & Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology, 39*(1), 40–59.

Schoch, R. R. (1992). Comparative ontogeny of early Permian branchiosaurid amphibians from southwestern Germany. Developmental stages. *Palaeontographica Abteilung A, 222,* 43–83.

Schoch, R. R. (2008). A new stereospondyl from the German Middle Triassic, and the origin of the Metoposauridae. *Zoological Journal of the Linnean Society, 152*(1), 79–113.

Schoch, R. R. (2013). The evolution of major temnospondyl clades: An inclusive phylogenetic analysis. *Journal of Systematic Palaeontology, 11*(6), 673–705.

Schoch, R. R., & Milner, A. R. (2000). *Handbook of Paleoherpetology—Temnospondyli II*. Munich: Dr. Friedrich Pfeil.

Schoch, R. R., & Milner, A. R. (2014). *Handbook of Paleoherpetology—Temnospondyli I*. Munich: Dr. Friedrich Pfeil.

Schoch, R. R., & Witzmann, F. (2009a). The temnospondyl *Glanochthon* from the Lower Permian Meisenheim Formation of Germany. *Special Papers in Palaeontology, 81,* 121–136.

Schoch, R. R., & Witzmann, F. (2009b). Osteology and relationships of the temnospondyl genus *Sclerocephalus*. *Zoological Journal of the Linnean Society, 157*(1), 135–168.

Wagner, G. P., & Altenberg, L. (1996). Perspective: Complex adaptations and the evolution of evolvability. *Evolution, 50*(3), 967–976.

Willmore, K. E., Young, N. M., & Richtsmeier, J. T. (2007). Phenotypic variability: Its components, measurement and underlying developmental processes. *Evolutionary Biology, 34*(3–4), 99–120.

Young, N. M., & Hallgrímsson, B. (2005). Serial homology and the evolution of mammalian limb covariation structure. *Evolution, 59*(12), 2691–2704.

Young, R. L., & Badyaev, A. V. (2006). Evolutionary persistence of phenotypic integration: influence of developmental and functional relationships on complex trait evolution. *Evolution, 60*(6), 1291–1299.

Zelditch, M. L., Swiderski, D. L., & Sheets, H. D. (2012). *Geometric morphometrics for biologists: A primer*. New York: Academic Press.

Zelditch, M. L., Wood, A. R., Bonett, R. M., & Swiderski, D. L. (2008). Modularity of the rodent mandible: Integrating bones, muscles, and teeth. *Evolution and Development, 10*(6), 756–768.

Zelditch, M. L., Wood, A. R., & Swiderski, D. L. (2009). Building developmental integration into functional systems: Function-induced integration of mandibular shape. *Evolutionary Biology, 36*(1), 71–87.

Affiliations

Celeste M. Pérez-Ben[1] · Raúl O. Gómez[1,2]

✉ Celeste M. Pérez-Ben
  celeste.perez.ben@gmail.com

[1] CONICET, Departamento de Ciencias Geológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria-Pabellón II, 1428 Buenos Aires, Argentina

[2] Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria-Pabellón II, 1428 Buenos Aires, Argentina