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Lucas J. Legendre, Nathalie Le Roy, Cayetana Martinez-Maza, Laëtitia Montes ...+2 more authors

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Lucas Legendre, Nathalie Le Roy, Cayetana Martinez-Maza, Laëtitia Montes, Michel Laurin, Jorge Cubo

Corresponding authors
Michel LAURIN
MNHN, bâtiment de géologie. BC 45. 43 rue Buffon, 75005 Paris, France
michel.laurin@upmc.fr
Jorge CUBO
UPMC, Univ. Paris 06, 4 place Jussieu, BC 19, F-75005, Paris, France
jorge.cubo_garcia@upmc.fr

Phylogenetic signal in bone histology of amniotes revisited

by

LUCAS LEGENDRE, NATHALIE LE ROY, CAYETANA MARTINEZ-MAZA,
LAETITIA MONTES, MICHEL LAURIN, JORGE CUBO

Running title: Phylogenetic signal in bone histology
L. Legendre *et al.*

Abstract

Legendre, L., Le Roy, N., Martinez-Maza, C., Montes, L., Laurin, M., Cubo, J. (2012). Phylogenetic signal in bone histology of amniotes revisited. *Zoologica Scripta* 00, 000-000. There is curretly a debate about the presence of a phylogenetic signal in bone histological data, but very few rigorous tests have fueled the discussions on this topic. Here we performed new analyses using a larger set of seven histological traits and including 25 taxa (nine extinct and sixteen extant taxa), using three methods: the phylogenetic eigenvector regression, the tree length distribution and the regressions on distance matrices. Our results clearly show that the phylogenetic signal in our sample of bone histological characters is strong, even after correcting for multiple testing. Most characters exhibit a significant phylogenetic signal according to at least one of our three tests, with the phylogeny often explaining 20 to 60% of the variation in the histological characters. Thus, we conclude that the phylogenetic comparative method should be systematically used in interspecific analyses of bone histodiversity to avoid problems of non-independence among observations.

Corresponding authors: *Michel Laurin, CNRS UMR 7207, MNHN, bâtiment de géologie. BC 45. 43 rue Buffon, 75005 Paris, France. E-mail : michel.laurin@upmc.fr and Jorge Cubo, UPMC, Univ. Paris 06, UMR 7193, ISTeP, 4 place Jussieu, BC 19, F-75005, Paris, France and CNRS, UMR 7193, ISTeP, 4 place Jussieu, BC 19, F-75005, Paris, France. E-mail: jorge.cubo_garcia@upmc.fr*

Lucas Legendre and Laetitia Montes, UPMC, Univ. Paris 06, UMR 7193, ISTeP, 4 placeJussieu, BC 19, F-75005, Paris, France and CNRS, UMR 7193, ISTeP, 4 place Jussieu, BC 19, F-75005, Paris, France. E-mails: lucas.legendre@upmc.fr, laetitia.montes-poloni@live.fr

Nathalie Le Roy, UMR CNRS 6282 Biogeosciences, Univ. Bourgogne, 6 bd Gabriel, Dijon 21000 France. E-mail : Nathalie.Le-Roy@u-bourgogne.fr

Cayetana Martinez-Maza, Department of Paleobiology, Museo Nacional de Ciencias Naturales – CSIC, Madrid, Spain. E-mail: martinezmaza.cayetana@gmail.com

Introduction

The putative presence of a phylogenetic signal in bone histological data has played a prominent role in the development of paleohistology. Paleohistology was born during the early XIXth century with the publication of the first observations of fossil samples (Agassiz 1833, 1844). The history of this discipline contains two well-delimited phases (Cubo and Laurin 2011). During the second half of the XIXth and the first half of the XXth centuries, paleohistologists were mainly interested in problems of taxon determination using fragments of bone tissue (*e.g.* Queckett 1849a, b, 1855). All these studies assumed that osteohistological variation contains diagnostic information and a phylogenetic signal. In fact, a number of bone histological traits are synapomorphies at different nodes of the vertebrate phylogeny. For instance, the presence of endochondral bone tissue in Osteichthyes (Janvier 1996) or the presence of acellular bone tissue in several teleosts (Meunier 2011). From the second half of the XXth century onwards, paleohistologists seemed no longer interested in utilizing bone tissue for systematics, and focused on paleobiology instead. These scientists used bone histological information to infer life history traits of extinct vertebrates, assuming that bone histodiversity is linked to functional parameters (*e.g.* Enlow & Brown 1956, 1957, 1958; Ricqlès 1975, 1976, 1977a, b, Sander 2000; Padian *et al* 2001; Horner *et al* 2001). This dichotomy between historicism and functionalism is unsatisfactory from a conceptual point of view because a given feature may simultaneously contain a phylogenetic signal (it may constitute a synapomorphy at a given node) and have a functional significance. Desdevises *et al.* (2003) developed a statistical method allowing to partition the variation of a trait into a phylogenetic component, a functional or ecological component, the covariation between these fractions, and finally an unexplained fraction. Cubo *et al.* (2005) applied this method to bone microstructural and histological traits and concluded that while phylogenetic signal was highly significant at the microstructural level of organization, it was significant for some histological traits, but not for others. This conclusion has been cited by many authors to argue that "the histological level of organization by itself may reflect at best a weak signal" (Ricqlès *et al* 2008) and that bone histodiversity mainly reflects functional

aspects (Buffrénil *et al* 2008). The aim of this paper is to test the presence of a phylogenetic signal in bone histodiversity of amniotes using a larger set of bone histological traits than previous studies and including extinct as well as extant taxa.

Material and Methods

Material

We analyzed the histological data set published by Cubo *et al.* (2012). It includes information from humeri, femora and tibiae of a sample of 52 specimens belonging to 16 extant species of amniotes, plus the following samples of extinct archosaurs: *Postosuchus* UCMP 28353 (humerus), *Calyptosuchus* UCMP 25914 (femur), *Rutiodon* UCMP 25921 (femur) and *Typothorax* A269 25905 (femur) among Crurotarsi, and *Lesothosaurus* QR 3076 (femur), *Maiasaura* MOR 005 (tibia), *Coelophysis* AMNH 27435 (tibia), *Allosaurus* UUVP 3694 (femur) and UUVP 154 (tibia), and *Troodon* MOR 748 (femur) among Ornithodira.

Methods

Phylogeny. A reference phylogeny is used in our analyses (Fig. 1). The divergence times are based mostly on fossil evidence. The topology for Testudines follows the established consensus according to which Cryptodira and Pleurodira are sister-taxa, as was established long ago (Gaffney & Meylan 1988). Our sample includes only three turtle terminal taxa, *Trachemys* (Emydidae), *Pelodiscus* (Trionychidae) and *Macrochelodina* (Pleurodira). For the squamates, the topology was compiled from Estes (1982), Estes *et al*. (1988), Rieppel (1988) and Caldwell (1999). The placement of Testudines is still controversial (Rieppel & Reisz 1999; Zardoya & Meyer 2001); therefore, we placed Testudines as the sister-group of Diapsida, as numerous paleontological studies have argued (Laurin & Reisz 1995; Lee 2001; Lyson *et al* 2010). Considering that the oldest known amniote (*Hylonomus lyelli*) comes from the late Bashkirian (Marjanović & Laurin 2007), we used a divergence time between mammals and sauropsids (last common ancestor of Amniota) of 310 Ma. Divergence times between lepidosaurs and

crocodylians (252 Ma) and between crocodiles and birds (last common ancestor of Archosauria, 243 Ma) were taken from Reisz & Müller (2004). The last two divergence times are reliable estimates because of the high quality of the fossil record before and after the first occurrence of these taxa (Reisz & Müller 2004). Divergence time between Lacertidae and Varanidae (189 Ma) and the age of the last common ancestor of dinosaurs (230 Ma) were respectively taken from Evans (2003) and Langer *et al*. (2010), both obtained from the fossil record. A few divergence times were taken from Pyron (2010), who used a molecular approach calibrated by the four well-constrained fossil dates obtained by Müller & Reisz (2005). Dates taken from Pyron (2010) include divergence times between Paleognathae and Neognathae (last common ancestor of modern birds, 112 Ma) and between *Anas* and *Turdus* (last common ancestor of Neognathae, 77 Ma). These molecular clock estimates are congruent with vicariance biogeography and fossil evidence, respectively (Laurin *et al* 2012).

Histology and microscopic observation. All histological measures were performed on transverse bone sections 100 +/- 10 μm thick, which were made across the diaphysis using a diamond-tip circular saw. Each thin section was ground and polished before being mounted on a side, and then observed using optical microscopy and digital imaging (Eclipse E600POL with a DXM 1200 Digital Eclipse Camera System; Nikon, Japan). Vascular orientation and density were measured with a magnification of 40x, whereas cellular variables were measured with a 400x magnification.

Ontogenetic control. Considering that there is a marked ontogenetic variation of bone histological features mainly linked to bone growth rate, we standardized our data acquisition by measuring bone histological features in regions formed during the phase of sustained high growth rate. Whereas in extant species sampled bone formed at sustained high growth rate is located at the bone periphery (specimens were actively growing when they were euthanized), in our samples from extinct taxa this region is located in the deep cortex (*i.e*., fossil specimens were ontogenetically older than those belonging to extant species).

Variables

Vascular orientation. Blood vessels in the bones were lost during sample preparation in extant species, and during the fossilization process in the extinct taxa. Thus, this variable measures the orientation of the cavities (called vascular canals) that contained the blood vessels and associated connective tissues (Fig. 2A, B). The orientation of each vascular cavity was determined using Image J. We inserted the largest ellipse that could fit into each vascular cavity. To improve repeatability, the orientation of each vascular cavity was measured using the radial index published by de Boef & Larsson (2007). The orientation of these cavities was computed as the angle between the major axis of each ellipse and a vector tangent to bone periphery. Thus, vascular canals running parallel to bone periphery have angles approaching 0°, and those running parallel to the radius of bone cross-section have angles approaching 90° (de Boef & Larsson 2007). Vascular canal orientation is a continuously varying trait that we transformed into discrete orientation classes: circular canals (C), which run roughly parallel to the bone periphery (0°+22.5°; 180°-22.5°); radial canals (R), which run roughly orthogonal to the bone periphery (90°+/- 22.5°); and oblique canals (O), *i.e*. those canals excluded from the intervals corresponding to radial canals and to circular ones. These types of vascular orientation are illustrated in de Margerie (2002), de Margerie *et al*. (2004) and de Boef & Larsson (2007). We used three variables to describe the major vascular orientations found on each bone section: proportion of circular canals (C/C+R+O), proportion of radial canals (R/C+R+O), and proportion of oblique canals (O/C+R+O). In avascular bones, the proportions of circular, radial and oblique canals were considered as zero.

Vascular density. Vascular density was measured by Cubo *et al*. (2005) as the ratio of total vascular canal area to primary bone area (Fig. 2C). Here we measured the number of canals divided by mm^2 because the osteons are not yet filled in our sample of extant taxa (the individuals were still growing). Sections showing a single vascular canal were considered to be avascular because this single vascular canal most probably corresponds to a blood vessel running from the periosteum to the endosteum (a “canal nourricier” oriented more or less radially).

Cellular variables. Cellular shape, size and density were carefully measured outside the osteons both in extant taxa (in which osteons are not yet filled because they are still growing) and in extinct taxa (in which osteons are already filled). Like vascular canals, osteocytes were lost during the preparation of bone samples in extant species and during the process of fossilization in extinct taxa (Fig. 2D). Thus, we measured the shape, size, and density of cavities (osteocyte lacunae) that contained bone cells (osteocytes). When possible in extant species (i.e., when the bone section contained enough osteocyte lacunae), we measured 120 osteocyte lacunae for each bone section (*i.e*. 30 lacunae measured in four areas in each the bone section – rostral, lateral, medial and caudal).

Cellular shape was quantified as the ratio between the major and the minor axes of these cavities ($0 < \text{shape} \leq 1$). The lacunae are perfectly circular in the plane of the section when the shape is equal to 1.

Cellular size was computed using the major and minor axes of osteocyte lacunae and assuming the geometry of an ellipse following the equation $\pi \times L/2 \times l/2$.

Cellular density was quantified as the number of lacunae divided by the surface of the bone section in mm^2.

All measurements were carried out using a microscope focused on a single layer of osteocyte lacunae. Thus, the measurements refer to a single layer of osteocyte lacunae whatever the thickness of the ground section. Cellular density was computed including all osteocyte lacunae of the selected single layer. Following Organ *et al*. (2007), only the largest osteocyte lacunae included in this layer were measured to compute cell size and shape, to ensure that cell lacunae were measured near the middle of their longitudinal axis.

Phylogenetic comparative methods

Three methods were used to test for phylogenetic signal. Obtained results were corrected for multiple testing.

Phylogenetic eigenvector regression. The phylogeny of our sample of amniotes (Fig. 1) was expressed in the form of principal coordinates (Diniz-Filho *et al* 1998) to be used as explanatory variables in tests of phylogenetic signal. Considering that we obtained as many principal coordinate axes as terminal taxa included in the analyses, a

selection procedure was necessary. We retained and used the phylogenetic principal coordinate axes significantly related to the dependent variable as explanatory variables (Desdevises *et al* 2003).

Regressions on Distance Matrices. This method was described by Mantel (1967). Firstly we computed pairwise phylogenetic (patristic) distances using the consensus phylogeny (Fig. 1). For each pair of species, the histological dissimilarity was quantitatively assessed using the absolute value of the difference between the character values. Two distance matrices were constructed: the phylogenetic distance matrix (the sum of branch lengths connecting two taxa, in Myr) and the histological dissimilarity matrix. Afterwards, the histological dissimilarity (the dependent variable) was regressed on the phylogenetic distance (the independent variable). The significance of the regression coefficient could not be tested using a parametric test because the values of the phylogenetic distance matrices (the independent variables) are not normally distributed, and a normal distribution is a fundamental condition of parametric testing. In these cases, significance of statistics must be tested through randomization tests (Harvey & Pagel 1991: 152–155). Therefore, the significance of the (R^2) parameter was tested by a permutation test (Mantel 1967) using Permute 3.4a9 (Casgrain 2005), a software that can perform regressions on distance matrices as described by Legendre *et al*. (1994). Each regression and its statistics were recomputed 9999 times by repeatedly randomizing the values of the histological dissimilarity matrix to obtain a null distribution against which to test the significance of the statistics of the regression on the original dataset.

Random Squared Tree_Length Distribution. A phylogenetic signal can also be detected in a character by determining if the character requires fewer steps on the reference phylogeny than on most randomly generated trees, provided that the phylogeny has been produced using other characters. In the case of continuous characters, squared length (rather than number of steps) of the character over the tree can be used (Maddison 1991). The squared length is the most appropriate statistic for a continuous character. It is the sum of the square of changes between each node or between nodes and terminal taxa. Squared change parsimony minimizes this statistic, and in the version that we used (weighted square-change parsimony, implemented in Mesquite), what is minimized is the sum over all branches of the squared change

divided by branch length (Maddison, 1991). The probability that the character values is distributed randomly with respect to the phylogeny is simply the proportion of random trees in which squared length is equal or less than on the reference tree. These simulations were performed by the TreeFarm package of modules of Mesquite (Maddison & Maddison 2011; Maddison *et al* 2011). The appropriate procedure is to randomly permute the taxa (along with their character values) on the tree, while holding the topology as well as the branch lengths constant (Laurin 2004). This procedure has the advantage of yielding random trees that have a branch length distribution identical to that of the reference tree. This is necessary because the squared length of a character over a tree depends on tree depth (Maddison 1991).

All these tests were performed for our whole sample (Amniota), as well as for three nested sub-groups: Sauropsida, Diapsida, and Archosauria. We could not test other subsets of our sampled taxa because the sample size would have been insufficient, resulting in very low power and hence, meaningless negative results.

Corrections for multiple testing. Given that we have seven characters, three bones, three tests, ,and four nested clades on which these tests were applied, we have performed 252 tests. Thus, corrections for multiple testing are required because at the customary 0.05 probability threshold, three false positives are expected. For this purpose, we have used the False Discovery Rate (FDR) analysis, which is reasonably easy to use and powerful, as it retains more significant results than classical Bonferroni corrections (Berjamini & Hochbert 1995; Curran-Everett 2000). This is why we have used it in our recent papers that included multiple tests (*e.g*. Laurin *et al* 2009).

Results

Most histological traits exhibit a significant phylogenetic signal according to at least one of our three tests (Table 1), at least for Amniota (15 bone by character combinations, out of 21, yielded significant results even after correction for multiple testing). For smaller, nested clades, the number of significant results was lower, presumably reflecting decreased power with a lower taxonomic sample size because the number of significant results is directly proportional to the number of included taxa (significant results for 12 bone by character combinations out of 21 for Sauropsida, but

only 10 for Diapsida and 4 for Archosauria). Among these traits, only femoral cell density becomes non-significant (with any of the three methods) among femoral variables after correction for multiple testing (False Discovery Rate analysis; Table 1). The probabilities yielded by tree length distribution on tibia were higher than those obtained from phylogenetic eigenvector regression or regressions on distance matrices (Table 1). Of the 34 probabilities that were <0.05 when taken in isolation, 26 remain significant after FDR analysis.

Bone histological variation explained by the phylogeny is in the order of 20–60%, as shown by the phylogenetic eigenvector regression analysis (Table 2, first column). Variation explained by the phylogeny obtained using regressions on distance matrices are lower, as expected, because this method underestimates the real values, as Legendre (2000) showed using simulations. Here, the regression coefficients obtained using phylogenetic eigenvector regression are always much higher than those obtained using regressions on distance matrices (Table 2), which is congruent with the findings of Legendre (2000).

Discussion

Mayr (1961) separates evolutionary biology (concerning historical, or ultimate, causation) from functional biology (tackling immediate, or proximate, causation). The nature of the evidence is comparative in evolutionary biology, whilst it is typically experimental in functional biology. Within evolutionary biology, systematists and functional morphologists are interested in different patterns. For the former, interested in the reconstruction of phylogenetic patterns, the functional adaptation to current conditions (autapomorphies) may mask a pure phylogenetic signal. For example, the autapomorphic flightless condition of the Galapagos cormoran is associated with a whole array of morphological changes (Cubo and Casinos, 1997) that may mask synapomorphies of more inclusive nodes (e.g. Phalacrocoracidae). For functional morphologists, phylogenetic patterns are factors that may explain why organisms do not appear to have reached optimal adaptation to current conditions. In « The shadow of forgotten ancestors differently constrains the fate of Alligatoroidea and Crocodyloidea »

Piras *et al*. (2009) suggest that the phylogenetic inheritance of a clade may determine its evolutionary fate. According to Seilacher (1970), a third set of factors (in addition to history and function) may contribute to explain evolutionary patterns: the properties inherent in the materials found in organs and their self-organization properties (with few genetic inputs). These three perspectives are not necessarily mutually exclusive.

In the field of bone biology, Cubo *et al*. (2005) found a significant phylogenetic signal at the microanatomical level of bone organization, but concluded that the histological level contained a lower signal. However, considering that Cubo *et al*. (2008, 2012) showed evidence for a significant phylogenetic signal in the variation of bone growth rate in amniotes, and that, according to Amprino's rule, bone histodiversity may reflect variation in bone growth rates (Amprino 1947; Montes *et al* 2010), we expected that bone histological variation contained a significant phylogenetic signal. Here we expand upon the analyses initiated by Cubo *et al*. (2005) using a larger set of histological traits and including extinct taxa.

Our results clearly show that the phylogenetic signal in the bone histological characters that we studied is strong, with the phylogeny often explaining 20 to 60% of the variation in the histological characters. The proportion of significant results appears to depend rather strongly on taxonomic sample size, reflecting the common and expected increased power at larger sample sizes. Nevertheless, our results do not imply that functional factors are unimportant. In fact, some of the variation explained by the phylogeny may represent covariation with functional factors (rather than purely phylogenetic variation), although variation partition analyses would be required to determine this. These are beyond the scope of this study, as they would require additional data (such as growth rate, metabolic rate, *etc*.). However, some evidence suggests that part of this phylogenetic signal represents covariation with functional factors, at least for the femur. Cubo *et al*. (2012) constructed a paleobiological inference model using extant taxa for estimating bone growth rate of extinct taxa (a functional factor according to Amptino's rule) from bone histological data. The response variable (*i.e*. the functional variable, bone growth rate) was significantly correlated with, and could be reliably inferred from predictor variables (bone histological traits). These results are evidence of a significant functional effect on bone histological variation, and are complementary to the evidence presented in this study for a significant phylogenetic

signal on the same traits. We conclude that, in view of the results reported above, the phylogenetic comparative method should be used in any study dealing with the interspecific variation of bone histology to avoid problems of non-independence among observations. This is unfortunately still not common practice. Some disciplines such as ecology and, to a lesser extent, vertebrate morphology, incorporated the phylogenetic comparative method soon after the initial development of this approach in the middle of the 1980s (see a review in Harvey & Pagel 1991). In contrast, the use of these methods is not yet generalized in other fields such as bone histology (e.g. de Buffrénil *et al* 2008), but we hope that this study will help motivate bone histologists to adopt these methods.

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Tables

Bone	Histological traits		n	Phylogenetic eigenvector regression	Tree length distribution
Femur	Cell density	Amniota	22	-	0.023
		Sauropsida	19	0.0002*	0.020
		Diapsida	16	0.002*	0.023
		Archosauria	12	0.040	0.110
	Cell size	Amniota	22	0.020*	0.0009*
		Sauropsida	19	-	0.0006*
		Diapsida	16	-	0.0006*
		Archosauria	12	0.011*	0.003*
	Cell shape	Amniota	22	0.012*	0.014*
		Sauropsida	19	0.026	0.019
		Diapsida	16	0.047	0.038
		Archosauria	12	-	0.056
	Vascular radial orientation	Amniota	22	0.015*	0.047
		Sauropsida	19	0.004*	0.050
		Diapsida	16	0.003*	0.025
		Archosauria	12	-	0.897
	Vascular oblique orientation	Amniota	22	0.001*	0.085
		Sauropsida	19	-	0.255
		Diapsida	16	-	0.560
		Archosauria	12	0.007*	0.221
	Vascular circular orientation	Amniota	22	0.010*	<0.0001*
		Sauropsida	19	0.0001*	0.0001*
		Diapsida	16	0.0001*	0.0008*
		Archosauria	12	-	0.578
	Vascular density	Amniota	22	0.001*	0.0002*
		Sauropsida	19	0.0001*	0.0002*
		Diapsida	16	0.0002*	0.001*
		Archosauria	12	0.062	0.028
Humerus	Cell density	Amniota	17	-	0.154
		Sauropsida	14	0.002*	0.161
		Diapsida	11	0.014	0.165
		Archosauria	7	0.054	0.119
	Cell size	Amniota	17	0.002*	0.140
		Sauropsida	14	0.010*	0.106
		Diapsida	11	0.037	0.084
		Archosauria	7	-	0.598
	Cell shape	Amniota	17	-	0.006*
		Sauropsida	14	0.0003*	0.004*
		Diapsida	11	0.0009*	0.004*
		Archosauria	7	0.037	0.046
	Vascular radial orientation	Amniota	17	0.008*	0.870

		Sauropsida	14	-	0.818
		Diapsida	11	-	0.768
		Archosauria	7	-	0.897
	Vascular oblique orientation	Amniota	17	0.003*	0.043
		Sauropsida	14	-	0.070
		Diapsida	11	-	0.051
		Archosauria	7	0.050	0.942
	Vascular circular orientation	Amniota	17	-	0.005*
		Sauropsida	14	0.0003*	0.007*
		Diapsida	11	0.0002*	0.005*
		Archosauria	7	0.050	0.713
	Vascular density	Amniota	17	-	0.012*
		Sauropsida	14	0.001*	0.018
		Diapsida	11	0.009*	0.013*
		Archosauria	7	-	0.248
Tibia	Cell density	Amniota	19	-	0.505
		Sauropsida	16	-	0.480
		Diapsida	13	-	0.581
		Archosauria	9	-	0.834
	Cell size	Amniota	19	0.045	0.388
		Sauropsida	16	-	0.472
		Diapsida	13	-	0.295
		Archosauria	9	-	0.205
	Cell shape	Amniota	19	0.073	0.283
		Sauropsida	16	-	0.298
		Diapsida	13	-	0.289
		Archosauria	9	-	0.138
	Vascular radial orientation	Amniota	19	-	0.839
		Sauropsida	16	-	0.860
		Diapsida	13	-	0.872
		Archosauria	9	-	0.483
	Vascular oblique orientation	Amniota	19	0.007*	0.396
		Sauropsida	16	0.004*	0.463
		Diapsida	13	0.042	0.624
		Archosauria	9	-	0.614
	Vascular circular orientation	Amniota	19	0.037	0.0007*
		Sauropsida	16	0.002*	0.0002*
		Diapsida	13	0.001*	0.0005*
		Archosauria	9	-	0.393
	Vascular density	Amniota	19	-	0.803
		Sauropsida	16	-	0.746
		Diapsida	13	-	0.683
		Archosauria	9	-	0.933

Table 1. Probability that the observed covariation between the histological data and the phylogeny is random. This is obtained using phylogenetic eigenvector regression, tree length distribution, and regressions from distance matrices. Phylogenetic signal is considered as significant at a 0.05 threshold when taken in isolation. However, only the P values marked with asterisks are still significant after correction for multiple testing (False Discovery Rate analysis). These data are available as Mesquite Nexus files in the supplementary materials (SOM 1–3).

Bone	Histological traits		n	Phylogenetic eigenvector regression	Regressions from distance matrices
Femur	Cell density	Amniota	22	-	0.001
		Sauropsida	19	0.666	0.005
		Diapsida	16	0.676	0.038
		Archosauria	12	0.327	0.073
	Cell size	Amniota	22	0.247	0.024
		Sauropsida	19	-	0.004
		Diapsida	16	-	0.011
		Archosauria	12	0.397	0.077
	Cell shape	Amniota	22	0.378	0.000
		Sauropsida	19	0.258	0.005
		Diapsida	16	0.252	0.0007
		Archosauria	12	-	0.003
	Vascular radial orientation	Amniota	22	0.247	0.028
		Sauropsida	19	0.834	0.071
		Diapsida	16	0.818	0.176
		Archosauria	12	-	0.004
	Vascular oblique orientation	Amniota	22	0.576	0.022
		Sauropsida	19	-	0.012
		Diapsida	16	-	0.0008
		Archosauria	12	0.536	0.002
	Vascular circular orientation	Amniota	22	0.286	0.117
		Sauropsida	19	0.908	0.250
		Diapsida	16	0.945	0.438
		Archosauria	12	-	0.0001
	Vascular density	Amniota	22	0.598	0.024
		Sauropsida	19	0.775	0.061
		Diapsida	16	0.811	0.438
		Archosauria	12	0.307	0.077
Humerus	Cell density	Amniota	17	-	0.002
		Sauropsida	14	0.767	0.025
		Diapsida	11	0.656	0.107
		Archosauria	7	0.527	0.355
	Cell size	Amniota	17	0.582	0.021
		Sauropsida	14	0.585	0.002
		Diapsida	11	0.408	0.001
		Archosauria	7	-	0.059
	Cell shape	Amniota	17	-	0.037
		Sauropsida	14	0.857	0.132
		Diapsida	11	0.813	0.370
		Archosauria	7	0.609	0.431
	Vascular radial orientation	Amniota	17	0.482	0.001

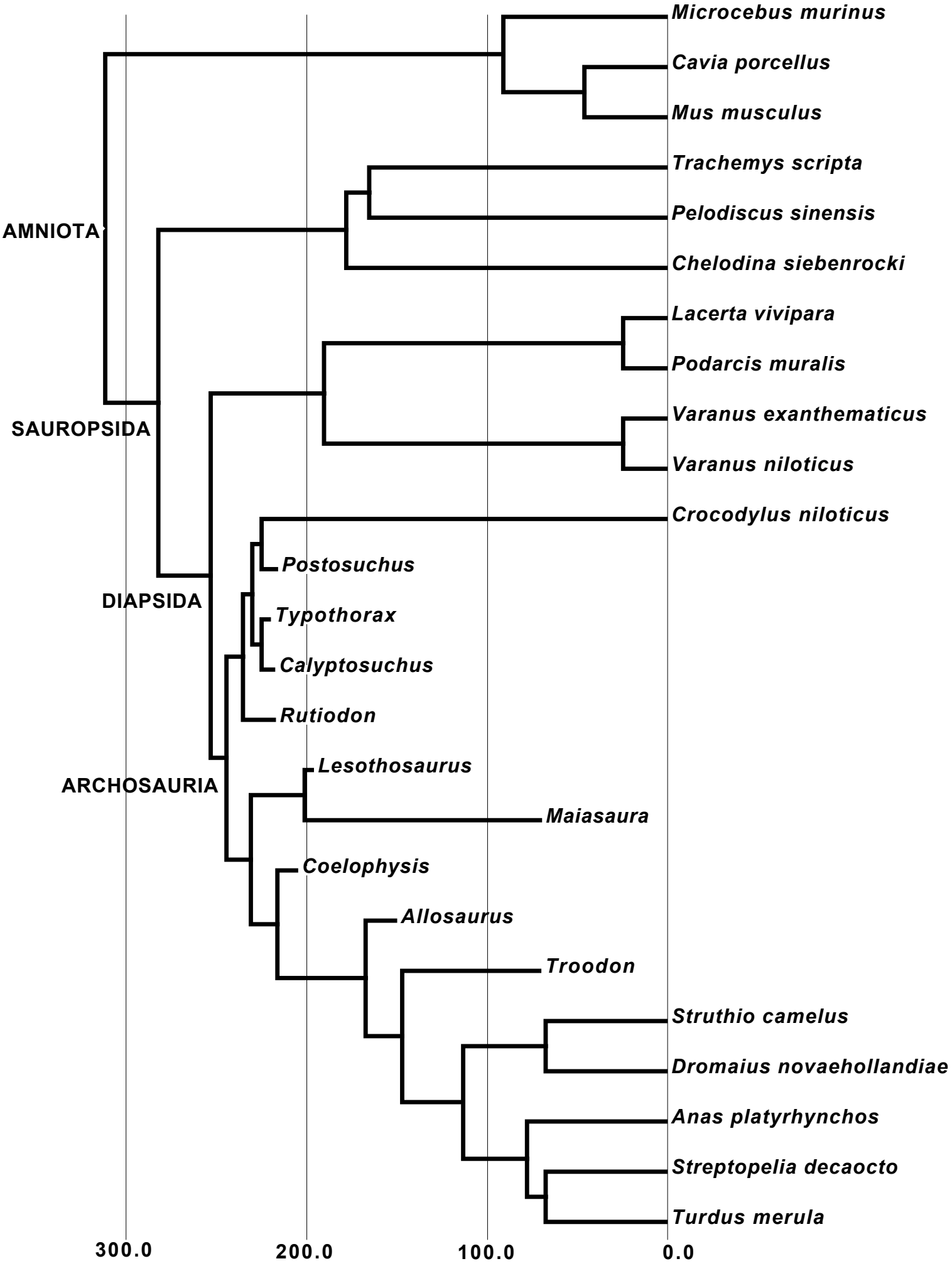
		Sauropsida	14	-	0.0002
		Diapsida	11	-	0.014
		Archosauria	7	-	0.146
	Vascular oblique orientation	Amniota	17	0.57	0.031
		Sauropsida	14	-	0.017
		Diapsida	11	-	0.038
		Archosauria	7	0.798	0.067
	Vascular circular orientation	Amniota	17	-	0.027
		Sauropsida	14	0.764	0.061
		Diapsida	11	0.886	0.247
		Archosauria	7	0.608	0.004
	Vascular density	Amniota	17	-	0.003
		Sauropsida	14	0.613	0.021
		Diapsida	11	0.625	0.136
		Archosauria	7	-	0.031
Tibia	Cell density	Amniota	19	-	0.033
		Sauropsida	16	-	0.008
		Diapsida	13	-	0.029
		Archosauria	9	-	0.002
	Cell size	Amniota	19	0.215	0.033
		Sauropsida	16	-	0.038
		Diapsida	13	-	0.004
		Archosauria	9	-	0.017
	Cell shape	Amniota	19	0.179	0.013
		Sauropsida	16	-	0.008
		Diapsida	13	-	0.007
		Archosauria	9	-	0.002
	Vascular radial orientation	Amniota	19	-	0.014
		Sauropsida	16	-	0.002
		Diapsida	13	-	0.021
		Archosauria	9	-	0.011
	Vascular oblique orientation	Amniota	19	0.308	0.034
		Sauropsida	16	0.058	0.559
		Diapsida	13	0.295	0.072
		Archosauria	9	-	0.014
	Vascular circular orientation	Amniota	19	0.219	0.013
		Sauropsida	16	0.067	0.539
		Diapsida	13	0.676	0.234
		Archosauria	9	-	0.004
	Vascular density	Amniota	19	-	0.094
		Sauropsida	16	-	0.077
		Diapsida	13	-	0.043
		Archosauria	9	-	0.060

Table 2. Covariation between bone histology and the phylogeny assessed as the R^2 values of histological variance explained by the tree, as obtained in test of phylogenetic signal using phylogenetic eigenvector regression and regressions on distance matrices. In phylogenetic eigenvector regression, we retained and used the phylogenetic principal coordinate axes significantly related to the dependent variable as explanatory variables. When no axes were retained, the analysis could not be performed. Note that the third phylogenetic signal test (tree length distribution) does not yield an explained variation, so it is not reported here.

Figure legend

Figure 1. Phylogeny (topology and divergence dates) including the species of the sample (modified after Cubo *et al* 2012). The bottom edge contains a time calibration in Ma.

Figure 2. Cross sections in long bone diaphyses of archosaurs in ordinary light. A. Two radial vascular canals (black arrows) in a mostly circular vascular pattern (white arrowheads), in *Dromaius novaehollandiae* (femur). B. Oblique vascular canals, in *Lesothosaurus* (femur). C Dense circular vascular pattern, in *Struthio camelus* (femur). D Osteocytes of *Postosuchus* (humerus) with fitting ellipses and major (MA) and minor (ma) axes figured to illustrate the measuring process of cell size (the ellipse area) and shape (the ma/MA ratio) in ImageJ. Scale bars : 1mm in 1, 2, 3 ; 0.05 mm in 4.


Microcebus murinus
Cavia porcellus
Mus musculus
Trachemys scripta
Pelodiscus sinensis
Chelodina siebenrocki
Lacerta vivipara
Podarcis muralis
Varanus exanthematicus
Varanus niloticus
Crocodylus niloticus
Postosuchus
Typothorax
Calyptosuchus
Rutiodon
Lesothosaurus
Maiasaura
Coelophysis
Allosaurus
Troodon
Struthio camelus
Dromaius novaehollandiae
Anas platyrhynchos
Streptopelia decaocto
Turdus merula
AMNIOTA
SAUROPSIDA
DIAPSIDA
ARCHOSAURIA
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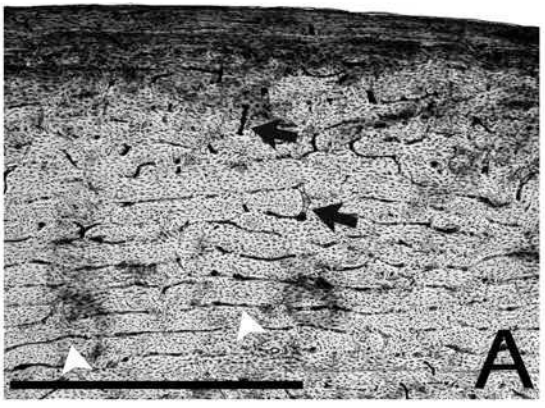
200.0
100.0
0.0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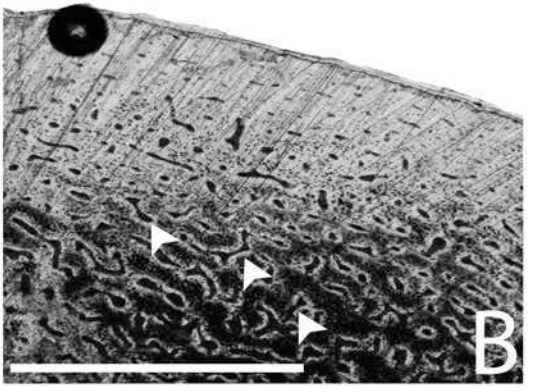
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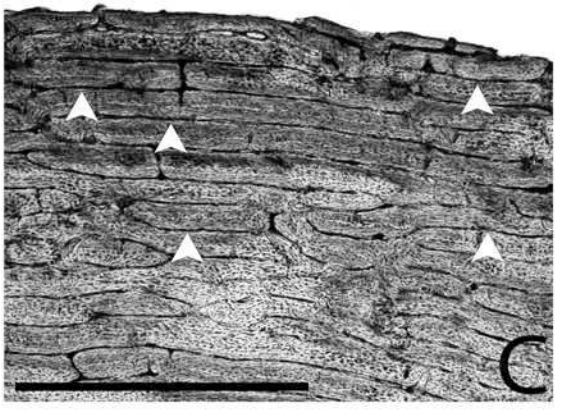
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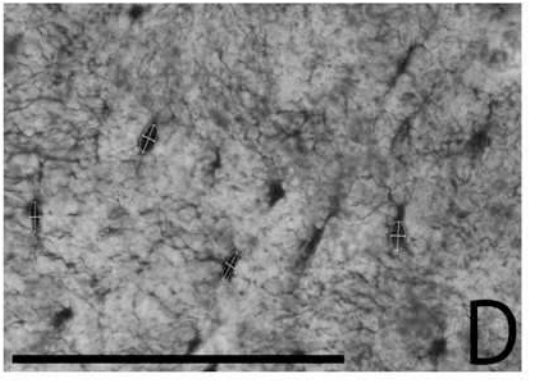
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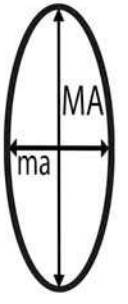
MA
ma