# Palaeohistological Evidence for Ancestral High Metabolic Rate in Archosaurs

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*Abstract*.—Metabolic heat production in archosaurs has played an important role in their evolutionary radiation during the Mesozoic, and their ancestral metabolic condition has long been a matter of debate in systematics and palaeontology. The study of fossil bone histology provides crucial information on bone growth rate, which has been used to indirectly investigate the evolution of thermometabolism in archosaurs. However, no quantitative estimation of metabolic rate has ever been performed on fossils using bone histological features. Moreover, to date, no inference model has included phylogenetic information in the form of predictive variables. Here we performed statistical predictive modeling using the new method of phylogenetic eigenvector maps on a set of bone histological features for a sample of extant and extinct vertebrates, to estimate metabolic rates of fossil archosauromorphs. This modeling procedure serves as a case study for eigenvector-based predictive modeling in a phylogenetic context, as well as an investigation of the poorly known evolutionary patterns of metabolic rate in archosaurs. Our results show that Mesozoic theropod dinosaurs exhibit metabolic rates very close to those found in modern birds, that archosaurs share a higher ancestral metabolic rate than that of extant ectotherms, and that this derived high metabolic rate was acquired at a much more inclusive level of the phylogenetic tree, among non-archosaurian archosauromorphs. These results also highlight the difficulties of assigning a given heat production strategy (i.e., endothermy, ectothermy) to an estimated metabolic rate value, and confirm findings of previous studies that the definition of the endotherm/ectotherm dichotomy may be ambiguous. [Archosaurs; bone histology; phylogenetic comparative methods; phylogenetic eigenvectors; resting metabolic rate; vertebrate palaeontology.]

Archosauria includes extant crocodiles and birds, as well as numerous extinct groups such as pterosaurs and non-avian dinosaurs. The ability of the latter group to produce metabolic heat has been a matter of controversy for the last four decades (Ostrom 1969; Bakker 1971); the finding of conclusive evidence on the occurrence of endothermy among non-avian dinosaurs would thus considerably change our understanding of the evolution of endothermy and a whole array of related characters among sauropsids (e.g., de Ricqlès et al. 2003). The classical hypothesis of endothermy being a synapomorphy of modern birds among sauropsids was challenged countless times during the 1970s, after the discovery of the dinosaurian origin of birds (Bakker 1974). These early studies, however, were all based on observations of overall morphological similarities between birds and non-avian dinosaurs; only qualitative features or variables indirectly related to metabolic rate were used without performing any quantitative estimation of thermometabolism (e.g., Russell 1965; Thulborn 1973). For this reason, evidence of endothermy in dinosaurs was considered inconclusive by most palaeontologists; dinosaurs were labeled ectothermic with probable homeothermy given their large size (McNab 1978, Benton 1979), and non-avian archosaurs were considered to be ectothermic by most palaeontologists (Reid 1987).

However, over the past decade, investigations on thermometabolism in extinct vertebrates have increased notably (Nespolo et al. 2011). Evidence for an endothermic ancestral condition at the archosaur node, and a reversal in modern-day crocodiles to an ectothermic state, has been raised in different fields of biology, including development (Seymour et al. 2004), physiology (Farmer and Sanders 2010), anatomy (Summers 2005), and palaeohistology (de Ricqlès et al. 2008).

It has been shown experimentally that bone tissue records a metabolic signal during growth: resting metabolic rate is related to bone growth rate, which, in turn, is related to bone histology (Montes et al. 2007). Thus, the analysis of bone histology of extinct archosauromorphs proves useful in understanding the origin and evolution of endothermy. A series of palaeohistological studies has provided a reconstruction of evolutionary patterns of bone growth rates in non-archosaurian archosauromorphs, whether qualitatively (de Ricqlès et al. 2008) or by quantifying the thickness between consecutive lines of arrested growth (Botha-Brink and Smith 2011). More recently, two studies used quantitative histological characters to build statistical predictive models and estimate bone growth rates of extinct archosauromorphs (Cubo et al. 2012; Legendre et al. 2013). These last attempts include significant methodological improvements (quantitative vs. qualitative inferences, optimization onto a calibrated phylogeny), but are perfectible.

On the one hand, no study to date has provided quantitative estimations of metabolic rate in extinct diapsids using bone histological features. Since the

relationship between bone growth rate and metabolic rate is statistically significant (Montes et al. 2007), previous inferences of bone growth rate provided indirect information on the evolution of metabolic rate in archosaurs, but they were not estimations of thermometabolism itself. Even though the increasing popularity of phylogenetic comparative methods (PCMs) has led in recent years to its use in many palaeontological studies (Benton 2015), very few have focused on inferring physiological parameters in extinct species. Several studies have estimated body mass in fossil archosauriformes using its empirical scaling relationship with anatomical parameters such as femoral length (Turner and Nesbitt 2013) or limb robustness (Benson et al. 2014), and reconstructed its evolutionary patterns along phylogenetic trees. These studies used body mass as a proxy for many key life parameters and discussed its influence on the adaptive radiation of archosauriformes, but did not provide significant information on metabolic rate in an evolutionary context. Hence, the direct estimation of RMR values in fossil archosaurs would allow us to identify various heat production strategies among archosaurs much more precisely than using bone growth rate or body mass as a proxy.

On the other hand, although phylogenetic signal is known to be a major explanatory factor on the variation of osteohistological variables (Cubo et al. 2005; Legendre et al. 2013, 2014), to date no inference model has included phylogenetic information in the form of predictive variables for a sample of extinct species. Phylogenetic prediction has been an important part of PCMs for more than a decade (see a review in Nunn and Zhu 2014). Since the original work of Garland and Ives (2000) using the software PDTREE, many studies have used phylogenetic generalized least squares (PGLS) to predict parameters such as genome size or feeding time in various clades of vertebrates, often including fossil species (e.g., Organ et al. 2007, 2011) and using either BayesTraits or R. The use of PGLS allows not only for the inclusion of an explicit evolutionary model, but also the output of a regression analysis in the form of a variance–covariance matrix that includes both the target species (i.e., species for which the variable of interest is to be estimated) and the predictor variable (i.e., independent variables in the regression). If there are no significant predictors in the model, predictions are equivalent to standard ancestral state reconstruction, with only phylogeny and an evolutionary model to estimate missing values for target species.

Another approach, phylogenetic eigenvector regression (PVR), originally developed by Diniz-Filho et al. (1998) to estimate phylogenetic signal, involves the extraction of phylogenetic eigenvectors from a phylogeny converted into a distance matrix and using them as explanatory variables in a regression model that could be used for phylogenetic prediction. However, this method has been criticized in recent years (e.g., Freckleton et al. 2011) for not including an explicit evolutionary model nor an accurate variable selection

procedure. Diniz-Filho et al. (2012) responded to this criticism by describing the influence of various models of evolution on the relationship between coefficients of determination and cumulative eigenvalues of eigenvectors. Nonetheless, phylogenetic eigenvector-based approaches have been far less investigated in recent years than PGLS in a Bayesian framework (Nunn and Zhu 2014).

Guénard et al. (2013) recently published a powerful new eigenvector-based approach to deal with phylogenetic information in predictive modeling: the structure of a calibrated phylogenetic tree is expressed as a set of eigenfunctions, termed phylogenetic eigenvector maps (PEMs), which depict a set of patterns of phenotypic variation among species from the structure of the phylogenetic tree. The construction of a PEM for a given trait involves weighting the edges (i.e., branches) of the phylogenetic tree on the basis of the among-species phylogenetic covariance matrix of this trait, using a steepness parameter $a$ related to Pagel's κ (Pagel 1999) and to the Ornstein–Uhlenbeck selection strength parameter α (Butler and King 2004) to describe the relationship between changes in trait values and branch lengths in the tree ($a = 0$ under purely neutral evolution; see Guénard et al. 2013). Thus, this procedure incorporates the underlying evolutionary model of a given data set before extracting PEMs from its corresponding phylogeny, which allows PEMs to outperform the original PVR as a predictive method (Diniz-Filho et al. 2015). A subset of eigenfunctions from a PEM can be selected to accurately predict phenotypic values of traits for species that are represented in a tree, but for which trait data are otherwise lacking—which is the case of fossil species for traits that can only be measured *in vivo*, such as metabolic rate. The investigation of metabolic rate in fossil archosauromorphs is thus a very fitting case study for this new method in the context of phylogenetic prediction for palaeontological data sets.

The purpose of this study is to build accurate predictive models using both phylogenetic information and osteohistological characters as independent variables to estimate mass-specific resting metabolic rate (mass-specific RMR) in a sample of archosauromorphs. These estimations will be useful for testing the hypothesis of ancestral endothermy in archosaurs (Seymour et al. 2004; de Ricqlès et al. 2008; Cubo et al. 2012; Legendre et al. 2013).

## MATERIAL AND METHODS

### *Sample and Reference Phylogeny*

The models were built using 57 specimens belonging to 14 extant species and 14 extinct species of tetrapods. The sample of extant species was originally assembled to test the statistical relationship between growth rate, resting metabolic rate, and bone histological features; RMR values are taken from Montes et al. (2007), for which all extant animals were injected with

fluorescent dyes for precise quantification of bone growth rate, and RMR values acquired *in vivo* (see also Cubo et al. 2012). All specimens in the sample are juveniles, and *in vivo* fluorescence marks on the sections correspond to the phase of sustained high growth rate ensuring ontogenetic control. All the information for all variables in the model (i.e., histological characters and metabolic rate) was measured on the same specimens. The bone histology sections were observed using a Zeiss Axiovert microscope and digitally imaged. Four histological quantitative characters were measured specifically for this study—vascular density, osteocyte density, osteocyte shape, and osteocyte area—on each bone section following a standardized procedure for each of them (Cubo et al. 2012).

Phylogenetic relationships between species were taken from published literature, following Cubo et al. (2012) and Legendre et al. (2013). The topology and branch length information for deep nodes in the phylogeny (i.e., Tetrapoda, Amniota, Sauropsida, Diapsida, Lepidosauria, Archosauria, Aves), as well as inner relationships between mammals, were taken from the Fossil Calibration Database (Benton et al. 2015). Inner relationships and branch lengths for lepidosaurs and turtles were taken from Conrad (2008) and Joyce et al. (2013), respectively. Phylogenetic relationships between non-archosaur archosauromorphs were taken from Nesbitt (2011), and divergence times for fossils in our sample were obtained from the Paleobiology Database (https://paleobiodb.org/; last accessed April 19, 2016).

The phylogenetic position of turtles among sauropsids has long been a matter of controversy. Until recently, most palaeontological studies supported the anapsid position of turtles, that is, as the sister group of all diapsids (e.g., Laurin and Reisz 1995; Zardoya and Meyer 2001). Rieppel and deBraga (1996), however, found morphological evidence for turtles being diapsids with modified anapsid skulls, and most molecular studies since then recovered a strong sister group relationship between turtles and archosaurs (e.g., Iwabe et al. 2005; Roos et al. 2007; Chiari et al. 2012; Crawford et al. 2012). Following the recent unveiling of unambiguously diapsid skulls in early stem turtles (Bever et al. 2015; Schoch and Sues 2015), we chose to position Testudines as the sister group to the Archosauromorpha clade in our phylogeny, according to the Archelosauria hypothesis suggested by Crawford et al. (2015).

### Predictive Modeling

The PEM approach was selected to estimate mass-specific RMR (in mL $O_2$ $h^{-1}$ $g^{-0.67}$) in this study, using bone histological data (Supplementary Tables 1–3 available on Dryad at http://dx.doi.org/10.5061/dryad.2853k; last accessed April 19, 2016) and phylogenetic information (Supplementary Phylogenies 1–4 available on Dryad). The value 0.67 is the allometric exponent, that is, the scaling of surface area on body mass for geometrically similar organisms. It is used to take into account the effect of body mass on mass-specific RMR (i.e., the dependent variable in our model), assuming that this effect is mediated by the fact that both the surface-to-volume ratio and the caloric loss per mass unit decrease as body mass increases (Withers 1992; White and Seymour 2005; Montes et al. 2007).

We used the R package MPSEM (Guénard et al. 2013) to convert the phylogeny into PEMs and predict values of the dependent variable for fossil species (see MPSEM package vignette for a tutorial on phylogenetic modeling). Three predictive models were compiled—one for each of the three elements (femur, humerus, and tibia), to maintain a strict frame of homology. Similar to PGLS-based phylogenetic predictions, PEMs allow for the inclusion, aside from phylogenetic eigenvectors, of auxiliary predictors significantly related to the response variable. Thus, the model selection procedure involved selecting the best set of variables among PEMs plus one of the four histological characters, since including more than one co-predictor might be a potential source of power loss considering our rather small sample size (Mundry 2014). The alternative hypothesis of a model with only PEMs and no auxiliary predictor was also tested, but a model including one of the histological variables as a co-predictor was always found to be more significant for all three bones. For each bone, the best model was selected on the basis of its AICc fit—that is, Akaike information criterion corrected for finite sample sizes (Burnham et al. 2011)—and cross-validated using leave-one-out cross-validation. Values of resting metabolic rate with 95% confidence intervals were then estimated for each fossil species in our sample (Fig. 1).

All three inference models showed high statistical significance ($R^2 > 0.97$; $p < 10^{-6}$). We thus obtained three mass-specific RMR estimations for specimens with all three limb bones included in the sample. However, mass-specific RMR is an organism-level parameter. In an individual with a given mass-specific RMR, some bones may grow faster than others (Castanet et al. 2004), and estimations can thus vary depending on the growth rate of the bone used to build the model. We used the mean value of the three estimations for each specimen (Table 1) in further analyses to ensure standardization when performing interspecific comparisons. Moreover, osteohistological features included in the model were measured in regions formed during the phase of sustained high bone growth rate to improve standardization (Cubo et al. 2012).

To investigate further into the evolution of high resting metabolic rates in amniotes, we reconstructed ancestral states of RMR from both observed and predicted values, using a phylogeny of our complete sample. Ancestral RMR values were estimated for all nodes using the function fastAnc from the R package phytools (Revell 2012); this function compiles maximum likelihood ancestral states with 95% confidence intervals assuming a Brownian motion model, and allows us to plot it on the original phylogeny (Fig. 2).
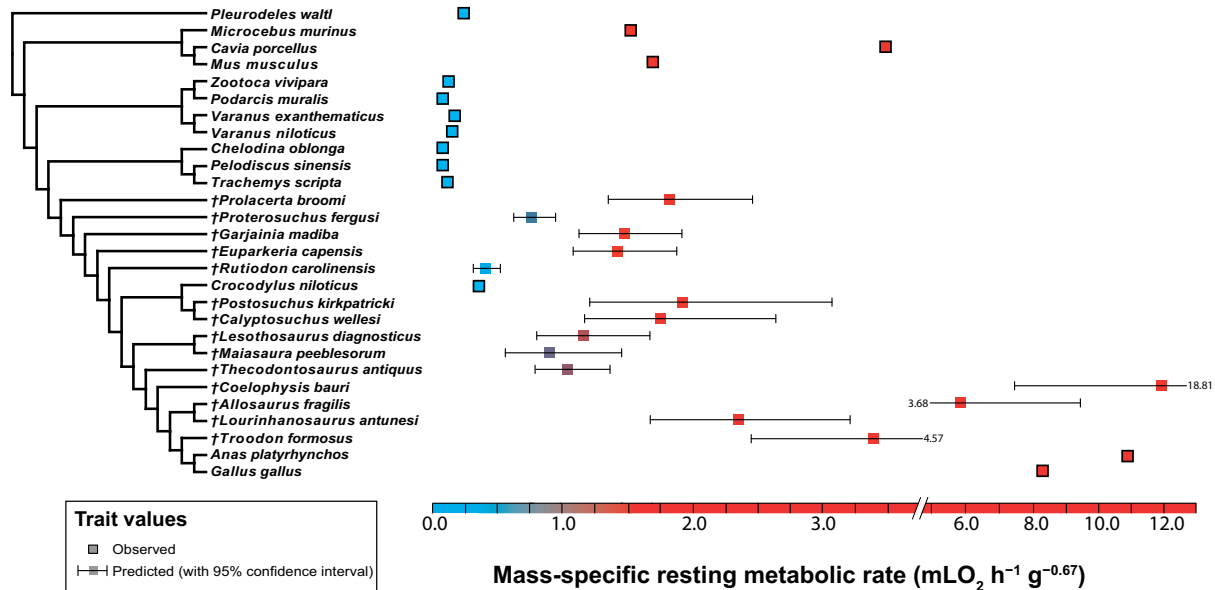
FIGURE 1. Mass-specific RMR for all species included in our sample. The color range on the axis represents the transition in values of resting metabolic rate between ectotherms (left) and endotherms (right); a gradient of intermediate tones is used for fossil species with intermediate values between those of extant ectotherms and endotherms in our sample (i.e., so-called "mesotherms").

TABLE 1. Resting metabolic rate (RMR) of extinct species in our sample

| Species | Lower limit (mL $O_2$ $h^{-1}$ $g^{-0.67}$) | Resting metabolic rate (mL $O_2$ $h^{-1}$ $g^{-0.67}$) | Upper limit (mL $O_2$ $h^{-1}$ $g^{-0.67}$) |
|---|---|---|---|
| *Prolacerta broomi* | 1.319254894 | 1.779574478 | 2.401309306 |
| *Proterosuchus fergusi* | 0.604057894 | 0.742796421 | 0.914306682 |
| *Garjainia madiba* | 1.093892708 | 1.428587942 | 1.865918168 |
| *Euparkeria capensis* | 1.05448569 | 1.388275876 | 1.827725139 |
| *Rutiodon carolinensis* | 0.308843779 | 0.395353151 | 0.506094433 |
| *Calyptosuchus wellesi* | 1.135032404 | 1.707702381 | 2.569307738 |
| *Postosuchus kirkpatricki* | 1.165933134 | 1.864455587 | 2.981470097 |
| *Lesothosaurus diagnosticus* | 0.78543557 | 1.130079749 | 1.630369802 |
| *Maiasaura peeblesorum* | 0.532978612 | 0.861329988 | 1.400681483 |
| *Thecodontosaurus antiquus* | 0.760912118 | 1.002164215 | 1.319906895 |
| *Coelophysis bauri* | 7.44819231 | 11.83642213 | 18.81005185 |
| *Lourinhanosaurus antunesi* | 1.624566141 | 2.285600254 | 3.215608472 |
| *Allosaurus fragilis* | 3.680724942 | 5.889620032 | 9.478280852 |
| *Troodon formosus* | 2.388193685 | 3.301842183 | 4.56502413 |

Note: Values of RMR, as well as lower and upper limits of the 95% confidence interval, are estimated by our predictive models based on PEMs and osteohistological features.

## RESULTS AND DISCUSSION

### The Metabolism of Early Archosaurs

The analysis of RMR estimations (Fig. 1) shows that, among non-archosaur archosauromorphs, *Prolacerta* presents a high value of mass-specific RMR (1.78 mL $O_2$ $h^{-1}$ $g^{-0.67}$) compatible with the hypothesis of an ancestral high metabolism at the archosauromorph node. Analyzing reconstructed ancestral states for RMR (Fig. 2) provides a better understanding of the evolution of metabolic rates among archosaurs and close outgroups: the archosauromorph node presents a relatively high metabolic rate (0.99 mL $O_2$ $h^{-1}$ $g^{-0.67}$) that may have been acquired by the last common ancestor of *Prolacerta* and *Gallus* and shared by most non-archosaur archosauromorphs, which is congruent with the high bone growth rates inferred in large archosauriforms such as *Garjainia* or *Erythrosuchus* from their histological profiles (Gross 1934; de Ricqlès et al. 2008; Botha-Brink and Smith 2011; Gower et al. 2014). *Garjainia* and *Euparkeria* show values of 1.43 and 1.39 mL $O_2$ $h^{-1}$ $g^{-0.67}$, respectively, which are comparable to those obtained by some small mammals in our sample and in previous studies—for example, White and Seymour (2003) obtained a RMR range of 0.91–19.02 mL $O_2$ $h^{-1}$ $g^{-0.67}$ (mean: 5.17 mL $O_2$ $h^{-1}$ $g^{-0.67}$) for a sample of 312 small mammals (M <100 g).

*Proterosuchus* (0.74 mL $O_2$ $h^{-1}$ $g^{-0.67}$) and the phytosaur *Rutiodon* (0.40 mL $O_2$ $h^{-1}$ $g^{-0.67}$) display much lower values; the RMR of *Rutiodon* is very close to that of *Crocodylus*, which is congruent with the strong similarities in morphology and lifestyle between phytosaurs and crocodilians. This finding supports a previous study by Cubo et al. (2012) who, using a different approach, inferred a bone growth rate for *Rutiodon* even lower than those measured in some extant ectotherms. Conversely, fossil pseudosuchians in our sample present very high mass-specific RMR values (1.71 mL $O_2$ $h^{-1}$ $g^{-0.67}$ for *Calyptosuchus* and 1.86 for *Postosuchus*) typical of extant endotherms. The ancestral metabolic rate estimated for pseudosuchians

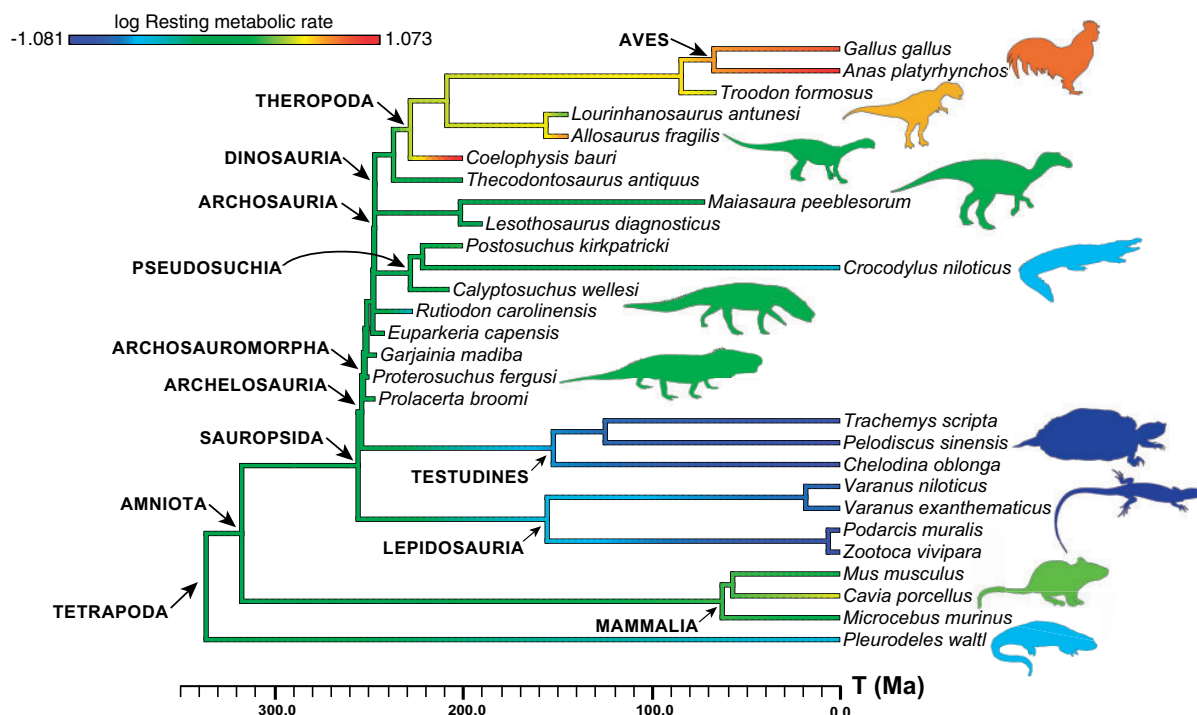FIGURE 2.    Ancestral state reconstruction of resting metabolic rate (in mL $O_2$ $h^{-1}$ $g^{-0.67}$, log-converted), figured as a color range (upper left corner), plotted on a calibrated phylogeny of our sample. Clades discussed in the clade are labeled.

is 1.47 mL $O_2$ $h^{-1}$ $g^{-0.67}$, which indicates an ancestral high metabolic rate for archosauromorphs, archosaurs, and pseudosuchians, and a reversion to a low heat production state in proterosuchids, phytosaurs, and extant crocodilians (Fig. 2). This reversion is likely linked with the acquisition of new predatory strategies associated with environmental constraints: most non-eusuchian pseudosuchians were terrestrial animals and shared anatomical features associated with active predation such as parasagittal members (e.g., large rauisuchids like *Postosuchus*), whereas crocodilians and phytosaurs are aquatic animals with a sit-and-wait predation strategy (Seymour et al. 2004, Nesbitt 2011). Although the terrestrial and/or aquatic lifestyle conditions of proterosuchids are still unresolved (Botha-Brink and Smith 2011), their morphology converges in many degrees with that of crocodilians, and is congruent with a similar predatory behavior (Smith and Botha 2005).

Most non-avian dinosaur species in our sample share high metabolic rate values compared with those of extant ectotherms (Fig. 1). We can observe a clear dichotomy between the heat production of herbivorous and carnivorous dinosaurs. Both ornithischian dinosaurs (*Lesothosaurus* and *Maiasaura*) and sauropodomorph *Thecodontosaurus* present intermediate mass-specific RMR values, around 1.00 mL $O_2$ $h^{-1}$ $g^{-0.67}$—*Maisaura* being the only dinosaur below that threshold, at 0.86 mL $O_2$ $h^{-1}$ $g^{-0.67}$. Conversely, theropod dinosaurs show

the highest values in the whole fossil sample, with *Allosaurus* and *Coelophysis* (5.89 and 11.84 mL $O_2$ $h^{-1}$ $g^{-0.67}$, respectively) having mass-specific RMR very close to those of modern birds.

This variation in RMR values results in an ancestral value of 1.17 mL $O_2$ $h^{-1}$ $g^{-0.67}$ estimated for the Dinosauria clade, a surprisingly low value compared with that obtained for Pseudosuchia. However, the metabolic rate of herbivorous dinosaurs in our sample might not be representative of the primitive condition shared by all ornithischians and sauropodomorphs. It has been argued that endothermy in dinosaurs was restricted to the smaller species, since very large dinosaurs such as sauropods or hadrosaurs would have required an impossibly large heart and lung ventilation rates too high to sustain mammalian- or avian-like metabolic rates, and because the internal temperature linked to body size may have been too elevated (McNab 1978; Benton 1979; Seebacher 2003; Seymour et al. 2004). In this context, the lower mass-specific RMR values observed in the large hadrosaurid *Maiasaura* compared with that of other dinosaurs in our sample is congruent with these observations. Conversely, the very high mass-specific RMR values found for theropod dinosaurs are congruent with the high systemic blood pressure inferred for such bipedal, active predators (Seymour 1976).

The low RMR value found for *Thecodontosaurus* might be linked to its basal phylogenetic position

among sauropodomorphs. The histological pattern of the *Thecodontosaurus* specimen in our sample consists of a succession of poorly vascularized fibrolamellar bone (suggesting a moderately high bone growth rate) and lamellar-zonal bone (compatible with a slow bone growth rate, probably after reaching sexual maturity). This pattern is typical of early sauropodomorphs of the Late Triassic; the very high sustained bone growth rate characteristic of the much larger sauropods was acquired during their radiation in the Early Jurassic (Sander et al. 2004). Furthermore, *Thecodontosaurus* was a small sauropodomorph (Benton et al. 2000) and has been described as a case of insular dwarfism (Whiteside and Marshall 2007), which could be an additional factor explaining its slow bone growth rate and low metabolic rate, as documented in *Magyarosaurus dacus*, another insular dwarf sauropod (Stein et al. 2010). Hence, the ancestral condition of metabolic rate in dinosaurs is likely to have been higher than recovered by these estimations.

In this perspective, the resting/basal metabolic rate of early archosaurs was probably close to those of extant endotherms, and many archosaurs most likely shared a series of physiological adaptations linked to true endothermy and previously considered to be synapomorphies of birds, as stated in many previous studies. For example, the presence of a four-chambered heart, similar to that of mammals and birds, was most probably ancestral for archosaurs and retained by ectothermic modern crocodiles, and allowed the separation of a high systemic blood pressure from a low pulmonary blood pressure. This may have allowed a high hematocrit (linked to high oxygen consumption), and high viscosity blood to flow through the body, as well as sufficient blood pressure to enable efficient ultrafiltration in the kidneys (Seymour et al. 2004). Similarly, the presence of unidirectional airflow in the lungs of extant crocodiles (Farmer 2015) and that of actively ventilated paranasal sinuses in many non-avian dinosaurs (Witmer and Ridgely 2008) support the hypothesis of a high, active metabolism associated with an important heat production as the ancestral condition of the archosaur node.

### *Phylogenetic Retrodictions in Palaeontology*

Overall, these results demonstrate the importance of phylogenetic retrodictions in palaeontological studies. PEMs are the latest contribution to a whole array of powerful estimation procedures that can provide a new insight into many palaeontological data sets, and the study of macroevolutionary patterns of extinct vertebrates in general has known a considerable expansion in recent years (review in Benton 2015). The use of various evolutionary models allows for the investigation of many lifetraits in different evolutionary scenarios that can easily be compared with one another; in this perspective, defining proper calibration procedures for trees that include fossil species has

become an important part of PCMs in palaeontology (Bapst 2014).

In this study, we only account for a Brownian motion model in our reconstruction of ancestral states (which is a realistic assumption, considering that the *a* parameter in the PEMs analysis was equal to zero). However, PEMs can also be used to perform such reconstructions with the same restricted maximum likelihood modeling approach already available for terminal taxa. The new function getAncGraphLocations has recently been added in the MPSEM package to perform such analyses (see MPSEM package vignette for more information), and additional functions to include auxiliary predictors and graphical representations of ancestral states on the phylogeny will soon be implemented. This method will thus likely be employed in further comparative analyses on palaeontological data sets in the near future, and expand the range of available techniques to describe the lifestyle of extinct vertebrates.

### *The Endotherm/Ectotherm Dichotomy*

In extant vertebrates, the clear gap of values in resting/basal metabolic rates between so-called "true" endotherms (i.e., birds and mammals) and other taxa has long been a straightforward way to discriminate endotherms from ectotherms and to define them as the two existing physiological strategies for heat production (e.g., Clarke and Pörtner 2010). However, this philosophy is oversimplified as there are many exceptions to this dichotomy. For example, Grady et al. (2014) built a predictive model of RMR based on ontogenetic growth using a sample of 381 species of vertebrates, and identified all 21 non-avian dinosaurs in their sample as being "mesothermic," that is, with RMR values intermediate between those of extant ectotherms and endotherms. A number of extant vertebrate species with intermediate metabolic rates are also quoted as being present-day mesotherms.

However, the physiological heat production strategy in these extant mesotherms is far from homologous in all of them, and many different strategies can be observed in each of them. Lamnid sharks and tunas, for example, display high RMR values compared with other non-tetrapods because they acquired independently many physiological adaptations to fast swimming, such as the ability to retain metabolic heat produced by continuous activity of red muscles during swimming to elevate their global body heat (Bernal et al. 2001; Sepulveda et al. 2008). Conversely, some small mesothermic mammals, such as echidnas or mouse lemurs (*Microcebus*), generate their own body heat through the uncoupling activity of proteins in mitochondria, just like endothermic mammals and birds. They display low resting metabolic rates compared with those of other mammals because of a high thermal lability, that is, a shift between an active phase with a preferred range of body temperature around 37 °C and a torpor phase with lower temperatures conditioned by environmental constraints

(Schmid et al. 2000). In fact, true endotherms do not share one single heat production strategy *sensu stricto*, since uncoupling proteins used to produce metabolic heat in mitochondria have evolved independently in mammals and birds (Mezentseva et al. 2008).

In our sample of fossil species, it is thus very difficult to unambiguously attribute a physiological strategy to an estimated RMR value, since we do not know the particular physiological adaptations that each of them could have evolved to acquire their individual resting metabolic rate. The many apomorphic strategies used by vertebrates to raise, lower, or maintain a constant body temperature correspond to various metabolic adaptations associated with very different physiological constraints. We cannot clearly infer these strategies in fossil species, nor can we interpret them as easily as a single homologous character state in a phylogenetic context. Using the endotherm/ectotherm dichotomy to describe such a mosaic of different heat production strategies may thus be misleading (Ruben 1995). Grady et al. (2014, p. 1268), while acknowledging the ambiguity of this dichotomy as being "overly simplistic," only change it into a trichotomy with the addition of a single mesothermic state, which is likely another oversimplification. A comprehensive review of all metabolic strategies in vertebrates without using such categories is yet to come, and the resting metabolic rates of extinct species are likely to be more accurately discussed as such rather than gathered together in arbitrary discrete categories.

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