

# Bone histology of Azendohsaurus laaroussii: Implications for the evolution of thermometabolism in Archosauromorpha

Jorge Cubo, Nour-Eddine Jalil

## ► To cite this version:

Jorge Cubo, Nour-Eddine Jalil. Bone histology of Azendohsaurus laaroussii: Implications for the evolution of thermometabolism in Archosauromorpha. Paleobiology, 2019, 45 (2), pp.317-330. 10.1017/pab.2019.13. hal-02969492

## HAL Id: hal-02969492 https://hal.sorbonne-universite.fr/hal-02969492

Submitted on 16 Oct 2020  $\,$ 

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1	Bone histology of Azendohsaurus laaroussii. Implications for the evolution of
2	thermometabolism in Archosauromorpha
3	
4	Jorge Cubo <sup>1</sup> * and Nour-Eddine Jalil <sup>2,3</sup>
5	
6	<sup>1</sup> Sorbonne Université, MNHN, CNRS, Centre de Recherche en Paléontologie – Paris (CR2P)
7	- 4 place Jussieu, BC 104, 75005 Paris, France.
8	E-mail: jorge.cubo_garcia@sorbonne-universite.fr
9	*Corresponding author
10	
11	<sup>2</sup> Muséum national d'Histoire naturelle, Sorbonne Université, CNRS, Centre de Recherche en
12	Paléontologie – Paris (CR2P) - 75005 Paris, France.
13	E-mail: nour-eddine.jalil@mnhn.fr
14	
15	<sup>3</sup> Laboratory of Biodiversity and Dynamic of Ecosystems, Department of Geology, Fac. Sci.
16	Semlalia, UCA, Marrakesh, Morocco
17	
18	

19 *Abstract.* This paper is aimed at constraining the phylogenetic frame of the acquisition of 20 endothermy by Archosauromorpha. We analyzed the bone histology of Azendohsaurus 21 *laaroussii*. Stylopodial and zeugopodial bones show three tissue types: (a) avascular lamellar 22 zonal bone formed at low growth rates; (b) a scaffold of parallel fibered bone containing 23 either small primary osteons or simple vascular canals; and (c) fibrolamellar bone formed at 24 high growth rates. We used quantitative histology to infer the thermometabolic regime 25 (endothermy versus ectothermy) of this taxon. We define endothermy as the presence of any 26 mechanism of non-shivering thermogenesis that increases both body temperature and resting 27 metabolic rate. Thus, estimating the resting metabolic rate of an extinct organism may be a 28 good proxy to infer its thermometabolic regime (endothermy versus ectothermy). High resting 29 metabolic rates have been shown to be primitive for the clade Prolacerta - Archosauriformes. 30 Therefore, we inferred the resting metabolic rates of Azendohsaurus laaroussii, a sister group 31 of this clade, and of 14 extinct related taxa, using Phylogenetic Eigenvector Maps. All the 32 inferences obtained are included in the range of variation of resting metabolic rates measured 33 in mammals and birds, so we can reasonably assume that all these taxa (including 34 Azendohsaurus) were endotherms. A parsimony optimization of the presence of endothermy 35 on a phylogenetic tree of tetrapods shows that this derived character state was acquired by the 36 last common ancestor of the clade Azendohsaurus - Archosauriformes, and that there is a 37 reversion in Crocodylia.

39

#### Introduction

40

The bone histology of non-archosaurian archosauromorphs has received great 41 42 attention in the last decade (Ricglès et al. 2008; Nesbitt et al. 2009; Botha-Brink and Smith 43 2011; Werning and Irmis 2011; Legendre et al. 2013, 2016; Ezcurra et al. 2014; Mukherjee 44 2015; Veiga et al. 2015; Werning and Nesbitt 2016; Jaquier and Scheyer 2017). Ricglès et al. (2008) suggested that the capacity of reaching and maintaining very high bone growth rates is 45 46 an apomorphic feature of archosauriforms. Botha-Brink and Smith (2011) showed that this 47 capacity may have been acquired in a more inclusive node, by the last common ancestor of 48 the clade *Prolacerta* - archosaurifoms, with a reversion in *Vancleavea* (see also Nesbitt et al. 49 2009). Padian and Horner (2002) hypothesized that "The types of tissue deposited in the 50 bones of extinct animals are the most direct evidence of basal metabolic rates, because they 51 directly reflect growth rates [...]. The sustained deposition of fast-growing bone tissues, as 52 displayed by mammals, birds and other dinosaurs, must reflect sustained high basal metabolic 53 rates». Montes et al. (2007) provided evidence for this hypothesis by showing that the 54 variation of bone growth rates explains significantly the variation of resting metabolic rates in 55 a sample of extant amniotes. They argued that this is because the high rates of protein 56 synthesis and degradation involved in the periosteal osteogenesis and endosteal osteolysis are 57 energy consuming (Montes et al. 2007). Consistently, Legendre et al. (2016) inferred resting 58 metabolic rates of a sample of Archosauromorpha using quantitative bone histology and 59 Phylogenetic Eigenvector Maps. Interestingly, they inferred a resting metabolic rate of Prolacerta included in the range of variation of extant mammals (Legendre et al. 2016). 60 61 Resting metabolic rate depends on bone growth rate (see above), but it depends also on the 62 presence of thermogenetic mechanisms. We define endothermy as the presence of any mechanism of non-shivering thermogenesis (e.g., Lowell and Spiegelman 2000; Rowland et 63

64	al. 2015; Nowack et al. 2017) that increases both body temperature and resting metabolic rate.
65	Thus, the resting metabolic rate of an extinct organism may be a good proxy to infer its
66	thermometabolic regime (endothermy versus ectothermy). Using this proxy, the results
67	obtained by Legendre et al. (2016) suggest that endothermy may have been acquired by the
68	last common ancestor of the clade Prolacerta - archosauriforms. The next step to better
69	constrain the phylogenetic frame of the acquisition of endothermy by Archosauromorpha
70	involves inferring the resting metabolic rate of Allokotosauria, the sister-group of the clade
71	Prolacerta – archosauriforms (Nesbitt et al. 2015; see also Ezcurra 2016). Therefore, we
72	analyzed the bone histology and inferred the resting metabolic rates of Azendohsaurus
73	laaroussii (Archosauromorpha, Allokotosauria; Nesbitt et al. 2015; Ezcurra 2016) using
74	Phylogenetic Eigenvector Maps.
75	
76	Material and methods
77	
78	Material We analyzed histologically skeletal remains from the non-archosaurifom
79	archosauromorph Azendohsaurus laaroussii from the Upper Triassic (Carnian) of the Argana
80	Basin (Morocco) deposited at the Paris Muséum National d'Histoire Naturelle (MNHN).
81	Azendohsaurus laaroussii Dutuit, 1972 was originally described on the basis of a dental
82	fragment and two isolated teeth specialized for plants feeding (Dutuit, 1972). On the basis on
83	comparison with Ornithischian dinosaurs such as Fabrosaurus and Scelidosaurus it was
84	attributed to a new ornithischian dinosaur, becoming one of the earliest dinosaurs (Dutuit
85	1972; Thulborn, 1973, 1974; Bonaparte, 1976; Galton, 1985, 1990; Weishampel, 1990;
86	Gauffre, 1993; Hunt and Lucas, 1994; Flynn et al. 1999). In addition to the craniodental
87	remains, hundreds of disarticulated and monospecific post-cranial remains have been
88	recovered from the locus typicus of Azendohsaurus laaroussii (Dutuit, 1976). The analysis of

these specimens argues strongly against a phylogenetic position inside Dinosauria (Jalil and
Knoll, 2002). Flynn et al. (2010) named the new species *Azendohsaurus madagaskarensis*from the Triassic of Madagascar and provided for the first time a comprehensive description
of the cranial anatomy of the genus. More recently, Nesbitt et al. (2015) provided a scrutiny
description the postcranial anatomy of *Azendohsaurus madagaskarensis* which is now one of
the best-known early archosauromorphs.

95 All the available post-cranial material in the MNHN collection attributed to 96 Azendohsaurus laaroussii is monotypic (Khaldoun, 2014). It was collected from the locus 97 typicus of Azendohsaurus laaroussii during the same initial field season (Dutuit, 1976), and 98 strongly resembles to the post-cranial skeleton of Azendohsaurus madagaskarensis (Fig. 1). 99 They can be confidently attributed to A. laaroussii. Among the diagnostic post-cranial 100 characters of the Malagasy form, only two can be checked on the available material of A. 101 laaroussii and both are present: a posteriorly expanded T-shaped interclavicle and 102 hyposphene-hypantra intervertebral articulations in anterior trunk vertebrae. This later 103 character-state was considered autapomorphic for A. madagaskarensis by Nesbitt et al. (2015) 104 but it may be a synapomorphy for the genus Azendohsaurus. The proportions of the studied 105 skeletal elements MNHN.F.ALM 435 (right humerus), MNHN.F.ALM 497 (left femur), and 106 MNHN.F.ALM 369 (right tibia), show that they are from different specimens.

107

*Histological methods.*- Skeletal elements were molded and cast prior to sectioning to preserve morphological information. Casts and bone remains after histological sampling were reposited at the Paleontology collection of the Paris Muséum National d'Histoire Naturelle where they are available upon request to the curator. Mid-diaphyses were embedded in epoxy resin and processed histologically following standard procedures (Lamm 2013). Transverse sections as well as longitudinal sections (at the anterior, posterior, dorsal and ventral sides for humerus and femur, and anterior, posterior, medial and lateral sides for the tibia) were
obtained and mounted on glass slides. They were analyzed and photographed in a Nikon
Eclipse E600POL microscope using normal light and cross-polarized light with lambda
compensator. Thin sections were reposited at the vertebrate hard tissues histological
collection of the Paris MNHN, where they are available upon request to the curator (thin
sections MNHN-F-HISTOS 2348 to 2362).

120

121 Qualitative histology.- The nomenclature and classification of bone tissues is based on 122 bone organization at different integration levels (Francillon-Vieillot et al. 1990). At the level 123 of bone matrix organization, three character states have been defined based on the 124 organization of collagen fibers: the presence (or absence) of woven-fibered bone matrix 125 (WB), of parallel-fibered bone matrix (PFB) and of lamellar bone matrix (LB). At the level of 126 organization of bone as a tissue, two character states have been defined based on the 127 organization of the collagen fibers, the vascular density, and the cyclicity of bone apposition: 128 the presence of lamellar-zonal bone (LZB) and the presence of the fibro-lamellar bone 129 (FLB). See Francillon-Vieillot et al. (1990) for a detailed description of these character states. 130 This pattern-oriented approach has been recently expanded towards a process-oriented view 131 based on the developmental origin of the components of bone tissue (Prondvai et al. 2014). In 132 process-oriented paleohistology, the developmental mechanisms involved in the formation of 133 bone tissue are inferred from the fine architecture of bone matrix. PFB and LB are formed by 134 the process of dynamic osteogenesis whereas WB is formed by the process of static 135 ossification (Ferretti et al. 2002; Palumbo et al. 2004; Marotti 2010; Prondvai et al. 2014; 136 Stein and Prondvai 2014; Cubo et al. 2017). Cortices can also have a composite nature 137 including (1) a scaffold of WB formed by the process of static osteogenesis and (2) centripetal 138 layers infilling primary osteons made of PFB or LB formed by the process of dynamic

osteogenesis. This last tissue type has been named woven-parallel complex by Prondvai et al.
(2014). In the context of process-oriented paleohistology, the widely used term of FLB is a
special case of woven-parallel complex in which there is abundant woven bone and dense
vascularity in the form of primary osteons (Prondvai et al. 2014).

143

*Quantitative histology.*- We quantified the vascular density (number of vascular canals
by square millimeter) in four orthogonal transects of the transverse sections of humerus,
femur and tibia of *Azendohsaurus laaroussii*. We used the dataset published by Legendre et
al. (2016) for comparative purposes. It contains the resting metabolic rates and the vascular
densities of humerus, femur and tibia of a sample of 13 extant species and 14 extinct species
of tetrapods. Data for *Mus musculus* were removed because this species was an outlier.

150

*Phylogenetic comparative methods.*- We tested whether vascular density explains a
significant fraction of the variation of resting metabolic rate using Phylogenetic Generalized
Least Squared regressions (PGLS, Grafen 1989), with the 'caper' package (Orme et al. 2013)
in R (R Core Team 2016). For this we used the dataset and the phylogeny published by
Legendre et al. (2016).

Moreover, we inferred the resting metabolic rates of *Azendohsaurus laaroussii* and those for 14 species of extinct tetrapods using phylogenetic eigenvector maps (Guenard et al. 2013) with the package MPSEM package (Guenard et al. 2013) in R (R Core Team 2016). For this we used our data from *Azendohsaurus laaroussii* and the dataset and the phylogeny published by Legendre et al. (2016).

Finally, we performed an optimization of the presence of fibrolamellar bone (using our data for *Azendohsaurus laaroussii* and published data for other Archosauromorpha) and an optimization of the presence of endothermy (using observed resting metabolic rates for extant

164	species and inferred resting metabolic rates for extinct ones as a proxy for the
165	thermometabolic regime) onto phylogenies of Archosauromorpha and Tetrapoda respectively
166	using parsimony in Mesquite (Maddison and Maddison 2015).
167	
168	Results
169	
170	Bone histology of Azendohsaurus laaroussii Supplementary Fig. 1 shows composite
171	images of the entire diaphyseal cross sections of humerus, femur and tibia. The medial region
172	of the transverse section of the tibia is avascular (Fig. 2A). The bone matrix appears red
173	(isotropic) under cross-polarized light with lambda compensator (Fig. 2B) and black in cross-
174	polarized light without lambda compensator (not shown). This can be interpreted either as
175	woven bone or as parallel-fibered bone with fibers perpendicular to the transverse plane of
176	section (Stein and Prondvai 2014). The longitudinal section of the same (medial) region is
177	also avascular (Fig. 2C). In the longitudinal section, the bone matrix appears anisotropic (blue
178	in the picture but it can be yellow depending on the orientation to the polarizers) under cross-
179	polarized light with lambda compensator (Fig. 2D). These complementary patterns of
180	interference suggest that the cortex is composed of parallel-fibered bone, with collagen fibers
181	running parallel to the longitudinal axis of the tibia. Osteocyte lacunae have a rounded aspect
182	in the transverse section (Fig. 2A, B) and an elongate spindle shape in the longitudinal section
183	(Fig. 2C, D). Their main axis is, thus, aligned with the collagen fibers of the surrounding
184	matrix, whereas the canaliculi are perpendicular (Fig. 2C, D). We can observe erosion cavities
185	lined by a coating of endosteal lamellar tissue around the medullary cavity (Fig. 2A, B).
186	The posterior region of the transverse section of the tibia is well vascularized (Fig.
187	2E). Vascular density is higher in the deep cortex than in the outer cortex. Vascular canals are
188	longitudinal: they are rounded in transverse section (Fig. 2E, F) and elongated (with the main

189 axis parallel to the main axis of the tibia) in the longitudinal section (Fig. 2G, H). The scarce 190 vascular canals of the outer cortex are simple primary canals, whereas those of the deep 191 cortex form small primary osteons (Fig. 2F). An avascular outer circumferential layer is 192 absent. The bone matrix is isotropic (it appears red) under cross-polarized light with lambda 193 compensator in the transverse section (Fig. 2F) and anisotropic (blue) in the longitudinal 194 section (Fig. 2H). This pattern suggests that bone matrix in the posterior region of the tibia is 195 composed of parallel-fibered bone, with collagen fibers running parallel to the longitudinal 196 axis of the tibia. Osteocyte lacunae have a rounded aspect in the transverse section (Fig. 2E, 197 F) and an elongate spindle shape in the longitudinal section (Fig. 2G, H). The anterior and the 198 lateral regions of the tibia are similar to the posterior region in all respects but one: the lateral 199 region contains a line of arrested growth split into two lines.

200 The stylopodial bones (femur and humerus) show a well developed vascularization in 201 the deep cortex mainly formed by vascular canals parallel to the bone periphery (circular 202 canals) connected through abundant radial anastomoses (Fig. 3A-D). This pattern of vascular 203 orientation is more regular in the femur (Fig. 3A, B) than in the humerus (Fig. 3C, D) but 204 vascular density decreases from the inner to the outer cortex in both cases. The bone matrix is 205 made of either a scaffold of woven bone containing primary osteons (fibro-lamellar bone; 206 e.g., Fig. 3E, F) or a scaffold of parallel fibered bone containing both simple vascular canals 207 and primary osteons (e.g. Fig. 3G, H). We can observe the anisotropic (blue in the picture) 208 aspect of the scaffold of bone matrix in a longitudinal section of the humerus under polarized 209 light suggesting that it is made of parallel fibered bone with collagen fibers oriented parallel 210 to the main axis of the bone (Fig. 3H). Please note that the matrix around vascular canals 211 appears isotropic (red) in this longitudinal section (Fig. 3H) whereas it appears anisotropoic 212 (blue in the picture) in the transverse section (Fig. 3G), suggesting that it is made of parallel 213 fibered bone forming primary osteons with collagen fibers perpendicular to the main axis of

the humerus. Avascular outer circumferential layers or lines of arrested growth are absent inboth femur and humerus (Fig. 3A-D).

216 In summary, vascular canals are more abundant in the deep cortex than in the outer 217 cortex in all three bones suggesting an ontogenetic decrease of bone growth rates (e.g., Fig. 218 2E). Considering that the avascular outer circumferential layer of parallel fibered bone is 219 absent (we observed vascular canals near the bone periphery in many regions, e.g., Fig. 2E), 220 and that lines of arrested growth are absent in all analyzed regions but one (the lateral region 221 of the tibia), we conclude that the analyzed specimens were subadults. The two lines of 222 arrested growth observed in the lateral region of the tibia probably correspond to the split of a 223 single line formed during bone cortical drift because they are absent in the anterior, posterior 224 and medial sides of the same section.

225

226 Inferring the resting metabolic rate of Azendohsaurus laaroussii.- First we tested 227 whether vascular density explains a significant fraction of the variation of resting metabolic 228 rate using the sample of extant tetrapods published by Legendre et al. (2016). For this we used 229 Phylogenetic Generalized Least Squared regressions (Grafen 1989). In all three cases 230 (humerus, femur and tibia), vascular density explained highly significant portions of the 231 variation of resting metabolic rate (85%, 73.3% and 78.9% respectively). Thus a priori we 232 can infer the resting metabolic rate of Azendohsaurus laaroussii confidently using the 233 vascular density values measured in the humerus, the femur and the tibia (65.56; 34.83 and 234 47.19 vascular canals by squared millimeter respectively) and the comparative data published 235 by Legendre et al. (2016).

236 Phylogenetic Eigenvector Maps (Guenard et al. 2013) includes an AIC procedure to 237 select the explanatory variables (here vascular density and/or the phylogeny) to be used to 238 infer the response variable (here the resting metabolic rate) of extinct taxa (here

239	Azendohsaurus laaroussii and the sample of extinct Archosauromorpha published by
240	Legendre et al. 2016). According to the AIC procedure, the variable that maximizes the R
241	squared and minimizes the AIC value should be selected. In the case of the humerus we
242	obtained the same R squared when using vascular density and the phylogeny and when using
243	only the phylogeny, but the AIC value was lower when using both vascular density and the
244	phylogeny (Fig. 4). We inferred a resting metabolic rate of $3.11 \text{ mL O}_2 \text{ h}^{-1} \text{ g}^{-0.67}$ and a
245	confidence interval of $2.87 - 3.36$ mL O <sub>2</sub> h <sup>-1</sup> g <sup>-0.67</sup> for <i>Azendohsaurus laaroussii</i> using the
246	phylogeny and vascular density of the humeurs (Fig. 4). In the case of the femur we found a
247	slightly higher R squared and a lower AIC value when using the phylogeny only (Fig. 5). We
248	inferred a resting metabolic rate of 2.20 mL $O_2\ h^{\text{-1}}\ g^{\text{-0.67}}$ and a confidence interval of 1.55 –
249	2.85 mL $O_2$ h <sup>-1</sup> g <sup>-0.67</sup> for <i>Azendohsaurus laaroussii</i> using only the phylogeny (this is
250	equivalent to an optimization; Fig. 5). Finally, in the case of the tibia we obtained again the
251	same R squared when using vascular density and the phylogeny and when using only the
252	phylogeny, but the AIC value was lower when using both vascular density and the phylogeny
253	(Fig. 6). We inferred a resting metabolic rate of 2.53 mL $O_2$ h <sup>-1</sup> g <sup>-0.67</sup> and a confidence interval
254	of $2.25 - 2.82$ mL O <sub>2</sub> h <sup>-1</sup> g <sup>-0.67</sup> for <i>Azendohsaurus laaroussii</i> using the phylogeny and the
255	vascular density of the tibia (Fig. 6).
256	
257	Discussion
258	
259	Evolution of fibrolamellar bone in Archosauromorpha The formation of
260	fibrolamellar bone is very energy consuming because it involves the combined action of static
261	osteogenesis (to form a scaffold of woven bone at high growth rates involving high rates of
262	protein synthesis) and dynamic osteogenesis (osteonal infilling with lamellar or non-lamellar
263	parallel fibered bone) (Prondvai et al. 2014). Therefore the capacity of reaching and

264 maintaining very high bone growth rates during ontogeny, recorded by the presence of 265 fibrolamellar bone in the bone cortex, may be a reliable proxy to infer the metabolic rate of 266 extinct animals (see the introduction). Four previous studies have analyzed the evolution of 267 the presence of fibrolamellar bone in Archosauromorpha in a phylogenetic context (Botha-268 Brink and Smith 2011; Mukherjee 2015; Veiga et al. 2015; Jaquier and Scheyer 2017). Here 269 we performed parsimony optimizations of this character state (Fig. 7 and Supplementary Figs. 270 2 and 3) using our observations on the bone histology of Azendohsaurus laaroussii, and the 271 observations on other Archosauromorpha published previously (summarized in Table 1). 272 We observed three bone tissue types in the humerus, femur and tibia of 273 Azendohsaurus laaroussii: (i) avascular lamellar zonal bone (e.g., Fig. 2A-D); (ii) a cortex 274 formed by a scaffold of parallel fibered bone (with collagen fibers parallel to the longitudinal 275 axis of the bone) containing either small primary osteons infilled by lamellar parallel fibered 276 bone (inner part of the cortex, Fig. 2F) or simple vascular canals (outer part of the cortex, Fig. 277 2F) and (iii) fibrolamellar bone (e.g., Fig. 3E-F). Therefore we coded presence of FLB for 278 Azendohsaurus (Fig. 7 and Supplementary Figs. 2 and 3). 279 We coded presence of FLB for Archosauria because this character state has been 280 reported both in Pseudosuchia (e.g., Ricglès et al. 2003; Padian et al. 2004; Nesbitt 2007; 281 Tumarkin-Deratzian 2007) and in Ornithodira (Ricglès et al. 2000; Padian et al. 2004; Cubo et 282 al. 2015). Consistently, Cubo et al. (2012) inferred a high bone growth rate for the last 283 common ancestor of archosaurs. Lepidosauromorpha has been chosen as an outgroup and is 284 characterized by the absence of this character state (Fig. 7 and Supplementary Figs. 2 and 3). We performed an optimization of the presence of FLB onto the phylogeny of 285 286 Archosauromorpha published by Nesbitt et al. (2015) (Fig. 7). Moreover, we carried out two 287 supplementary optimizations of the presence of FLB using the phylogenies of 288 Archosauromorpha published by Pritchard et al. (2015) (Supplementary Fig. 2) and Ezcurra

(2016) (Supplementary Fig. 3). All three analyses showed a flickering on and off pattern that
prevents inferring the primitive condition for this clade. Moreover, the parsimony method
fails to find the condition for the three more inclusive (basal) nodes of the clade. The only
robust conclusion obtained in the three analyses is congruent with that published by BothaBrink and Smith (2011): the fibrolamellar bone was acquired by the last common ancestor of *Prolacerta* - archorsauriforms, with a reversion in *Vancleavea* (Fig. 7 and Supplementary
Figs. 2 and 3).

296

297 *Constraining the phylogenetic frame of the acquisition of endothermy by* 298 Archosauromorpha.- The resting metabolic rate may be a good proxy to infer the 299 thermometabolic regime (endothermy versus ectothermy) of extinct organisms because 300 thermogenesis is very energy consuming (see the introduction). Legendre et al. (2016) used 301 this proxy in Archosauromorpha and concluded that endothermy may have been acquired by 302 the last common ancestor of the clade Prolacerta - archosauriforms. We inferred the resting 303 metabolic rate of a member of the sister group of this clade (Azendohsaurus laaroussii : 304 Allokotosauria) as the next step to better constrain the phylogenetic frame of the acquisition 305 of endothermy by Archosauromorpha. Moreover, we inferred resting metabolic rate values for 306 the sample of 14 extinct Archosauromorpha analyzed by Legendre et al. (2016). The values 307 inferred for all these taxa are included in the range of variation measured in the sample of 308 extant endotherms (mammals and birds) (Fig. 4-6). Considering that thermogenesis is very 309 energy consuming, we can reasonably assume that all these taxa were endotherms. A parsimony optimization of the presence of endothermy in the whole sample (including extant 310 311 taxa of known thermometabolism and extinct taxa of inferred thermometabolism) shows that 312 endothermy was acquired twice, by mammals and by the last common ancestor of the clade Azendohsaurus – archosauriforms, and that within this last clade there is a reversion in 313

314	Crocodylia (Fig. 8). Considering that many archosauriforms have been found in the Permian
315	and that endothermy may have been acquired in a more inclusive clade (Azendohsaurus -
316	archosauriforms), this key evolutionary event took place probably in the Permian. Bernard et
317	al. (2010) inferred fully developed endothermy for ichthyosaurs and plesiosaurs and incipient
318	endothermy for mosasaurs using a geochemical approach. These conclusions are congruent
319	with those obtained using a paleohistological approach (Buffrénil and Mazin 1990 for
320	ichthyosaurs; Fleischle et al. 2018 for plesiosaurs; and Houssaye et al. 2013 for mosasaurs).
321	Thermogenetic mechanisms have also been described in Actinopterygii: at the extraocular
322	muscles that warm the eyes and brain in billfishes (Xiphioidei) and in the butterfly kingfish
323	Gasterochisma melampus (Scombridae: Gasterochismatinae) (Davesne et al. 2018). Future
324	research on the biochemical basis of thermogenesis in extant taxa and on paleobiological
325	inference of resting metabolic rates in extinct taxa are needed to elucidate the origin and the
326	evolutionary patterns of endothermy in Osteichthyes.
327	
328	Acknowledgements
329	
330	We thank Ronan Allain, curator of paleontology at the Paris MNHN, for giving us the
331	permission to perform histological analyses in the right humerus MNHN.F ALM435, the left
332	femur MNHN.F ALM497 and the right tibia MNHN.F ALM369 of Azendohsaurus
333	laaroussii. We also thank Damien Germain, curator of the vertebrate hard tissues histological
334	collection of the Paris MNHN, for having included the thin sections produced in this study in
335	that collection. Thanks also to Hayat Lamrous for the preparation of the thin sections, to
336	Philippe Loubry for the photos and to Sophie Fernandez for the drawings. Finally, we thank
337	Holly Woodward and an anonymous reviewer for their constructive comments.
220	

### References

341	Bernard, A., C. Lécuyer, P. Vincent, R. Amiot, N. Bardet, N., E. Buffetaut, G. Cuny, F.
342	Fourel, F. Martineau, J. M. Mazin and A.Prieur 2010: Regulation of body
343	temperature by some mesozoic marine reptiles. Science 328 : 1379–1382.
344	Bonaparte, J. F. 1976: Pisanosaurus mertii Casamiquela and the origin of the Ornithischia.
345	Journal of Paleontology 50: 808-820.
346	Botha-Brink, J., and R. M. H. Smith. 2011: Osteohistology of the Triassic Archosauromorphs
347	Prolacerta, Proterosuchus, Euparkeria, and Erythrosuchus from the Karoo Basin of
348	South Africa. Journal of Vertebrate Paleontology 31:1238-1254.
349	Buffrenil, V. de and J.M. Mazin 1990: Bone histology of the ichthyosaurs : comparative data
350	and functional interpretation. Paleobiology 16: 435-447.
351	Cubo, J., N. Le Roy, C. Martinez-Maza, and L. Montes. 2012: Paleohistological estimation of
352	bone growth rate in extinct archosaurs. Paleobiology 38:335-349.
353	Cubo, J., H. Woodward, E. Wolff, and J. R. Horner. 2015: First reported cases of
354	biomechanically adaptive bone modeling in non-avian dinosaurs. Plos One
355	10:e0131131.
356	Cubo, J., M. Hui, F. Clarac, and A. Quilhac. 2017: Static osteogenesis does not precede
357	dynamic osteogenesis in periosteal ossification of Pleurodeles (Caudata, Amphibia)
358	and Pogona (Squamata, Lepidosauria). Journal of Morphology 278:621-628.
359	Davesne, D., F. J. Meunier, M. Friedman, R. B. J. Benson and O. Otero 2018: Histology of
360	the endothermic opah (Lampris sp.) suggests a new structure-function relationship in
361	teleost fish bone. Biology Letters 14: 20180270.
362	http://dx.doi.org/10.1098/rsbl.2018.0270
363	Dutuit, JM. 1972: Découverte d'un Dinosaure ornithischien dans le Trias supérieur de l'Atlas
364	occidental marocain. Comptes Rendus de l'Académie des Sciences, Paris 275: 2841-
365	2844.
366	Dutuit, JM. 1976: Introduction à l'étude paléontologique du Trias continental marocain.
367	Description des premiers Stégocéphales recueillis dans le Couloir d'Argana (Atlas
368	occidental). Mémoires du Muséum National d'Histoire Naturelle, N. S. C 36: 1-253.
369	Ezcurra, M. D. 2016: The phylogenetic relationships of basal archosauromorphs, with an
370	emphasis on the systematics of proterosuchian archosauriforms. Peerj 4:e1778.
371	Ezcurra, M. D., T. M. Scheyer, and R. J. Butler. 2014: The Origin and Early Evolution of

372 Sauria: Reassessing the Permian Saurian Fossil Record and the Timing of the 373 Crocodile-Lizard Divergence (vol 9, e89165, 2014). Plos One 9:e97828. 374 Ferretti, A., C. Palumbo, M. Contri, and G. Marotti. 2002: Static and dynamic osteogenesis: 375 two different types of bone formation. Anatomy and Embryology 206:21-29. 376 Fleischle, C. V., T. Wintrich and P. M. Sander 2018: Quantitative histological models suggest 377 endothermy in plesiosaurs. PeerJ, 6, 25. https://doi.org/10.7717/peerj.4955 378 Flynn, J.J., Nesbitt, S.J., Parrish, J.M., Ranivoharimana, R. and Wyss, A.R. 2010: A new 379 species of Azendohsaurus (Diapsida : Archosauromorpha) from the Triassic Isalo 380 Group of southestern Madagascar : cranium and mandible. Palaeontology: 53, 669-381 688. 382 Flynn, J. J., Parrish, J. M., Rakotosamimanana, B., Simpson, W. F., Whatley, R. B. & Wyss 383 and A. R. 1999: A Triassic fauna from Madagascar, including early dinosaurs. 384 Science 286: 763-765. 385 Francillon-Vieillot, H., V. de Buffrénil, J. Castanet, J. Geraudie, F. Meunier, J. Y. Sire, L. 386 Zylberberg, and A. de Ricglès. 1990: Microstructure and mineralization of vertebrate 387 squeletal tissues. Pp.471-548 in Skeletal Biomineralization: Patterns, Processes and 388 Evolutionary Trends. Van Nestrand Reinhold, New York. 389 Galton, P. M. 1985: Diet of Prosauropod dinosaurs from the Late Triassic and Early Jurassic. 390 Lethaia 18: 105-123. 391 Galton, P. M. 1990: Basal Sauropodomorpha-Prosauropoda, In D. B. Weishampel, P. Dodson & H. Osmólska (eds.), The Dinosauria, pp. 320-344. University of California Press, 392 393 Berkeley. 394 Gauffre, F. -X. 1993: The prosauropod dinosaur Azendohsaurus laaroussii from the Upper 395 Triassic of Morocco. Palaeontology 36(4): 897-908. 396 Grafen, A. 1989: The Phylogenetic Regression. Philosophical Transactions of the Royal 397 Society of London Series B-Biological Sciences 326:119–157. 398 Gross, W. 1934: Die Typen des mikroskopischen Knochenbaues bei fossilen Stegocephalen 399 und reptilien. Zeitschrift für Anatomie und Entwicklungsgeschichte 203:731-764. 400 Guenard, G., P. Legendre, and P. Peres-Neto. 2013: Phylogenetic eigenvector maps: a 401 framework to model and predict species traits. Methods in Ecology and Evolution 402 4:1120-1131. 403 Houssaye, A., J. Lindgren, R. Pellegrini, A. H. Lee, D. Germain and M. J. Polcyn 2013: 404 Microanatomical and histological features in the long bones of mosasaurine 405 mosasaurs (Reptilia, Squamata) - Implications for aquatic adaptation and growth

406 rates. PLoS ONE, 8(10): 1-12. https://doi.org/10.1371/journal.pone.0076741 407 Hunt, A. P. and Lucas, S.P. 1994: Ornithischian dinosaurs from the Upper Triassic of the 408 United States. In N. C. Fraser & H. -D. Sues (eds.), In the Shadow of the Dinosaurs. 409 Early Mesozoic Tetrapods, pp.227-241. Cambridge University Press, Cambridge. 410 Jaquier, V. P., and T. M. Scheyer. 2017: Bone Histology of the Middle Triassic Long-Necked 411 Reptiles Tanystropheus and Macrocnemus (archosauromorpha, Protorosauria). 412 Journal of Vertebrate Paleontology 37:e1296456. 413 Khaldoun, F. 2014: Les vertébrés du Permien et du Trias du Maroc (Bassin d'Argana, Haut 414 Atlas Occidental) avec la réévaluation d'Azendohsaurus laaroussii (Reptilia, 415 Archosauromorpha) et la description de Reptilia Moradisaurinae et Rhynchosauria 416 nouveaux : Anatomie, relations phylogénétiques et implications biostratigraphiques. 417 342pp. PhD thesis, University Cadi Ayyad, Marrakech, Morocco (unpublished) 418 Lamm, E. T. 2013: Preparation and Sectioning of Specimens. Pp.55-160 in Bone Histology 419 of Fossil Tetrapods: Advancing Methods, Analysis, and Interpretation. University of 420 California Press, Berkeley, CA. 421 Legendre, L. J., L. Segalen, and J. Cubo. 2013: Evidence for High Bone Growth Rate in 422 Euparkeria Obtained Using a New Paleohistological Inference Model for the 423 Humerus. Journal of Vertebrate Paleontology 33:1343–1350. 424 Legendre, L. J., G. Guenard, J. Botha-Brink, and J. Cubo. 2016: Palaeohistological Evidence 425 for Ancestral High Metabolic Rate in Archosaurs. Systematic Biology 65:989–996. 426 Lowell, B. B., and B. M. Spiegelman. 2000. Towards a molecular understanding of adaptive 427 thermogenesis. Nature 404:652-660. 428 Maddison, W. P., and D. R. Maddison. 2015: Mesquite: a modular system for evolutionary 429 analysis. p. 430 Marotti, G. 2010: Static and dynamic osteogenesis. Italian journal of anatomy and 431 embryology = Archivio italiano di anatomia ed embriologia 115:123–126. 432 Montes, L., N. Le Roy, M. Perret, V. De Buffrenil, J. Castanet, and J. Cubo. 2007: 433 Relationships between bone growth rate, body mass and resting metabolic rate in 434 growing amniotes: a phylogenetic approach. Biological Journal of the Linnean 435 Society 92:63-76. 436 Mukherjee, D. 2015: New Insights from Bone Microanatomy of the Late Triassic 437 Hyperodapedon (archosauromorpha, Rhynchosauria): Implications for 438 Archosauromorph Growth Strategy. Palaeontology 58:313–339. 439 Nesbitt, S. 2007: The Anatomy of Effigia okeeffeae (Archosauria, Suchia), Theropod-Like

- 440 Convergence, and the Distribution of Related Taxa. Bulletin of the American441 Museum of Natural History 302:1-84.
- Nesbitt, S. J., M. R. Stocker, B. J. Small, and A. Downs. 2009: The osteology and
  relationships of Vancleavea campi (Reptilia: Archosauriformes). Zoological Journal
  of the Linnean Society 157:814–864.
- 445 Nesbitt, S. J., J. J. Flynn, A. C. Pritchard, J. M. Parrish, L. Ranivoharimanana, and A. R.
  446 Wyss. 2015: Postcranial Osteology of *Azendohsaurus Madagaskarensis* (?middle to
  447 Upper Triassic, Isalo Group, Madagascar) and Its Systematic Position Among Stem
- 448 Archosaur Reptiles. Bulletin of the American Museum of Natural History:1–126.
- Nowack, J., S. Giroud, W. Arnold and T. Ruf. 2017. Muscle non-shivering thermogenesis and
  its role in the evolution of endothermy. Frontiers in Physiology 8. Article 889.
- 451 Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2013:
  452 Caper: Comparative Analyses of Phylogenetics and Evolution in R.
- Padian, K., and J. R. Horner. 2002: Typology versus transformation in the origin of birds.
  Trends in Ecology and Evolution 17:120-124.
- Padian, K., J. R. Horner, and A. De Ricqles. 2004: Growth in small dinosaurs and pterosaurs:
  The evolution of archosaurian growth strategies. Journal of Vertebrate Paleontology
  24:555–571.
- Palumbo, C., M. Ferretti, and G. Marotti. 2004: Osteocyte dendrogenesis in static and
  dynamic bone formation: An ultrastructural study. Anatomical Record Part aDiscoveries in Molecular Cellular and Evolutionary Biology 278A:474–480.
- Pritchard, A. C., A. H. Turner, S. J. Nesbitt, R. B. Irmis, and N. D. Smith. 2015: Late Triassic
  tanystropheids (Reptilia, Archosauromorpha) from northern New Mexico (Petrified
  Forest Member, Chinle Formation) and the biogeography, functional morphology,
  and evolution of Tanystropheidae. Journal of Vertebrate Paleontology 35(2),
- 465 e911186.
- Prondvai, E., K. H. W. Stein, A. de Ricqles, and J. Cubo. 2014: Development-based revision
  of bone tissue classification: the importance of semantics for science. Biological
  Journal of the Linnean Society 112:799–816.
- 469 R Core Team. 2016: R: A Language and Environment for Statistical Computing. R
  470 Foundation for Statistical Computing, Vienna, Austria, p.
- 471 Ricqlès, A. de. 1976: On bone histology of fossil and living reptiles, with comments on its
  472 functional and evolutionary significance. Pp.126–150 *in* Morphology and Biology of
  473 Reptiles. Linnean Society of London and Academic Press, London.

474 Ricqlès, A. de, K. Padian, and J. R. Horner. 2003: On the bone histology of some Triassic 475 pseudosuchian archosaurs and related taxa. Annales de Paléontologie 89:67-101. 476 Ricqlès, A. de, K. Padian, J. R. Horner, and H. Francillon-Vieillot. 2000: Palaeohistology of 477 the bones of pterosaurs (Reptilia : Archosauria): anatomy, ontogeny, and 478 biomechanical implications. Zoological Journal of the Linnean Society 129:349-385. 479 Ricqlès, A. de, K. Padian, F. Knoll, and J. R. Horner. 2008: On the origin of high growth rates 480 in archosaurs and their ancient relatives: Complementary histological studies on 481 Triassic archosauriforms and the problem of a "phylogenetic signal", in bone 482 histology. Annales De Paleontologie 94:57-76. 483 Rowland, L. A., N. C. Bal, and M. Periasamy. 2015. The role of skeletal-muscle-based 484 thermogenic mechanisms in vertebrate endothermy. Biological Reviews 90:1279-485 1297. 486 Stein, K., and E. Prondvai. 2014: Rethinking the nature of fibrolamellar bone: an integrative 487 biological revision of sauropod plexiform bone formation. Biological Reviews 488 89:24-47. 489 Thulborn, R. A. 1973: Teeth of Ornithischian dinosaurs from the Upper Jurassic of Portugal. 490 Serviços Geológicos de Portugal, Memoria 22: 89-134. 491 Thulborn, R. A.1974: A new heterodontosaurid dinosaur (Reptilia: Ornithischia) from the 492 Upper Triassic Red Beds of Lesotho. Zoological Journal of the Linnean Society of 493 London 55: 151-175. 494 Tumarkin-Deratzian, A. R. 2007: Fibrolamellar bone in wild adult Alligator mississippiensis. 495 Journal of Herpetology 41:341–345. 496 Veiga, F. H., M. B. Soares, and J. M. Sayao. 2015: Osteohistology of hyperodapedontine 497 rhynchosaurs from the Upper Triassic of Southern Brazil. Acta Palaeontologica 498 Polonica 60:829-836. 499 Weishampel, D. B. 1990: Dinosaurian distribution. In D. B. Weishampel, P. Dodson & H. 500 Osmólska (eds.), The Dinosauria, pp. 63-139. University of California Press, 501 Berkeley. 502 Werning, S., and R. B. Irmis. 2011: Reconstructing growth of the basal archosauromorph 503 Trilophosaurus. Integrative and Comparative Biology 51:E147–E147. 504 Werning, S., and S. J. Nesbitt. 2016: Bone histology and growth in Stenaulorhynchus 505 stockleyi (Archosauromorpha: Rhynchosauria) from the Middle Triassic of the 506 Ruhuhu Basin of Tanzania. Comptes Rendus Palevol 15:163–175. 507

508	Figure captions
509	
510	FIGURE 1. Azendohsaurus laaroussii Dutuit 1972, (Argana Basin, Irohalène Member,
511	Carnian) : 1 & 2: MNHN.F.ALM 586 left humerus (1) and mould of MNHN.F. 435 right
512	humerus (2) in dorsal (a), ventral (b), anterior (c), proximal (e) and distal (f) views; 3 & 4:
513	MNHN.F. ALM 498 and 502, two ends of the same left femur (3) and mould of
514	MNHN.F.ALM 497 left femur (4) in ventral (a), dorsal (b) and posterior (c) views; 5 & 6:
515	MNHN.F. ALM 398 right tibia (5) and MNHN.F. 369 mould of left tibia (6) in medial (a) and
516	lateral (b) views. The arrow-heads show the level of the thin sections. Abbreviations : a.fi,
517	articulation for fibula; cap, capittellum; cc, cnemial crest; dp, deltopectoral crest; ect,
518	ectepicondyle; gr, groove; int, internal trochanter; tro, trochlea.
519	
520	FIGURE 2. Transverse (A, B, E, F) and longitudinal (C, D, G, H) sections of the medial (A-D)
521	and the posterior (E-H) regions of the right tibia MNHN.F ALM369 of Azendohsaurus
522	laaroussii under normal light (A, C, E, G) and cross-polarized light with lambda compensator
523	(B, D, F, H). Periosteum is on the left. Arrows: primary osteons. Arrowheads: erosion cavities
524	lined along its periphery by a coating of endosteal lamellar tissue. Scale bars: 300 $\mu$ m.
525	
526	FIGURE 3. Transverse sections of the left femur MNHN.F ALM497 (A, anterior region; B,
527	posterior region; E, F, postero-dorsal region), and the right humerus MNHN.F ALM435 (C,
528	anterior region; D, posterior region; G, posterior region with higher magnification), and a
529	longitudinal section of the same humerus (H, posterior region) of Azendohsaurus laaroussii
530	under normal light (A-E) and cross-polarized light with lambda compensator (F-H).
531	Periosteum is on the left in A and C, on the right in B and D and in the bottom in G.

532 Endosteum is in the bottom in E, F, H. Arrow: primary osteon. Arrowhead: woven bone.
533 Scale bars: 1 mm in A-D; 300 μm in E-H.

534

FIGURE 4. Resting metabolic rates quantified in a sample of extant tetrapods and inferred in a sample of extinct Archosauromopha using the phylogeny and the vascular density of the humerus. Data for *Azendohsaurus laaroussii* were obtained in this study and data for other taxa as well as the phylogeny were taken from Legendre et al (2016). Resting metabolic rate inferred for *Azendohsaurus, Prolacerta, Garjainia* and *Euparkeria* are in the range of variation of extant mammals, whereas the value inferred for *Postosuchus* is in the range of variation of extant birds.

542

FIGURE 5. Resting metabolic rates quantified in a sample of extant tetrapods and inferred in a
sample of extinct Archosauromopha using the phylogeny only. Data for *Azendohsaurus laaroussii* were obtained in this study and data for other taxa as well as the phylogeny were
taken from Legendre et al (2016). Resting metabolic rate inferred for *Azendohsaurus*, *Proterosuchus* and *Maiasaura* are in the range of variation of extant mammals, values
inferred for *Calyptosuchus*, *Lesothosaurus*, *Allosaurus* and *Troodon* are in the range of
variation of extant birds, whereas value inferred for *Rutiodon* is intermediate.

550

FIGURE 6. Resting metabolic rates quantified in a sample of extant tetrapods and inferred in a sample of extinct Archosauromopha using the phylogeny and the vascular density of the tibia. Data for *Azendohsaurus laaroussii* were obtained in this study and data for other taxa as well as the phylogeny were taken from Legendre et al (2016). Resting metabolic rate inferred for *Azendohsaurus, Prolacerta, Proterosuchus, Garjainia, Lesothosaurus, Maiasaura* and *Thecodontosaurus* are in the range of variation of extant mammals, the value inferred for

*Allosaurus* is in the range of variation of extant birds, whereas value inferred for *Lourinhanosaurus* is intermediate.

559

560 FIGURE 7. Optimization using parsimony of the presence of fibrolamellar bone in the 561 stylopodial and zeugopodial bones of the Archosauromorpha analyzed to date (data 562 summarized in Table 1). The topology is based on Nesbitt et al (2009) for Archosauriformes, 563 and on Nesbitt et al (2015) for non-archosauriform Archosauromorpha with the exception of 564 the phylogenetic position of Aenigmastropheus parringtoni that has been taken from Ezcurra 565 et al (2014). The minimum age of taxa has been taken from the Paleobiology Database. Black 566 branches indicate presence of fibrolamellar bone, white branches indicate absence and black 567 and white branches indicate uncertain character state. 568

FIGURE 8. Optimization using parsimony of the presence of endothermy in a sample of
tetrapods. The presence of endothermy was inferred in extinct tetrapods using resting
metabolic rates estimated through phylogenetic eigenvector maps. Red corresponds to the
presence of endothermy and blue to the presence of ectothermy. The phylogeny has been
taken from Legendre et al (2016).