

Research



**Cite this article:** Clarac F, Scheyer TM, Desojo JB, Cerda IA, Sanchez S. 2020 The evolution of dermal shield vascularization in Testudinata and Pseudosuchia: phylogenetic constraints versus ecophysiological adaptations. *Phil. Trans. R. Soc. B* **375**: 20190132. <http://dx.doi.org/10.1098/rstb.2019.0132>

Accepted: 1 October 2019

One contribution of 15 to a theme issue 'Vertebrate palaeophysiology'.

**Subject Areas:**

palaeontology, physiology

**Keywords:**

acidosis buffering, cutaneous respiration, heat transfer, historical constraints

**Author for correspondence:**

François Clarac

e-mail: [francois.clarac@ebc.uu.se](mailto:francois.clarac@ebc.uu.se)

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.4782582>.

# The evolution of dermal shield vascularization in Testudinata and Pseudosuchia: phylogenetic constraints versus ecophysiological adaptations

François Clarac<sup>1</sup>, Torsten M. Scheyer<sup>2</sup>, Julia B. Desojo<sup>3</sup>, Ignacio A. Cerda<sup>4</sup> and Sophie Sanchez<sup>1,5</sup>

<sup>1</sup>Department of Organismal Biology, Subdepartment of Evolution and Development, Uppsala University, Norbyvägen 18A, 752 36 Uppsala, Sweden

<sup>2</sup>Paleontological Institute and Museum, University of Zurich, Karl Schmid-Strasse 4, 8006 Zurich, Switzerland

<sup>3</sup>CONICET, División Paleontología Vertebrados, Museo de La Plata, Paseo del Bosque s/n°, B1900FWA La Plata, Argentina

<sup>4</sup>CONICET, Argentina y Instituto de Investigación en Paleobiología y Geología, Universidad Nacional de Río Negro, Museo Carlos Ameghino, Belgrano 1700, Paraje Pichi Ruca (predio Marabunta), 8300 Cipolletti, Río Negro, Argentina

<sup>5</sup>European Synchrotron Radiation Facility, 71 Avenue des Martyrs, CS-40220, 38043 Grenoble Cedex, France

FC, 0000-0001-8247-8507; TMS, 0000-0002-6301-8983; JBD, 0000-0002-2739-3276

Studies on living turtles have demonstrated that shells are involved in the resistance to hypoxia during apnea via bone acidosis buffering; a process which is complemented with cutaneous respiration, transpharyngeal and cloacal gas exchanges in the soft-shell turtles. Bone acidosis buffering during apnea has also been identified in crocodylian osteoderms, which are also known to employ heat transfer when basking. Although diverse, many of these functions rely on one common trait: the vascularization of the dermal shield. Here, we test whether the above ecophysiological functions played an adaptive role in the evolutionary transitions between land and aquatic environments in both Pseudosuchia and Testudinata. To do so, we measured the bone porosity as a proxy for vascular density in a set of dermal plates before performing phylogenetic comparative analyses. For both lineages, the dermal plate porosity obviously varies depending on the animal lifestyle, but these variations prove to be highly driven by phylogenetic relationships. We argue that the complexity of multi-functional roles of the post-cranial dermal skeleton in both Pseudosuchia and Testudinata probably is the reason for a lack of obvious physiological signal, and we discuss the role of the dermal shield vascularization in the evolution of these groups.

This article is part of the theme issue 'Vertebrate palaeophysiology'.

## 1. Introduction

The vertebrate post-cranial dermal skeleton is composed of bony scutes which ossify within the dermis [1–4]. The presence of these bony elements varies taxonomically, and the resulting shield morphology results from both the shape and the relative position of the dermal plates. These bones can be juxtaposed or articulated as observed in stem archosaurs [5,6], in pseudosuchians [7–9] and some squamates [10]; they can also be fused as in turtles [11,12] and xenarthrans [13,14] or be isolated as in some ornithischian and sauropod dinosaurs [15–18].

Continuous shields of osteoderms (e.g. Aetosauria, Xenarthra) or bony scutes (e.g. Testudinata) have mostly been considered for their protective

aspects against predators [19–23]. However, experimental investigations on turtles have shown that their dermal shield would also have ecophysiological functions. Indeed, the bone tissues composing the shield would be able to buffer the acidosis which is caused by blood pH decrease after both the blood CO<sub>2</sub> pressure has increased and the lactic acid (lactate) has been produced via fermentation during prolonged apnea [24–28]. Bone acidosis buffering consists of supplying mineral elements such as (1) bicarbonates that can bind to the free protons which are dissolved in the blood plasma (due to respiratory acidosis) and (2) calcium that can complex with the lactate and thus inhibits its acidity (in answer to metabolic acidosis) [29].

Such a physiological process has also been identified in the osteoderms of crocodylians [30] which are known to be semi-aquatic animals, derived from terrestrial ancestors [5,8,31]. In addition, crocodylian osteoderms are also involved in heat transfer with the environment during emerged and semi-emerged basking periods [17,23,32] via the enclosed vessels of which blood flow is controlled by cardiac activity and vasomotion, thus regulating the distribution of heat to the vital organs [33–35]. Even though no specific studies have yet been performed on this aspect in the testudinans, their dermal shield must also be involved in heat transfer [36], since it covers the majority of the body surface while enclosing peripheral blood vessels within bone cavities [37,38].

We quantified the post-cranial dermal bone vascular area as a proxy to assess the number and size of the blood vessels that are both enclosed within bony cavities and closely in contact with the apical bone surface when a superficial ornamentation is present. Indeed, the sculptural elements that compose the bone ornamentation are known to provide vascular openings contributing to the dermal plate global vascularization by conducting blood vessels to the overlying soft dermis [39] as observed in pseudosuchians [40], tryonichids [37] and helochelydrids [41,42]. We then analysed the data with phylogenetic comparative methods (in Pseudosuchia and Testudinata) to reveal whether the post-cranial dermal bone vessel proliferation is: (1) influenced by the phylogeny and (2) correlated with lifestyle transitions unrelated to the phylogenetic relationships.

## 2. Material and methods

### (a) Sampling strategy

We studied 31 cross sections of dermal bones coming from different parts of the shell of both extant and extinct testudinatan species (dry bones and well-preserved fossils) from museum collections or published articles (table 1). The cross sections are transverse and pass by the centre of the sampled bones. The taxonomic affiliation and lifestyle attributes of the fossil forms could be identified unambiguously based on anatomical features. We classified the specimens into three categories depending on their lifestyle: terrestrial, freshwater and marine. When there was no ambiguity regarding the taphonomy (post-mortem transportation), the nature of the sediment was also used as a clue to infer their living environment (e.g. marine versus fresh water; electronic supplementary material, S1).

We sampled 32 cross sections of pseudosuchian osteoderms. The taxa were categorized based on two different lifestyles: terrestrial and semi-aquatic (table 1*b*). We decided not to distinguish the marine animals from the freshwater semi-aquatic forms as they have a similar amphibious ambush predator

lifestyle [43,44]. The pelagic marine forms from the Jurassic (the metryorhynchids) [45,46] had completely lost the osteoderm shield and are therefore not suitable for this study (this aspect is discussed below). Extinct pseudosuchians are categorized based on the orientation of their skull neurosensory organs and limb postures as reviewed in a previous article [47].

### (b) Data acquisition

We produced photographs of each cross section before segmenting and rendering them binary in black and white with Adobe Photoshop CC 2015 (electronic supplementary material, S2 and S3), so that the bone matrix appears in black and the empty cavities appear in white. The ornamentation of dermal bones is often made of crests separated by pits, which systematically host large clusters of blood vessels from the soft dermis. These blood vessels are intimately associated with the dermal bone vascularization as they pierce its surface [39] and connect vessel clusters in the internal cavities (spongiosa) via these vascular openings at the surface of the bone [37,39]. As pseudosuchians [48], tryonichids [37] and helochelydrids [41] possess ornamented post-cranial dermal bones whose dermis vascularization is directly related to the dermal bone internal vascularization. Although these pits are large, they obviously do not over-estimate the porosity of the bone as they are fully filled in by a great number of blood vessels [39]. For that reason, we decided to include the space of these pits into our measurements. To do so, we connected the top of the crests of the ornamentation with a virtual black line of one pixel in the most parsimonious way to embed the surface of the pits into the vascular measurements (electronic supplementary material, S3). We exported the pictures in TIFF format (electronic supplementary material, S2) and analysed them with Bone profiler [49] in order to measure the area occupied by the empty spaces proportionally to the entire area covered by bone and vascular spaces (as detailed in electronic supplementary material, S3).

### (c) Phylogenetic comparative analyses

For both Pseudosuchia and Testudinata, time-scaled phylogenetic relationships of the sampled specimens were reconstructed in Mesquite [50] by relying on published references [5,41,42,51–63] (figure 1). We then traced the evolution of the post-cranial dermal bone vascular area using the least-squared parsimony to calculate the ancestral states for each clade. In order to test the influence of the phylogeny (i.e. the historical constraint) on the vascular area of the post-cranial dermal skeleton, we exported the trees in NEX format into R [64] and we further computed both Pagel's  $\lambda$  [65] and Blomberg's  $K$  [66] after uploading the 'caper' package [67,68]. Finally, we tested the correlation between the post-cranial dermal bone vascular area and the corresponding lifestyle for each taxon using a phylogenetic ANOVA. This is a statistical test that reveals a correlation between quantitative and qualitative data while retracting the influence of the phylogeny, which is quantified either by  $K$  or  $\lambda$ —we decided to consider both options [68] (table 2).

## 3. Results

### (a) Evolution of vascular density in the osteoderms of Pseudosuchia

We first tested whether the variability of the vascular area—proportionally to the dermal bone area—was inherited from the phylogenetic relationships of the studied species. Phylogenetic tests showed that the vascular area in the osteoderms of the pseudosuchians is significantly influenced by the phylogeny, as both the Blomberg's  $K$  and the Pagel's  $\lambda$  tests are

**Table 1.** Description of the sample, (a) Testudinata and (b) Pseudouchia. TMM: Texas Memorial Museum (Austin USA); FMNH: Field Museum of Natural History (Chicago, USA); MCNA: Museo de Ciencias Naturales de Alava (Vitoria-Gasteiz, Spain); YPM: Yale Peabody Museum (New Haven, USA); UPUAM: Unidad de Paleontología, Universidad Autónoma de Madrid (Spain); WU-SILS-RH: Waseda University (Tokyo, Japan); NSMT: National Museum for Nature and Science of Tokyo (Japan); ZIN PH: Zoological Institute (Russian Academy of Sciences, Saint Petersburg); FPDM: Fukui Prefectural Dinosaur Museum (Katsuyama City, Fukui Prefecture, Japan); UA: Université d'Antananarivo (Madagascar); SMNS: Smithsonian Institution; BSPG: Bayerische Staatssammlung für Paläontologie und Geologie, München, Germany; PFFO: Petrified Forest National Park, USA; ISI: Indian Statistical Institute (Calcutta, India); UCMP: University of California, Museum of Paleontology (Berkeley, USA); MNHN: Muséum National d'Histoire Naturelle; IPB: Instituto de Paleontología (Bonn, Germany); NMS: Naturmuseum Solothurn, Switzerland; MCL: Musée des confluences (Lyon, France); PVL: Colección de Paleovertebrados del Instituto Miguel Lillo (Tucumán, Argentina); n.a.: non-attributed.

	porosity	lifestyle	region	ornamentation	age	collection number
(a) Testudinata						
<i>Hesperotestudo</i> sp.	0.07	terrestrial	flat osteoderm	no	Pleistocene	TMM 30967-1010.1
<i>Hesperotestudo</i> sp.	0.21	terrestrial	spiked osteoderm	no	Pleistocene	TMM 30967-1010.2
<i>Terapene carolina tringuis</i>	0.40	terrestrial	neural	no	extant	FMNH 211806
<i>Terapene carolina tringuis</i>	0.22	terrestrial	costal (right)	no	extant	FMNH 211806
<i>Dorkata vasconica</i>	0.21	freshwater	costal	no	Barremian	MCNA 14366
<i>Dorkata vasconica</i>	0.24	freshwater	neural	no	Barremian	MCNA 14372
<i>Podocnemis erythrocephala</i>	0.15	freshwater	sample costal	no	extant	YPM 11853
<i>Solenys</i> sp.	0.07	terrestrial	costal fragment	yes	Maastrichtian	UPUAM-14001
<i>Solenys vermicalata</i>	0.14	terrestrial	costal fragment	yes	Maastrichtian	MCNA 15047
<i>Solenys vermicalata</i>	0.16	terrestrial	shell fragment	yes	Maastrichtian	MCNA 15046
<i>Carettochelys insculpta</i>	0.10	freshwater	costal (right 7th)	yes	extant	WU-SILS RH1044
<i>Pelodiscus sinensis</i>	0.11	freshwater	costal	yes	extant	NSMT-H 6600
Trionychidae indet.	0.11	freshwater	costal	yes	Aptian-Albian	ZIN PH 102
Trionychidae indet.	0.10	freshwater	costal	yes	early Cenomanian	ZIN PH 122
Trionychidae indet.	0.09	freshwater	costal	yes	Barremian–Aptian	FPDM V0127
<i>Bothremys barberi</i>	0.31	marine	costal	no	Campanian	FM P27406 (FMNH)
<i>Bothremys barberi</i>	0.32	marine	costal	no	Campanian	FM P27406 (FMNH)
<i>Bothremys barberi</i>	0.30	marine	neural	no	Campanian	FM P27406 (FMNH)
<i>Caretta caretta</i>	0.39	marine	costal	no	extant	FMNH 98963
<i>Caretta caretta</i>	0.33	marine	hyoplastron	no	extant	FMNH 98963
<i>Archelon ischyros</i>	0.26	marine	shell fragment	no	Late Cretaceous	YPM 1783
<i>Plesiochelys</i> sp.	0.18	marine	neural	no	Kimmeridgian	NMS 8730
<i>Taphrosphys sulcatus</i>	0.34	marine	costal	no	Maastrichtian	YPM 40288
<i>Taphrosphys sulcatus</i>	0.35	marine	neural	no	Maastrichtian	YPM 40288

(Continued.)

**Table 1.** (Continued.)

	porosity	lifestyle	region	ornamentation	age	collection number
<i>Genochelys stenoporus</i>	0.36	marine	neural	no	Campanian	FM PR 442
<i>Geochelone elegans</i>	0.14	terrestrial	costal	no	extant	IPB 561-C
<i>Geochelone elegans</i>	0.10	terrestrial	costal	no	extant	IPB 561-C
<i>Geochelone elegans</i>	0.07	terrestrial	neural	no	extant	IPB 561-C
<i>Hesperotestudo crassiscuta</i>	0.19	terrestrial	neural	no	Pleistocene	ROM 5540
<i>Hesperotestudo crassiscuta</i>	0.30	terrestrial	plaston fragment	no	Pleistocene	ROM 5541
<i>Hesperotestudo crassiscuta</i>	0.28	terrestrial	shell fragment	no	Pleistocene	ROM 5542
<i>(b) Pseudosuchia</i>						
<i>Araripesuchus tsangatsangana</i>	0.05	terrestrial	n.a.	yes	Late Cretaceous	UA 9966
<i>Batrachotomus kupferzellensis</i>	0.01	terrestrial	paramedian pre-caudal	yes	Late Ladinian	SMNS 80317
<i>Prestosuchus chiniquensis</i>	0.05	terrestrial	sacral paramedian	yes	Late Ladinian/Early Carnian	BSPG ASXXV7
<i>'Prestosuchus' loritatus</i>	0.04	terrestrial	pre-caudal paramedian	yes	Late Ladinian/Early Carnian	BSPG ASXXV46d
<i>Rauisuchus tiradentes</i>	0.16	terrestrial	pre-caudal paramedian	yes	Late Carnian/Early Norian	BSPG ASXXV121b
<i>Revueltosaurus</i> sp.	0.04	terrestrial	paramedian	yes	Norian	PEFO 33787
<i>Tikisuchus romeri</i>	0.14	terrestrial	pre-caudal paramedian	yes	Carnian	ISI R 305/ 1
<i>Simosuchus clarki</i>	0.1	terrestrial	n.a.	no	Late Cretaceous	UA 9965
<i>Simosuchus clarki</i>	0.07	terrestrial	n.a.	no	Late Cretaceous	UA 9965
<i>Yarasuchus deccanensis</i> (Avenmetatarsalia)	0.10	terrestrial	pre-caudal paramedian	yes	Anisian	ISI R 334
<i>Alligator mississippiensis</i>	0.15	semi-aquatic	n.a.	yes	extant	SMNS 10481b
<i>Allognathosuchus wartheni</i>	0.13	semi-aquatic	n.a.	yes	Wasatchian	UCMP 113731
<i>Crocodylus niloticus</i>	0.13	semi-aquatic	dorsal	yes	extant	MNHN-AC- 1920.90, PC
<i>Diplocynodon</i> sp.	0.23	semi-aquatic	n.a.	yes	Eocene–Miocene	IPB R144 / 1
<i>Diplocynodon remensis</i>	0.24	semi-aquatic	nuchal	yes	Thanetian	MNHN. F. No number
<i>Machimosaurus hugii</i>	0.22	semi-aquatic	n.a.	yes	Late Jurassic	SMNS 81608
<i>Sarcosuchus imperator</i>	0.24	semi-aquatic	n.a.	yes	Upper Cretaceous	MNHN.F. GDF 380
<i>Steneosaurus</i> sp.	0.07	semi-aquatic	n.a.	yes	Late Jurassic	NMS 752
<i>Steneosaurus jugleri</i>	0.12	semi-aquatic	n.a.	yes	Late Jurassic	NMS 7152
<i>Paleosuchus trigonatus</i>	0.29	semi-aquatic	n.a.	yes	extant	MCL 420003939
<i>Protocaiman peligrensis</i>	0.13	semi-aquatic	n.a.	yes	Danian	UCMP 131693
<i>Telesaurus cadomensis</i>	0.22	semi-aquatic	n.a.	yes	Bathonian	MNHN Histo 1960

(Continued.)

Table 1. (Continued.)

	porosity	lifestyle	region	ornamentation	age	collection number
<i>Goniopholis</i> sp.	0.2	semi-aquatic	n.a.	yes	Oxfordian-Berriasian	MNHN Histo 1727
<i>Borealosuchus</i> sp.	0.17	semi-aquatic	n.a.	yes	Campanian-Ypresian	UCMP 133903
<i>Mahajangasuchus insignis</i>	0.17	semi-aquatic	n.a.	yes	Campanian	UA 9962
<i>Brachychampsa montana</i>	0.22	semi-aquatic	n.a.	yes	Maastrichtian	UCMP 133901
<i>Osteolaemus tetraspis</i>	0.12	semi-aquatic	n.a.	yes	extant	MNHN-AC-1991.4488
<i>Paleosuchus palpebrosus</i>	0.11	semi-aquatic	n.a.	yes	extant	MNHN.AC-1909.204
<i>Caiman crocodilus</i>	0.27	semi-aquatic	nuchal	yes	extant	Sorbonne Université - NA
<i>Borealosuchus wilsoni</i>	0.18	semi-aquatic	n.a.	yes	Ypresian	UCMP 131696
<i>Paratypothorax</i> sp.	0.25	terrestrial	paramedian	yes	Late Triassic	PEFO 5030
<i>Aetosaurus scagliai</i>	0.05	terrestrial	paramedian	yes	Late Triassic	PVL 2073

significant ( $p$ -values of less than 0.05; table 2). The fact that the  $\lambda$ -value of 0.99 is very close to the maximum ( $\lambda = 1$ ) means that the vascular area covaries in direct proportion with the species' shared evolutionary history through a Brownian motion on the phylogeny [65,69]. The  $K$ -test shows a significant  $p$ -value (table 2) and thus emphasizes the tendency of closely related species to share a similar osteoderm vascular area. Nevertheless, as the  $K$ -value itself clearly remains below 1, the phylogeny must not be the only component that explains the resulting evolutionary pattern of vascular area variability within Pseudosuchia [66,70].

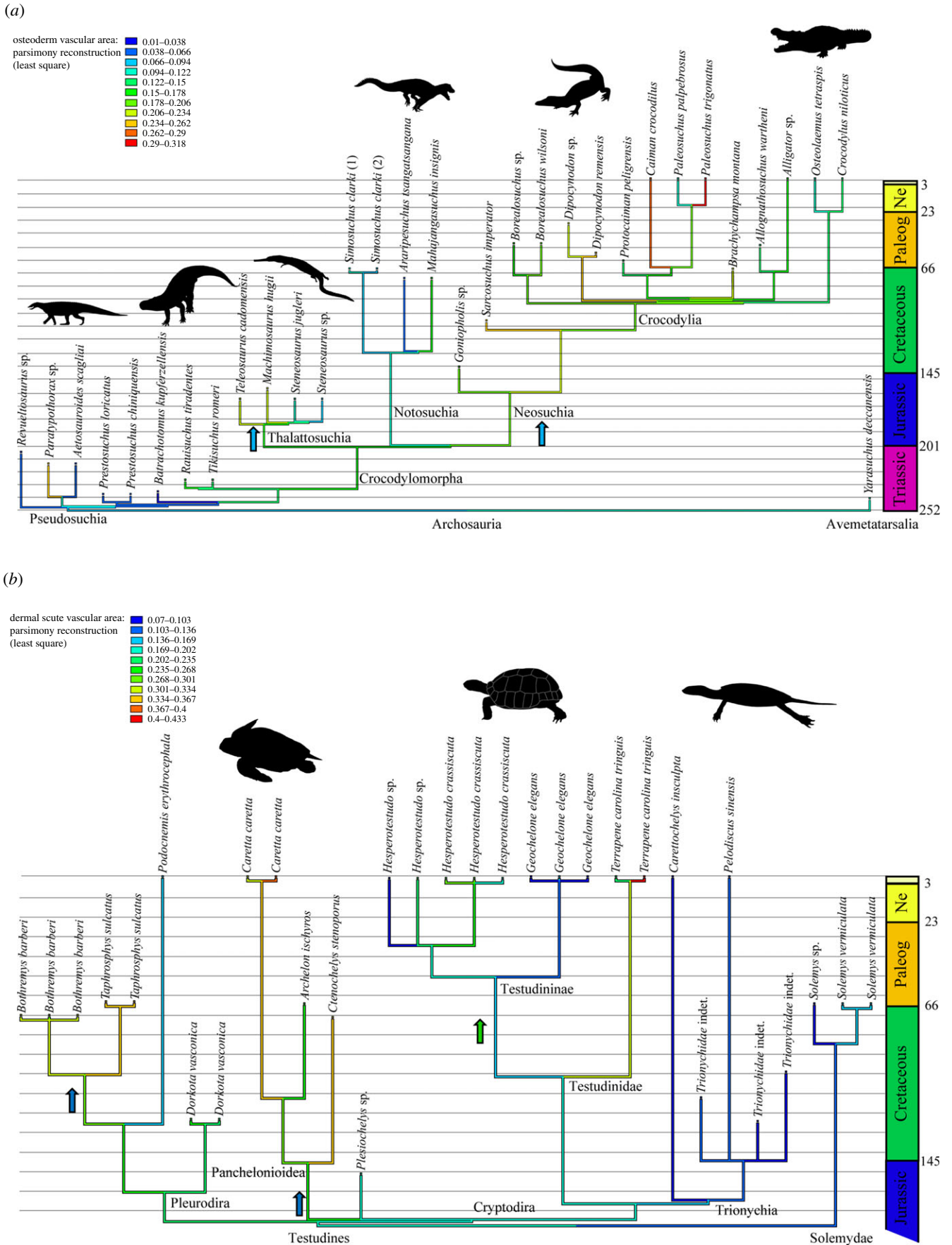
A first glimpse of the vascular density distribution would suggest that the lifestyle of the studied taxa could partly explain this variability. Boxplots were calculated to illustrate the distribution of the vascular cross-sectional area in the osteoderms of semi-aquatic and terrestrial pseudosuchians. Semi-aquatic forms exhibit a larger vascular area (proportionally to their dermal bone area) than terrestrial pseudosuchians (while showing a more pronounced apical ornamentation; table 2 and figure 2a; electronic supplementary material, S2). Indeed, although both datasets show an equal standard deviation ( $s.d._{\text{terrestrial}} = 0.07$ ;  $s.d._{\text{semi-aquatic}} = 0.06$ ), the mean value of the osteoderm vascular area is equal to 0.09 in the terrestrial forms, whereas it is twice as high in the semi-aquatic pseudosuchians (mean = 0.18).

In order to test whether this distribution could partly explain the variability of vascular density in Pseudosuchia, we performed a phylogenetic ANOVA that takes into account the phylogenetic signal. The results present no significant correlation between the osteoderm vascular area and the pseudosuchian lifestyle (table 2).

## (b) Evolution of vascular density in the shell of Testudinata

We evaluated the vascular areas of turtle shell in a phylogenetic context. They show that the dermal shell bone vascular density is significantly influenced by the phylogeny since both the Blomberg's  $K$  and the Pagel's  $\lambda$  tests are significant ( $p$ -values of less than 0.05; table 2). However, both the  $\lambda$  and the  $K$  show lower values ( $\lambda = 0.83$ ;  $K = 0.09$ ) than for the pseudosuchian osteoderm vascular area ( $\lambda = 0.99$ ;  $K = 0.41$ ). We deduce that the phylogeny explains to a lesser extent the variability of shell vascular density in testudinata than in pseudosuchians (figure 1b).

As presented with boxplots (figure 2b) and in table 2, the testudinatan dermal bone vascular area seems to score higher values than in the pseudosuchian osteoderms although the standard deviation is equal, with the exception of terrestrial testudinatan, whose vascular density varies in a larger spectrum around a mean value of 0.18 ( $s.d. = 0.10$ ). Unlike semi-aquatic pseudosuchians (which are most often found in freshwater environments), the freshwater turtle dermal bones show a lower vascular density (mean = 0.14) than terrestrial forms (mean = 0.18). The presence of ornamentation in both Trionychia and in Helochelydridae does not seem to influence the global turtle dermal bone vascularity as these bones still score a low vascular area (all values are lower than 0.16; table 2; see Material and methods). As a third category, the marine turtles, which are fully aquatic (with a very brief terrestrial excursion on land for females to lay eggs), present a high average value of shell vascular area (mean = 0.31) with a



**Figure 1.** (a) Reconstruction of the osteoderm vascular area on the phylogeny of Pseudosuchia using a least-square reconstruction. The phylogeny was reconstructed and time-scaled according to published references [5,51–58]. The light blue arrows represent the transitions from a terrestrial to a semi-aquatic lifestyle. (b) Reconstruction of the dermal scute vascular area on the phylogeny of Testudinata using a least-square reconstruction. The phylogeny was reconstructed and time-scaled according to published references [41,42,59–63]. The dark blue arrows represent the transitions from a freshwater to a marine lifestyle. The green arrow represents a transition from a freshwater to a terrestrial lifestyle. Regarding the dermal plates, which belong to the same species or specimen, we decided to separate them from a 1 Myr-old last hypothetical common ancestor: a systematic error which is below 1% when considering the total branch length within the phylogeny timescale (180 Ma for the testudinans and 250 Ma for the pseudosuchians). Paleog, Paleogene, Ne, Neogene.

**Table 2.** Statistical results. s.d.: standard deviation; max: maximum value; min: minimum value.

Pseudosuchia			Testudinata		
phylogenetic analyses	result/value	p-value	phylogenetic analyses	result/value	p-value
<i>K</i>	significant/ <i>K</i> = 0.41	0.004	<i>K</i>	significant/ <i>K</i> = 0.09	0.003
ANOVA( <i>K</i> )	non-significant	0.21	ANOVA( <i>K</i> )	non-significant	0.6658
$\lambda$	significant/ $\lambda$ = 0.99	$1.97 \times 10^{-5}$	$\lambda$	significant/ $\lambda$ = 0.83	$2.48 \times 10^{-5}$
ANOVA( $\lambda$ )	non-significant	0.21	ANOVA( $\lambda$ )	non-significant	0.6658
statistical results			statistical results		
lifestyle	terrestrial	semi-aquatic	terrestrial	freshwater	marine
mean porosity	0.09	0.18	0.18	0.14	0.31
median	0.06	0.19	0.16	0.11	0.33
s.d.	0.07	0.06	0.10	0.06	0.06
min	0.01	0.07	0.07	0.09	0.18
max	0.25	0.29	0.40	0.24	0.39

standard deviation similar to that of the freshwater turtles (figure 2*b* and table 2).

A phylogenetic ANOVA was performed and shows that the vascular area in the testudinatan post-cranial dermal bones was not significantly correlated with their lifestyle (table 2), despite these discrete boxplot distributions.

## 4. Discussion

### (a) Pseudosuchian osteoderm vascularization: historical constraints versus ecophysiological adaptations

Our results show that the variability of the osteoderm vascularization correlates with the phylogenetic relationships within Pseudosuchia. Although the lifestyle seems to partly explain the rest of the correlation factor according to the global distribution of the data, our phylogenetic ANOVA revealed no significant correlation between osteoderm vascular variability and lifestyle. The high osteoderm vascularity in the semi-aquatic forms was therefore likely the result of a historical constraint (as evidenced by the significant values of  $\lambda$  and *K*) rather than an ecological adaptation based on natural selection. Nevertheless, some recent studies on living species have shown that the bone cavities in the crocodylian osteoderms reveal an enclosed vascular proliferation [39], which is involved in acidosis buffering during prolonged apnea [30], as well as in heat transfer during emerged and semi-emerged basking periods [23]. Therefore, we cannot refute the existence of such physiological mechanisms in the extinct crocodylomorphs who shared the same semi-aquatic ambush predator behaviour as the extant crocodylians: the extinct neosuchians (e.g. *Sarcosuchus imperator*, *Goniopholis* sp.) [31,71,72] and the teleosaurids [45,55,73].

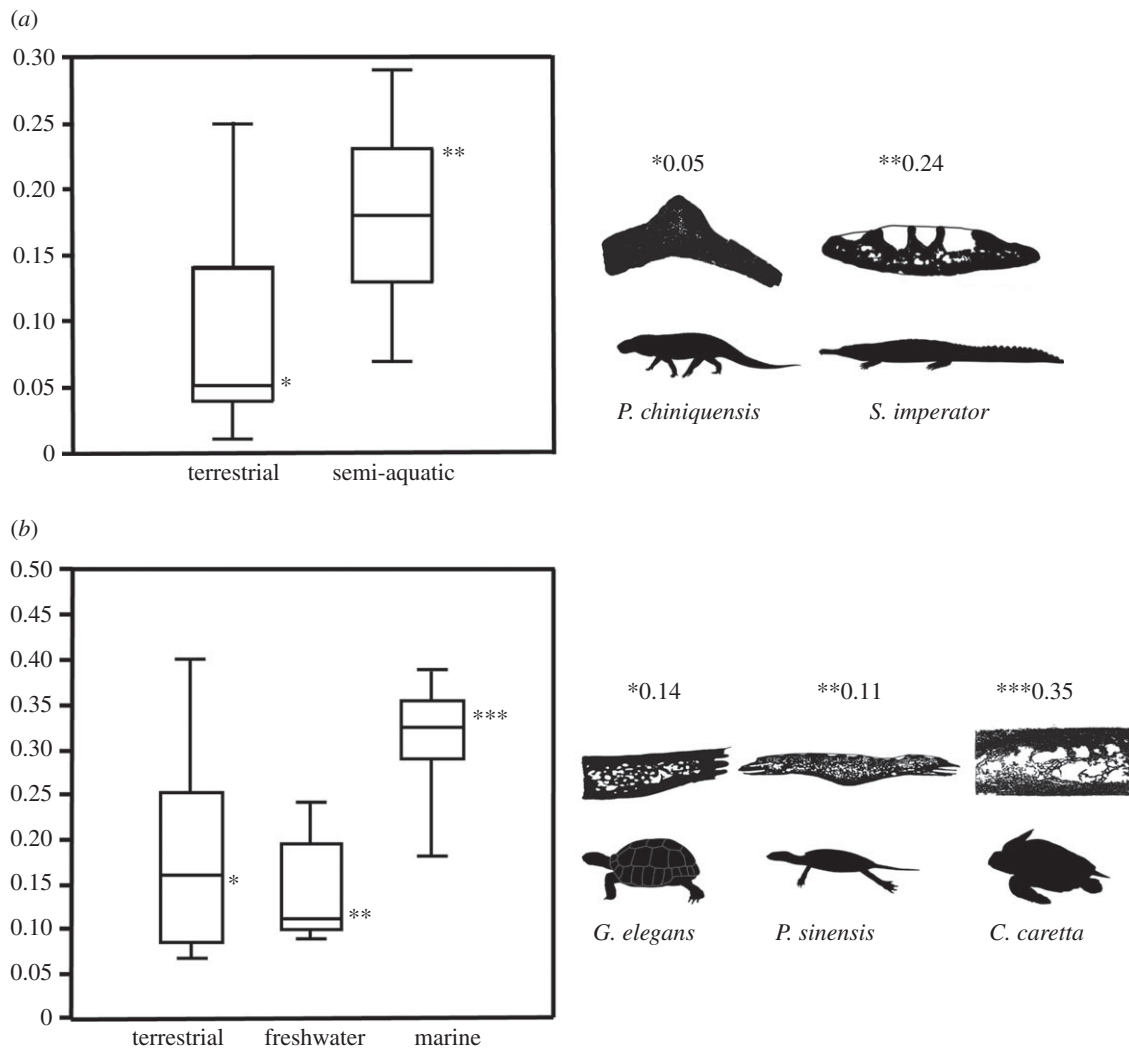
Regarding the thalattosuchians that adopted a pelagic lifestyle involving long-term apneas (Metriorhynchidae; [44–46]), the loss of the dermal shield must have negatively impacted their performance in bone acidosis buffering. Nevertheless, other pathways can buffer acidosis via the involvement of soft tissues [74]. Such mechanisms have already been observed in marine birds and mammals [75–77]. Contrary to extant

crocodylians, marine birds and mammals are very active swimmers. Their lack of oxygen due to apnea essentially affects their appendicular musculature. To compensate for the acidity increase, limb muscles synthesize a protein (carnosine) [78–80] which complexes with protons and thus buffers the intracellular acidosis in muscle tissues where free oxygen concentration is the lowest. As the fossil forms—metriorhynchids—probably were active sea predators, as evidenced by the presence of a tail fluke and swimming paddles [45,46], we can assume that metabolic acidosis buffering could have involved the muscular system as in extant marine birds and mammals. However, the reasons for the metriorhynchids to have lost their osteoderm shield remain unknown. This loss could reflect a complex conjuncture involving both phylogenetic and structural constraints influencing the development of the dorsal shield in disregard of its physiological implication(s) (i.e. weight loss, flexibility along the anteroposterior axis, etc. [45,46,81]).

### (b) Testudinatan shell vascularization: historical constraints versus ecophysiological adaptations

Likewise, our results show that the testudinatan shell vascular density is essentially constrained by the phylogeny despite the noticeable differences in the mean values of vascular area between taxa belonging to different lifestyle categories (table 2 and figure 2*b*).

Higher porosity is encountered in the marine forms. It probably provides a dense vascular system, which facilitates long-term apnea via bone acidosis buffering since this function is essential to sea turtles, of which only the females emerge on land for nesting [82]. Most of their feeding habits rely on a vegetarian or omnivorous diet from the sea bottom [83]. Density reduction due to the lightening of the shell bone perforated by a large number of vascular canals obviously increases their buoyancy and intensifies their effort to dive and remain at the bottom of the sea. Because the control of buoyancy is moderated by the lungs [84,85], we strongly suspect that the porosity of the shell could be better explained as the result of physiological functions



**Figure 2.** (a) Boxplot of the osteoderm vascular area in the pseudosuchians. (b) Boxplot of the dermal scute vascular area in the testudinans. The four quartiles represent the dispersion of the values for each lifestyle.

such as bone acidosis buffering than in relation to biomechanics.

Unlike the marine forms, the freshwater turtles do not exhibit a high shell bone porosity although they are known to perform bone acidosis buffering during prolonged apnea and while hibernating in freezing and/or anoxic conditions [24–30,86]. Some freshwater species such as the trionychids seem to have developed a different strategy to withstand long duration apneas. Indeed, in comparison with the other freshwater testudinans, the trionychids are known to have a lower performance in bone acidosis buffering as they are less tolerant to anoxia [87]. Instead, they exchange blood gases with those dissolved in the surrounding water using pharyngeal, cloacal and cutaneous respiration [88]. Although gas exchanges are not possible through scales of keratin in sauropsids (including crocodylians and testudinans), this mechanism is rendered possible in trionychids by the secondary loss of their superficial keratin layer [63]. It is worth mentioning that the use of cutaneous respiration in *Trionychia* correlates with a rare expression of shell apical ornamentation in testudines. As illustrated in previous studies [37], the pits which compose the trionychid shell sculpture always house one or several vascular openings which provide a proliferation of superficial vessels as in crocodylian ornamented osteoderms [39]. This configuration provides a large blood-vessel network for gas exchanges in cutaneous respiration [89].

Even if heat exchange with the environment is vital for testudinans [90], which are ectotherms, this function does not seem to correlate with the evolutionary pattern of shield vascularization (considering the vascular area as a proxy). Indeed, both freshwater and terrestrial turtle dermal bones globally show a lower vascular density than marine forms, although temperature variations in the sea are much narrower than on land or in freshwater environments.

In conclusion, the dermal shield of the testudinans seems to play multiple physiological roles which differently concern: (1) marine turtles (acidosis buffering during prolonged apnea); (2) freshwater turtles (cutaneous respiration and/or acidosis buffering in response to prolonged apnea or hibernation in anoxic or freezing conditions, heat transfer when basking); and (3) terrestrial tortoises (heat transfer). Therefore, it seems unlikely that any resulting combination of these functions represents the primary determinant of morphology once we have considered the influence of the phylogenetic relationships (historical constraints).

### (c) Evolutionary trends in Pseudosuchia and Testudinata

Pseudosuchians and testudinans have been repeatedly defined as sister taxa according to several phylogenetic reconstructions [91–97]. Among amniotes, these two groups are the main ones to have developed a large post-cranial dermal



skeleton which is known to be used in both acidosis buffering and heat transfer. This pattern leads us to wonder if this ability of the post-cranial dermal bones to perform ecophysiological functions results from a phylogenetic heritage or consists of a functional analogy. The vascular density in the post-cranial dermal skeleton increased when pseudosuchians transitioned to a semi-aquatic lifestyle in the Early Jurassic and when turtles transitioned to a marine lifestyle (within Pleurodira during the Cretaceous, within Cryptodira during the Jurassic and maybe in some early testudinatan species such as *Eileanchelys waldmani* and *Heckerochelys romani* for which the assumed marine lifestyle is still debated [38,98]; figures 1 and 2). All these transitions probably induced bone acidosis buffering to balance prolonged apnea, which directly depends on the bone vascularization inside the shell cavities [29].

Even though the vascularization in post-cranial dermal bones must also be involved in heat transfer due to its peripheral location on the body [20,35], testudinatan and pseudosuchians nevertheless have evolved through very different thermal metabolism patterns. Indeed, the pseudosuchians derive from an endothermic ancestor [99–103] whereas ectothermy is a plesiomorphic condition in Testudinata [38,104]. The increase in dermal bone vascularization relates to heat transfer in the semi-aquatic crocodylomorphs [23,39,47] but this process does not explain the observed pattern in the testudinatan shell as the terrestrial forms score lower relative vascular area although they are the most exposed to external thermal variations.

The functional role(s) of bone ornamentation may also differ between the turtles and the crocodylians. Indeed, even though the vascular openings within the ornamental pits must play a role in cutaneous respiration in soft-shell turtles, this function is not possible in the crocodylians since their entire body is covered by a layer of keratine [31]. The function(s) of bone ornamentation in Crocodylomorpha must instead concern acidosis buffering and heat transfer via the housing of vessel clusters straight over the bone apical surface in connection with the blood vessels underneath, which are enclosed in the bone cavities within the osteoderm core (spongiosa).

As a conclusion, we suggest that the vascular plasticity of the post-cranial dermal bones in both Testudinata and Pseudosuchia probably helped these clades make major evolutionary shifts by offering various pathways to oxygen and/or heat management. Despite the fact that upshifts in vascular density often relate to an increased frequency of internal low oxygen due to a freshwater or marine lifestyle, we do not exclude that vascular density also relates to other vital functions as well as historical and structural constraints which drive the development and morphology of the dermal plates [105]. The complexity of multi-functional roles of the post-cranial dermal skeleton in both pseudosuchians and testudinatan might be a reason why our phylogenetic ANOVA revealed no relation between ‘vascular area’ and ‘ecology’ despite obvious differences between the lifestyle categories. Our results however demonstrate that the advanced development of a post-cranial skeleton in these groups was crucial for the survival and dispersal of these taxa in various ecological niches. This major evolutionary step should be more thoroughly investigated.

**Data accessibility.** The data are accessible in electronic supplementary material files.

**Authors' contributions.** F.C. computed and analysed the data before writing the first version of the manuscript. S.S. funded this study and gave important input regarding the interpretation of the data. T.M.S. provided his expertise on the testudinatan anatomy and phylogenetic relationships. J.B.D. and I.A.C. provided their expertise on the histology and on the natural history of the pseudosuchians. All authors worked on the finalization of the manuscript.

**Competing interests.** We declare we have no competing interests.

**Funding.** This work was supported by a grant from the Vetenskapsrådet awarded to S.S. (grant no. 2015–04335). T.M.S. acknowledges support by the Swiss National Science Foundation (grant no. 205321\_162775).

**Acknowledgements.** We thank Damien Germain (curator of the paleohistology collection in the Muséum National d' Histoire Naturelle; MNHN) for giving us access to the sample of European pseudosuchian specimens as well as the curators of the museums and collections who granted access to materials used in the previous studies on the histology of armoured animals.

## References

- Dubansky BH, Dubansky BD. 2018 Natural development of dermal ectopic bone in the American alligator (*Alligator mississippiensis*) resembles heterotopic ossification disorders in humans. *Anat. Rec.* **301**, 56–76. (doi:10.1002/ar.23682)
- Gilbert SF, Loredo GA, Brukman A, Burke AC. 2001 Morphogenesis of the turtle shell: the development of a novel structure in tetrapod evolution. *Evol. Dev.* **3**, 47–58. (doi:10.1046/j.1525-142x.2001.003002047.x)
- Vickaryous MK, Hall BK. 2008 Development of the dermal skeleton in *Alligator mississippiensis* (Archosauria, Crocodylia) with comments on the homology of osteoderms. *J. Morphol.* **269**, 398–422. (doi:10.1002/jmor.10575)
- Vickaryous MK, Sire JY. 2009 The integumentary skeleton of tetrapods: origin, evolution and development. *J. Anat.* **214**, 441–464. (doi:10.1111/j.1469-7580.2008.01043.x)
- Nesbitt SJ. 2011 The early evolution of archosaurs: relationships and the origin of major clades. *Bull. Am. Mus. Nat. Hist.* **352**, 1–292. (doi:10.1206/352.1)
- Cerda IA, Desojo JB, Trotteyn MJ, Scheyer TM. 2015 Osteoderm histology of Proterochampsia and Doswelliidae (Reptilia: Archosauriformes) and their evolutionary and paleobiological implications. *J. Morphol.* **276**, 385–402. (doi:10.1002/jmor.20348)
- Cerda IA, Desojo JB. 2011 Dermal armour histology of aetosaurs (Archosauria: Pseudosuchia), from the Upper Triassic of Argentina and Brazil. *Lethaia* **44**, 417–428. (doi:10.1111/j.1502-3931.2010.00252.x)
- Irmis RB, Nesbitt SJ, Sues HD. 2013 Early Crocodylomorpha. *Geol. Soc. London Spec. Publ.* **379**, 275–302. (doi:10.1144/SP379.24)
- Burns ME, Vickaryous MK, Currie PJ. 2013 Histological variability in fossil and recent alligatoroid osteoderms: systematic and functional implications. *J. Morphol.* **274**, 676–686. (doi:10.1002/jmor.20125)
- Anjan B, Bhullar S. 2008 Osteoderms of the California legless lizard *Anniella* (Squamata: Anguillidae) and their relevance for considerations of miniaturization. *Copeia* **4**, 785–793. (doi:10.1643/CG-07-189)
- Acrai B, Wagner HD. 2013 Micro-structure and mechanical properties of the turtle carapace as a biological composite shield. *Acta Biomater.* **9**, 5890–5902. (doi:10.1016/j.actbio.2012.12.023)
- Chen IH, Yang W, Meyers MA. 2015 Leatherback sea turtle shell: a tough and flexible biological design. *Acta Biomater.* **28**, 2–12. (doi:10.1016/j.actbio.2015.09.023)

13. Vickaryous MK, Hall BK. 2006 Osteoderm morphology and development in the nine-banded armadillo, *Dasypus novemcinctus* (Mammalia, Xenarthra, Cingulata). *J. Morphol.* **267**, 1273–1283. (doi:10.1002/jmor.10475)
14. Wolf D, Kalthoff DC, Sander PM. 2012 Osteoderm histology of the Pamphathiidae (Cingulata, Xenarthra, Mammalia): implications for systematics, osteoderm growth, and biomechanical adaptation. *J. Morphol.* **273**, 388–404. (doi:10.1002/jmor.11029)
15. Scheyer TM, Sander PM. 2004 Histology of ankylosaur osteoderms: implications for systematics and function. *J. Vertebr. Paleontol.* **24**, 874–893. (doi:10.1671/0272-4634)
16. Main RP, de Ricqlès A, Horner JR, Padian K. 2005 The evolution and function of thyreophoran dinosaur scutes: implications for plate function in stegosaurs. *Paleobiology* **31**, 291–314. (doi:10.1666/0094-8373)
17. Farlow JO, Hayashi S, Tattersall GJ. 2010 Internal vascularity of the dermal plates of *Stegosaurus* (Ornithischia, Thyreophora). *Swiss. J. Geosci.* **103**, 173–185. (doi:10.1007/s00015-010-0021-5)
18. Carrano MT, D'Emic MD. 2015 Osteoderms of the titanosaur sauropod dinosaur *Alamosaurus sanjuanensis* Gilmore, 1922. *J. Vertebr. Paleontol.* **35**, e901334. (doi:10.1080/02724634.2014.901334)
19. Sun CY, Chen PY. 2013 Structural design and mechanical behavior of alligator (*Alligator mississippiensis*) osteoderms. *Acta Biomater.* **9**, 9049–9064. (doi:10.1016/j.actbio.2013.07.016)
20. Chen IH, Yang W, Meyers MA. 2014 Alligator osteoderms: mechanical behavior and hierarchical structure. *Mater. Sci. Eng. C* **35**, 441–448. (doi:10.1016/j.msec.2013.11.024)
21. Broeckhoven C, Diedericks G, Mouton PLFN. 2015 What doesn't kill you might make you stronger: functional basis for variation in body armour. *J. Anim. Ecol.* **84**, 1213–1221. (doi:10.5061/dryad.7h4r3)
22. Du Plessis A, Broeckhoven C, Yadroitsev I, Yadroitsava I, le Roux SG. 2018 Analyzing nature's protective design: the glyptodont body armor. *J. Mech. Behav. Biomed.* **82**, 218–223. (doi:10.1016/j.jmbbm.2018.03.037)
23. Clarac F, Quilhac A. 2019 The crocodylian skull and osteoderms: a functional exaptation to ectothermy? *Zoology* **132**, 31–40. (doi:10.1016/j.zool.2018.12.001)
24. Jackson DC, Heisler N. 1982 Plasma ion balance in submerged anoxic turtles at 3°C: the role of calcium lactate formation. *Res. Physiol.* **49**, 159–174. (doi:10.1016/0034-5687(82)90071-8)
25. Jackson DC, Goldberger Z, Visuri S, Armstrong RN. 1999 Ionic exchanges of turtle shell in vitro and their relevance to shell function in the anoxic turtle. *J. Exp. Biol.* **202**, 503–520.
26. Jackson DC. 2000 Living without oxygen: lessons from the freshwater turtle. *Comp. Biochem. Physiol. A* **125**, 299–315. (doi:10.1016/S1095-6433(00)00160-4)
27. Jackson DC, Crocker CE, Ultsch GR. 2000 Bone and shell contribution to lactic acid buffering of submerged turtles *Chrysemys picta bellii* at 3°C. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **278**, R1564–R1571. (doi:10.1152/ajpregu.2000.278.6.r1564)
28. Jackson DC, Ramsey AL, Paulson JM, Crocker CE, Ultsch GR. 2000 Lactic acid buffering by bone and shell in anoxic softshell and painted turtles. *Phys. Chem. Zool.* **73**, 290–297. (doi:10.1086/316754)
29. Jackson DC. 2004 Surviving extreme lactic acidosis: the role of calcium lactate formation in the anoxic turtle. *Resp. Physiol. Neurobiol.* **144**, 173–178. (doi:10.1016/j.resp.2004.06.020)
30. Jackson DC, Andrade D, Abe AS. 2003 Lactate sequestration by osteoderms of the broad-nose caiman, *Caiman latirostris*, following capture and forced submergence. *J. Exp. Biol.* **206**, 3601–3606. (doi:10.1242/jeb.00611)
31. Trutnau L, Sommerlad R. 2006 *Crocodylians their natural history and captive husbandry*. Frankfurt am Main, Germany: Edition Chimaira.
32. Seidel MR. 1979 The osteoderms of the American alligator and their functional significance. *Herpetol. Leag.* **35**, 375–380.
33. Seebacher F, Franklin CE. 2004 Integration of autonomic and local mechanisms in regulating cardiovascular responses to heating and cooling in a reptile (*Crocodylus porosus*). *J. Comp. Physiol. B* **174**, 577–585. (doi:10.1007/s00360-004-0446-0)
34. Seebacher F, Franklin CE. 2007 Redistribution of blood within the body is important for thermoregulation in an ectothermic vertebrate (*Crocodylus porosus*). *J. Comp. Physiol. B* **177**, 841–848. (doi:10.1007/s00360-007-0181-4)
35. Grigg GC, Alchin J. 1976 The role of the cardiovascular system in thermoregulation of *Crocodylus johnstoni*. *Physiol. Zool.* **49**, 24–36. (doi:10.1086/physzool.49.1.30155674)
36. Tattersall DJ, Cadena V. 2010 Insights into animal temperature adaptations revealed through thermal imaging. *Imaging Sci. J.* **58**, 261–268. (doi:10.1179/136821910X12695060594165)
37. Scheyer TM, Sander PM, Joyce WG, Böhme W, Witzel U. 2007 A plywood structure in the shell of fossil and living soft-shelled turtles (Trionychidae) and its evolutionary implications. *Org. Divers. Evol.* **7**, 136–144. (doi:10.1016/j.ode.2006.03.002)
38. Scheyer TM, Sander PM. 2007 Carapace bone histology in the giant pleurodiran turtle *Stupendemys geographicus*: phylogeny and function. *Proc. R. Soc. B* **274**, 1885–1893. (doi:10.1098/rspb.2007.0499)
39. Clarac F, Buffrénil V, Cubo J, Quilhac A. 2018 Vascularisation in ornamented osteoderms: physiological implications in ectothermy and amphibious lifestyle in the crocodylomorphs? *Anat. Rec.* **301**, 175–183. (doi:10.1002/ar.23695)
40. Buffrénil V, Clarac F, Fau M, Martin S, Martin B, Pellé E, Laurin M. 2015 Differentiation and growth of bone ornamentation in vertebrates: a comparative histological study among the Crocodylomorpha. *J. Morphol.* **276**, 425–445. (doi:10.1002/jmor.20351)
41. Joyce WG, Chapman SD, Moody RTJ, Walker CA. 2011 The skull of the solemydid turtle *Helochelydra nopcsai* from the Early Cretaceous of the Isle of Wight (UK) and a review of Solemydidae. *Palaentology* **86**, 75–97. (doi:10.1111/j.1475-4983.2011.01075.x)
42. Joyce WG. 2017 A review of the fossil record of basal Mesozoic turtles. *Bull. Peabody Mus. Nat. Hist.* **58**, 65–113. (doi:10.3374/014.058.0105)
43. Young MT, de Andrade MB, Cornéed JJ, Steel L, Foffa D. 2014 Re-description of a putative Early Cretaceous 'teleosaurid' from France, with implications for the survival of metriorhynchids and teleosaurids across the Jurassic-Cretaceous Boundary. *Hist. Biol.* **27**, 947–953. (doi:10.1016/j.annpal.2014.01.002)
44. Fanti F, Miyashita T, Cantelli L, Mnasri F, Dridi J, Contessi M, Cau A. 2016 The largest thalattosuchian (Crocodylomorpha) supports teleosaurid survival across the Jurassic-Cretaceous boundary. *Cret. Res.* **61**, 263–274. (doi:10.1016/j.cretres.2015.11.011)
45. Bufféret E. 1982 Radiation évolutive, paléocécologie et biogéographie des crocodyliens méso-suchiens. *Mém. Soc. Géol. France N S* **142**, 1–88.
46. Young MT, Brusatte SL, Ruta M, de Andrade MB. 2010 The evolution of Metriorhynchoidea (Mesoeucrocodylia, Thalattosuchia): an integrated approach using geometric morphometrics, analysis of disparity, and biomechanics. *Zool. J. Linnean Soc.* **158**, 801–859. (doi:10.1111/j.1096-3642.2009.00571.x)
47. Clarac F, de Buffrénil V, Brochu CA, Cubo J. 2017 The evolution of bone ornamentation in Pseudosuchia: morphological constraints versus ecological adaptation. *Biol. J. Linn. Soc.* **121**, 395–408. (doi:10.1093/biolinnean/blw034)
48. Clarac F, Souter T, Cornette R, Cubo J, de Buffrénil V. 2015 A quantitative assessment of bone area increase due to ornamentation in the Crocodylia. *J. Morphol.* **276**, 1183–1192. (doi:10.1002/jmor.20408)
49. Gironodot M, Laurin M. 2003 Bone Profiler: a tool to quantify, model, and statistically compare bone-section compactness profiles. *J. Vertebr. Paleontol.* **23**, 458–461. (doi:10.1671/0272-4634(2003)023[0458:bpattq]2.0.co;2)
50. Maddison WP, Maddison DR. 2011 Mesquite: a modular system for evolutionary analysis. Version 2.7.5. See <http://mesquiteproject.org>.
51. Jouve S. 2009 The skull of *Teleosaurus cadomensis* (Crocodylomorpha; Thalattosuchia). *J. Vertebr. Paleontol.* **29**, 88–102. (doi:10.1671/039.029.0129)
52. de Andrade MB, Edmonds R, Benton MJ, Schouten R. 2011 A new Berriasian species of *Goniopholis* (Mesoeucrocodylia, Neosuchia) from England, and a review of the genus. *Zool. J. Linnean Soc.* **163**, S66–S108. (doi:10.1111/j.1096-3642.2011.00709.x)
53. Pol D, Nascimento PM, Carvahlo AB, Riccomini C, Pires-Domingue RA, Zaher H. 2014 A new notosuchian from the late Cretaceous of Brazil and the phylogeny of advanced notosuchians. *PLoS ONE* **9**, e93105. (doi:10.1371/journal.pone.0093105)
54. Pol D, Leardi JM. 2015 Diversity patterns of Notosuchia (Crocodyliformes, Mesoeucrocodylia) during the Cretaceous of Gondwana. In *Reptiles*

- extintos-volumen en homenaje a zulma a gasparini. Publicacion electronica de la asociacion paleontologica Argentina 15(1)* (eds M Fernandez, Y Herrera), pp. 172–186. Buenos Aires, Argentina: Asociación Paleontológica Argentina.
55. Young MT *et al.* 2014 Revision of the Late Jurassic telesaurid genus *Machimosaurus* (Crocodylomorpha, Thalattosuchia). *R. Soc. open sci.* **1**, 140222. (doi:10.1098/rsos.140222)
  56. Wilberg EW. 2015 What's in an outgroup? The impact of outgroup choice on the phylogenetic position of Thalattosuchia (Crocodylomorpha) and the origin of Crocodyliformes. *Syst. Biol.* **64**, 621–637. (doi:10.1093/sysbio/syv020)
  57. Nesbitt SJ *et al.* 2019 The earliest bird-line archosaurs and the assembly of the dinosaur body plan. *Nature* **544**, 484–544. (doi:10.1038/nature22037)
  58. Bona P, Ezcurra MD, Barrios F, Blanco MVF. 2018 A new Paleocene crocodylian from southern Argentina sheds light on the early history of caimanines. *Proc. R. Soc. B* **285**, 20180843. (doi:10.1098/rspb.2018.0843)
  59. Meylan PA, Sterrer W. 2000 *Hesperotestudo* (Testudines: Testudinidae) from the Pleistocene of Bermuda, with comments on the phylogenetic position of the genus. *Zool. J. Linnean. Soc.* **128**, 51–76. (doi:10.1006/zjls.1998.0199)
  60. Kear BP, Lee MSY. 2006 A primitive protostegid from Australia and early sea turtle evolution. *Biol. Lett.* **2**, 116–119. (doi:10.1098/rsbl.2005.0406)
  61. Joyce WG, Parham JF, Lyson TR, Warnock RCM, Donoghue PCJ. 2013 A divergence dating analysis of turtles using fossil calibrations: an example of best practices. *J. Paleontol.* **87**, 612–634. (doi:10.1666/12-149)
  62. Sterli J, Pol D, Laurin M. 2013 Incorporating phylogenetic uncertainty on phylogeny-based palaeontological dating and the timing of turtle diversification. *Cladistics* **9**, 232–246. (doi:10.1111/j.1096-0031.2012.00425.x)
  63. Nakajima Y, Danilov IG, Hirayama R, Sonoda T, Scheyer TM. 2017 Morphological and histological evidence for the oldest known softshell turtles from Japan. *J. Vertebr. Paleontol.* **37**, e1278606. (doi:10.1080/02724634.2017.1278606)
  64. R Development Core Team. 2012 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for statistical Computing. (<http://www.r-project.org/>)
  65. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884. (doi:10.1038/44766)
  66. Blomberg SP, Garland T. 2002 Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *J. Evol. Biol.* **15**, 899–910. (doi:10.1046/j.1420-9101.2002.00472.x)
  67. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012 *The caper package: comparative analysis of phylogenetics and evolution in R. R package version 0.5.2*. London, UK: CRAN.R Project.
  68. Revell LJ. 2012 Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)
  69. Garland Jr T, Dickerman AW, Janis CM, Jason AJ. 1993 Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**, 265–292. (doi:10.1093/sysbio/42.3.265)
  70. Freckleton RP. 2012 Fast likelihood calculations for comparative analyses. *Methods Ecol. Evol.* **3**, 940–947. (doi:10.1111/j.2041-210X.2012.00220.x)
  71. Blomberg SP, Garland Jr T, Ives AR. 2003 Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745. (doi:10.1111/j.0014-3820.2003.tb00285.x)
  72. Sereno PC, Larsson HC, Sidor CA, Gado B. 2001 The giant crocodyliform *Sarcosuchus* from the Cretaceous of Africa. *Science* **294**, 1516–1519. (doi:10.1126/science.1066521)
  73. Buscalioni AD, Piras P, Vullo R, Signore M, Barbera C. 2011 Early eusuchia crocodylomorpha from the vertebrate-rich Plattenkalk of Pietraroia (Lower Albian, southern Apennines, Italy). *Zool. J. Linnean Soc.* **163**, S199–S227. (doi:10.1111/j.1096-3642.2011.00718.x)
  74. Hua S, de Buffrénil V. 1996 Bone histology as a clue in the interpretation of functional adaptations in the Thalattosuchia (Reptilia, Crocodylia). *J. Vertebr. Paleontol.* **16**, 703–717. (doi:10.1080/02724634.1996.10011359)
  75. Bickler PE, Buck LT. 2007 Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. *Annu. Rev. Physiol.* **69**, 145–170. (doi:10.1146/annurev.physiol.69.031905.162529)
  76. Brix O, Condo SG, Lazzarino G, Clémenti ME, Scatena R, Giardina B. 1989 Arctic life adaptation-III. The function of whale (*Balaenoptera acutorostrata*) hemoglobin. *Comp. Biochem. Physiol.* **94B**, 139–142. (doi:10.1016/0305-0491(89)90024-2)
  77. Lestyk KC, Folkow LP, Blix AS, Hammil MO, Burns JM. 2009 Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity. *J. Comp. Physiol. B* **179**, 985–996. (doi:10.1007/s00360-009-0378-9)
  78. Andrews RD, Enstipp MR. 2016 Diving physiology of seabirds and marine mammals: relevance, challenges and some solutions for field studies. *Comp. Biochem. Physiol. A* **202**, 38–52. (doi:10.1016/j.cbpa.2016.07.004)
  79. Artioli GG, Gualano B, Smith A, Stout J, Lancha Jr AH. 2010 The role of  $\beta$ -alanine supplementation on muscle carnosine and exercise performance. *Med. Sci. Sports Exerc.* **42**, 1162–1173. (doi:10.1249/MSS.0b013e3181c74e38)
  80. Baguet A, Koppo K, Pottier A, Derave W. 2010  $\beta$ -Alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. *Eur. J. Appl. Physiol.* **108**, 495–503. (doi:10.1007/s00421-009-1225-0)
  81. Boldyrev AA, Aldini G, Derave W. 2013 Physiology and pathophysiology of carnosine. *Physiol. Rev.* **93**, 1803–1845. (doi:10.1152/physrev.00039.2012)
  82. Clarac F, Souter T, Cubo J, de Buffrénil V, Brochu C, Cornette R. 2016 Does skull morphology constrain bone ornamentation? A morphometric analysis in the Crocodylia. *J. Anat.* **229**, 292–301. (doi:10.1111/joa.12470)
  83. Lutz PL, Musick JA, Wyneken J. 2002 *The biology of the sea turtles, vol. 2*. Boca Raton, FL: CRC Press.
  84. Broderick AC, Godley BJ, Hays GC. 2001 Trophic status drives interannual variability in nesting numbers of marine turtles. *Proc. R. Soc. Lond. B* **268**, 1481–1487. (doi:10.1098/rspb.2001.1695)
  85. Hochscheid S, Bentivegna F, Speakman JR. 2003 The dual function of the lung in chelonian sea turtles: buoyancy control and oxygen storage. *J. Exp. Mar. Biol. Ecol.* **297**, 123–140. (doi:10.1016/j.jembe.2003.07.004)
  86. Hochscheid S, McMahon CR, Bradshaw CJA, Maffucci F, Bentivegna F, Hays GC. 2007 Allometric scaling of lung volume and its consequences for marine turtle diving performance. *Comp. Biochem. Phys. A* **148**, 360–367. (doi:10.1016/j.cbpa.2007.05.010)
  87. Dinkelacker SA, Costanzo JP, Lee Jr RE. 2005 Anoxia tolerance and freeze tolerance in hatchling turtles. *J. Comp. Physiol. B* **175**, 209–217. (doi:10.1007/s00360-005-0478-0)
  88. Reese SA, Jackson DC, Ultsch GR. 2003 Hibernation in freshwater turtles: softshell turtles (*Apalone spinifera*) are the most intolerant of anoxia among North American species. *J. Comp. Physiol. B* **173**, 263–268. (doi:10.1007/s00360-003-0332-1)
  89. Dunson WA. 1960 Aquatic respiration in *Trionyx spinifer asper*. *Herpetologica* **16**, 277–283.
  90. Feder ME, Burggren WW. 1985 Cutaneous gas exchange in vertebrates: design, patterns, control and implications. *Biol. Rev.* **60**, 1–45. (doi:10.1111/j.1469-185X.1985.tb00416.x)
  91. Hochscheid S, Bentivegna F, Speakman JR. 2002 Regional blood flow in sea turtles: implications for heat exchange in an aquatic ectotherm. *Physiol. Biochem. Zool.* **75**, 66–76. (doi:10.1086/339050)
  92. Kumazawa Y, Nishida M. 1999 Complete mitochondrial DNA sequences of the green turtle and blue-tailed mole skink: statistical evidence for archosaurian affinity of turtles. *Mol. Biol. Evol.* **16**, 784–792. (doi:10.1093/oxfordjournals.molbev.a026163)
  93. Zardoya R, Meyer A. 1998 Complete mitochondrial genome suggests diapsid affinities of turtles. *Proc. Natl Acad. Sci. USA* **95**, 14 226–14 231. (doi:10.1073/pnas.95.24.14226)
  94. Zardoya R, Meyer A. 2001 The evolutionary position of turtles revised. *Naturwissenschaften* **88**, 193–200. (doi:10.1007/s001140100228)
  95. Cao Y, Sorenson MD, Kumazawa Y, Mindell DP, Hasegawa M. 2000 Phylogenetic position of turtles among amniotes: evidence from mitochondrial and nuclear genes. *Gene* **259**, 139–148. (doi:10.1016/S0378-1119(00)00425-X)
  96. Hedges SB, Poling LL. 1999 A molecular phylogeny of reptiles. *Science* **283**, 998–1001. (doi:10.1126/science.283.5404.998)

97. Crawford NG, Faircloth BC, McCormackn JE, Brumfield RT, Winker K, Travis C, Glenn TC. 2012 More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biol. Lett.* **8**, 783–786. (doi:10.5061/dryad.75nv22qj)
98. Joyce WG. 2015 The origin of turtles: a paleontological perspective. *J. Exp. Zool. B Mol. Dev. Evol.* **324B**, 181–193. (doi:10.1002/jez.b.22609)
99. Scheyer TM, Danilov IG, Sukhanov VB, Syromyatnikova EV. 2014 The shell bone histology of fossil and extant marine turtle revisited. *Biol. J. Linn. Soc.* **112**, 701–718. (doi:10.1111/bij.12265)
100. Riquès A, Padian K, Horner JR. 2003 On the bone histology of some Triassic pseudosuchian archosaurs and related taxa. *Ann. Paleontol.* **89**, 67–101. (doi:10.1016/S0753-3969(03)00005-3)
101. Riquès A, Padian K, Knoll F, Horner JR. 2008 On the origin of high growth rates in archosaurs and their ancient relatives: complementary histological studies on Triassic archosauriforms and the problem of a 'phylogenetic signal' in bone histology. *Ann. Paleontol.* **94**, 57–76. (doi:10.1016/j.annpal.2008.03.002)
102. Seymour RS, Bennett-Stamper CL, Johnston SD, Carrier DR, Grigg GC. 2004 Evidence for endothermic ancestors of crocodiles at the stem of archosaur evolution. *Physiol. Biochem. Zool.* **77**, 1051–1067. (doi:10.1086/422766)
103. Legendre L, Segalen L, Cubo J. 2013 Evidence for high bone growth rate in *Euparkeria* obtained using a new paleohistological inference model for the humerus. *J. Vertebr. Paleontol.* **33**, 1343–1350. (doi:10.1080/02724634.2013.780060)
104. Legendre L, Guenard G, Botha-Brink J, Cubo J. 2016 Palaeohistological evidence for ancestral high metabolic rate in archosaurs. *Syst. Biol.* **65**, 989–996. (doi:10.1093/sysbio/syw033)
105. Lyson TR, Rubidge B, Scheyer TM, de Queiroz K, Schachner ER, Smith RM, Botha-Brink J, Bever GS. 2016 Fossorial origin of the turtle shell. *Curr. Biol.* **14**, 1887–1894. (doi:10.1016/j.cub.2016.05.020)
106. Seilacher A. 1970 Arbeitskonzept zur Konstruktionsmorphologie. *Lethaia* **3**, 393–396. (doi:10.1111/j.1502-3931.1970.tb00830.x)