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Origin of the unique ventilatory apparatus of turtles

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The turtle body plan differs markedly from that of other vertebrates and serves as a model system for studying structural and developmental evolution. Incorporation of the ribs into the turtle shell negates the costal movements that effect lung ventilation in other air-breathing amniotes. Instead, turtles have a unique abdominal-muscle-based ventilatory apparatus whose evolutionary origins have remained mysterious. Here we show through broadly comparative anatomical and histological analyses that an early member of the turtle stem lineage has several turtle-specific ventilation characters: rigid ribcage, inferred loss of intercostal muscles and osteological correlates of the primary expiratory muscle. Our results suggest that the ventilation mechanism of turtles evolved through a division of labour between the ribs and muscles of the trunk in which the abdominal muscles took on the primary ventilatory function, whereas the broadened ribs became the primary means of stabilizing the trunk. These changes occurred approximately 50 million years before the evolution of the fully ossified shell.

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Extant amniotes (the clade comprising mammals, turtles, birds, crocodylians and lepidosaurs) ventilate their lungs using a diversity of mechanisms (Fig. 1a). The most common of these solutions involves expansion and contraction of the ribcage effected by the intercostal muscles, either primarily, as in lepidosaurs (squamates and tuataras) or to supplement previously accessory structures that eventually became primary (for example, mammalian diaphragm)^{1,2}. Costal ventilation is impossible in turtles because their intercostal muscles are lost during embryogenesis³ and their ribs are bound in a typically rigid shell⁴. Instead, turtles employ a unique apparatus mainly involving two paired, antagonistic abdominal (hypaxial) muscles: *M. transversus* (including the ‘*M. diaphragmaticus*’ of others^{5,6}; see results) and *M. obliquus abdominis*^{6,7} (Figs 1b,c, 2). The *M. transversus* includes two bellies that originate separately from the cranial (*M. t. thoracis*) and caudal (*M. t. abdominis*) portions of the shell and run towards one another ventrally before merging via a tendinous aponeurosis. The muscle thus serves as a sling-like functional unit that largely embraces the coelomic cavity and thereby the viscera. Contraction of the *M. transversus* initiates

expiration by pressing the ventrally positioned viscera against the dorsally positioned lungs, which increases intrapulmonary pressure^{7,8}. Inspiration is effected by the posteriorly positioned *M. obliquus abdominis*, which when relaxed exhibits a concave, dome-like shape in the caudal flank region between the hind limbs and the bridge of the shell (Fig. 1b,c). Contraction and flattening of the *M. obliquus abdominis* initiates inspiration by pulling the *M. transversus* and underlying peritoneum caudoventrally, which decreases intrapulmonary pressure^{6,7} (Fig. 1b,c).

In most amniotes, the dorsal ribs and hypaxial muscles serve the dual function of ventilating the lungs and stabilizing the trunk against torsional forces generated during locomotion^{8,9}. Turtles have divided these functions, with the dorsal ribs only stabilizing the trunk and the hypaxial muscles exclusively effecting ventilation^{10–12}; however, it is unclear how and when such a division could have evolved. The need to maintain respiratory function and the ancestral role of the dorsal ribs in lung ventilation dictate that either the unique abdominal muscle apparatus or an alternative ventilatory mechanism was in place in the turtle stem lineage before the fusion of the ribs^{11,12}.

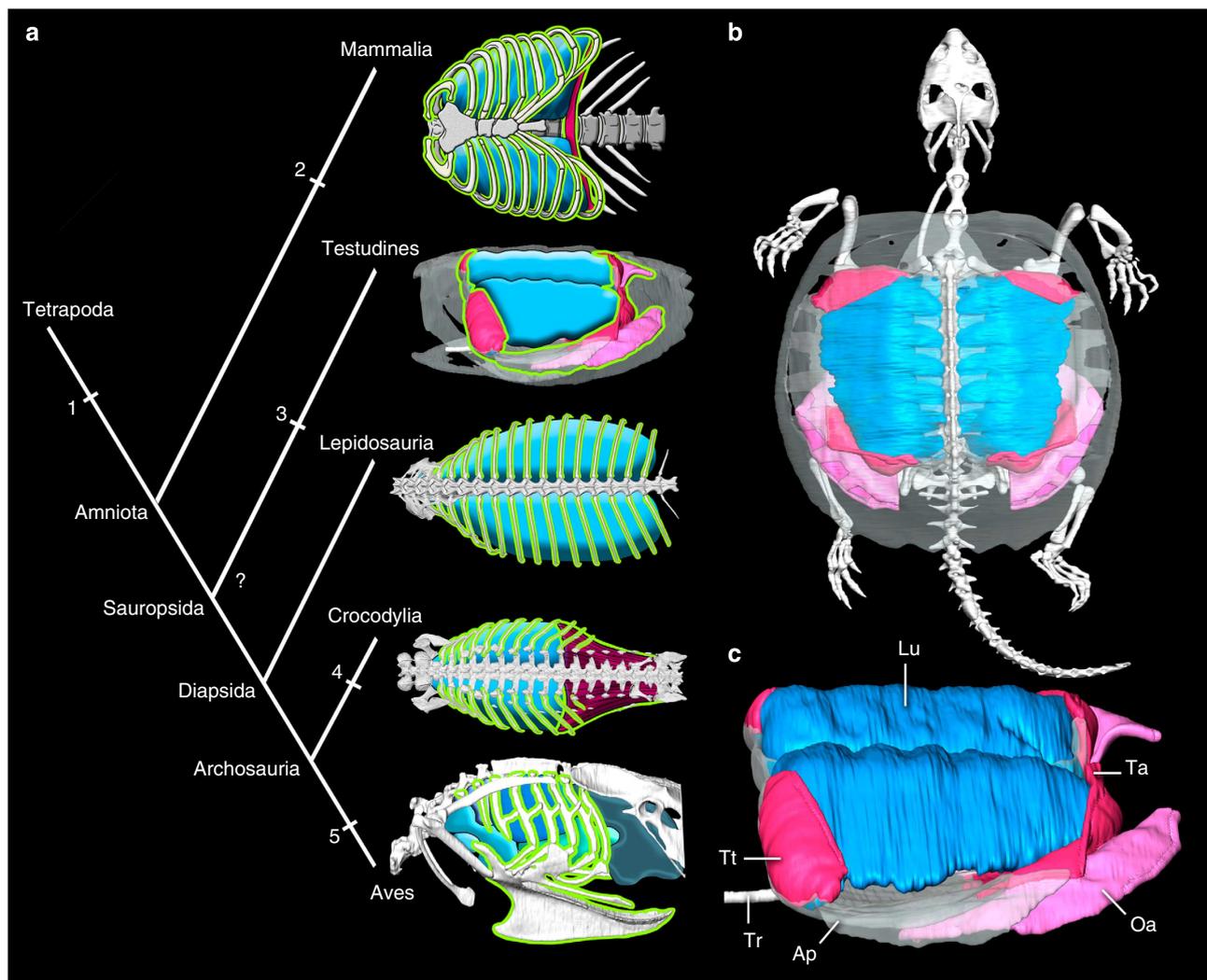


Figure 1 | Evolution of ventilatory mechanisms in Amniota. (a) Plesiomorphic costal ventilation (1) is employed in Lepidosauria (for example, *V. exanthematicus*) and in taxa with a diaphragm (Mammalia: *Hylobates* sp.; 2), hepatic piston (Crocodylia: *A. mississippiensis*; 4) and sternal pump (*G. domesticus*; 5), but not in turtles (Testudines: *C. serpentina*), which have a unique abdominal muscle-based mechanism (3). Phylogeny is derived from morphology^{4,17,43,44}. Molecular results switch the positions of Testudines and Lepidosauria^{45–47} but do not affect our inferences regarding the evolution of lung ventilation in turtles. (b,c) Digital reconstruction of the skeleton, lungs and hypaxial muscles of *C. serpentina* (b, dorsal view; c, dorsolateral view). Ap, tendinous aponeurosis; Lu, lung; Oa, *M. obliquus abdominis*; Ta, *M. transversus abdominis*; Tr, trachea; Tt, *M. transversus thoracis*.

To understand the origin of the pulmonary ventilatory mechanism of turtles, we homologize the hypaxial muscles used in extant turtle respiration with those found in other tetrapods. We then analyse the trunk anatomy of two early stem turtles, *Odontochelys semitestacea* and *Eunotosaurus africanus*. Finally, we compare the dorsal rib histology of *E. africanus* to that of extinct and extant amniotes to determine if the highly modified hypaxial musculature used by modern turtles to ventilate their lungs was already present in *E. africanus*. These analyses indicate that the early stem-group turtle *E. africanus* has several turtle-specific lung ventilation characters. Our results place the origin of major portions of the unique lung ventilatory apparatus of extant turtles shortly after the divergence of the turtle stem lineage from those of other extant reptiles and approximately 50 million years before the oldest known fully developed shell. Our data indicate that as the dorsal ribs broadened over time they became more effective at supporting the trunk during locomotion but less effective for costal ventilation. Simultaneously, the hypaxial muscles were gradually freed from trunk support during locomotion and delegated purely to ventilating the lung. Thus,

an easing of structural constraints through division of function (divergent specialization) between the dorsal ribs and the musculature of the body wall facilitated the evolution of both the novel turtle lung ventilation mechanism and the turtle shell.

Results

Homology of the respiratory muscles in turtles. The body wall of amniotes is composed of a complex arrangement of muscles that are subdivided into epaxial and hypaxial groups¹³. We compared the hypaxial musculature from 55 turtle specimens from 24 species, whose phylogenetic distribution represents the diversity of crown turtles, with that of one tuatara, *Sphenodon punctatus* (Fig. 2 and Supplementary Methods for list of specimens and taxa analysed). In most amniotes, the hypaxial muscles play a crucial role in respiration as well as in locomotion, which results in a mechanical conflict between these two functions^{8,9}. Of the hypaxial muscles, which are divided into layers with different fibre orientations¹³, the *M. obliquus abdominis* and *M. transversus abdominis* are found in all

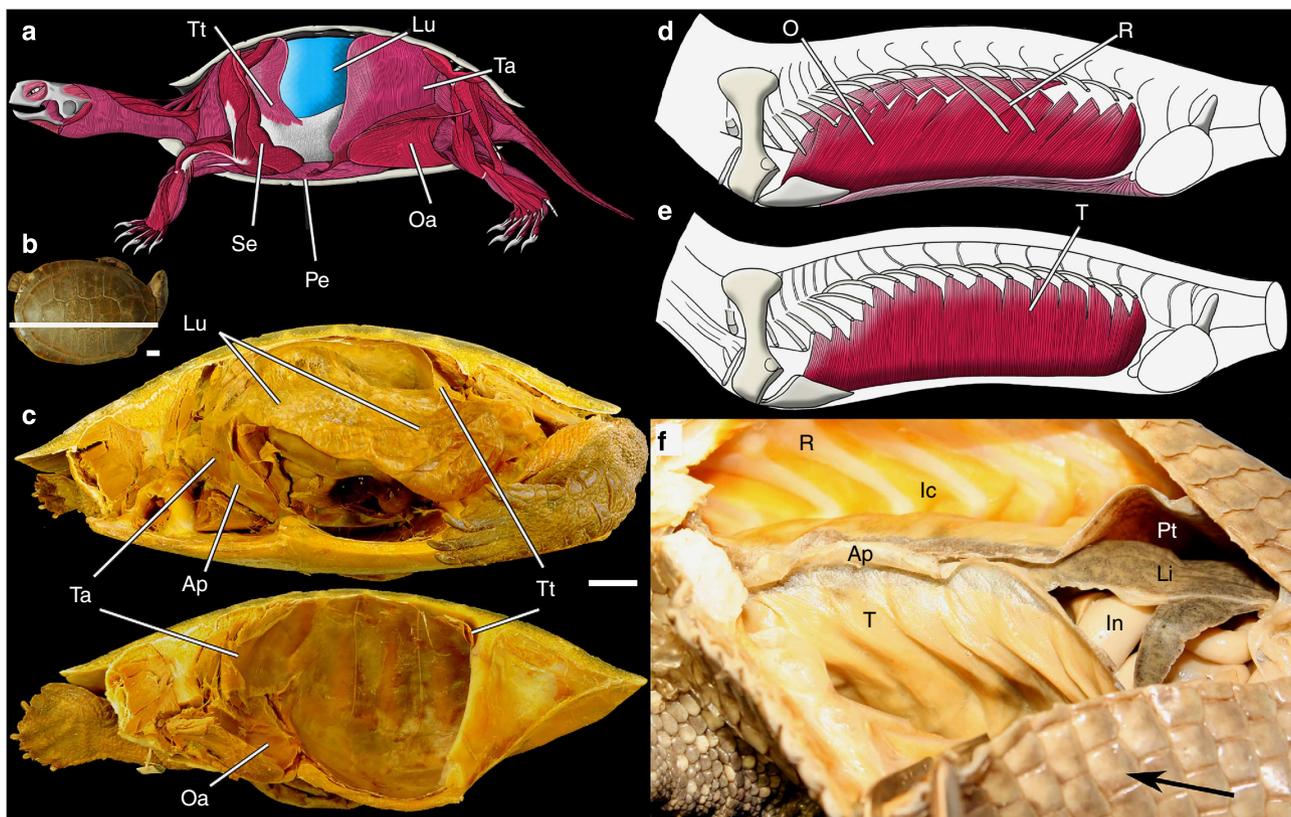


Figure 2 | Comparison of the deep hypaxial muscles in turtles and tuatara *Sphenodon punctatus*. (a) Diagrammatic image of a European pond turtle, *Emys orbicularis* (Emydidae), illustrating the cup-like form of the caudal *M. obliquus abdominis* (Oa) and the cranial *M. serratus* (Se). The *M. transversus thoracis* (Tt) originates on dorsal rib three and encloses the cranial portion of the lung (Lu). The *M. transversus abdominis* (Ta) originates from the dorsal ribs five to eight and inserts into a tendinous aponeurosis ventrally. Redrawn from Bojanus⁵. (b) *Emydura subglobosa* (Chelidae) (USNM 574459) with white line showing where the shell was cut. Scale bar, 2 cm. (c) Lateral view with the shell cut longitudinally (top), showing the major respiratory muscles, lungs and viscera (except the liver, which has been removed) *in situ*. The *M. transversus thoracis* (Tt) originates on dorsal rib three and inserts into a tendinous aponeurosis (Ap) that lies superficial to the peritoneum. Right portion of the shell (bottom) showing the origination sites for the major respiratory muscles. The *M. transversus abdominis* (Ta) originates on dorsal rib five and inserts into a tendinous aponeurosis ventrally. Scale bar, 2 cm. (d) In *S. punctatus*, the *M. obliquus* (O) forms a continuous sheath between the shoulder and pelvic girdle and originates from tendinous sheaths attached to each dorsal rib (R). (e) The *M. transversus* (T) of *S. punctatus* is the deepest layer of the hypaxial body wall muscles, and is situated just superficial to the peritoneum. It extends between the shoulder and pelvic girdles and originates from the ventral surface of each bony rib just dorsal to the cartilaginous uncinat processes. The fibres of the three cranial-most bundles insert on the dorsolateral margin of the sternum, whereas those of the remaining bundles insert into a midventral tendinous aponeurosis (Ap). (f) Ventrolateral view from the anatomical right side of the trunk in *S. punctatus* (ZSM 1318/2006; snout-vent length = 192 mm) showing the *M. transversus* (T) inserting ventrally into a tendinous aponeurosis (Ap). The peritoneum is opened in the abdominal region revealing the liver (Li) and intestine (In). Ic: *M. intercostalis*. Arrow points cranially.

Table 1 | Primary respiratory muscles of turtles.

	M. transversus thoracis	M. transversus abdominis	M. pulmonalis	M. obliquus abdominis	M. serratus
Topography	Embraces the anterior portion of the body cavity; often extends around the anterior portion of the lung	Embraces the posterior portion of the body cavity	Directly envelops the lung	Stretches along the posterior flanks between the hind legs and the shell	Stretches along the anterior flanks between the front legs and the shell
Description	Sling-like	Sling-like	Thin sheet	Cup-shaped	Cup-shaped
Function	Expiration; presumably also buoyancy control	Expiration; presumably also buoyancy control	Unknown; presumably expiration and/or buoyancy control	Inspiration	Inspiration and locomotion
Synonymy					
Townson ⁵¹	NA	Muscle of expiration	NA	Muscle of inspiration	NA
Bojanus ⁶	M. diaphragmaticus	M. transversus abdominis	NA	M. obliquus abdominis	M. serratus magnus
Rathke ⁵²	Not specifically named, but not M. diaphragmaticus	M. transversus abdominis	NA	M. obliquus internus abdominis	NA
Ogushi ¹⁷	M. tensor pleuro-peritonei	M. tensor pleuro-peritonei	NA	M. abdominis lateralis	M. carapaco-scapulo-coracoideus
Hansemann ⁵³	M. diaphragmaticus	M. transversus abdominis	M. pulmonalis	M. obliquus abdominis	NA
George and Shah ⁵⁴	diaphragmaticus	transverse abdominis	'Striated muscle'	oblique abdominis	serratus magnus
Ashley ⁵⁵	NA	transverse abdominis	NA	oblique abdominis	serratus magnus
Shah ¹⁵	M. diaphragmaticus	M. transversus abdominis	M. striatum pulmonale	M. obliquus abdominis	M. serratus magnus
Gans and Hughes ¹¹	NA	M. transversus abdominis	NA	M. obliquus abdominis	M. serratus magnus; M. testocoracoideus; M. serratus major
Gaunt and Gans ⁷	M. diaphragmaticus	M. transversus abdominis	NA	M. obliquus abdominis	M. testocoracoideus
Duncker ³⁶	M. transversus thoracis	abdominis	NA	NA	NA
Landberg et al. ¹²	diaphragmaticus	transverse abdominis	striatum pulmonale	oblique abdominis	NA
Landberg et al. ¹³	diaphragmaticus	transverse abdominis	NA	oblique abdominis	NA
Lambertz et al. ¹⁶	M. diaphragmaticus	NA	NA	NA	NA

NA, not applicable

Note that Gans and Hughes¹¹ and Gaunt and Gans⁷ have shown that there is an additional suite of accessory muscles of the shoulder and pelvic girdles that are associated with ventilation. However, all of them are involved in the movements of the extremities, which in turn cause alterations of intracoelomic pressure and air flow. With the exception of the M. serratus, these are omitted from the present table as they are regarded as of secondary relevance for breathing.

turtles¹⁴ and are active during lung ventilation^{6,10}. Other hypaxial muscles, such as the *Mm. intercostales*, are reduced and eventually lost during embryonic ontogeny⁷.

The general topography and innervation of the turtle M. obliquus abdominis and M. transversus abdominis are similar to those found in *Sphenodon punctatus* (Fig. 2 and Supplementary Figs 1 and 2) and we agree with previous authors^{6,10,14} on the homology of these muscles (see Table 1 and Supplementary Note 1 for a detailed discussion on the homology of each muscle used in respiration in turtles). However, the homology of the 'M. diaphragmaticus,' a name proposed by Bojanus⁵ and used by subsequent authors^{6,10,14}, is problematic. Unfortunately, muscles with identical names but doubtful homology have been described in turtles, mammals and crocodylians⁶ thus confounding the evolutionary origin of the muscle in turtles. The M. diaphragmaticus of mammals and that of crocodylians are caudal to the lungs, whereas the 'M. diaphragmaticus' of turtles is cranial and ventral to the lungs, and there is widespread agreement that these muscles are not homologous across the three groups². Rather the 'M. diaphragmaticus' of turtles shares several points of morphological identity with the M. transversus thoracis of other amniotes: (i) it has the same innervation

(posterior (= ventral, see Supplementary Note 1) rami of the spinal nerves); (ii) it is positioned superficial to the peritoneum; (iii) it inserts into a tendinous aponeurosis that stretches over the entire width of the ventral peritoneum (as does the M. transversus abdominis posteriorly) and (iv) it originates from the ventral side of the cranial portion of the dorsal ribs (generally dorsal ribs two and three)^{5,15,16} (Fig. 2 and Supplementary Figs 1 and 2). Thus, we consider the 'M. diaphragmaticus' of turtles to be homologous to the amniote M. transversus thoracis (see Table 1 and Supplementary Note 1).

Trunk skeleton of stem turtles. We analysed the trunk anatomy of two early stem-group turtles, both of which have unfused ribs, by comparing them to extant turtles and a lepidosaur (Fig. 3 and Supplementary Methods 1 for list of specimens and taxa analysed). The older *E. africanus* (Middle Permian, 260 mya)^{4,17–20} shares several unambiguous derived characters with stem- and crown-group turtles, including paired gastralia and a greatly reduced trunk region (nine vertebrae) with nine pairs of broad and strongly curved dorsal ribs that are T-shaped in cross-section^{4,17–20} (Fig. 3a–c). The ribs overlap sequentially and for

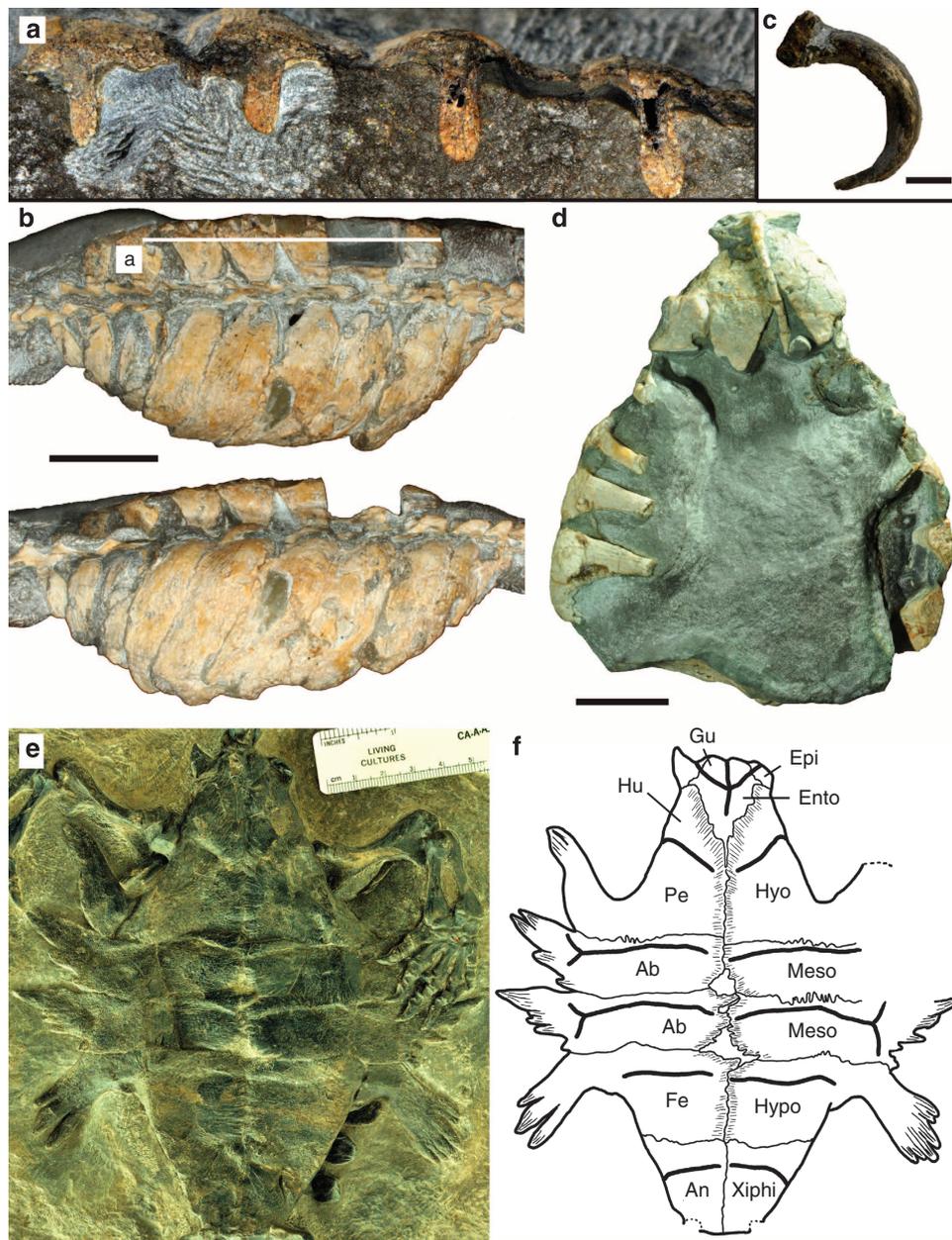


Figure 3 | The trunk anatomy of the early stem turtles *E. africanus* and *O. semitestacea*. (a) Cross-section through dorsal ribs three to six along a natural break in *E. africanus* specimen CM 86-341. (b) Two photographs of *E. africanus* specimen CM 86-341 in dorsolateral views showing how each rib overlaps the rib caudal to it for the majority of its length. White line marks ribs in part (a). Scale bar, 2 cm. (c) Right dorsal rib six of *E. africanus* specimen BP/1/4090 demonstrating the strong curvature of the dorsal ribs. Scale bar, 1 cm. (d) Ventral view of *E. africanus* specimen CM 777 that shows the unfinished and blunt to slightly concave distal tips of the dorsal ribs, indicating the presence of cartilaginous ventral components. Scale bar, 2 cm. (e, f) The plastron of *O. semitestacea* (IVPP V13240) was covered in keratinous scutes (e) photograph and (f) line drawing exhibiting a pattern similar to that seen in *P. quenstedti*. Bold lines represent sulci between scutes and thin lines represent sutures between bones. Scute names are given on the left: Ab, abdominal scute; An, anal scute; Fe, femoral scute; Gu, gular scute; Hu, humeral scute; Pe, pectoral scute. Bone names are given on the right: Ento, entoplastron bone; Epi, epiplastron bone; Hyo, hyoplastron bone; Hypo, hypoplastron bone; Meso, mesoplastron bone; Xiphi, xiphiplastron bone.

the majority of their length (Fig. 3b). The rib heads are weakly bicapitate and articulate with the cranial half of the neural arch and the centrum, with the tuberculum and capitulum in a nearly vertical alignment²⁰. This arrangement restricts costal movement to a cranio-caudal plane (bucket handle-like). The ribs have blunt and unfinished distal tips, indicating retention of cartilaginous ventral components (Fig. 3d).

Like *E. africanus*, *O. semitestacea* (Late Triassic, 220 mya)²¹ has nine cranio-caudally broadened ribs. Similar to crown turtles, the ribs are relatively straight (that is, less curved ventrolaterally), are

single headed and have finished distal apices indicating loss of the ventral cartilages^{4,21}. *O. semitestacea* has a fully developed plastron consisting of paired gastralia and dermal shoulder girdle bones (clavicles and interclavicle) similar to that found in crown turtles. The original morphological description by Li *et al.*²¹ made no mention of scutes, that is, the keratinous plates that are found in all known extant and extinct shelled-turtles, with the exception of at least three groups that have secondarily lost them (represented by the extant Trionychidae, *Carettochelys insculpta* and *Dermochelys coriacea*)²². Although turtle scutes are

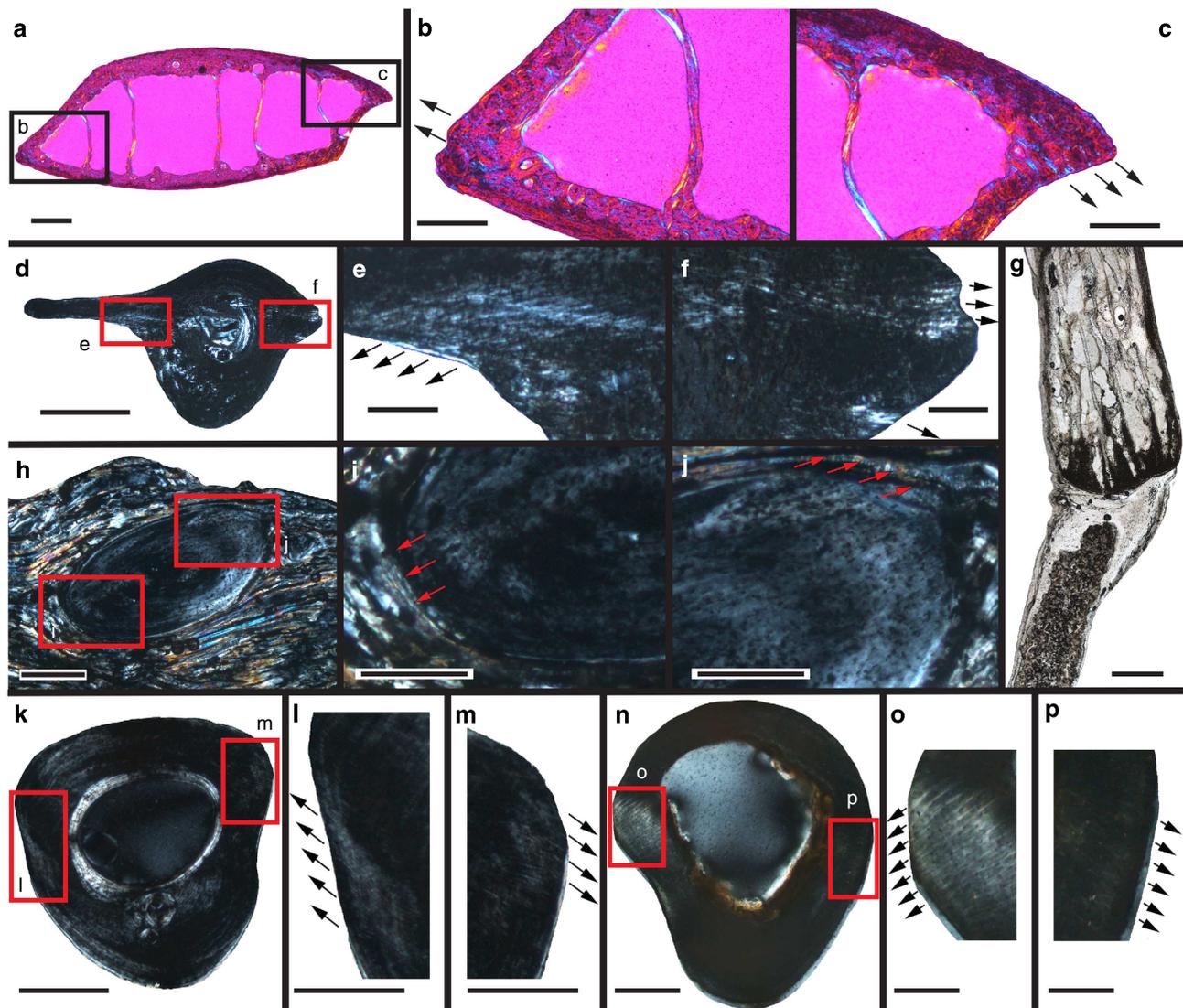


Figure 4 | Histology of extant amniote ribs. Histological sections of amniote ribs demonstrating the presence of Sharpey's fibers (ShF) on both the cranial and caudal edges at the attachment sites of *Mm. intercostales* attachment sites. (**a-c**) Histological cross-section of a dorsal rib of a specimen (PIMUZ uncatalogued) of *Capreolus capreolus* (Mammalia) under polarized light with close-up images of the cranial (**b**) and caudal (**c**) edges of the rib. Scale bar, 1.0 mm in **a** and 500 μ m in **b,d**. (**d-f**) Histological cross-section of a dorsal rib of a specimen (PIMUZ uncatalogued) of *Gavialis gangeticus* (Archosauria) cross-polarized light (**d**; scale bar, 1.0 mm) with close-up images of the cranial (**e**) and caudal (**f**) edges of the rib. Scale bar, 125 μ m in **e,f**. (**g**) Histological longitudinal section of the distal end of a dorsal rib of a specimen (PIMUZ uncatalogued) of *A. mississippiensis* (Archosauria) with calcified cartilage attached showing the blunt nature of the bony dorsal rib for attachment of ventral cartilaginous rib (compare with **d**). Scale bar, 1.0 mm. (**h-j**) Histological cross-section of gastralian of PIMUZ (uncatalogued specimen) of *A. mississippiensis* embedded in abdominal musculature under cross-polarized light (**h**; scale bar, 500 μ m) with close-up images of the cranial (**i**) and caudal (**j**) edges of the rib. Scale bar, 250 μ m in **i, j**. (**k-m**) Histological cross-section of a dorsal rib of YPM 11160 (*Corucia zebra*; Squamata) under cross-polarized (**k**; scale bar, 500 μ m) light with close-up images of the cranial (**l**) and caudal (**m**) edges of the rib. Scale bar, 250 μ m in **l,m**. (**n-p**) Histological cross-section of a dorsal rib of YPM11216 (*Furcifer pardalis*; Squamata) under cross-polarized (**n**; scale bar, 1 mm) light with close-up images of the cranial (**o**) and caudal (**p**) edges of the rib. Scale bar, 125 μ m in **o, p**. Black and red arrows show where ShFs are visible.

not usually preserved in the fossil record, they leave distinctive sulci on the dorsal surface of the shell that are readily observed in fossilized shell bones. Examination of the paratype of *O. semitestacea* (IVPP V13240) revealed the presence of such sulci on the plastron (Fig. 3e,f) forming a pattern similar to that of other stem turtles, including *Proganochelys quenstedti*²³. The presence of sulci on the dorsal ribs could not be determined. However, of the three described *O. semitestacea* specimens, only two have dorsal portions of the ribs prepared. In addition, these two specimens (IVPP V15639, IVPP V15653) are not well-preserved relative to IVPP V13240, and the absence of sulci could

reflect poor preservation rather than true morphology. There are currently no known turtles (crown or stem) that have scutes on the plastron but not the carapace (or vice versa).

Amniote rib histology. To determine if the highly modified hypaxial musculature (Fig. 2) used by modern turtles to ventilate their lungs was present in *E. africanus*, we compared its dorsal rib histology based on 3 individuals (eight ribs in total) to that of 47 extinct and extant taxa representing all major amniote clades (Supplementary Data 1). Sharpey's fibres (ShFs), a component of

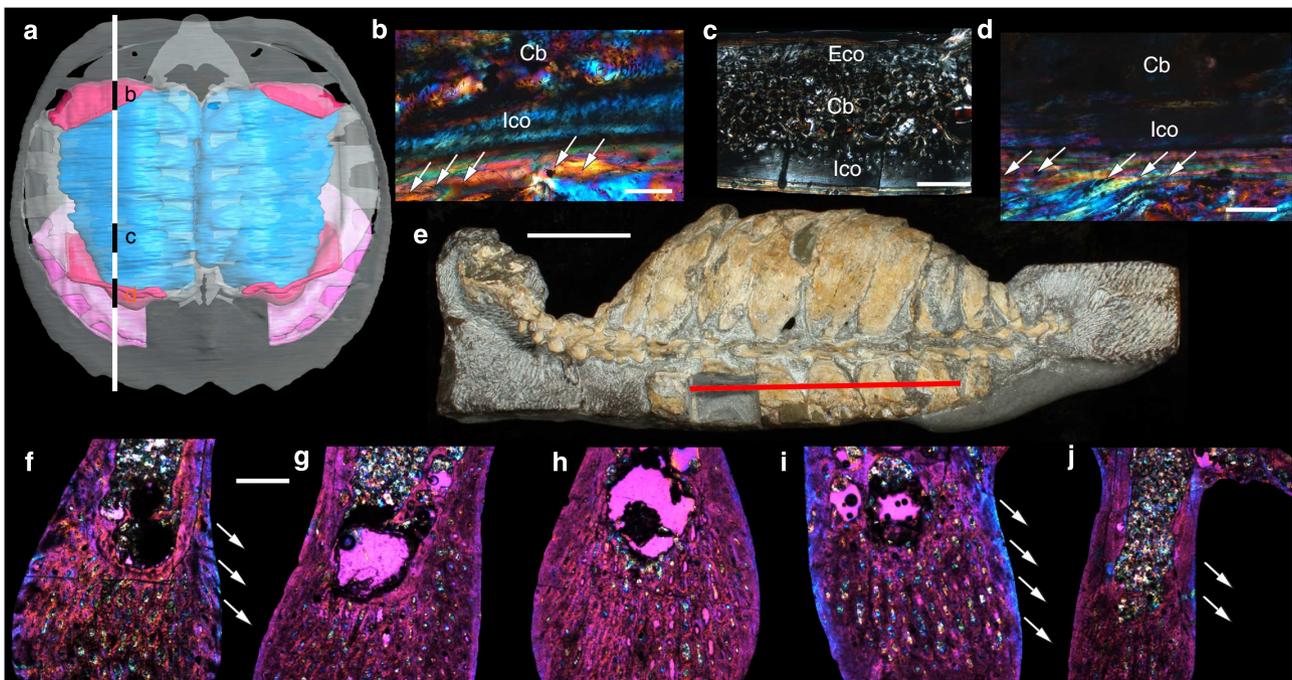


Figure 5 | Histological comparisons of the ribs of a crown turtle and the stem turtle *E. africanus*. (a) Three-dimensional digital model of a crown turtle (*C. serpentina*) in dorsal view highlighting the shell, lungs (blue) and hypaxial muscles (red and pink). (b–d) Histological sections of *C. serpentina* (USNM 324328) ribs taken along the length of the carapace (black/white line) show a modified connectivity of the *M. transversus abdominis* in which Sharpey's fibres (ShF; white arrows) are present cranially (b; dorsal rib two, scale bar, 0.2 mm) and caudally (d; dorsal rib five, scale bar, 0.2 mm) but not in the other dorsal ribs (c; for example, three, six and so on, scale bar, 1 mm). (e–j) Histological sections of *E. africanus* (CM86-341) (e), scale bar, 2 cm) (f–j) ribs taken along the length of the trunk (red line) show ShF (white arrows) on the caudal edge only of dorsal ribs three (f), six (i) and seven (j), but on neither edge of dorsal ribs four (g) and five (h). (f–j) Scale bar, 500 μ m for each rib (f–j). Cb, cancellous bone; Eco, external cortex; Ico, internal cortex.

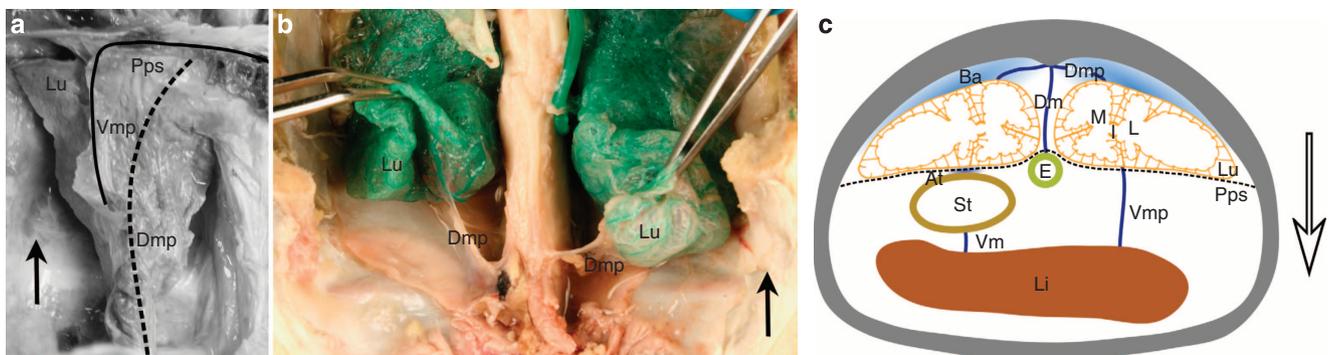


Figure 6 | The coelomic connections of the lungs in turtles. (a) Right lung (Lu) of a specimen (ZMB uncatalogued; carapace length = 270 mm) of *C. serpentina* in ventral view indicating the course of the dorsal (Dmp) and ventral (Vmp) mesopneumonia. Modified after Lambertz *et al.*¹⁵ (b) Both lungs of a wet specimen of *Trachemys scripta* (Rheinische Friedrich-Wilhelms-Universität Bonn uncatalogued; carapace length = 170 mm) in ventral view showing the dorsal mesopneumonia. Modified after Lambertz and Perry⁴⁸. (c) Schematized cross-section through the trunk of a turtle in caudal view. The lungs are suspended dorsally via either the dorsal mesopneumonia or a broadened attachment (Ba). The liver (Li) attaches directly to the right lung via the ventral mesopneumonium and indirectly to the left lung via the ventral mesentery (Vm) through the stomach (St), which is broadly attached (At) to the left lung. A postpulmonary septum (Pps) is present to varying degrees of completeness and provides further support for the lungs. Gravity (white arrow) acting on the viscera, particularly the liver, can expand the lungs and passively generate inspiratory airflow. E, esophagus; Dm, dorsal mesentery; I, intrapulmonary bronchus; L, lateral chamber of the lung; M, medial chamber of the lung. Figure (c) modified from Gräper³⁵.

the tendinous insertion of muscle into bone, can indicate the presence/absence of muscular attachments in extinct taxa^{4,24–26}. All of the non-chelonian extant taxa examined ($N=36$) possess intercostal muscles, and all of them exhibited ShF on both the cranial and caudal sides of the ribs (Fig. 4). Most of the non-chelonian extinct taxa examined (Supplementary Fig. 3a,b) also preserved ShF on both the cranial and caudal sides of the ribs

(also see Supplementary Fig. 4) including a pareiasaur (Supplementary Fig. 3c–h) from the same stratigraphic horizon as *E. africanus* and thus controlling for diagenetic alterations. Unfortunately, the rib histology from two broad ribbed parareptile taxa, *Milleretta rubidgei* and *Pumilioparia pricei*, was diagenetically altered such that the development and presence/absence of ShF could not be determined. A common snapping

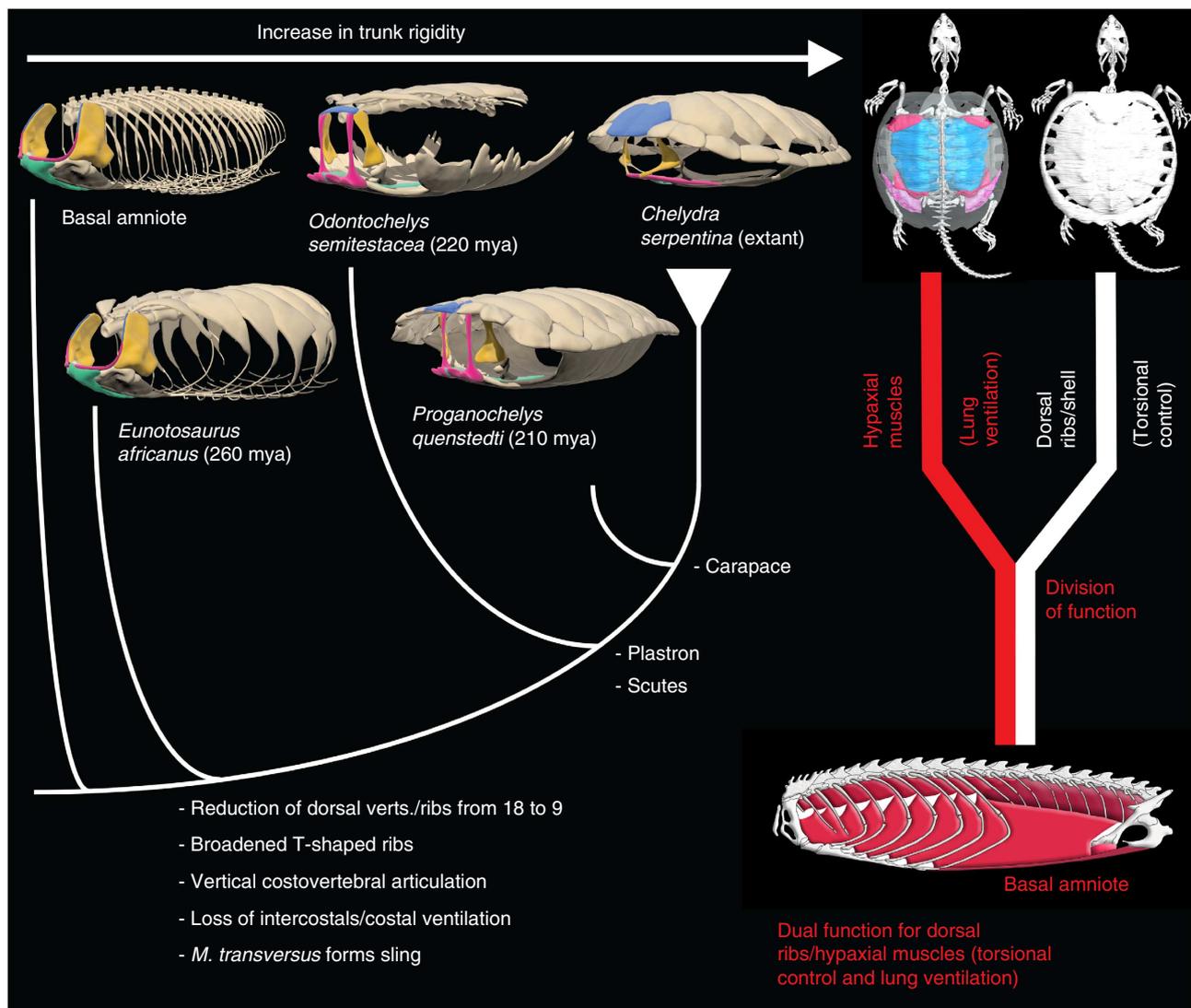


Figure 7 | Evolutionary scenario for the origin of the unique lung ventilation apparatus of turtles. Phylogeny (left) of stem turtles^{4,17} showing increasing trunk rigidity towards the crown (Note: the plastron is formed by the fusion of gastralia, interclavicle and clavicles, whereas the carapace is formed by the fusion of ribs, vertebrae, cleithra and ossified osteoderms found along the perimeter of the shell)⁴⁹. Our results suggest that the early stem lineage of turtles evolved a division of function in which the dorsal ribs took on a purely stabilizing role, whereas abdominal muscles became specialized for ventilating the lungs (right). Homology of the shoulder girdle elements follows Lyson *et al.*⁴⁹ Basal amniote redrawn and modified from Carrier⁵⁰. Clavicle: pink; cleithrum: blue; interclavicle: green; scapula: yellow.

turtle (*Chelydra serpentina*; Fig. 5a), which serves as a negative and positive control, lacked ShF where intercostal muscles have been lost and exhibited ShF where muscles are retained (for example, origin points of *M. transversus*; Fig. 5b–d).

Dorsal rib histology from three individuals (CM86-341 ribs 3–7 (Fig. 5e–j); NHM PV R 4949 rib 7; BP/1/7024 ribs 5–6 (Supplementary Fig. 4)) of *E. africanus* revealed that the cranial margins of all sampled ribs (3–7) lack ShF. ShFs are present along the caudal margins of dorsal ribs three (Fig. 5f), six (Fig. 5i) and seven (Fig. 5j) but not four and five (Fig. 5g–h and Supplementary Fig. 4).

Discussion

The shortened trunk, cranio-caudally broadened dorsal ribs (each of which forms a strongly curved arch that overlaps the rib posterior to it for most of its length), and costovertebral articulations of *E. africanus* indicate a rigid trunk skeleton (Fig. 3a–d). The trunk of *O. semitestacea* was stiffened further by

a fully ossified plastron covered with keratinous scutes (Fig. 3e,f). Such an inflexible body wall is ineffective for generating changes in intrapulmonary pressure²⁷ sufficient for ventilation, indicating that the plesiomorphic amniote costal ventilation mechanism was already abandoned in the most recent common ancestor of *E. africanus* and crown-group turtles.

In addition, the lack of ShF on the cranial margins of dorsal ribs 3–7 (Fig. 5) suggests the absence of intercostal muscles in *E. africanus*⁴. The inflexible trunk, combined with the loss of intercostal muscles in *E. africanus*⁴, indicates that costally mediated ventilation was replaced early in the turtle stem lineage. This situation presents two possibilities: either a fundamentally different ventilatory mechanism evolved and was later replaced by the one seen in crown turtles, or more parsimoniously, the mechanism in crown turtles was partially or fully developed in *E. africanus* and the last common ancestor that it shared with crown turtles.

It is unlikely that early stem-group turtles relied upon gular or buccal pumping (ventilation effected by muscles of the mouth

and/or throat), as this mode of breathing requires a large mouth-to-body size ratio²⁷ not present in *E. africanus* or *O. semitestacea*. Cutaneous respiration (including cloacal²⁸ and pharyngeal^{29,30}, which supplements lung breathing in some modern turtles, is also unlikely, as it requires both permeable skin and an aquatic environment³¹. *E. africanus*, however, was terrestrial¹⁹ and at least the plastron of *O. semitestacea* was covered by keratinous scutes (Fig. 3e,f).

Rather, the histology of *E. africanus* provides strong evidence that part of the derived abdominal muscle-based lung-ventilation mechanism found in crown turtles was already in place early in the turtle stem lineage. In *E. africanus*, ShFs are present along the caudal margins of dorsal ribs three, six and seven but not four and five (Fig. 5e–j). The *M. transversus* of most modern turtles originates from ribs two and three, as well as ribs six and seven, but not in the mid-trunk (ribs four and five), where connections are present in non-chelonian amniotes (Fig. 2 and Supplementary Figs 1 and 2). The remarkable correspondence of the ShF pattern between *E. africanus* (Fig. 5e–j) and crown turtles (Fig. 5a–d) supports the hypothesis that the derived muscular sling, formed by the *M. transversus* and used by modern turtles to expire, was already present in this Middle Permian form.

Unlike the *M. transversus*, strong osteological correlates for the derived hypaxial muscle used in turtle inspiration, the *M. obliquus abdominis*, do not exist and the inverted dome-like morphology found in crown turtles cannot be established in *E. africanus*. However, ancestrally the *M. obliquus* extends between the pelvic and shoulder girdles attaching to each dorsal rib via tendinous sheets, as in *Sphenodon punctatus* (Fig. 2d). The absence of ShF on dorsal ribs four and five indicates these plesiomorphic connections were not present in *E. africanus* so that perhaps the modified arrangement of this muscle seen in extant turtles was also present in this early stem turtle. The connection of the *M. obliquus* with the gastralia is unknown, so it is not possible to determine if they supported an inverted, cup-shaped *M. obliquus*, which powers active inspiration in crown turtles. In addition, if ShFs are found in the gastralia of *E. africanus*, it would not be possible to determine if they represent the *M. obliquus* or other abdominal muscles (for example, *M. rectus*) that also have their origination and/or insertion points here³².

Active inspiration in *E. africanus* was likely not primarily driven by the broadened dorsal ribs as they create a largely rigid body wall and furthermore, the intercostal muscles used to actively move the ribs to create volumetric changes in the coelomic cavity are lacking. However, the dorsal ribs are not sutured together, as in crown turtles, and limited movement was possible, indicating that some costal ventilation may have been possible. This costal ventilation may have been effected by the cartilaginous ventral ribs known to be present in *E. africanus* (Fig. 3d). Such a duplication of inspiratory function (ribs and hypaxial muscles) in *E. africanus* would allow for subsequent specialization of the *M. obliquus* and eventually further specialization of the ribs and gastralia, which are the primary shell elements in crown turtles.

In addition, passive inspiration was likely possible in the terrestrial *E. africanus* and may have helped facilitate the transition in active inspiration from the ancestral state involving both the intercostal and hypaxial (abdominal) muscles to the highly derived hypaxial muscle-based mechanism found in turtles. Whereas active inspiration in turtles is effected by hypaxial musculature (mainly the *M. obliquus abdominis*), experimental data on snapping turtles (*C. serpentina*) indicate that inspiration can occur passively on land, via a gravity-based mechanism⁶. Specifically, the reduced plastron of *C. serpentina* provides only limited support for the weight of the viscera on

land, where the buoyant force of water does not offset gravity. The viscera, part of which are attached to the ventral portion of the lungs (Fig. 6), were shown experimentally to passively pull downward on the latter, particularly after they have been lifted dorsally during expiration⁶. Dorsal attachment to the shell (Fig. 6) prevents the entire lung from being pulled ventrally. Rather, only the ventral portion moves, increasing the volume of the pliable lung, thereby reducing intrapulmonary pressure and affecting inspiration.

To better understand passive inspiration mechanism in turtles, it is necessary to consider their pulmonary anatomy and how the lungs are situated in the coelomic cavity. The lungs of all turtles are multi-chambered, with individual chambers branching off from a central intrapulmonary bronchus in a hierarchical sequence³³. Plesiomorphically, there is a row of larger lateral and smaller medial chambers (Fig. 6c). The intrapulmonary bronchus thus lies closer to the vertebral axis of the animal than does the sagittal midline of the lung (Fig. 6c). Dorsally, the visceral pleura adhering to the surface of the lungs connects to the parietal pleura either via discrete mesopneumonia or through a broadened attachment³⁴ (Fig. 6). When dorsal mesopneumonia are present, they are situated over the portion of the lung that houses the intrapulmonary bronchus, thus providing mechanical stabilization for this central conductive structure (Fig. 6). Such an anatomical configuration forces the central intrapulmonary airway, which has additional cartilage reinforcements in most taxa³⁵, to be open at all times. In addition to these dorsal connections, the lungs are connected to other viscera on their ventral surfaces (Fig. 6). In most turtles, the right lung attaches via the ventral mesopneumonium directly to the liver. Cranially, the left lung is broadly attached to the stomach, which in turn is connected to the liver via the ventral mesentery (Fig. 6). In both cases, the ventral connections coincide closely with the course of the central intrapulmonary bronchus. A gravity induced pull of the liver hence acts indirectly (via the various inter-organ connections) on the relatively immobile regions of the lung. This passive gravitational mechanism can thereby achieve an opening of the airways combined with a reduction in intrapulmonary pressure, resulting in inspiration.

A similar passive inspiratory mechanism is plausible in terrestrial *E. africanus*, whose ventral trunk skeleton consists of paired gastralia⁴, which, like the slender plastron found in *C. serpentina*, provide a lesser degree of support for the weight of the viscera on land—particularly after lifting of the viscera by the contraction of the sling-like *M. transversus*—than found in a turtle with a more robust plastron. Given the coelomic integration of the lungs among extant amniotes, and turtles in particular^{15,36–38}, we can infer a similar set of inter-organ connections for *E. africanus*. The gravity-induced ventral pull of the viscera would passively open the lungs, as documented in *C. serpentina* on land, and increase the volume of the pliable lung, resulting in lower intrapulmonary pressures and passive inspiration. Even though the actual nature of the *M. obliquus abdominis* cannot be reconstructed with certainty for *E. africanus* or any other extinct stem group representative of turtles (see above), our functional approximation of its respiratory apparatus allows us to recognize this passive mode of inspiration as a plausible alternative or at least additional solution, which serves as a minimum explanation for how the animal could have supplied its metabolic demand for oxygen.

Our findings suggest that the early stem lineage of turtles (at least as early as *E. africanus*) deviated from the basal amniote body plan (in which locomotion and breathing are coupled) and evolved a division of function in which the ribs took on a stabilizing role, whereas the abdominal muscles became specialized to ventilate the lungs (Fig. 7). The plausibility of this

scenario is bolstered by a similar, albeit less extreme, division of labour among broad-ribbed mammals (for example, the anteater *Cyclopes didactylus*; the lorises *Arctocebus calabarensis*; the tree shrew *Ptilocercus lowii*)³⁹. As the ribs of early stem turtles began to broaden and overlap, they became more effective at stabilizing the trunk (Fig. 7) but less effective contributors to pulmonary ventilation. This shift liberated the *M. transversus*, *M. obliquus* and *Mm. intercostales* from their functions in trunk stabilization, allowing them either to specialize for ventilation (*M. transversus* and *M. obliquus*) or to be lost (*Mm. intercostales*; Fig. 3). Once ventilation no longer required flexibility of the trunk, a constraint on the dorsal ribs was removed, allowing them to further broaden, fuse and eventually form a rigid carapace, as seen in turtles more crownward than *Odontochelys*. This model, which is based on an easing of structural constraints through a division of function (divergent specialization), places the origin of the unique lung ventilatory apparatus of extant turtles shortly after the divergence of turtles from other reptiles, well within the Paleozoic and at least 50 million years before the oldest known fully developed shell, from the Late Triassic (Middle Norian, *Proterochersis robusta*)⁴⁰.

Methods

Material analysed. The hypaxial musculature from 55 turtle specimens from 24 species, whose phylogenetic distribution covers the basal divergence within crown turtles, was dissected and compared with that of one tuatara, *Sphenodon punctatus* (Supplementary Methods 1 for list of specimens analysed). The skeletal morphology from the trunk region from 37 *E. africanus* and 3 *O. semitestacea* specimens was examined (Supplementary Methods 1 for list of specimens analysed). Eight dorsal ribs from three *E. africanus* specimens were sectioned for histology and compared with that of 46 extinct and extant taxa representing all major amniote clades (Supplementary Data 1). In addition, two *C. serpentina* carapaces were sectioned for histology (Supplementary Data 1). Specimens of *C. serpentina* (deceased), *Varanus exanthematicus* (live), *Alligator mississippiensis* (live) and *Gallus domesticus* (deceased) were imaged using computed tomography (CT).

Histology. The petrographic thin sections (see Supplementary Data 1 for list of taxa) were prepared using standard procedures⁴¹ and analysed using a LEICA DM 2500 M composite microscope, equipped with a LEICA DFC420 C digital camera and Nikon Eclipse 50i Polarizing microscope, equipped with a DS-Fi1 digital camera. Processing and preparation of images was accomplished using Adobe Photoshop and Illustrator, and CorelDraw.

The description of histological structures follows Francillon-Vieillot *et al.*⁴² and Sanchez *et al.*²⁵ for recognition of entheses and extrinsic fibres within the bone. Sharpey's fibres are herein identified as conspicuous fibres and coarser fibre bundles that extend obliquely into the cortical periosteally deposited bone tissue. In cross-polarized light (and cross-polarized light using a lambda compensator), these appear as fibre bundles that show different extinction patterns compared with the surrounding bone tissue because of different fibre orientation.

Computed tomography. The CT scans were performed at the University of Utah South Jordan Medical Center in accordance with and approved by the University of Utah Institutional Animal Care and Use Committee. Live unsexed animals (subadult *V. exanthematicus* (female) and subadult *A. mississippiensis* (sex unknown)) were scanned during a natural apnea; deceased specimens (adult *G. domesticus* (female) and adult *C. serpentina* (sex unknown)) were intubated and the lungs artificially inflated by hand with a 60-cc syringe. The individuals were CT scanned at 100 kVp and 400 MA with a slice thickness of 0.6 mm at intervals of 0.4 mm (with 0.2 mm overlap). Three-dimensional digital models were segmented in Avizo version 7.1 (<http://www.vsg3d.com/avizo/standard>) by hand, using a Wacom Intuos4 pen tablet. The images were edited and modified into figures in Adobe Photoshop.

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Author contributions

T.R.L. designed the project. T.R.L., E.R.S., J.B.-B., T.M.S., M.L. and B.R. collected data. All authors analysed data and contributed in preparing the manuscript.

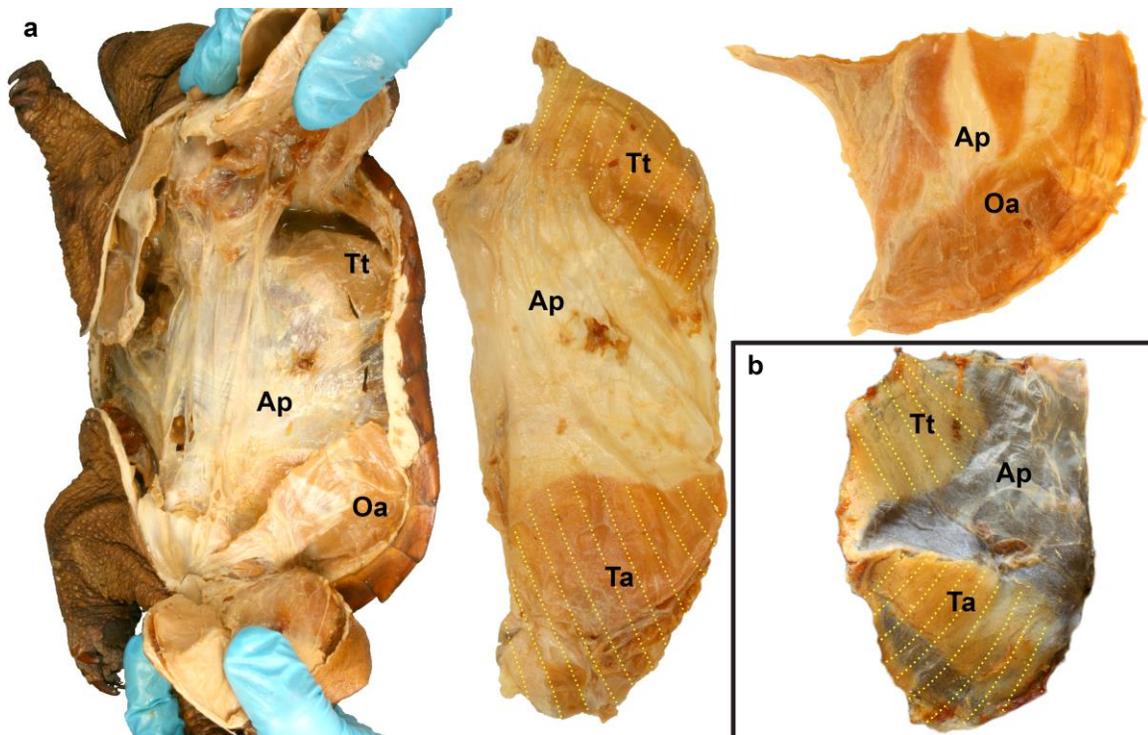
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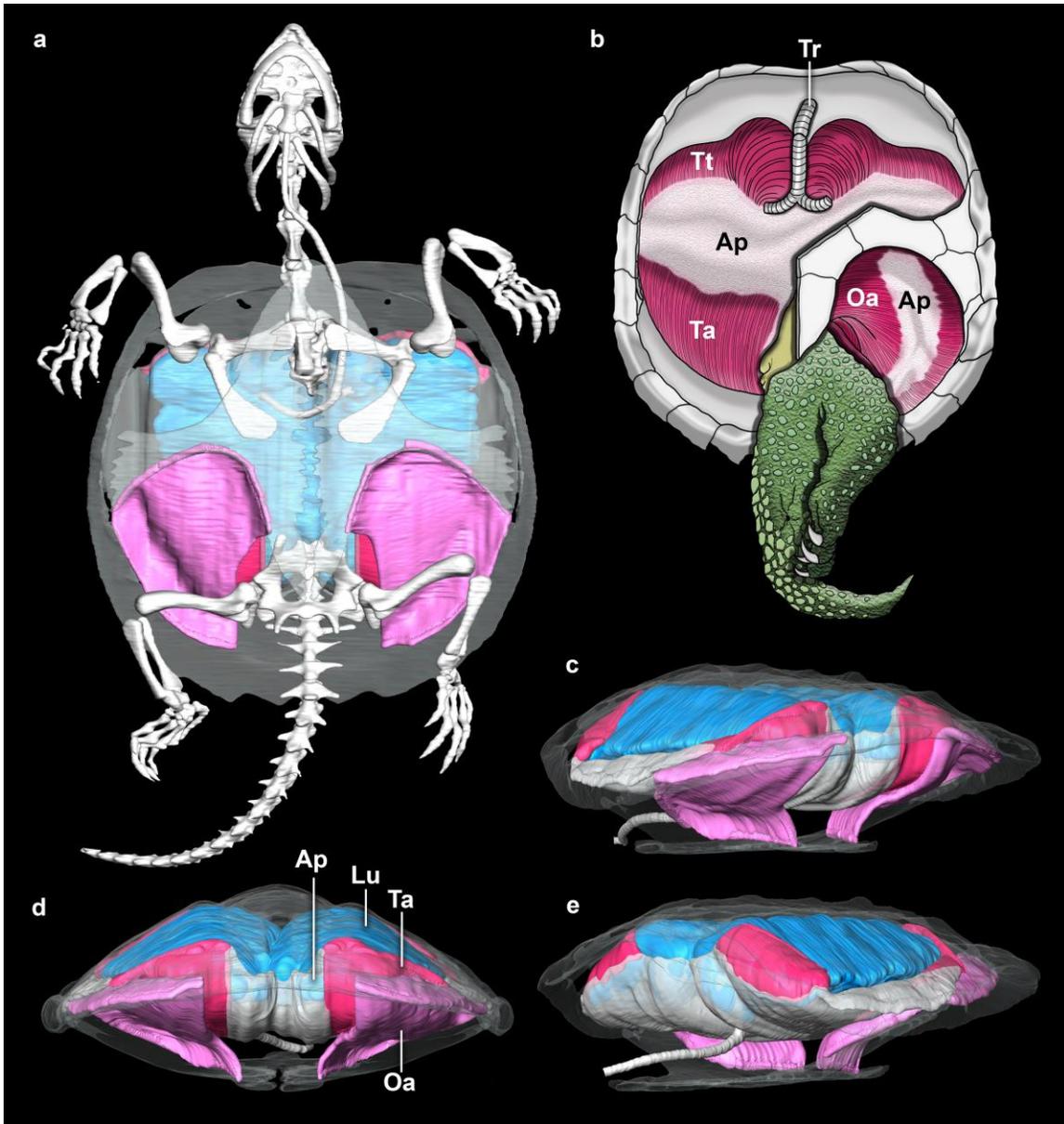
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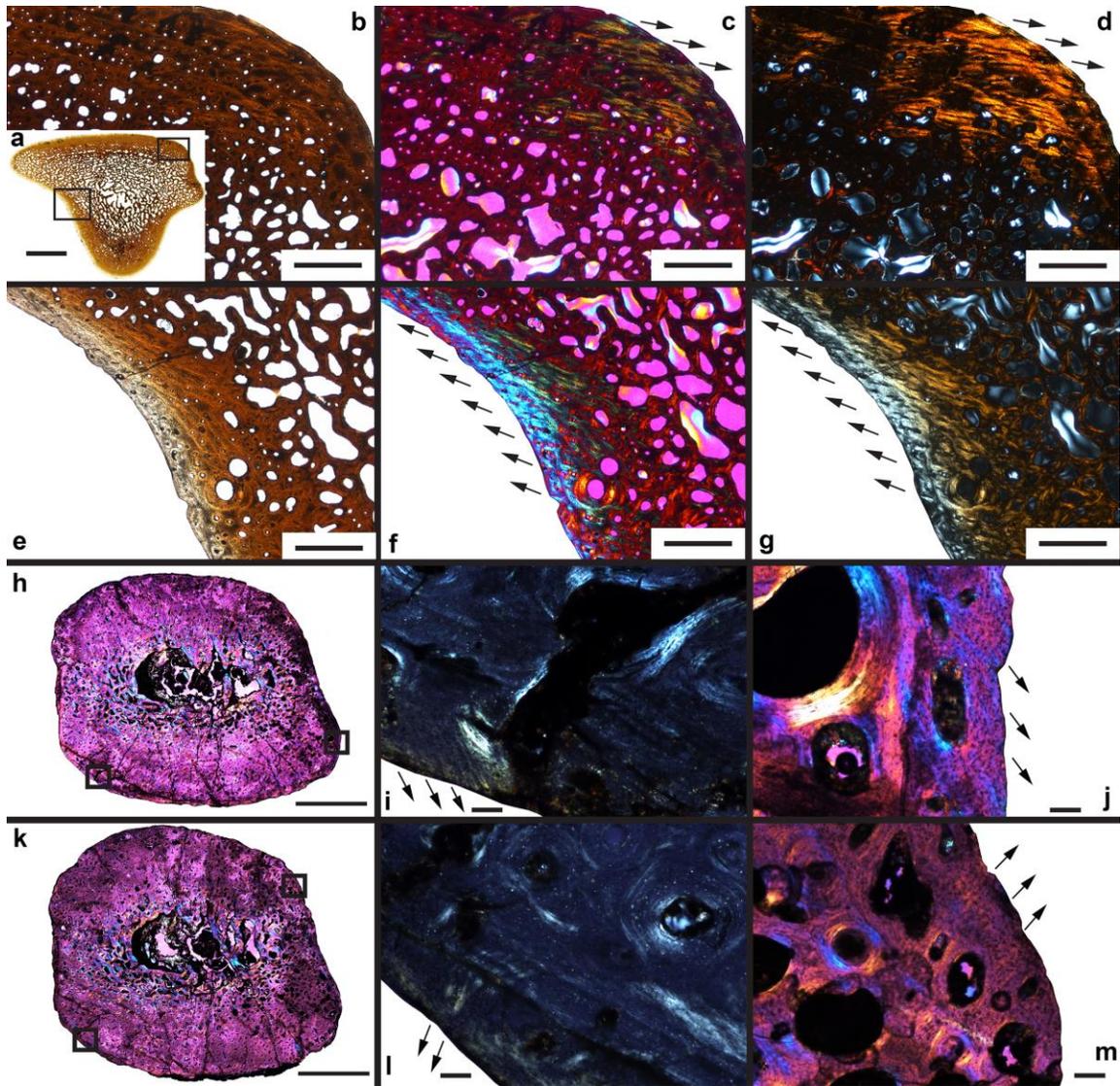
Supplementary Figure 1: Respiratory muscles of cryptodire turtles. a, Ventral view of a snapping turtle, *Chelydra serpentina* (Chelydridae) (ZMB uncataloged), with the plastron removed (**a** left), showing the major respiratory muscles *in situ*. The *M. transversus thoracis* (Tt) originates on thoracic ribs two and three and the *M. transversus abdominis* (Ta) originates on thoracic ribs five and six; both insert into a tendinous aponeurosis (Ap) that lies superficial to the peritoneum. The excised left ventral portion of the peritoneum (**a** middle) with the associated *M. transversus thoracis* and *M. transversus abdominis* overlain with dotted lines which indicate the orientation of the muscle fibers. The excised left *M. obliquus abdominis* (**a** right) is composed of distinct muscle bellies, each of which inserts into a tendinous aponeurosis (Ap). **b**, Ventral view of a Chinese softshelled turtle, *Pelodiscus sinensis* (Trionychidae) (PIMUZ lab#2009.72IW; Carapace length = 82 mm), with the excised ventral portion of the peritoneum with the orientation of muscle fibers indicated by dotted lines showing the

major expiratory muscles *in situ*. The *M. transversus thoracis* and *M. transversus abdominis* are well developed in this turtle and, at least laterally, nearly completely enclose the peritoneum. The *M. transversus thoracis* originates on thoracic ribs 2-4 and the *M. transversus abdominis* originates on thoracic ribs 5-8.



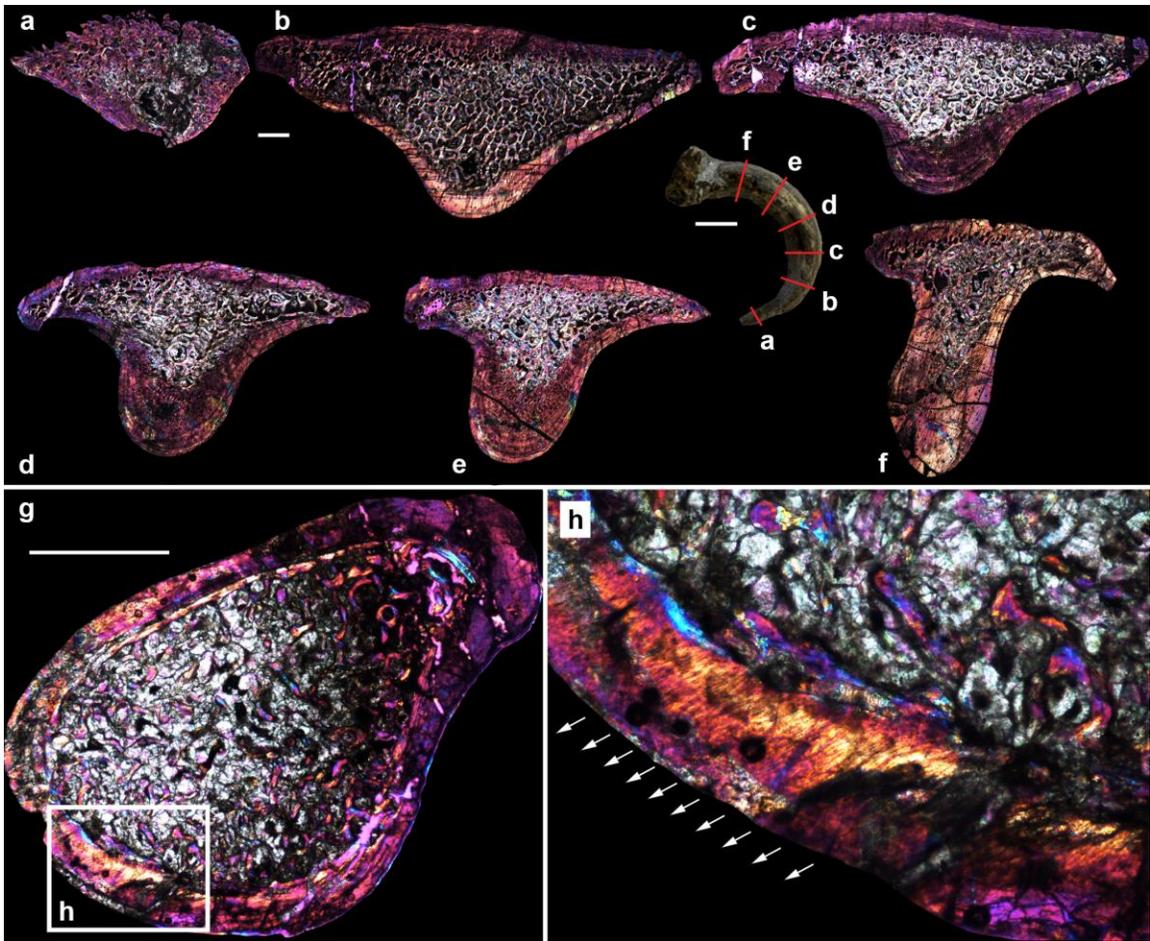
Supplementary Figure 2: Respiratory muscles of a snapping turtle, *Chelydra serpentina* (Cryptodira: Chelydridae). **a**, Computed tomography rendering of a specimen (Uncatalogued University of Utah) in ventral view with different regions segmented: skeleton (white and gray), lung (blue), *M. obliquus abdominis* (pink) and *M. transversus* (red). **b**, Diagram of the muscular ventilatory apparatus in ventral view with the plastron removed. Redrawn from Mitchell and Morehouse⁸. **c-e**, Digital surface models of the shell, lungs, trachea and ventilatory muscles in left caudolateral (**c**), caudal

(**d**), and left craniolateral (**e**) views with different anatomical components segmented as in **a**. Lu = lung; Oa = *M. oblique abdominis*; Ta = *M. transversus abdominis*; Tr = trachea; Ap = tendinous aponeurosis; Tt = *M. transversus thoracis*.



Supplementary Figure 3. Histological preparations from extinct amniotes showing the presence of Sharpey's fibers (ShF) on both the cranial and caudal edges of the dorsal ribs that have *Mm. intercostales* attachment sites. a-g, Histological cross-section of a dorsal rib of a specimen (MRF 254; Scale bar = 5 mm) of *Thescelosaurus neglectus* (Archosauria) in normal light with close-up images of the cranial (b-d) and caudal edges (e-g) in normal (b, e), polarized (c, f), and cross-polarized (d, g) light. and caudal (c) edges of the rib. Scale bar = 1 mm. h-m, Histological cross-section of a dorsal rib of a specimen (BP/1/7280; Scale bar = 5 mm in h and k) of *Pareiasaurus* sp.

(Parareptilia) under polarized light with close-up images of the cranial (**i, j**) and caudal edges (**l, m**) under cross-polarized (**i, l**) and polarized (**j, m**) light. Scale bar = 200 μm .



Supplementary Figure 4: Histological preparations of the right fifth dorsal rib and a limb fragment of a specimen (BP/1/7024) of *Eunotosaurus africanus* in cross section under polarized light. a-f, The right fifth dorsal rib shows no signs of Sharpey's fibers (ShF) along the entire length of the rib. Scale bar = 1 cm. g, h, A limb fragment from the same specimen preserves ShF (white arrows in close-up view in h) and indicates the lack of ShF in the dorsal rib is not the result of diagenetic alterations. Scale bar = 1 cm.

Supplementary Discussion : Homology and terminology of the respiratory muscles in turtles

The body wall of amniotes is composed of a complicated set of muscles that is subdivided into an epaxial and a hypaxial group¹³. The hypaxial group plays a crucial role in breathing, but is also involved in locomotion, which results in a mechanical conflict between respiration and locomotion^{8,9}. Of these hypaxial muscles, which are divided into layers with different fiber orientations¹³, the abdominal *M. obliquus* and abdominal *M. transversus* are responsible for lung ventilation in extant turtles. Other hypaxial muscles, such as the *Mm. intercostales*, are reduced and eventually lost in embryonic ontogeny (Fig. 1b to c, 2, and Supplementary Figs. 1-2)³. The *M. obliquus* and *M. transversus* are found in all turtles¹⁴ and are active during lung ventilation⁶. See Table 1 for a summary of the main muscles and their synonymy.

M. obliquus

In most extant amniotes, the abdominal *M. obliquus* occurs as a *M. obliquus externus* lateral to the ribs and as a *M. obliquus internus* medial to them. Both *M. obliquus* groups can be further subdivided¹³. The *M. obliquus*-complex extends between the shoulder and pelvic girdles and originates from tendinous sheaths attached to each dorsal rib (Fig. 2d) in tuataras (*Sphenodon punctatus*)³². A nearly identical condition is found in squamates^{32,56} and is inferred to represent the basal amniote condition². The *M. obliquus* is innervated by the caudal intercostal nerves^{S1}.

In turtles a single element from the abdominal *M. obliquus* group, usually designated the *M. obliquus abdominis*⁵ (but see the *M. abdominis lateralis* of Ogushi¹⁶),

is present (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2). Although Rathke⁴⁵ homologized the muscle in question to the *M. obliquus internus* (as *M. obliquus internus abdominis*), its derivation, in part, from the *M. obliquus externus* cannot be excluded. Indeed, the fragmentary appearance of the chelonian *M. obliquus abdominis* (Supplementary Fig. 1a) suggests it may derive from multiple sources, although additional developmental data are needed. The chelonian *M. obliquus abdominis* is a highly modified, inverted cup-shaped muscle located cranial to each hindlimb that originates on the caudolateral portion of the carapace and plastron and inserts on the aponeurosis of the *M. transversus*⁶ (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2) with a dorsal attachment to the ventral surface of the caudal aspect of the *M. transversus*. As in other amniotes, it is innervated by the caudal intercostal nerves⁵.

M. transversus

In tetrapods, the abdominal *M. transversus* is the deepest layer of the hypaxial body wall muscles. In *Sphenodon punctatus* the *M. transversus* (Fig. 2e to f) lies just superficial to the peritoneum, separated by the transversalis fascia, and extends continuously between the first dorsal rib that connects to the sternum through the last pre-pelvic rib³². The *M. transversus* originates via tendinous sheaths from the ventral surface of each bony rib. The fibers of the three cranial-most bundles insert on the dorsolateral margin of the sternum, whereas those of the remaining bundles insert into a tendinous aponeurosis that stretches over the ventral body midline, covering the peritoneum⁵² (Fig. 2e to f) A nearly identical condition is found in squamates^{32,56} and was inferred to represent the ancestral amniote condition². The *M. transversus* can be further subdivided

into the *M. transversus thoracis* and *M. transversus abdominis*^{32,56,S2} both of which are innervated by the ventral rami of the spinal nerves³².

In most species of turtles, a tendinous aponeurosis separates the thoracic and abdominal elements of the *M. transversus* (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2). However, in some species (e.g. *Pelodiscus sinensis*; Supplementary Fig. 1b), the two elements nearly converge medially to form a complete muscular sheath, resembling the plesiomorphic condition (e.g. *Sphenodon punctatus*, Fig. 2e).

Like most previous authors, we identify the caudal portion of the *M. transversus* as the *M. transversus abdominis*^{5,7,11,12,14,48} (but see Ogushi¹⁶ who termed the entire sheath in *P. sinensis* the *M. tensor pleuro-peritonei*). The *M. transversus abdominis* originates from the posterior portion of the carapace (generally dorsal ribs five, six, or seven) and inserts into a tendinous aponeurosis ventrally (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2). The tendinous aponeurosis lies just superficial to the peritoneum. The *M. transversus abdominis* is innervated by the posterior (= ventral, see below) rami of the spinal nerves in turtles^{5,16,34}.

The turtle *M. transversus thoracis* originates on the ventral side of the cranial portion of the carapace (generally dorsal ribs two and three), and encapsulates the anterior portion of the coelomic cavity and thereby also the cranial portion of the lungs^{36,37} (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2). The *M. transversus thoracis* inserts ventrally into a tendinous aponeurosis that stretches over the entire width of the ventral peritoneum, similarly to the *M. transversus abdominis*. The *M. transversus thoracis* is innervated by the posterior (= ventral, see below) rami of the spinal nerves (Bojanus⁵ termed the muscle “*M. diaphragmaticus*”). Ogushi¹⁶ described a corresponding

innervation for the cranial portion of his “*M. tensor pleuro-peritonei*” (of which our *M. transversus thoracis* represents the anterior portion) and further stated that *Pelodiscus sinensis* has an anterior and a posterior ramus of the associated spinal nerve, the latter innervating the “*M. tensor pleuro-peritonei*.” He furthermore convincingly demonstrated the homology of these rami with the dorsal (= turtle anterior) and ventral (= turtle posterior) rami of other vertebrates, thus revealing another source for confusion regarding the homology of this muscle. Due to the upright posture of humans, human anatomists refer to the dorsal rami of the spinal nerves as the “posterior rami” and the ventral rami of the spinal nerves as the “anterior rami”^{S3}; the spatial terminology is the other way around in quadrupeds, including turtles.

A muscle named “*M. diaphragmaticus*” was recognized by Bojanus⁵ in *Emys orbicularis* (Fig. 2a). This muscle originates from the same position as the *M. transversus thoracis* (see above) and at least the dorsal portion also runs superficial to the peritoneum^{5,15,16} (Fig. 2a). However, it does not extend as far ventrally along the peritoneum as does the *M. transversus thoracis* of *Pelodiscus sinensis*. Instead it only dorsally and cranially (and partly laterally) envelops the cranial part of the lung. It is also found in the intracoelomic *Septum postpulmonale* directly ventral to the lungs and thus lies within the coelomic cavity^{15,36,37}. Unfortunately, muscles with identical names but doubtful homology have been described in turtles, mammals and crocodiles². The *M. diaphragmaticus* of mammals and crocodylians is caudal to the lungs, whereas the “*M. diaphragmaticus*” of turtles is cranial and ventral to the lungs (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2) and there is widespread agreement that these muscles are not homologous among the three groups². Rather the “*M. diaphragmaticus*” of turtles has a

similar origination on the ventral portion of the dorsal ribs, a similar position superficial to the peritoneum, and the same innervation pattern^{5,15,16} (Fig. 1b to c, 2a to c, and Supplementary Figs. 1-2) as the *M. transversus thoracis* of other amniotes, and we therefore consider it homologous to that muscle.

Other muscles used in turtle respiration:

One muscle associated with the shoulder girdle, the *M. serratus* (Fig. 2a), is also directly involved in turtle lung ventilation (Supplementary Discussion 1). This muscle was named *M. serratus magnus* by Bojanus⁵ and *M. carapaco-scapulo-coracoideus* by Ogushi¹⁶. We recommend maintaining use of the name *M. serratus* until embryological data disprove its homology to the *M. serratus* of other tetrapods, which not only exhibits a corresponding topography but is also known to serve an inspiratory function in humans^{S3}. Also the *M. pectoralis* (Fig. 2a), through its ability to retract the anterior extremities, can be recruited for ventilation. The *M. pectoralis* is only one example of a diverse suite of girdle-associated, mainly locomotory muscles, that can contribute to chelonian breathing¹⁰.

Finally, some turtles have a very thin and enigmatic muscle that is directly associated with the lungs. The *M. pulmonalis* of Hansemann⁴⁶ = *M. striatum pulmonale* of George & Shah⁴⁷ lies beneath the “*M. diaphragmaticus*” (= *M. transversus thoracis*). Its homology is unknown and its function has never been studied.

Supplementary Methods : List of material analyzed by the authors

Institutional Abbreviations: AM = Albany Museum, Grahamstown, South Africa; BP/1/ = Evolutionary Studies Institute (formerly the Bernard Price Institute), University of the Witwatersrand, Johannesburg, South Africa; BMNH = The Natural History Museum, London, UK; CM = Council for Geosciences, Pretoria, South Africa; GPIT = Paläontologische Lehr- und Schausammlung, University of Tübingen, Germany; IVPP = Institute for Vertebrate Paleontology and Paleoanthropology, Beijing, China; MRF = Marmarth Research Foundation, Marmarth ND, USA; NMQR = National Museum, Bloemfontein, South Africa; PIMUZ = Paläontologisches Institut und Museum der Universität Zürich, Zurich, Switzerland; SAM-PK = Iziko Museums of South Africa, Cape Town, South Africa; SMNS = Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany; YPM = Yale Peabody Museum of Natural History, Yale University, New Haven CT, USA; USNM = National Museum of Natural History, Washington DC, USA; ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; ZMB = Museum für Naturkunde, Berlin, Germany; ZSM = Zoologische Staatssammlung München, Munich, Germany

EXTANT TAXA

Dissections:

TESTUDINES: CRYPTODIRA

Carettochelyidae: <i>Carettochelys insculpta</i>	ZFMK 94265, fluid specimen. ZFMK 94266, fluid specimen. ZSM 374/2001, fluid specimen. Uncatalogued fresh specimen, donated by M. Baur (Reptilienauffangstation München, Munich, Germany) to M. Lambertz.
Chelydridae: <i>Chelydra serpentina</i>	USNM 324328, fluid specimen. USNM 334981, fluid specimen. ZMB uncatalogued, five fluid specimens (Fig. 6a and Supplementary Fig. 1a). University of Utah uncatalogued, fresh specimen (Fig. 1, b to c and Supplementary Fig. 2).
<i>Macrochelys temminckii</i>	ZMB uncatalogued, fluid specimen.
Emydidae: <i>Clemmys guttata</i>	ZFMK uncatalogued, fluid specimen.
<i>Trachemys scripta</i>	Rheinische Friedrich-Wilhelms-Universität Bonn uncatalogued, four fluid specimens (Fig. 6b).

Geoemydidae	<i>Sacalia quadriocellata</i>	ZFMK uncatalogued, fluid specimen.
Kinosternoidea	<i>Claudius angustatus</i>	ZMB uncatalogued, five fluid specimens.
	<i>Kinosternon acutum</i>	SMNS 3742, fluid specimen.
	<i>Kinosternon baurii</i>	SMNS 3744, fluid specimen.
		ZFMK uncatalogued, two fluid specimens.
	<i>Kinosternon flavescens</i>	ZSM 335/1978, fluid specimen.
	<i>Kinosternon hirtipes</i>	ZSM uncatalogued, two fluid specimens.
	<i>Kinosternon leucostomum</i>	SMNS 7166, fluid specimen.
	<i>Kinosternon scorpioides</i>	Uncatalogued fresh specimen, donated by Rheinische Friedrich-Wilhelms-Universität Bonn to M. Lambertz. SMNS 4658, fluid specimen.
	<i>Staurotypus triporcatus</i>	ZMB uncatalogued, fluid specimen.
	<i>Sternotherus carinatus</i>	ZSM 438/2001, fluid specimen.
		ZSM 439/2001, fluid specimen.
	<i>Sternotherus minor</i>	ZSM 440/2001, fluid specimen.
	<i>Sternotherus odoratus</i>	ZMB uncatalogued, two fluid specimens.
Platysternidae	<i>Platysternon megacephalum</i>	ZSM 380/2001, fluid specimen.
		ZMB uncatalogued, four fluid specimens.

		Uncatalogued fresh specimen, donated by M. Baur (Reptilienauffangstation München, Munich, Germany) to M. Lambertz.
Testudinidae	<i>Geochelone elegans</i>	ZFMK uncatalogued, fluid specimen.
	<i>Testudo hermanni</i>	Rheinische Friedrich-Wilhelms-Universität Bonn uncatalogued, four fresh specimens.
Trionychidae	<i>Pelodiscus sinensis</i>	PIMUZ lab#2009.72IW, fluid specimen (Supplementary Fig. 1b).
TESTUDINES: PLEURODIRA		
Chelidae	<i>Chelus fimbriatus</i>	ZFMK uncatalogued, fluid specimen
	<i>Emydura subglobosa</i>	USNM 574459, fluid specimen (Fig. 2, b to c).
Podocnemidae	<i>Podocnemis unifilis</i>	Uncatalogued fresh specimen, donated by A. S. Abe to M. Lambertz.
LEPIDOSAURIA: RHYNCHOCEPHALIA		
	<i>Sphenodon punctatus</i>	ZSM 1318/2006, fluid specimen (Fig. 2f).
FOSSIL MATERIAL		
<i>Eunotosaurus africanus</i> :		
	AM 5999:	impression of a nearly complete articulated specimen with an articulated shoulder girdle.
	BP/1/7198:	disarticulated dorsal ribs and vertebrae.
	BP/1/7027:	fragmentary dorsal ribs and vertebrae.
	BP/1/7024:	posterior two thirds of an axial skeleton (Fig. 3c and Supplementary Fig. 4).
	BP/1/6218:	posterior two thirds of an axial skeleton and portions of the pelvis.
	BP/1/5677:	fragmentary dorsal ribs and vertebrae.
	BP/1/3514:	disarticulated dorsal vertebrae and ribs.
	BP/1/3515:	disarticulated dorsal vertebrae and ribs.
	BMNH R 1968 (Holotype):	disarticulated dorsal vertebrae and associated dorsal ribs and associated limb elements.
	BMNH R 4949:	disarticulated dorsal vertebrae and associated dorsal ribs.

BMNH R 4054: nodule containing articulated dorsal vertebrae and ribs.
BMNH R 49424: nodule containing articulated dorsal vertebrae and ribs.
BMNH R 49423: highly eroded nodule containing partial dorsal vertebrae and ribs.
CM 71: articulated dorsal vertebrae and ribs.
CM 86-341: beautifully preserved partial skull, completely articulated neck with a few cervical ribs, and complete carapace (nine dorsal vertebrae and nine pairs of dorsal ribs) (Figs. 3, a to b and Fig. 4, e to j).
CM 775: articulated posterior dorsal vertebrae and ribs.
CM 777: articulated skull, neck, elongate cervical ribs, shoulder girdle, limb elements, and cranial half of carapace including dorsal vertebrae and ribs (Fig. 3d).
NMQR 3299: mostly complete skeleton including an articulated shoulder girdle.
NMQR 3466: isolated dorsal rib.
NMQR 3474: impression of a mostly articulated skeleton.
NMQR 3486: isolated dorsal rib.
NMQR 3490: isolated dorsal rib.
NMQR 3500: isolated dorsal rib.
Fransie Pienaar Museum, Prince Albert, 2014/269: mostly complete skeleton including an articulated shoulder girdle and pelvis.
SAM-PK-K207: plastically deformed, articulated dorsal ribs and vertebrae.
SAM-PK-K1132: mostly complete series of dorsal ribs and vertebrae with partial shoulder girdle.
SAM-PK-K1133: complete shell with articulated shoulder girdle.
SAM-PK-K7611: plastically deformed, articulated dorsal ribs and vertebrae.
SAM-PK-K7670: highly weathered nodule with mostly complete skeleton including cranial two-thirds dorsal ribs and vertebrae, impressions of the cervical vertebrae, and an impression of the skull.
SAM-PK-K7909: weathered nodule complete shell with articulated neck, impression of the skull, and complete shoulder girdle.
SAM-PK-K7910: plastically deformed, articulated series of dorsal ribs and vertebrae.
SAM-PK-K7911: mid-series section of articulated dorsal vertebrae and ribs.
SAM-PK-K4328: impression of back two-thirds of an articulated specimen, including dorsal ribs, vertebrae, partial pelvis, and first few caudal vertebrae.
SAM-PK-K11954: highly weathered nodule containing mid-section with dorsal vertebrae and ribs.
SAM-PK-K1509: mid-series section of articulated dorsal vertebrae and ribs.
SAM-PK-K1673: isolated dorsal rib.
USNM 23099: disarticulated dorsal ribs, vertebrae, limb elements, pelvic girdle, and carpal and tarsal elements.

Odontochelys semitestacea:

IVPP V15639: holotype, complete articulated skeleton.
IVPP V13240: paratype, complete articulated skeleton (Fig. 3, e to f).
IVPP V15653: referred specimen, incomplete disarticulated skeleton.

Proganochelys quenstedti

GPIT (uncataloged holotype): internal mold of shell showing distinct T-shaped
(cross-section) ribs.

SMNS 10012: partial carapace with internal surface and external surface preserved in
separate pieces.

SMNS 15759: crushed skull, mandibles, and right half of shell.

SMNS 16980: nearly complete skeleton.

SMNS 17203: carapace and plastron.

SMNS 17204: carapace and plastron.

Supplementary References:

1. Putz, R., in *Benninghoff Anatomie - Makroskopische Anatomie, Embryologie und Histologie des Menschen, Band 1: Zellen- und Gewebelehre, Entwicklungsbiologie, Bewegungsapparat, Herz-Kreislauf-System, Immunsystem, Atem- und Verdauungsapparat*, (eds Drenckhahn, D. & Zenker, W.) 245–324 (Urban & Schwarzenberg, München-Wien-Baltimore, 1994)
2. Bhullar, B.-A. S. A reevaluation of the unusual abdominal musculature of squamate reptiles (Reptilia: Squamata). *Anat. Rec.* **292**, 1154–1161
3. Drenckhahn, D. in *Benninghoff Anatomie - Makroskopische Anatomie, Embryologie und Histologie des Menschen, Band 1: Zellen- und Gewebelehre, Entwicklungsbiologie, Bewegungsapparat, Herz-Kreislauf-System, Immunsystem, Atem- und Verdauungsapparat*, (eds Drenckhahn, D. & Zenker, W.) 405–470 (Urban & Schwarzenberg, München-Wien-Baltimore, 1994)

Extended Data Table 1: List of taxa from which histological data was obtained

SPECIMEN	ACCESSION NUMBER	HIGHER TAXONOMY	ELEMENTS SAMPLED	ADDITIONAL DATA
Testudines				
<i>Eunotosaurus africanus</i>	CM86-341	stem turtle	dorsal ribs 3-7 (Fig. 4f-j)	Pootjie Member, <i>Pristerognathus</i> Assemblage Zone, Beaufort Group, SA
<i>Eunotosaurus africanus</i>	NHM PV R 4949	stem turtle	dorsal rib 7 (Lyson et al., 2013, Fig. S2)	"Karoo Formation, South Africa"
<i>Eunotosaurus africanus</i>	BP/1/7024	stem turtle	dorsal ribs 5-6, limb elements (Supplementary Fig. 4)	Abrahamskraal Formation, <i>Tapinocephalus</i> Assemblage Zone, Beaufort Group, SA
<i>Eubaena hatcheri</i>	MRF 457	Baenidae	right dorsal ribs 5-6	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Eubaena hatcheri</i>	MRF 486	Baenidae	dorsal ribs 2-3	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Eubaena hatcheri</i>	MRF 443	Baenidae	dorsal ribs 8-9	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Eubaena hatcheri</i>	MRF 345	Baenidae	dorsal ribs 8-9	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Eubaena hatcheri</i>	MRF 7	Baenidae	dorsal rib 2 or 4?	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Chelydra serpentina</i>	USNM 324328	Chelydridae	complete carapace (nuchal, dorsal ribs 2-9, peripheral 1&11) (Fig. 4)	extant
<i>Chelydra serpentina</i>	University of Utah	Chelydridae	complete carapace (nuchal, dorsal ribs 2-9, peripheral 1&11)	extant
<i>Apalone spinifera</i>	YPM HERR 010586	Trionychidae	dorsal rib 7 (Lyson et al., 2013, Fig. S2)	extant
Crocodylia				
<i>Alligator mississippiensis</i>	University of Utah	Alligatoridae	gastralia embedded in abdominal wall musculature (Fig. 5h-j)	extant
<i>Alligator mississippiensis</i>	PIMUZ uncat.	Alligatoridae	dorsal ribs 1-2 with distal calcified cartilage (Fig. 5g)	extant
<i>Gavialis gangeticus</i>	PIMUZ uncat.	Gavialidae	anterior dorsal rib (Fig. 5d-f)	extant
Choristodera				
Champsosauridae indet.	MRF uncat.	Champsosauridae	two dorsal ribs	Hell Creek Fm., Late Cretaceous, North Dakota
Dinosauria				
<i>Thescelosaurus</i> sp.	MRF 254	Hypsilophodontidae	four dorsal ribs (Supplementary Fig. 3a,b)	Hell Creek Fm., Late Cretaceous, North Dakota
Ceratopsidae indet.	MRF uncat.	Ceratopsidae	dorsal rib	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Triceratops</i> sp.	MRF uncat.	Ceratopsidae	dorsal rib	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Edmontosaurus</i> sp.	MRF 566.15	Hadrosauridae	dorsal rib (proximal part)	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Edmontosaurus</i> sp.	MRF 541	Hadrosauridae	dorsal rib	Hell Creek Fm., Late Cretaceous, North Dakota
<i>Edmontosaurus</i> sp.	MRF 695.1	Hadrosauridae	dorsal rib	Hell Creek Fm., Late Cretaceous, North Dakota
Aves				
<i>Maleagris gallopavo</i>	PIMUZ uncat.	Phasianidae	sternal ribs and lateral part of sternum	extant
Mammalia				
<i>Capreolus capreolus</i>	PIMUZ uncat.	Cervidae	dorsal rib of female specimen (Fig. 5a-c)	extant
<i>Odocoileus virginianus</i>	PIMUZ uncat.	Cervidae	three dorsal ribs	extant
<i>Bison bison</i>	PIMUZ uncat.	Bovidae	three dorsal ribs	extant
<i>Cylopes didactylus</i>	YPM MAM 007543	Cyclopedidae	dorsal rib (Lyson et al., 2013, Fig. S2)	extant
Squamata				
<i>Uma scoparia</i>	YPM HERR 010388	Phrynosomatidae	dorsal rib	extant
<i>Scincus mitranus</i>	YPM HERR 010450	Scincidae	dorsal rib	extant
<i>Gecko gecko</i>	YPM HERR 010629	Gekkonidae	dorsal rib	extant
<i>Coleonyx variegatus</i>	YPM HERR 010976	Gekkonidae	dorsal rib	extant
<i>Elgaria multicarinata</i>	YPM HERR 011028	Anguidae	dorsal rib	extant
<i>Norops sagrei</i>	YPM HERR 011048	Polychrotidae	dorsal rib	extant
<i>Furcifer verrucosus</i>	YPM HERR 011101	Chamaeleonidae	dorsal rib	extant
<i>Corucia zebrata</i>	YPM HERR 011160	Scincidae	dorsal rib (Fig. 5k-m)	extant
<i>Furcifer pardalis</i>	YPM HERR 011216	Chamaeleonidae	dorsal rib (Fig. 5n-p)	extant
<i>Hemidactylus bowringii</i>	YPM HERR 011282	Gekkonidae	dorsal rib	extant
<i>Hypsilurus dilophus</i>	YPM HERR 011356	Agamidae	dorsal rib	extant
<i>Teratoscincus microlepis</i>	YPM HERR 012182	Gekkonidae	dorsal rib	extant
<i>Adolfus jacksoni</i>	YPM HERR 013015	Lacertidae	dorsal rib	extant
<i>Phrynosoma platyrhinos</i>	YPM HERR 013377	Phrynosomatidae	dorsal rib	extant
<i>Lialis jicari</i>	YPM HERR 013496	Pygopodidae	dorsal rib	extant
<i>Uromastyx dispar</i>	YPM HERR 013523	Agamidae	dorsal rib	extant
<i>Rhampholeon breviceauda</i>	YPM HERR 013566	Chamaeleonidae	dorsal rib	extant
<i>Chalcides sepsoides</i>	YPM HERR 013593	Scincidae	dorsal rib	extant
<i>Mesaspis moreletii</i>	YPM HERR 013661	Anguidae	dorsal rib	extant
<i>Polychrus marmoratus</i>	YPM HERR 014950	Polychrotidae	dorsal rib	extant
<i>Ophisaurus attenuatus</i>	YPM HERR 016026	Anguidae	dorsal rib	extant
<i>Celestus warreni</i>	YPM HERR 016070	Anguidae	dorsal rib	extant
<i>Leiolepis</i> sp.	YPM HERR 016604	Agamidae	dorsal rib	extant
<i>Corucia zebrata</i>	YPM HERR 016623	Scincidae	dorsal rib	extant
<i>Sceloporus jarrovii</i>	YPM HERR 016655	Phrynosomatidae	dorsal rib	extant
<i>Draco maculatus</i>	YPM HERR 017451	Agamidae	dorsal rib	extant
<i>Aspidoscelis tigris</i>	YPM HERR 017509	Teiidae	dorsal rib	extant
<i>Physignathus lesueurii</i>	YPM HERR 016670	Agamidae	dorsal rib (Lyson et al., 2013, Fig. S2)	extant
<i>Bronchocela cristatella</i>	YPM HERR 017870	Agamidae	dorsal rib	extant
Parareptilia				
<i>Pareiasaurus</i>	BP/1/7280	Pareiasauria	dorsal rib (Supplementary Fig. 3c-h)	Abrahamskraal Formation, <i>Tapinocephalus</i> Assemblage Zone, Beaufort Group, SA
<i>Milleretta rubidgei</i>	BP/1/2040	Millerettidae	dorsal rib	Balfour Formation, <i>Dicynodon</i> Assemblage Zone, Beaufort Group, SA
<i>Pumiliopareia pricei</i>	BP/1/81	Pareiasauria	dorsal rib and osteoderm	Balfour Formation, <i>Dicynodon</i> Assemblage Zone, Beaufort Group, SA