Evolution of the turtle bauplan: the topological relationship of the scapula relative to the ribcage

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The turtle shell and the relationship of the shoulder girdle inside or 'deep' to the ribcage have puzzled neontologists and developmental biologists for more than a century. Recent developmental and fossil data indicate that the shoulder girdle indeed lies inside the shell, but anterior to the ribcage. Developmental biologists compare this orientation to that found in the model organisms mice and chickens, whose scapula lies laterally on top of the ribcage. We analyse the topological relationship of the shoulder girdle relative to the ribcage within a broader phylogenetic context and determine that the condition found in turtles is also found in amphibians, monotreme mammals and lepidosaurs. A vertical scapula anterior to the thoracic ribcage is therefore inferred to be the basal amniote condition and indicates that the condition found in therian mammals and archosaurs (which includes both developmental model organisms: chickens and mice) is derived and not appropriate for studying the developmental origin of the turtle shell. Instead, among amniotes, either monotreme mammals or lepidosaurs should be used.

1. INTRODUCTION

The turtle shell is often regarded as an evolutionary innovation (*sensu* [1]) and the two most commonly cited features unparalleled among other tetrapods are the turtle shell [2–4] and the relationship of the scapula relative to the dorsal ribs [3]. The turtle scapula is regularly referenced as being uniquely located 'inside' the dorsal ribcage [2,5,6], or at least 'deep' to the ribcage [7–9]. This topological relationship, however, is confounded by the fact that the scapula is located inside the turtle *shell*, of which only a portion is formed by the turtle's ribcage, whereas the rest is composed of intramembranously ossified dermis [4]. Indeed, the dorsal ribcage is often equated with the shell [3, p. 3] and the putatively unique relationship between the scapula and the ribcage is used as evidence for turtles

appearing to have broken basic rules of the vertebrate body plan [3]. The turtle shell is therefore regarded as an evolutionary innovation: neither arising from conspicuous changes to the reptile body plan [7,8,10–12] nor from a hopeful monster [9,13].

Recent developmental and fossil evidence have shed considerable light on the scapula's relationship relative to the dorsal ribcage. As was initially noted by Ruckes [14] in 1929 and expanded upon by more recent developmental biologists [2,6–8,15], the turtle scapula does not move posteriorly during development to a position within or deep to the ribcage (contra [16]), but rather the dorsal ribs have a unique dorsolateral growth trajectory into a more superficial position within the dermis. The ribs later spread apart from one another distally to obtain a fan-like arrangement. The dermis finally encapsulates the ribs together with parts of the shoulder girdle [3]. Of note, the fan-like arrangement is exaggerated in the highly derived softshell turtle *Pelodiscus sinensis*, which is used as the primary model organism for understanding the development of the turtle shell [2,15–17]. As in other amniotes, the turtle shoulder girdle maintains its position relative to the muscle plate throughout development via a unique muscle folding mechanism, in some cases maintaining ancestral connectivities and in others creating novel ones [15]. However, the entire pectoral girdle remains anterior to the cartilaginous first dorsal rib throughout ontogeny, and only becomes encapsulated within the *shell* upon intramembranous ossification of the nuchal bone, the dorsal ribs and neural spines of the carapace [18]. Indeed, as has long been noted by skilled anatomists [19], the scapula remains anterior to the first dorsal rib even in adult turtles. The newly discovered stem turtle with a protoshell, *Odontochelys semitestacea* from the early Late Triassic of China, also has a scapula that lies anterior to the ribcage, further supporting the idea that the turtle scapula does not lie *within* the ribcage [3,20].

While it is clear that the scapula of turtles does not lie within the ribcage, its vertical orientation anterior to the ribcage still appears unique. Kuratani *et al.* [3] argue that two factors contribute to the placement of the scapula 'beneath' the ribcage: (i) axial arrest of the ribs, and (ii) the inability of the scapula to form a 'scapular blade' (i.e. a blade-like process that lies on top of the ribcage). While the axial arrest of the dorsal ribs is undoubtedly a key transition in the origin of the turtle carapace [2,3,6–8,16], the putatively unique *topological* position of the turtle scapula has not been critically examined. The slender, rod-like morphology of the turtle scapula is clearly derived, relative to the massive, rectangular shape found in basal reptiles (e.g. *Paleothyris acadiana*, *Petrolacosaurus kansensis*, etc.), but it is unclear whether the topological position anterior to the dorsal ribcage is unique and whether it represents an important transition in the origin of the turtle bauplan [3,7,8]. We analyse the topological position of the scapula in a representative sample of extant and fossil amniotes to determine the basal condition for Amniota. Given the central role that the topological position of the scapula plays in the evolutionary model for the origin of the turtle carapace [3,15], it is important to critically analyse this feature within a broader phylogenetic context.
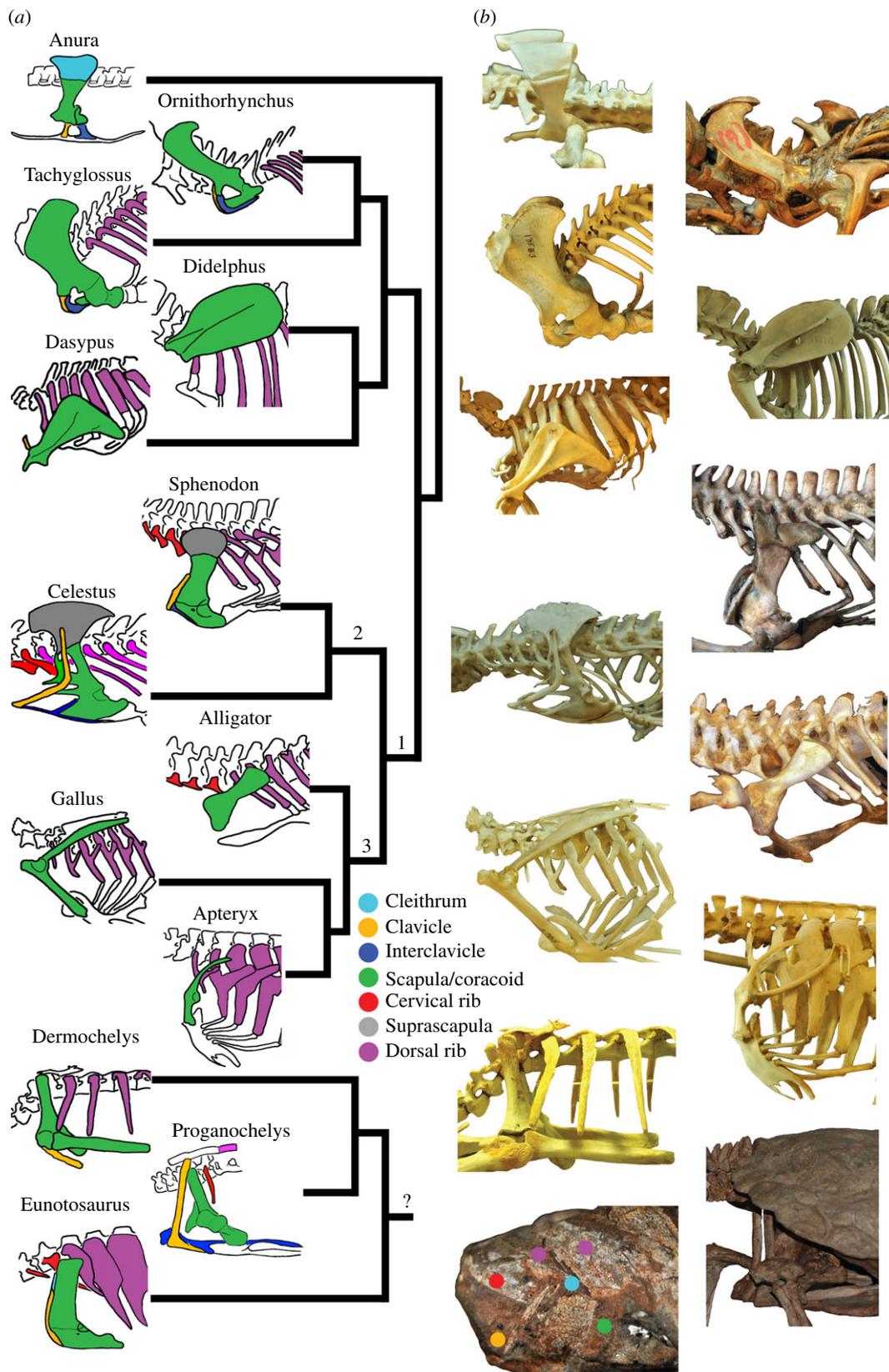
Figure 1. (*a*) Illustrations and (*b*) photographs of the shoulder girdle of taxa representing each of the major amniote clades (Mammalia, Monotremata, Theria, Testudines, Lepidosauria, Squamata, Archosauria, Aves and Crocodylia). The scapula is situated dorsal to the ribcage in therian mammals and archosaurs, including the two developmental model organisms: the house mouse and the domestic chicken. A vertically oriented scapula, anterior to the ribcage is found in turtles, the basal most stem turtle *Eunotosaurus africanus* [21], the stem turtle *Proganochelys quenstedti*, lepidosaurs, monotreme mammals and the amphibian *Rana catesbeiana*, indicating this is the basal amniote condition. The numbers represent the three hypotheses for turtle origins: 1, turtles as sister to diapsids (lepidosaurs + archosaurs) is supported by most morphological [21,22] and developmental data [23]; 2, turtles as sister to lepidosaurs is supported by some morphological [24] and molecular data [25]; 3, turtles as sister to archosaurs is supported by nuclear and mitochondrial genetic data [26].

2. MATERIAL AND METHODS

A large sample of articulated skeletons of extant and extinct amniotes was studied and photographed. Sampling was undertaken to cover the greatest possible tree space in order to allow for reconstruction of ancestral conditions for each major amniote clade (Monotremata and Theria for Mammalia, Cryptodira and Pleurodira for Testudines; *Sphenodon* and Squamata for Lepidosauria; Aves and Crocodylia for Archosauria). Species are included only if the shoulder girdle is preserved in articulation, thereby reflecting as closely as possible the topological position of all elements *in vivo*. A complete list of species used is provided in the electronic supplementary material.

3. RESULTS AND DISCUSSION

The development of extant turtles, as well as the gross morphology of extant and fossil turtles, indicate that the scapula is universally oriented vertically and situated anterior to the ribcage. This position appears to be ancestral for amniotes, as among extant taxa it is found in amphibians, monotreme mammals and lepidosaurs (figure 1), and among fossil taxa is found in early tetrapods (e.g. *Ariekanerpeton sigalovi*), stem amniotes (e.g. *Limnoscelis paludis*) and early reptiles (e.g. *Aerosaurus wellesi*) [27,28]. Scapular blades that cover the dorsal ribs, by contrast, occur convergently in therian mammals and archosaurs and the scapular blades are convergently derived from axial somites [29,30]. Ancestrally, the scapulae of tetrapods are braced by dermal bones: the clavicles, interclavicle and cleithra. This plesiomorphically remains the case in turtles, where the scapula is surrounded by the nuchal, the entoplastron (interclavicle) and the epiplastra (clavicles). In monotreme mammals, the scapulae are strongly braced ventrally by the pre- and post-coracoids, the clavicles and the interclavicle. In therian mammals, by contrast, which typically lack all dermal components of the shoulder girdle, the scapular blade extends onto the ribcage and is stabilized by skeletal muscles (m. serratus anterior (AS), m. rhomboidei and m. levator scapulae [30]). A similar condition is found in archosaurs, where the scapula extends onto the ribcage and is affixed to the ribcage via the AS and the m. levator scapulae-rhomboid complex (*sensu* [15]).

Other 'shelled' amniotes exhibit both topological relationships of the scapula relative to the ribcage, indicating that this character is not correlated with the presence of a shell. Shelled sauropterygian reptiles from the Triassic, such as *Henodus chelyops* or *Sinosaurosphargis yunguiensis*, resemble turtles by having a vertically oriented scapula situated inside the shell, but placed anterior to the ribcage [31,32]. By contrast, therian armadillos or archosaurian ankylosaurs exhibit a scapular blade that is situated on top of the ribcage but underneath the shell.

The realization that a vertically oriented scapula which is situated anterior to the ribcage and that does not overlap the trunk ribs is plesiomorphic for amniotes has important implications for the evolutionary model regarding the origin of the turtle shell. Current hypotheses for the developmental evolution of the turtle shell are largely based on a single model organism, the trionychid *Pelodiscus sinensis*, and comparisons are made only to the development of two other model amniotes: the domestic chicken (an archosaur) and the house mouse (a therian mammal). Both species have long, posterodorsally directed scapular blades that are located on top of the ribcage. Thus, the position of the turtle scapula is seen as a uniquely derived feature, when in fact this topological relationship represents the basal amniote condition from which the conditions in the mouse and chicken later evolved. Although issues of sampling must be taken into consideration, it is apparent that the derived condition found in therian mammals and archosaurs (which includes both developmental model organisms: chickens and mice) is not an appropriate outgroup for studying the developmental origin of the turtle shell. Instead, among amniotes either monotreme mammals or lepidosaurs should be used.

1 Gauthier, J. A., Nesbitt, S. J., Schachner, E. R., Bever, G. S. & Joyce, W. G. 2011 A new bipedal stem crocodilian, early archosaur phylogeny, and innovation in archosaur locomotion. *Bull. Peabody Mus. Nat. Hist.* **52**, 107–126. (doi:10.3374/014.052.0102)

2 Gilbert, S. F., Loredo, G. A., Brukman, A. & Burke, A. C. 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)

3 Kuratani, S., Kuraku, S. & Nagashima, H. 2011 Evolutionary developmental perspective for the origin of turtles: the folding theory for the shell based on the developmental nature of the carapacial ridge. *Evol. Dev.* **13**, 1–14. (doi:10.1111/j.1525-142X.2010.00451.x)

4 Scheyer, T. M., Brullmann, B. & Sánchez-Villagra, M. R. 2008 The ontogeny of the shell in sidenecked turtles, with emphasis on the homologies of costal and neural bones. *J. Morphol.* **269**, 1008–1021. (doi:10.1002/jmor.10637)

5 Gilbert, S. F., Bender, G., Betters, E., Yin, M. & Cebra-Thomas, J. A. 2007 The contribution of neural crest cells to the nuchal bone and plastron of the turtle shell. *Integr. Comp. Biol.* **47**, 401–408. (doi:10.1093/icb/icm020)

6 Cebra-Thomas, J., Tan, F., Sistla, S., Estes, E., Bender, G., Kim, C. & Gilbert, S. F. 2005 How the turtle forms its shell: a paracrine hypothesis of carapace formation. *J. Exp. Zool.* **304B**, 558–569. (doi:10.1002/jez.b.21059)

7 Burke, A. C. 1989 Development of the turtle carapace: implications for the evolution of a novel bauplan. *J. Morphol.* **199**, 363–378. (doi:10.1002/jmor.1051990310)

8 Burke, A. C. 1991 The development and evolution of the turtle body plan. Inferring intrinsic aspects of the evolutionary process from experimental embryology. *Am. Zool.* **31**, 616–627. (doi:10.1093/icb/31.4.616)

9 Rieppel, O. 2001 Turtles as hopeful monsters. *BioEssays* **23**, 987–991. (doi:10.1002/bies.1143)

10 Burke, A. C. 2009 Turtles … again. *Evol. Dev.* **11**, 622–624. (doi:10.1111/j.1525-142X.2009.00369.x)

11 Hall, B. K. 1998 *Evolutionary developmental biology*, 2nd edn. London, UK: Chapman & Hall.

12 Rieppel, O. 2009 How did the turtle get its shell? *Science* **325**, 154–155. (doi:10.1126/science.1177446)

13 Theißen, G. 2006 The proper place of hopeful monsters in evolutionary biology. *Theory Biosci.* **124**, 349–369. (doi:10.1016/j.thbio.2005.11.002)

14 Ruckes, H. 1929 The morphological relationships between the girdles, ribs, and carapace. *Ann. NY Acad. Sci.* **13**, 81–120. (doi:10.1111/j.1749-6632.1929.tb55192.x)

15 Nagashima, H., Sugahara, F., Takechi, M., Ericsson, R., Kawashima-Ohya, Y., Narita, Y. & Kuratani, S. 2009 Evolution of the turtle body plan by the folding and

creation of new muscle connections. *Science* **325**, 193–196. (doi:10.1126/science.1173826)

16 Kuraku, S., Usuda, R. & Kuratani, S. 2005 Comprehensive survey of carapacial ridge-specific genes in turtle implies co-option of some regulatory genes in carapace evolution. *Evol. Dev.* **7**, 3–17. (doi:10.1111/j.1525-142X.2005.05002.x)

17 Nagashima, H., Kuraku, S., Uchida, K., Ohya, Y. K., Narita, Y. & Kuratani, K. 2007 On the carapacial ridge in turtle embryos: its developmental origin, function, and the chelonian body plan. *Development* **134**, 2219–2226. (doi:10.1242/dev.002618)

18 Sánchez-Villagra, M. R., Müller, H., Sheil, C. A., Scheyer, T. M., Nagashima, H. & Kuratani, S. 2009 Skeletal development in the Chinese soft-shelled turtle *Pelodiscus sinensis* (Reptilia: Testudines: Trionychidae). *J. Morphol.* **270**, 1381–1399. (doi:10.1002/jmor.10766)

19 Ogushi, K. 1911 Anatomische Studien an der japanischen dreikralligen Lippenschildkröte (*Trionyx japonicus*). *Morpholog. Jahrbuch* **43**, 1–106.

20 Li, C., Wu, X.-C., Rieppel, O., Wang, L.-T. & Zhao, J. 2009 Ancestral turtle from the Late Triassic of southwestern China. *Nature* **456**, 497–501. (doi:10.1038/nature07533)

21 Lyson, T. R., Bever, G. S., Bhullar, B.-A. S., Joyce, W. G. & Gauthier, J. A. 2010 Transitional fossils and the origin of turtles. *Biol. Lett.* **6**, 830–833. (doi:10.1098/rsbl.2010.0371)

22 Gauthier, J., Kluge, A. G. & Rowe, T. 1988 Amniote phylogeny and the importance of fossils. *Cladistics* **4**, 105–209. (doi:10.1111/j.1096-0031.1988.tb00514.x)

23 Werneburg, I. & Sánchez-Villagra, M. R. 2009 Time of organogenesis support basal position of turtles in the amniote tree of life. *BMC Evol. Biol.* **9**, 82. (doi:10.1186/1471-2148-9-82)

24 Rieppel, O. & deBragga, M. 1996 Turtles as diapsid reptiles. *Nature* **384**, 453–455. (doi:10.1038/384453a0)

25 Lyson, T. R., Sperling, E. A., Heimburg, A. M., Gauthier, J. A., King, B. L. & Peterson, K. J. 2012 microRNAs support a turtle + lizard clade. *Biol. Lett.* **8**, 104–107. (doi:10.1098/rsbl.2011.0477)

26 Hugall, A. F., Foster, R. & Lee, M. S. Y. 2007 Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. *Syst. Biol.* **56**, 543–563. (doi:10.1080/10635150701477825)

27 Laurin, M. 1996 A reevaluation of *Ariekanerpeton*, a Lower Permian seymouriamorph (Tetrapoda: Seymouriamorpha) from Tadzhikistan. *J. Vert. Paleontol.* **16**, 653–665. (doi:10.1080/02724634.1996.10011355)

28 Langston, W. & Reisz, R. R. 1981 *Aerosaurus wellesi*, new species, a varanopseid mammal-like reptile (Synapsida: Pelycosauria) from the Lower Permian of New Mexico. *J. Vert. Paleontol.* **1**, 73–96. (doi:10.1080/02724634.1981.10011881)

29 Huang, R., Zhi, Q., Patel, K., Wilting, J. & Christ, B. 2000 Dual origin and segmental organisation of the avian scapula. *Development* **127**, 3789–3794.

30 Valasek, P., Theis, S., Krejci, E., Grim, M., Maina, F., Shwartz, Y., Otto, A., Huang, R. & Patel, K. 2010 Somitic origin of the medial border of the mammalian scapula and its homology to the avian scapula blade. *J. Anat.* **216**, 482–488. (doi:10.1111/j.1469-7580.2009.01200.x)

31 Rieppel, O. 2002 The dermal armor of the cyamodontoid placodonts (Reptilia, Sauropterygia): morphology and systematic value. *Fieldiana Geol.* **46**, 1–41.

32 Li, C., Rieppel, O., Wu, X.-C., Zhao, L.-J. & Wang, L.-T. 2011 A new Triassic marine reptile from southwestern China. *J. Vert. Paleontol.* **31**, 303–312. (doi:10.1080/02724634.2011.550368)