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Homology of the enigmatic nuchal bone reveals novel reorganization of the shoulder girdle in the evolution of the turtle shell

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SUMMARY The turtle shell represents a unique modification of the ancestral tetrapod body plan. The homologies of its approximately 50 bones have been the subject of debate for more than 200 years. Although most of those homologies are now firmly established, the evolutionary origin of the dorsal median nuchal bone of the carapace remains unresolved. We propose a novel hypothesis in which the nuchal is derived from the paired, laterally positioned cleithra—dorsal elements of the ancestral tetrapod pectoral girdle that are otherwise retained among extant tetrapods only in frogs. This hypothesis is supported by origin of the nuchal as paired, mesenchymal condensations likely derived from the neural crest followed by a unique two-stage pattern of ossification. Further support is drawn from the establishment of the nuchal as part of a highly conserved “muscle scaffold” wherein the cleithrum (and its

evolutionary derivatives) serves as the origin of the *Musculus trapezius*. Identification of the nuchal as fused cleithra is congruent with its general spatial relationships to other elements of the shoulder girdle in the adult morphology of extant turtles, and it is further supported by patterns of connectivity and transformations documented by critical fossils from the turtle stem group. The cleithral derivation of the nuchal implies an anatomical reorganization of the pectoral girdle in which the dermal portion of the girdle was transformed from a continuous lateral-ventral arc into separate dorsal and ventral components. This transformation involved the reduction and eventual loss of the scapular rami of the clavicles along with the dorsal and superficial migration of the cleithra, which then fused with one another and became incorporated into the carapace.

INTRODUCTION

The turtle shell stands out as one of the most distinctive and morphologically novel structures in all of Vertebrata. Homology of the approximately 50 distinct bones comprising the turtle shell has been a source of vigorous debate throughout the history of comparative biology (Geoffroy Saint-Hilaire 1809; Meckel 1824; Cuvier 1825; Vallén 1942; Scheyer et al. 2008). Much consensus has emerged. The costal and neural series of the carapace are compound structures that include components preformed in cartilage (i.e., endochondral) considered homologous to dorsal ribs and vertebrae, respectively, plus an extensive neomorphic intramembranous portion (i.e., bone deposited directly in the fibrous dermis; Fig. 1) (Cuvier 1800; Scheyer et al. 2008). The wholly intramembranous peripherals and

pygals are accepted as neomorphs unique to turtles (Rathke 1848; Ivashchenko 1987). These structures do not ossify within scales and thus are not considered osteoderms. The entoplastron and epiplastra are derived from dermal components (interclavicle and clavicles, respectively) of the ancestral tetrapod shoulder girdle (Owen 1849; Vallén 1942; Cherepanov 1997; Gilbert et al. 2001). Like the costals and neurals of the carapace, the entoplastron and epiplastra have an additional neomorphic component of intramembranous bone. The remaining plastral elements are wholly intramembranous and may be homologs of the gastralia of ancestral tetrapods (Fig. 1) (Gilbert et al. 2007).

The most enigmatic element is the anterior median nuchal of the carapace (Fig. 1). This bone has been homologized with several different elements of the ancestral tetrapod skeleton, but

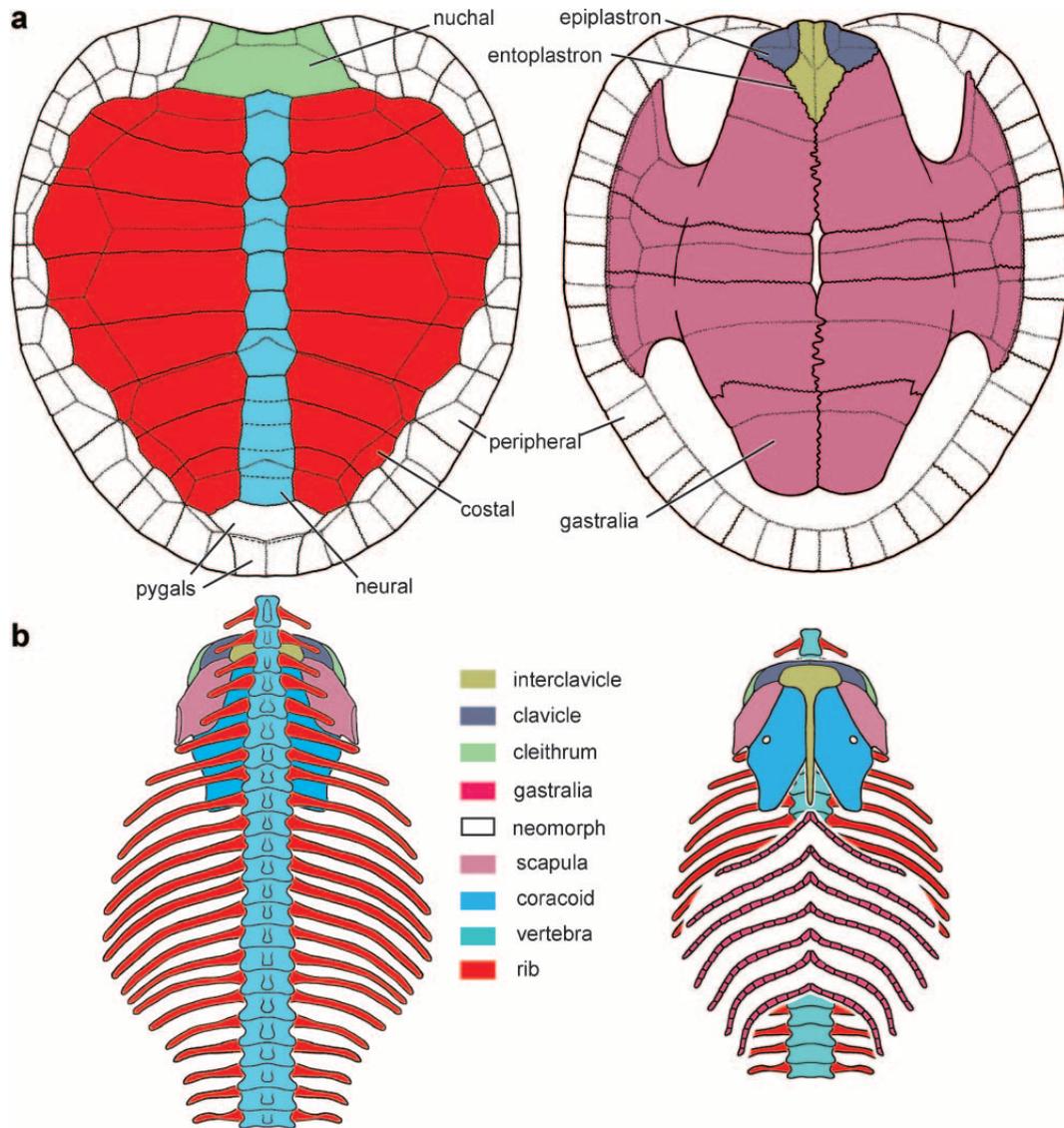


Fig. 1. Individual homology hypotheses for the approximately 50 bones found in the turtle shell based on comparison with the ribs, vertebrae, and shoulder girdle of an early amniote. The addition of unique intramembranous ossification (i.e., bone deposited directly in the fibrous dermis) to each of the bones found in the turtle shell, and their accordingly modified morphology, resulted in turtle-specific names for all of these bones. (a) Turtle shell in dorsal (left) and ventral (right) views based on the stem turtle *Kayentachelys aprix*. (b) Early amniote vertebrae, ribs, and shoulder girdle in dorsal (left) and ventral (right) views loosely based on the stem amniote *Solenodonsaurus janenschii* (Laurin and Reisz 1999).

none of these hypotheses has proved satisfactory. Based on its mid-line position dorsal to the thoracic vertebrae, early assessments of the nuchal concluded that it represents a modified vertebral neural spine (Geoffroy Saint-Hilaire 1809; Meckel 1824; Cuvier 1825). Subsequent workers who studied the highly modified nuchals of snapping turtles, which bear rib-like (i.e., costiform processes) processes, argued that the nuchal was part of the eighth cervical rib (Baur 1887) or a modified “costoneural” plate (Boulenger 1889). Vallén (1942; also see Nilsson 1945) analyzed the early development of turtles and

noted that the nuchal forms from paired anlagen. Based on this observation, as well as his acceptance of Jaekel’s (1915) proposed homology of the dorsal processes of the epiplastra with the cleithra, he argued that the nuchal was homologous with the supracleithra, dermal shoulder girdle elements no longer considered present in any crown tetrapod (e.g., Vorobyeva and Schultze 1991; Ahlberg et al. 2008). Currently, the most widely accepted hypothesis is that, like the pygals and peripherals, the nuchal is neomorphic (Rathke 1848; Ivashchenko 1987; Scheyer et al. 2008). Unlike the peripherals and pygals, however, the

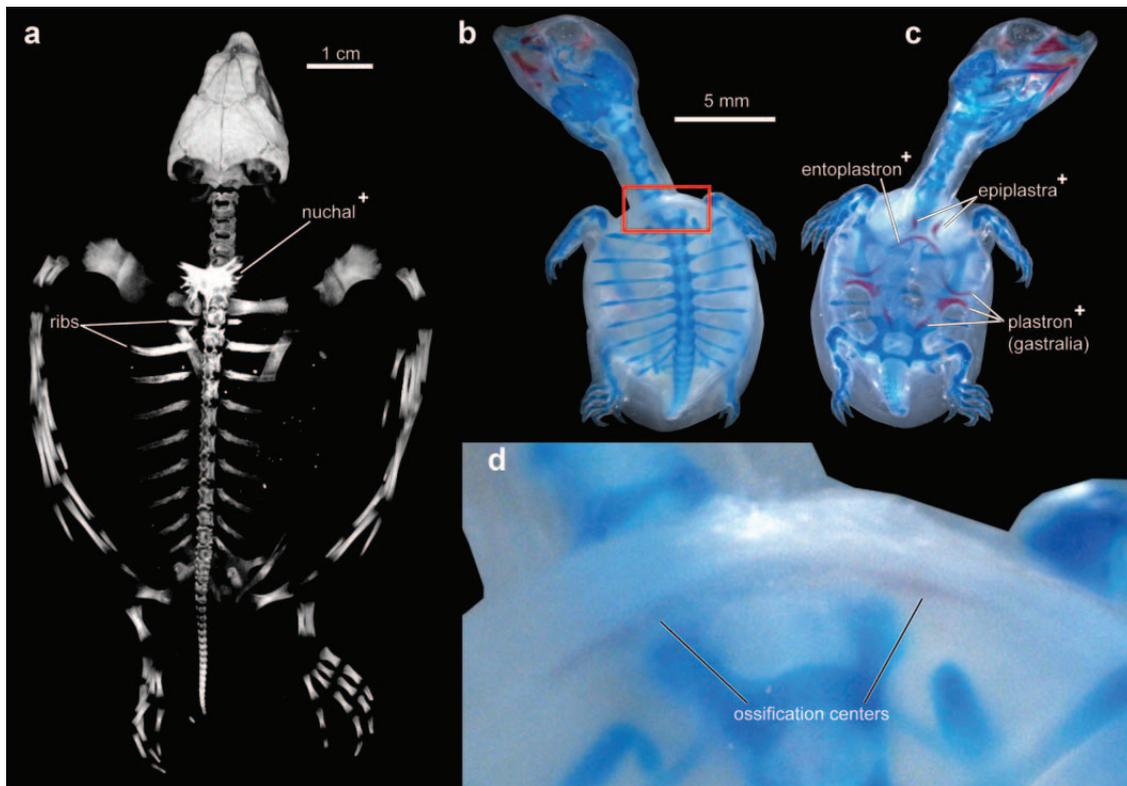


Fig. 2. The nuchal bone is never lost in turtles and its development is unlike that of any other bone in the carapace but is similar to that of the dermal shoulder girdle. (a) Computed tomography scan of a hatchling leather back turtle (*Dermochelys coriacea*) showing the loss of the intramembranous bone in the neural and costal bones, as well as the loss of all the pygal and peripheral bones, but the presence of the nuchal bone. (b and c) *Pelodiscus sinensis* embryos at Tokita and Kuratani (TK) (Tokita and Kuratani 2001) stage 21 showing the paired mesenchymal condensations and ossification centers not found in any other carapacial bone, but found in other neural crest derived bones of the shoulder girdle (Sánchez-Villagra et al., 2009). (c) Close-up of the nuchal region from *P. sinensis* showing the paired mesenchymal condensations and ossification centers. The nuchal ossifies shortly after the epiplastron (clavicle) and entoplastron (interclavicle), but long before the pygals and peripherals (Sánchez-Villagra et al. 2009). + denotes bones inferred to be, at least in part, derived from the neural crest (Clark et al. 2001; Gilbert et al., 2007).

nuchal is never lost phylogenetically. Trionychid turtles have lost the peripherals and pygals, but retain the nuchal. Leatherback sea turtles, *Dermochelys coriacea*, lack the peripherals, pygals, and all the intramembranous contributions to the costal and neural series but retains the nuchal (Fig. 2a). This disparate evolutionary fate suggests the nuchal is part of a different developmental network.

Given the problems with all previously proposed hypotheses concerning the homology of the nuchal, we reconsidered this question by examining diverse sources of data, both existing and new. We ultimately reject previous hypotheses and based on multiple lines of evidence outlined below we propose a novel hypothesis: that the nuchal is a derived form of the cleithra, paired dorsal elements of the dermal shoulder girdle in ancestral tetrapods, and reflects the evolutionary dorsomedial migration and fusion of those elements. We provide corroboration for this hypothesis by integrating developmental data with observations of muscle connectivity, topology, and innervation, as well as the

fossil record. Derivation of the nuchal bone from ancestrally paired cleithra indicates that the dermal shoulder girdle underwent a novel and extensive reorganization along the turtle stem lineage involving a polarization into dorsal and ventral components through the incorporation of different elements into the carapace and plastron.

Development

The development of the nuchal distinguishes it from all other carapacial bones, including the peripherals and pygals. First, immunological data (cell lineage analysis has not yet been done on turtle embryos) strongly support the neural crest origin of the nuchal, an origin shared with the plastral bones, including the epiplastra and entoplastron (Fig. 2) (Clark et al. 2001; Gilbert et al. 2007), homologs of the clavicles and interclavicle in the shoulder girdle of ancestral tetrapods (Fig. 1). Second, unlike the other midline carapacial elements, the nuchal develops from

paired mesenchymal condensations each of which contains a separate ossification center (Fig. 2b and d) (Vallén 1942; Sánchez-Villagra et al. 2009). This paired beginning is absent from the ontogenies of the midline neurals and pygals and is congruent with an interpretation of the nuchal as reflecting the evolutionary fusion of what were once paired elements. The paired beginning of the nuchal was first observed by Vallén (1942) and led him to conclude the nuchal was homologous with the supracleithra. Third, these ossification centers first appear in the embryo shortly after those of the epiplastra and entoplastron, and well before the strictly post-hatching appearance of the other intramembranous carapacial bones (Fig. 2b and c) (Sánchez-Villagra et al. 2009). Finally, the nuchal ossifies in a distinctive two-stage pattern characterized by bilateral calcium deposition within an initially thin bar of condensed cells in the dermis, followed by a posterolateral expansion of a loose lattice-work of bone that maintains topographical contiguity with the scapula in adults (Gilbert et al. 2007; Sánchez-Villagra et al. 2009). These characteristics are shared, not with the peripherals, pygals or any other carapacial element, but with the plastral bones, including the girdle-derived epiplastra and entoplastron, homologs of the dermal clavicles and interclavicle of the ancestral tetrapod shoulder girdle (Fig. 1) (Matsuoka et al. 2005). Thus, there is a striking lack of distinctive developmental features shared by the nuchal and other carapacial bones.

Instead, several of the above-mentioned features of the nuchal are shared not with other carapacial bones but with dermal elements of the shoulder girdle and suggest a common evolutionary origin. The widely accepted identification of the turtle epiplastra and entoplastron as modified clavicles and interclavicle, respectively, leaves the cleithra as the only potential homolog for the nuchal among the dermal shoulder girdle elements. The only extant tetrapods previously considered to retain cleithra as separate elements are frogs (Shearman 2005; Matsuoka et al. 2005), however, cleithra were present in members of stem lineages of caecilians, salamanders, mammals, and diapsids (Matsuoka et al. 2005). In addition, *Eumotosaurus africanus*, the initial description of which as a possible turtle relative (Seeley 1892; Watson 1914) has been corroborated by recent morphological, histological, and phylogenetic analyses, has cleithra (Lyson et al. 2010, in press; Lyson and Joyce 2012; Supplementary Material).

Muscle connectivity

Muscle connectivity is an important criterion for establishing homology (Owen 1843). Indeed, a recent article formalized this approach by performing cell lineage analyses of the embryogenesis of the shoulder girdle and craniocervical regions in osteichthyans (Matsuoka et al. 2005). That study identified a population of neural crest cells interpreted as the “ghost” of the cleithrum within the scapular spine and associated connective tissue of mammals (Matsuoka et al. 2005). The inference of

homology between this cell population and the cleithrum was based on the postulate that muscle attachments in the craniocervical region are highly conserved. For example, in all osteichthyans (including tetrapods) that retain the cleithrum, this bone serves as the origin of the *Musculus trapezius (cucullaris)*—a muscle that inserts on the back of the skull and is innervated by the vagus and spinal accessory nerves (Matsuoka et al. 2005). The *Musculus sternocleidomastoideus*, which also is innervated by the vagus and accessory nerves and inserts on the back of the skull, originates from the clavicle and interclavicle. Thus, this phylogenetically highly conserved “scaffold” of branchial (gill-arch derived) muscle connections and innervations predicts that the *M. sternocleidomastoideus* will originate on the turtle epiplastra (clavicles) and entoplastron (interclavicle) and insert on the back of the skull. If the epiplastra and entoplastron conserve the origins of the *M. sternocleidomastoideus*, then a positive control is established for testing the homology of the turtle nuchal and tetrapod cleithra based on the origin of the *M. trapezius*.

The highly modified body plan of turtles has led to anatomists giving unique, turtle-specific names not only to each bone in the shell (Fig. 1) but also to many of the muscles that attach to the shell (Table 1). In contrast, we first identified the *M. sternocleidomastoideus* and *M. trapezius* based on their superficial positions within a distinctive fascial layer, their oblique relationships to the deeper muscles of the neck, their insertions, and their innervation (Dioga et al. 2008). To avoid circularity in our argument, we did not identify these muscles based on their originations (Fig. 3). The neck musculature of seventeen extant turtle species that phylogenetically bracket the cryptodire, pleurodire, and turtle crowns were dissected with the specific aim of finding the *M. sternocleidomastoideus* and *M. trapezius* using the criteria outlined above (see Supplementary Information for list of turtles dissected).

In agreement with numerous previous authors (see Table 1), we identified two paired superficial muscles, lying in their own fascial layers, cross cutting the deeper neck muscles, originating from the nuchal and epiplastra/entoplastron, and inserting on the back of the skull. However, unlike previous authors who gave these muscles turtle-specific names (Table 1), we propose their homology with the *M. trapezius* and *M. sternocleidomastoideus*. The *M. sternocleidomastoideus* was identified in all examined specimens as a ventrally positioned (Fig. 3a–c), superficial muscle surrounded by the inner investing layer of deep cervical fascia (confirmed in serial sections of *Chrysemys picta* [MCZ H. E.C 1096]; Fig. 3b) and oriented obliquely to the deeper cervical muscles (Fig. 3a, c, and f), and innervated by the spinal accessory nerve (Table 1; Fig. 3a) (Bojanus 1819–1821). The strap-like sternocleidomastoid in turtles inserts on the squamosal and/or parietal at the back of the skull (Fig. 3c and e) and, as predicted by current hypotheses of shell homology, originates from the epiplastra (clavicles) and entoplastron (interclavicle) (Fig. 3c). A superficial muscle identified here as the *M. trapezius* was present

Table 1. List of names used by previous authors for the *M. trapezius* and *M. sternocleidomastoideus* (n.a. = not applicable)

	<i>M. trapezius</i>	<i>M. sternocleidomastoideus</i>
Bojanus (1819–1821)	Splenius capitis	<i>Sternomastoideus</i>
Meckel (1828)	“Kappenmuskel” (i.e., <i>M. trapezius</i>)	<i>Sternomastoideus</i>
Rathke (1848)	Cucullaris	n.a.
Fürbringer (1874)	n.a.	<i>Capiti-plastralis</i>
Ogushi (1911)	n.a.	<i>M. plastro-squamosus</i>
Vallois (1922)	<i>M. testo-capitis medialis</i>	n.a.
George and Shah (1954)	n.a.	<i>Rectus capitis</i>
George and Shah (1955)	n.a.	<i>Rectus capitis cervico-plastralis</i>
Shah (1963)	<i>testocapitis</i>	<i>Rectus capitis cervico-plastralis</i>
Schumacher (1973)	n.a.	<i>M. plastro-squamosus</i>
Yeow and Peterson (1986)	<i>Testo-capitis</i>	<i>Plastro-squamosus</i>
Herrel et al. (2008)	<i>m. testocapitis</i>	n.a.

in turtles of all examined clades except Trionychidae (where its absence is most parsimoniously inferred as a derived loss). The muscle is invested in its own fascia (Fig. 3b, c, e, f, and g) and runs obliquely to the deeper *serratus* group for much of its length (Fig. 3c, f, and g). Corroborating its identification as the trapezius, this muscle is innervated by the intricately intertwined vagus/spinal accessory complex (Chase and Ranson 1914), as well as cervical nerves III, IV, VI, VII, and VIII (Fig. 3a) (Bojanus 1819–1821). The insertion of the turtle trapezius lies consistently along the parietal and/or squamosal, and its origin resides along the anteroventral surface of the nuchal (Fig. 3c, d, f, and g).

The patterns of muscle connectivity and innervation support the accepted hypotheses that the epiplastron and entoplastron of the turtle shell are derived, respectively, from the clavicles and interclavicle of ancestral tetrapods. It also provides telling support for the hypothesis that the nuchal is a modified form of the originally paired cleithra, which have fused and, like the clavicles and interclavicle, become incorporated into the shell.

Skeletal topography and transitional fossils

Finally we used similarity in topography (Remane 1952) and the fossil record to test our nuchal/cleithra hypothesis. In addition to serving as the single point of origination for the *M. trapezius* (Matsuoka et al. 2005), the cleithrum in early amniotes is characterized topographically by its superficial position along the anterodorsal margin of the scapula and by its ventral contact with the scapular ramus of the clavicle (Fig. 4). The nuchal of crown turtles exhibits neither of these contacts, although the general spatial relationships—superficial, anterodorsal to the scapula, and dorsal to the clavicle (epiplastron)—are the same. In crown turtles, loss of contact with the clavicle is the result of loss of the clavicle’s scapular ramus (ascending process of the epiplastron). We examined species from the turtle stem group

(see Supplementary Information for list of examined specimens) (Joyce et al. 2006; Lyson et al. 2010) to determine whether the known fossil record preserves the morphological transformations required if the nuchal is homologous with the cleithrum of early amniotes.

Like other extinct crown amniotes that possess a cleithrum, the putative stem turtle *Eunotosaurus africanus* (see Lyson et al. 2010, in press; Lyson and Joyce 2012) preserves a small splint-like cleithrum in contact with the anterodorsal portion of the scapula and with a small ventral contact with the underlying rod-like scapular ramus of the clavicle (Fig. 4). Of the three known specimens of the oldest uncontroversial stem turtle, *Odontochelys semitestacea*, only one is preserved in dorsal view. Unfortunately, this specimen is flattened and the anterior margin of the shell and shoulder girdle are jumbled together, making it impossible to determine the presence or absence of either an ancestral small, splint-like cleithrum like that of *E. africanus* or the derived cleithrum (nuchal) of crown turtles (Li et al. 2008; Rieppel 2013) or an intermediate morphology. However, it is clear that *O. semitestacea* exhibits a strong pillar-like scapular ramus of the clavicle (i.e., ascending process of the epiplastron) found in more crown-ward stem turtles such as *Proganochelys quenstedti* and *Proterochersis robusta*. The nuchal region in *P. quenstedti* and *P. robusta* lies dorsal to the clavicles, contacts the scapula ventrally, and exhibits contact with the scapular ramus of the clavicle (Gaffney 1990), which are expected if the nuchal is homologous with the cleithrum. In the more crown-ward stem turtle, *Kayentachelys aprix* (we follow the phylogenetic inference of Joyce 2007; Sterli and Joyce 2007; Sterli 2008, 2010; Sterli and de la Fuente 2011; Anquetin 2012, which places *Kayentachelys* outside of the crown, but see Gaffney and Jenkins 2010 for an alternative placement), the scapular ramus of the clavicle is reduced so that it no longer contacts the nuchal (Joyce et al. 2006; Joyce 2007). Thus, the known fossil record preserves, to a remarkable degree,

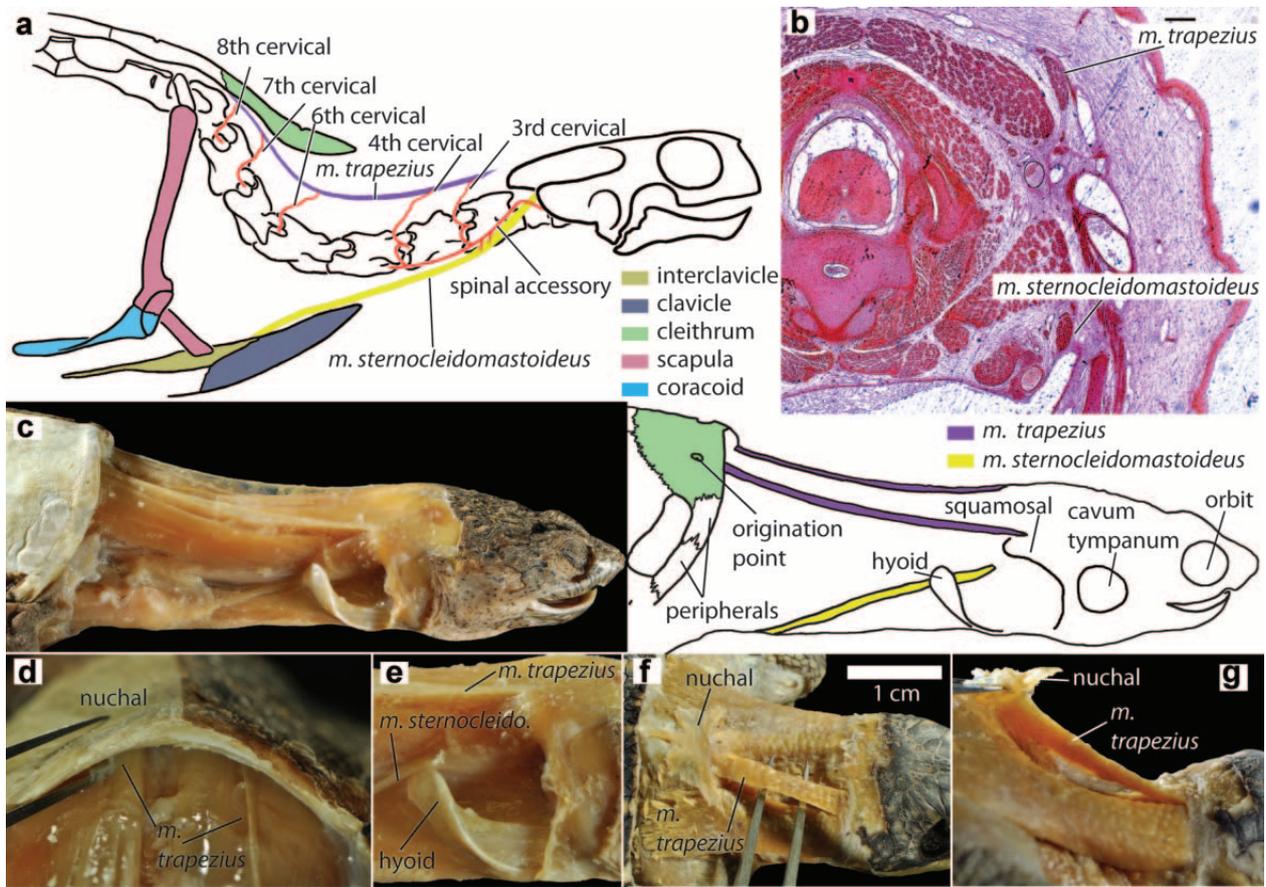


Fig. 3. The turtle *M. sternocleidomastoideus* and *M. trapezius* are identified based on: (1) their innervation by the spinal accessory nerve and cervical nerves III, IV, VI, VII, and VIII; (2) their superficial position within a distinctive fascial layer; and (3) their oblique relationships to the deeper muscles of the neck (Dioga et al. 2008), and not on the topology of their origins and insertions. (a) As in other osteichthyans, the turtle trapezius is innervated by the spinal accessory nerve and cervical nerves III, IV, VI, VII, and VIII, whereas the turtle sternocleidomastoid is innervated by the spinal accessory nerve. (b) Thin section from a 27 mm embryo of *Chrysemys picta* showing the superficial position within a distinctive fascial layer of the trapezius and sternocleidomastoid. (c–g) Dissections of 17 turtles reveals that muscles meeting these criteria are present in both cryptodires (*Chelydra serpentina* (c–e) and *Dermochelys coriacea* (f and g) and pleurodires. (d) Close-up of the shell of *C. serpentina* in anterior view showing the origination of the *M. trapezius* on the nuchal bone. (e) Close-up of the posterior skull of *C. serpentina* in lateral view showing the insertion of the *M. trapezius* and *M. sternocleidomastoideus* on the back of the skull along the parietal/squamosal suture. (f) Dorsal view of the neck of *Dermochelys coriacea* showing the superficial position of the *M. trapezius*, which runs obliquely to the deeper neck muscles, originates from the nuchal bone and inserts on the back of the skull along the parietal/squamosal suture. (g) Close-up of the neck of *D. coriacea* in lateral view with the nuchal bone lifted up to show the origination of the *M. trapezius* on the anteroventral surface of the nuchal bone.

the transformations required to isolate the cleithra (nuchal) above from the other dermal elements, particularly the clavicles (epiplastra), of the pectoral girdle, to arrive at the morphology diagnostic of the skeleton of crown turtles.

DISCUSSION

Neural crest origin, paired mesenchymal precursors and ossification centers, timing of ossification, muscle connectivity, topography relative to other skeletal elements, and transitional fossil morphologies all support the hypothesis that the unpaired

median nuchal of the postembryonic turtle carapace is derived from the paired cleithra of ancestral tetrapods. Consilience among diverse datasets is viewed as strong support for this seemingly radical hypothesis. This level of evidentiary support is comparable to that supporting the homology of turtle epiplastra and entoplastron with the clavicles and interclavicle, respectively—a hypothesis that has not been seriously questioned because its initial proposal by Gegenbaur (1898) more than a century ago. These data outweigh the evidence supporting the recent hypothesis that cleithra are retained in stem turtles as the “epiplastral processes,” which was based largely on a putative articular connection between these structures and the epiplastra

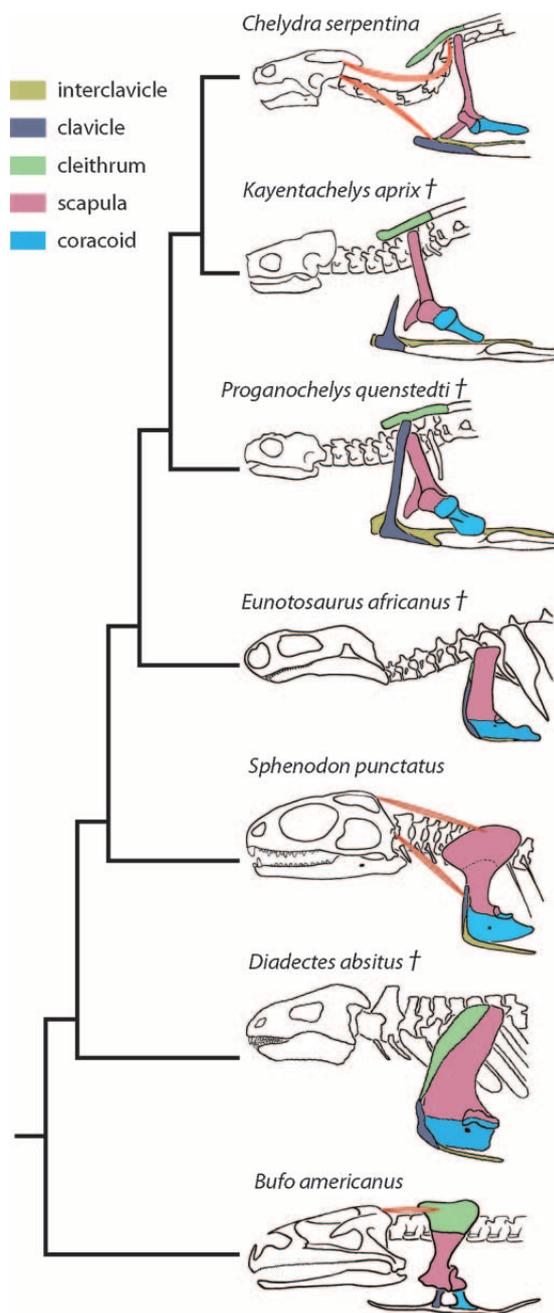


Fig. 4. Tetrapod phylogeny showing the topology and both the skeletal and muscle (for extant species only) connectivity for each of the shoulder girdle bones. While the topology and skeletal connectivity of the cleithrum in crown turtles is markedly different than that in other amniotes, the fossil record preserves most transformations required to isolate the cleithrum above from the other dermal skeletal elements of the pectoral girdle below, as in the skeleton of crown turtles. † denotes extinct species. See Supplementary Information regarding cleithra in *Eunotosaurus africanus*.

in a single fossil specimen of *Kayentachelys aprix* (Joyce et al. 2006). We herein interpret the “articular” surface as the point of origination for the *m. sternocleidomastodeus*. The remaining *K. aprix* specimens have no sign of a suture between the epiplastra and the reduced dorsal process of the clavicle (Joyce et al. 2006), indicating the process is not a cleithrum (contra Joyce et al. 2006), but rather part of the clavicle, the ascending process of the epiplastron (see Gaffney 1990 for a similar interpretation). In addition, the data provided herein constitute multiple lines of evidence corroborating an alternative to the hypothesis that the nuchal is a neomorphic element, a conclusion that should only be invoked when no evidence of homology with known elements exists. It is not surprising that the evolutionary relationship between the turtle nuchal and tetrapod cleithrum was long overlooked, given the extensive nature of the transformation (dorsal and medial migration, incorporation into the shell, fusion of paired lateral ossifications to form a single median element). The relationship was also likely obscured by the unique body plan of turtles, which made anatomists reluctant to hypothesize homologies for many different anatomical features and thus to give those features turtle-specific names (as e.g., see Table 1 for a list of the names that have been applied to the turtle *M. trapezius* and *M. sternocleidomastodeus*). However, despite the unique body plan of turtles, many of their anatomical features retain topographical relationships, connections, innervations, etc. from more general vertebrate and tetrapod body plans that provide evidence of their evolutionary origins, and the nuchal is no exception.

The homology of the nuchal with the cleithra bears upon the contentious issue of turtle relationships (see Lyson and Gilbert 2009 for a summary). Several datasets each strongly support three different hypotheses of turtle relationships: turtles as sister to diapsids (lepidosaurs + archosaurs) is supported by most morphological (Gauthier et al. 1988a,b; Laurin and Reisz 1995; Lee 1995, 2001; Lyson et al. 2010) and developmental data (Werneburg and Sánchez-Villagra 2009), turtles as sister to lepidosaurs is supported by some morphological (Rieppel and deBraga 1996; deBraga and Rieppel 1997; Hill 2005) and microRNA data (Lyson et al. 2012), and turtles as sister to archosaurs is supported by nuclear and mitochondrial nucleotide sequence data (Hugall et al. 2007; Shen et al. 2011; Crawford et al. 2012; Chiari et al. 2012). Taken at face value, the presence of a cleithrum in turtles is most parsimoniously explained by the hypothesis that turtles diverged from other reptiles before the origin of crown diapsids, all of which lack this element (Lyson et al. 2010). Alternatively, if turtles are crown diapsids (i.e., sister either to archosaurs or to lepidosaurs), then either early members of the archosaur and lepidosaur stem groups possessed cleithra that remain undiscovered, or cleithra re-evolved in the stem lineage of turtles.

Homology of the nuchal with the cleithra of ancestral tetrapods reveals a unique anatomical and developmental

transformation of the pectoral girdle in turtles. Dorsal migration and eventual fusion of the cleithra, along with loss of the scapular rami of the clavicles, resulted in wide separation of the cleithra and clavicles, elements that were once intimately associated. The result is an anatomical configuration in which the neural crest-derived, intramembranous elements of the shoulder girdle bracket the endochondral elements dorsally and ventrally and form the anterior portion of the shell. The incorporation of the formerly deep dermal shoulder girdle elements into a superficially situated protective shell is unique to turtles among amniotes. This arrangement is all the more striking in that it is unusual for structures to migrate from one mesenchyme-derived connective tissue or fascial layer to another. Most evolutionary transformations involve the folding or accommodation of those layers instead, as seen in some of the deeper musculature of the turtle shoulder girdle (Nagashima et al. 2009). The ancestral tetrapod cleithrum is embedded within trunk musculature, with muscular attachments to both its deep and superficial surfaces (Ecker and Haslam 1889), as are the clavicle and interclavicle, which lie deep to the superficial slip of the *m. rectus abdominis* containing the gastralia (from which the remainder of the turtle plastron is derived) (Byerly 1925). In turtles, these bones are part of the shell and lie just below the skin with no intervening muscle or extra connective tissue layers. In addition, in contrast to the U-shaped configuration of the dermal elements of the ancestral tetrapod shoulder girdle, those of turtles are distinctly separated into dorsal and ventral components. Evolutionarily, separation was achieved by the reduction and eventual loss of the scapular rami of the clavicles, with the remainder moving superficially and becoming incorporated into the carapace; additionally, the cleithra migrated dorsally and superficially before fusing with one another and becoming incorporated into the carapace.

This extensive transformation is an evolutionary innovation fundamental to the formation of the unique morphology of the shell of turtles. The split girdle of turtles represents a reorganization of the functionally integrated shoulder girdle that is also part of a key evolutionary innovation fundamental to the origin and adaptive success of the turtle radiation. Its importance is indicated by the persistence and global distribution of the complete turtle shell including girdle elements since the early Mesozoic (Gauthier et al. 2011).

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Supplementary Material

Additional supporting information may be found in the online version of this article from the publisher's web-site.

Figure S1. Photographs of *Eunotosaurus africanus* (SAM K 1133) in dorsal (top left) and lateral (top right) with a close-up (bottom right) of the shoulder girdle region showing the vertical nature of the scapula rostral to the dorsal ribs. A small, but distinct, cleithrum is also present.