

Transitional fossils and the origin of turtles

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The origin of turtles is one of the most contentious issues in systematics with three currently viable hypotheses: turtles as the extant sister to (i) the crocodile–bird clade, (ii) the lizard–tuatara clade, or (iii) Diapsida (a clade composed of (i) and (ii)). We reanalysed a recent dataset that allied turtles with the lizard–tuatara clade and found that the inclusion of the stem turtle *Proganochelys quenstedti* and the ‘parareptile’ *Eumotosaurus africanus* results in a single overriding morphological signal, with turtles outside Diapsida. This result reflects the importance of transitional fossils when long branches separate crown clades, and highlights unexplored issues such as the role of topological congruence when using fossils to calibrate molecular clocks.

Keywords: turtle; Diapsida; molecular clock; transitional fossil; *Eumotosaurus africanus*; *Odontochelys semitestacea*

1. INTRODUCTION

The position of turtles among amniotes remains one of the oldest and most contentious problems in vertebrate systematics. Three hypotheses are viable (figure 1): turtles are the extant sister to (i) the crocodile–bird clade (Cao *et al.* 2000; Hugall *et al.* 2007), (ii) the lizard–tuatara clade (Rieppel & deBragga 1996; deBraga & Rieppel 1997; Rieppel & Reisz 1999; Li *et al.* 2009), or (iii) Diapsida (Gauthier *et al.* 1988; Lee 1997, 2001; Lee *et al.* 2008; Werneburg & Sánchez-Villagra 2009). The first hypothesis is supported by most molecular-based analyses; the others are derived from morphological studies that include the wide range of fossils seldom available to molecular systematists. The seemingly disparate nature of the morphology-based trees has led some to dismiss morphological data in favour of the more consistent molecular signal (Hedges & Poling 1999; Tsuji & Müller 2009).

2. MATERIAL AND METHODS

We reanalysed the most recent version of the morphological dataset that supports turtles as the extant sister to the lizard–tuatara clade (Li *et al.* 2009). Two extinct species—the unambiguous stem turtle *Proganochelys*

quenstedti and the ‘parareptile’ *Eumotosaurus africanus*, long considered a possible close relative of turtles (Watson 1914)—were added to the otherwise unaltered dataset (see the electronic supplementary material for material analysed and character scorings for these two species). We performed a maximum parsimony analysis on the augmented dataset using PAUP 4.0b10 (Swofford 2001) with all characters unordered and unweighted as in the original analysis (Rieppel & deBragga 1996). ‘Seymouriidae’ and ‘Diadectomorpha’ were specified as the outgroup taxa and a heuristic search using the tree-bisection-reconnection algorithm was used. Minimum branch lengths were set to collapse. A second analysis included seven additional characters (see electronic supplementary material for character definitions and character scorings) and was analysed with the same parameters outlined above. Support for the *Eumotosaurus*–turtle node was measured by calculating Bremer support values (Bremer 1994) and bootstrap frequencies (Felsenstein 1985), with 10 000 bootstrap replicates and 100 random sequence addition replicates.

Given that both major amniote matrices (e.g. Lee 2001; Li *et al.* 2009) converge on the same signal—that ‘parareptiles’ are stem turtles—we then added the near-crown stem turtles *Odontochelys semitestacea* and *P. quenstedti* to the otherwise unchanged and most recent ‘parareptile’ dataset (Müller & Tsuji 2007) (see electronic supplementary material for character scorings for these taxa). ‘Seymouria’, ‘Limnoscelidae’ and ‘Diadectidae’ were specified as the outgroup taxa and a heuristic search was performed with the same parameters outlined above.

3. RESULTS

The first two analyses resulted in four most parsimonious trees (MPTs) (662 steps in the former and 681 steps in the latter analysis) and a strict consensus tree was calculated (simplified strict consensus tree is shown in figure 1). The last analysis resulted in two MPTs (483 steps), differing only in possible relationships among a few very incomplete ‘parareptile’ species from the Russian Permian and a strict consensus tree was calculated. Support for each node was calculated as outlined in the previous analysis (figure 2).

4. DISCUSSION

Both analyses nest turtles within ‘parareptiles’ as the extant sister to Diapsida (figure 1) (see electronic supplementary material for discussion on nomenclature). Thus, although some morphological support exists for all three hypotheses, the overriding signal in the major morphological datasets actually agree and converge on turtles as nested within ‘parareptiles’ and not diapsids (figure 1). The basal-most undisputed stem turtles *O. semitestacea* and *P. quenstedti* possess all six unequivocal synapomorphies listed by Tsuji & Müller (2009) as diagnosing ‘Parareptilia’: absence of a lacrimal–nasal contact, absence of a caniniform region, shortened postorbital region, single median embayment of the posterior margin of the skull roof, the absence of a supraglenoid foramen and the absence of a subtemporal process of the jugal. In addition, turtles possess several other characters used to diagnose ‘parareptiles’ including a solid prefrontal–palatine contact, a dorsally expanded quadratojugal, and a jaw articulation at the level of, or slightly posterior to, the occiput (Tsuji & Müller 2009). When these two stem turtles were analysed in the most recent ‘parareptile’ phylogeny, there was strong support for an exclusive *Eumotosaurus*–turtle clade as sister to the *Nyctiphruetus*, Bolosauridae, Procolophonoidea, Pareiasauria and ‘*Nycteroleter*’ clade (figure 2).

The marked topological shift in the Li *et al.* (2009) analysis following the addition of two extinct species reflects the importance of transitional fossils when

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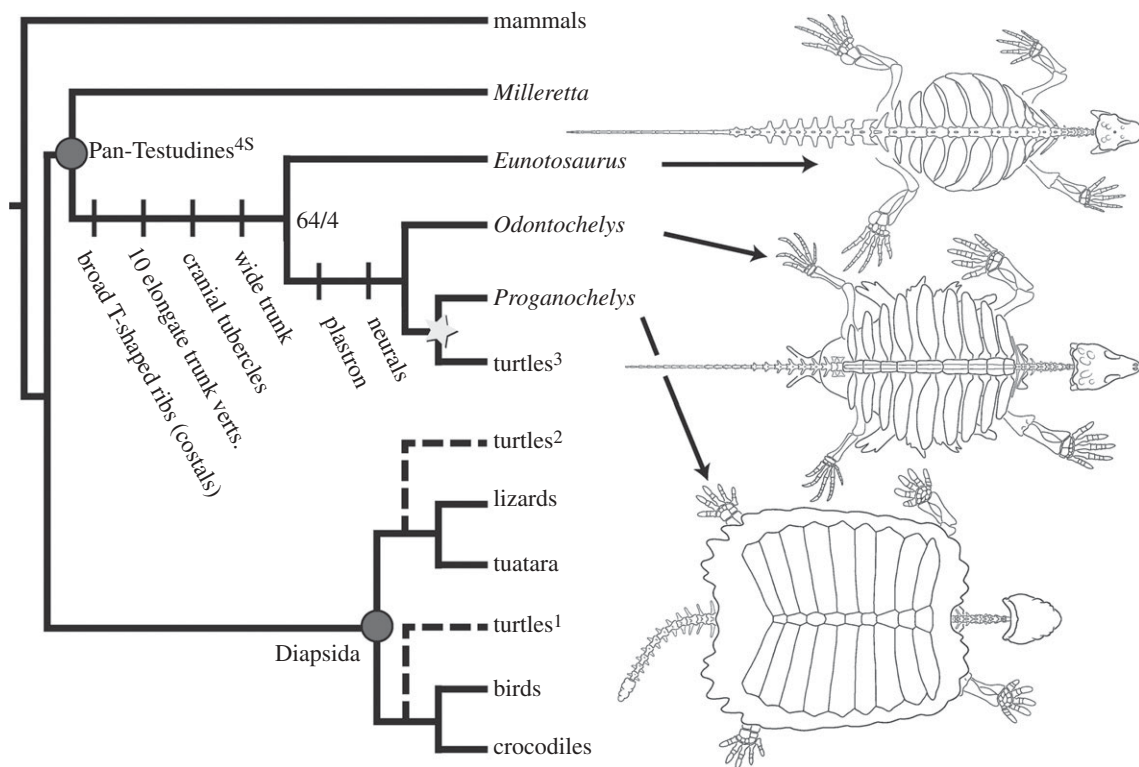


Figure 1. The position of turtles based on molecular (1: e.g. Hugall *et al.* 2007) and morphological datasets (2: e.g. deBraga & Rieppel 1997; 3: Gauthier *et al.* 1988). The addition of key fossils eliminates the apparent disagreement among morphological datasets in support of turtles outside Diapsida (3). The Permian ‘parareptile’ *Eunosaurus* shares uniquely derived features with turtles that help fill important gaps in the evolutionary origin of the turtle shell. Bootstrap (top) and Bremer (bottom) support values are provided for the *Eunosaurus*-turtle clade. Star indicates complete shell.

long branches separate the origins of major crown clades (Gauthier *et al.* 1988). *Eunosaurus* exhibits several turtle characters not previously considered in global analyses of reptile phylogeny, but, when added to the dataset of Li *et al.* (2009), increases statistical support for an exclusive *Eunosaurus*-turtle clade. These characters include T-shaped, abutting ribs that taper to finished apices (indicating loss of cartilaginous ventral rib segments and, perhaps, the sternum), 10 elongate dorsal vertebrae, cranial tubercles and a wide body form. Many of these features were noted previously (Watson 1914; Cox 1969; Gow 1997), but without consideration of transitional near-crown stem turtles, the establishment of a shared evolutionary origin for them remained elusive.

The broadened ribs of *Eunosaurus*, like those of *Odontochelys*, *Proganochelys* and crown turtles, appear to exhibit metaplastic ossification of the dermis, albeit more so in the latter two taxa than the former two (Cox 1969; Li *et al.* 2009). The eccentric position of the expanded portion of the ribs suggests that the primary endochondral rib ossifications themselves are not expanding, but are joined by a second ossification. In taxa whose expanded ribs are the result of simple expansion of embryonic costal chondrifications, expansion is central and the ribs are lenticular, not T-shaped, in cross section (Jenkins 1970; Daeschler *et al.* 2006). In addition, the dorsal surfaces of the expanded portions bear a rough texture characteristic of intradermal ossification (Watson 1914; Cox 1969; Vickaryous & Hall 2008). The phylogenetic position of *Eunosaurus* as a stem-turtle thus establishes the acquisition of this

style of rib ossification as an early event in the origin of the turtle shell. This transformation was followed by incorporation of portions of the shoulder girdle (Gegenbaur 1898) and gastralia (Gilbert *et al.* 2007) into a fully ossified plastron, and by the appearance of neurals, transformations that occurred during the approximately 44 Myr separating the Late Permian *Eunosaurus* and Late Triassic *Odontochelys* (figure 1). The approximately 5 Myr that separate *Odontochelys* and *Proganochelys* suggest completion of the shell may have occurred relatively rapidly. The sequence of evolutionary events derived from the fossil record is largely mirrored by the observed sequence of ossification in turtle embryos (Sánchez-Villagra *et al.* 2009), a sequence that also supports turtles as being outside Diapsida (Werneburg & Sánchez-Villagra 2009).

The placement of *Eunosaurus* along the turtle stem has implications for the ancestral ecology of turtles. A marine origin is inferred when turtles are considered to be closely related to extinct marine sauropterygian diapsids (deBraga & Rieppel 1997). The near shore marine sediments preserving *Odontochelys* support that inference (Li *et al.* 2009). However, the front limb proportions, shape of shell and histological data suggest that other near-crown stem turtles (e.g. *P. quenstedti* and *Palaeochersis talampayensis*) were terrestrial (Joyce & Gauthier 2004; Scheyer & Sander 2007). In addition, *Eunosaurus* (and successive outgroups) lacks obvious aquatic adaptations and is only known from terrestrial sediments (Gow 1997). Because a turtle-diapsid sister group is now recovered by the same dataset that previously supported a

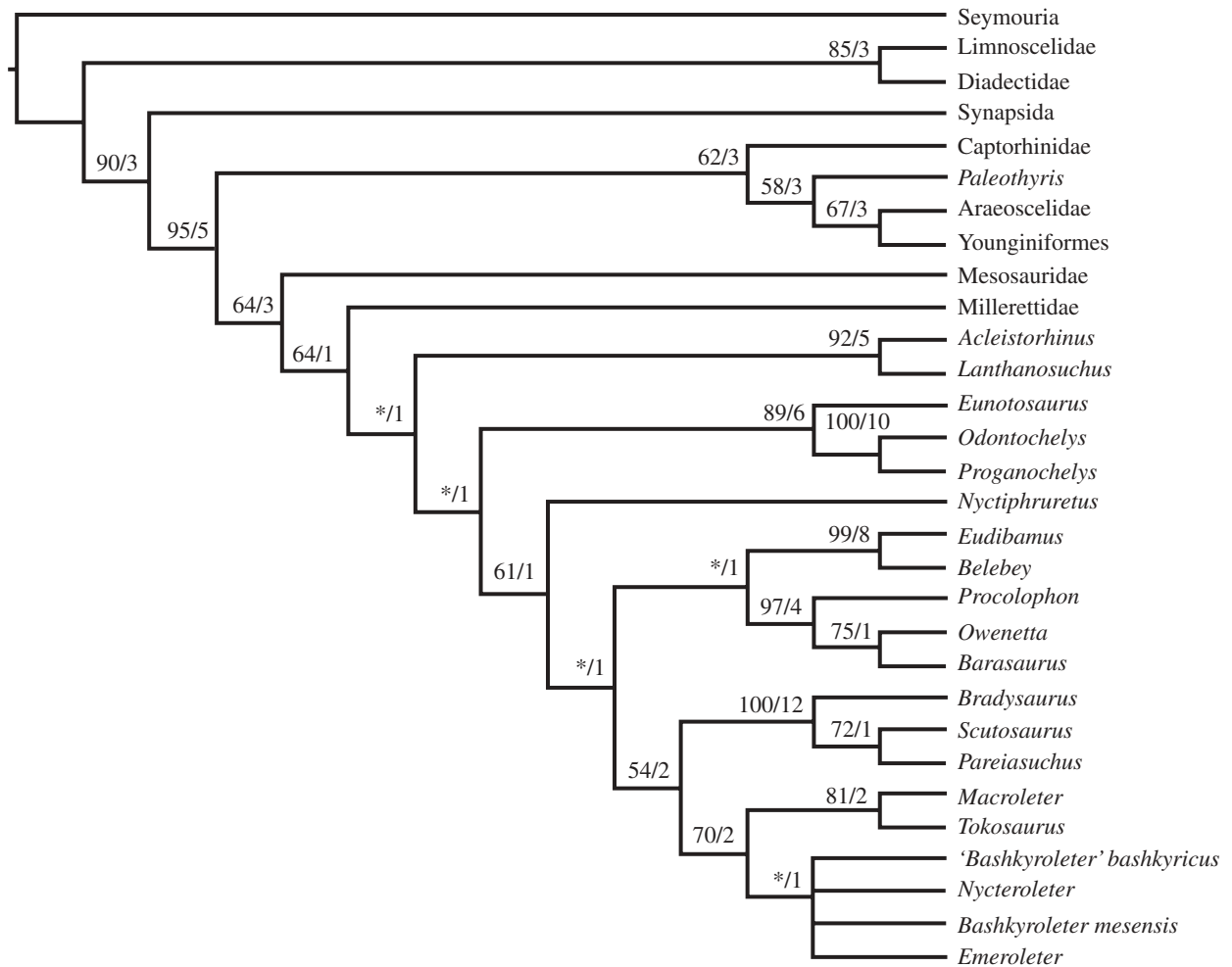


Figure 2. Strict consensus cladogram of two trees of 483 steps showing the phylogenetic relationships of 'parareptiles' when turtles are included in the analysis. CI = 0.4079, RI = 0.6720. Bootstrap (top) and Bremer (bottom) support values are provided for each node. The asterisk (*) indicates a bootstrap value under 50%.

marine origin of turtles along the lizard–tuatara stem, morphological and molecular-based phylogenies both support a terrestrial origin of stem turtles. The putative marine ecology of *Odontochelys* is inferred as independently derived and not ancestral to the subsequent radiation of turtles (Reisz & Head 2008), the crown of which appears to have originated in freshwater (Joyce & Gauthier 2004).

Our finding that morphological datasets place turtles outside Diapsida is at odds with most recent molecular work. Currently, the molecular datasets show strong support for a turtle + archosaur relationship, yet few morphological features support this clade (Rieppel 2000; Bhullar & Bever 2009). Additionally, a recent combined analysis including morphological and molecular data also concluded that turtles are outside Diapsida (Lee *et al.* 2008). More importantly, both morphological *and* molecular data are sensitive to taxon sampling, homology issues, rate heterogeneity and missing data owing to evolutionary change (Lee *et al.* 2008). The implications of these shared complexities have yet to be fully explored. For example, the logical conclusion that fossils cannot be used to calibrate points in molecular clock studies for groups whose monophyly is not recovered by both morphological and molecular

datasets is a previously underemphasized reality, which has important consequences for dating the Tree of Life—a pursuit that requires the integration of fossil and molecular data. Reptile phylogeny presents a nearly ideal platform for the continued critical analysis of these issues.

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