MicroRNAs support a turtle + lizard clade

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Despite much interest in amniote systematics, the origin of turtles remains elusive. Traditional morphological phylogenetic analyses place turtles outside Diapsida—amniotes whose ancestor had two temporal regions in the temporal region of the skull (among the living forms the tuatara, lizards, birds and crocodilians)—and allied with some unfenestrate-skulled (anapsid) taxa. Nonetheless, some morphological analyses place turtles within Diapsida, allied with Lepidosauria (tuatara and lizards). Most molecular studies agree that turtles are diapsids, but rather than aligning them with lepidosaurs, instead place turtles near or within Archosauria (crocodilians and birds). Thus, three basic phylogenetic positions for turtles with respect to extant Diapsida are currently debated: (i) sister to Diapsida, (ii) sister to Lepidosauria, or (iii) sister to, or within, Archosauria. Interestingly, although these three alternatives are consistent with a single unrooted four-taxon tree for extant reptiles, they differ with respect to the position of the root. Here, we apply a novel molecular dataset, the presence versus absence of specific microRNAs, to the problem of the phylogenetic position of turtles and the root of the reptilian tree, and find that this dataset unambiguously supports a turtle + lepidosaur group. We find that turtles and lizards share four unique miRNA gene families that are not found in any other organisms’ genome or small RNA library, and no miRNAs are found in all diapsids but not turtles, or in turtles and archosaurs but not in lizards. The concordance between our result and some morphological analyses suggests that there have been numerous morphological convergences and reversals in reptile phylogeny, including the loss of temporal fenestrae.

Keywords: turtle; microRNA; amniote

1. INTRODUCTION

The phylogenetic position of turtles remains labile, owing in part to the fact that, while many primitive cranial features suggest a basal position outside Diapsida...
Figure 1. The inter-relationships among the major groups of reptiles. Using an unrooted tree, it is possible to show that although each of the three previous hypotheses concerning turtle relationships—morphology 1 [1–3], morphology 2 [4–7] and the molecular results [10–12]—agree on the topology, they disagree on the position of the root (arrows).

4. DISCUSSION

Turtles as sister group to lizards are recovered in some morphological analyses [4–7] and this is supported by some, mostly postcranial, characters including the fusion of the astragalus and calcaneum in postnatal ontogeny [25]. However, a diapsid affinity also requires several morphological reversals in turtles, including the closure of the temporal fenestrae [25]. In addition, it suggests that molecular analyses that recover an archosaur affinity might be the result of a systematic artefact caused by the attraction of the long-branched lizards towards the outgroup [8].

The consilience between at least some morphological apomorphies and the evolutionary acquisition of miRNAs suggests the validity of the sister group relationship between lepidosaurs and turtles. Hence, we propose ‘Ankylopodia’ (‘fused foot’ in reference to the fused ankle bones—astragalus and calcaneum—shared by lepidosaurs and turtles) as the name of the Lepidosauria + Testudines crown clade, which is defined as the last common ancestor of *Chrysemys picta* and *A. carolinensis* and all of its descendants living or extinct (electronic supplementary material, file S4). If Lepidosauria + Testudines is indeed a monophyletic group, then this implies significant convergence in molecular (nuclear and mitochondrial), developmental and morphological characters, including the loss of temporal fenestrae, in the early evolutionary history of turtles. Further, our study suggests that lizards are the appropriate outgroup comparison for understanding the origin of the turtle body plan.

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Figure 2. microRNAs support a turtle–lizard relationship. (a) Structures and alignments of the mature sequences for three of the 35 families analysed phylogenetically, the reptile-specific miR-1677, the archosaur-specific miR-1791 and miR-5390, a novel miRNA shared between Anolis carolinensis and C. pictus. Mature sequences within the context of the miRNA hairpin are shown in grey, changes in the mature sequence with respect to the reference sequence, either chicken or lizard, are shown in bold. (b) To arbitrate among these competing hypotheses (see figure 1), eight amniote taxa were scored for the presence/absence of 35 miRNA families with the frog *Xenopus laevis* as the outgroup. Using a combination of small RNA library sequencing coupled with genomic searches (electronic supplementary material, file S1), we find that the turtle *C. picta* shares four miRNA families with the lizard *A. carolinensis* that are not found elsewhere in the animal kingdom, supporting the rooting position of ‘morphology 2’ in figure 1 [4–7]. Bremer support indexes are indicated at each node. Character changes on branch: grey boxes, acquisition of miRNA family; filled triangle, loss of miRNA family.


