

The impact of the fossils in molecular clocks: an example using chelid turtles

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Background. The clade Chelidae (Testudines, Pleurodira) is a group of fresh water turtles with representatives in Australasia and South America. Its diversity of extant and fossil species is characterized by two recognized morphotypes: the long-necked and the short-necked chelids. So far, the phylogenies constructed over Chelidae differ depending on the information source. While morphology recovers one monophyletic group of long-necked chelids (with South American and Australasian species), the molecular data split the group into South American and Australasian chelids, both as monophyletic sister groups and containing long-necked species. The consequences of this conflict imply the emergence of long-necked chelids (i) one time before the final breakup of Southern Gondwana ($\cong 35$ Mya) or (ii) independently after this event.

Methods. Using BEAST, a set of molecular clock analyses was performed. Seven of these analyses correspond to the molecular hypothesis and thirteen to the morphological hypothesis. So, ten fossils were used as calibration points in different combinations for each hypothesis. The results were statistically compared performing ANOVA and the global similarity was inspected by a hierarchical cluster analysis (HCA).

Results. *Molecular hypothesis:* all the analyses produced an age of the origin of Chelidae, and rising of long neck, older than 35 Mys. Divergence times in the South American clade were generally older than the observed in the Australasian clade. The result of the HCA was: analyses 2, 4 and 5 form a group and the analyses 3, 6 and 7 form another group; the analysis 1 is close related to this last. *Morphological hypothesis:* the origin of the clade of long-necked chelids predated the 35 Mys in all the analyses except one; however the *Chelodina* group resulted younger than this age in all the analyses. The HCA yielded two main groups of molecular clock analyses (1, 3, 7, 8, 9, 13 and 2, 4, 6, 10, 11, 12) and one analysis (5) clearly

separated of these two. The ANOVA resulted in significant differences for all estimated nodes in both phylogenetic hypotheses.

Discussion. Our set of molecular clock analyses suggests an early diversification of the chelid turtles and the raising of the long-necked chelids before the final breakup of Southern Gondwana. However, the appearance of this trait one time or as evolutionary convergence still depends on which phylogenetic scenario is taken into account. Furthermore, our results indicate that the number of calibration points not necessarily improve the precision of estimated nodes. Instead the “quality” of the fossils used as calibrations and its position in the phylogeny, have appreciable impact not only over this parameter, but also over the global evolutionary rate along the tree.

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