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Complex phylogeography in *Rhinoclemmys melanosterna*: conflicting mitochondrial and nuclear evidence suggests past hybridization (Testudines: Geoemydidae)

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Abstract

We examined differentiation within the Colombian wood turtle *Rhinoclemmys melanosterna*, and among *R. melanosterna* and the closely allied species *R. diademata*, *R. funerea* and *R. punctularia*, based on 1060 base pairs of the mitochondrial *cyt b* gene. We also assessed the phylogenetic relationships among these species using 2050 bp of mtDNA (partial *cyt b*, 12S and 16S genes) and 3620 bp of nuclear DNA (partial *Rag 1*, *Rag 2*, *C-mos*, *R35* and *ODC* genes). There is considerable phylogeographic structuring within *R. melanosterna*, with seven distinct clades distributed across the species' range. These clades correlate to some extent with previously described differences in the dorsal pattern of head coloration. Individual and combined analyses of mitochondrial and nuclear DNA indicated contradictory relationships among *R. melanosterna*, *R. diademata*, *R. funerea* and *R. punctularia*. Mitochondrial DNA sequences revealed *R. melanosterna* to be non-monophyletic with respect to *R. diademata*, *R. funerea* and *R. punctularia*. In contrast, *R. melanosterna* constituted a well-supported monophyletic clade using nuclear DNA. This conflict between mitochondrial and nuclear data suggests past gene flow among the allopatric and parapatrically distributed species *R. melanosterna*, *R. diademata*, *R. funerea* and *R. punctularia*. Compared to the other *Rhinoclemmys* species, the taxa under study are weakly differentiated. To assess their taxonomic status, further research is warranted using additional nuclear markers and additional samples of *R. diademata*, *R. funerea* and *R. punctularia*. For the time being, a continued classification of *R. melanosterna*, *R. diademata*, *R. funerea* and *R. punctularia* as distinct species is justified owing to their allopatric and parapatric distributions, and to conserve the established usage of names that is based on morphological and karyotypic differentiation.

Key words: Molecular phylogeny, mtDNA, nDNA, phylogeography, *Rhinoclemmys diademata*, *Rhinoclemmys funerea*, *Rhinoclemmys punctularia*, South America

Introduction

The advent of molecular genetic methods has contributed significantly to an enhanced understanding of the diversity and phylogenetic relationships of turtles and tortoises (Fritz & Havaš 2007). However, although only approximately 320 extant species are currently recognized (Fritz & Havaš 2007; Fritz 2011), their phylogeographic differentiation is often completely unknown. This is especially true for species occurring in the Neotropics (Central and South America), where relatively few phylogeographic studies have been performed so far (for a review see Vargas-Ramírez *et al.* 2012a). Such studies are crucial for unravelling evolutionarily significant units (Moritz 1994) that may correspond to unrecognized taxa or cryptic species (for chelonians, e.g., Fritz *et al.* 2005, 2008, 2011, 2012a; Prashag *et al.* 2007, 2011; Vargas-Ramírez *et al.* 2010; Stuckas & Fritz 2011) and have relevance for conservation. Moreover, such investigations elucidate underlying historical events and processes that may have shaped the current genetic diversity (e.g., Vargas-Ramírez *et al.* 2010, 2012b; Fritz *et al.* 2012b, c).