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On the Reclassification of the *Terrapene* (Testudines: Emydidae): A Response to Fritz & Havaš

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The American box turtles (*Terrapene* spp.) consist of four historically recognized species based primarily on morphological data (Minx 1996): *Terrapene carolina* (Linnaeus), *T. ornata* (Agassiz), *T. nelsoni* (Stejneger), and *T. coahuila* (Schmidt & Owens). Each of these species is polytypic except for *T. coahuila*. Recently, the Placyk lab re-assessed the classification of *Terrapene* using molecular phylogenetic data, and suggested elevating a clade consisting of *T. carolina triunguis* (Agassiz)*, T. c. mexicana* (Gray)*, and T. c. yucatana* (Boulenger) to a distinct species: *Terrapene mexicana* ssp. (Martin *et al.* 2013). Our dataset included all *Terrapene* species and almost all of the subspecies (except for *T. nelsoni klauberi*), as well as large sample sizes and a wide geographic sampling for both mitochondrial (mt) and nuclear (nuc) DNA. The mtDNA *Cytochrome b* (*Cytb*) gene and a nucDNA intron from the *Glyceraldehyde-3-phosphate-dehydrogenase* (*GAPD*) gene were used for phylogenetic analyses, and indicated that the *T. mexicana* clade was highly divergent from the remainder of the original *T. carolina* clade. In addition, the *Cytochrome c oxidase subunit 1* (*COI*) gene, which is used in many animals for DNA barcoding, was sequenced and used to calculate pairwise percent DNA sequence divergence between taxa. The barcoding data also strongly supported our suggested classification revisions. However, because possible introgression was detected for some of the individuals, *T. mexicana* was not recognized as a distinct species in a recent assessment of Testudines taxonomic updates (Fritz & Havaš 2013). The purpose of this treatise is to suggest that the possible presence of introgression seen in Martin *et al.* (2013) does not warrant disregarding *T. mexicana* as a unique species.

For many years, debates over species concepts have been prevalent in biology. In his seminal book *On the Origin of Species*, Darwin (1859) described the process of speciation as "uniformitarian," where species are defined as entities displaying morphological gaps from other species, despite in some cases being able to live in sympatry, and the concept assumes speciation occurs along a continuum (for a discussion, see Mallet 2008). Intraspecific varieties, which often contain distinctive features or characters, will eventually become separate species, but it is not clear exactly when this might happen. With the development of the "Modern Synthesis" (Huxley 1942), Mayr (1963) and Dobzhansky (1937) forwarded the Biological Species Concept (BSC), which defines species in terms of reproductive isolation. One problem with the BSC, however, is that it does not account for hybridization and introgression, which for many taxonomic groups are currently being considered evolutionary processes that are more important than previously believed.

Hybridization and introgression are thought to commonly occur in ~10-30% of animal and plant species, especially between closely related species that are found in sympatry (Mallet 2005, 2008; Abbott *et al.* 2013). It has been argued that taxonomic entities capable of coexisting in sympatry, regardless of whether gene flow is occurring, should be considered distinct species (Harrison 1998; Coyne & Orr 2004; Abbott *et al.* 2013). Furthermore, in some cases sympatric taxa inhabiting natural hybrid zones, such as the plains gartersnake (*Thamnophis radix*) and Butler's gartersnake (*T. butleri*) can remain distinct for divergently selected loci, despite extensive gene flow (Fitzpatrick *et al.* 2008). Kapfer *et al.* (2013) also recently found evidence of hybridization between Butler's gartersnake and the more distantly related common gartersnake (*T. sirtalis*). Thus, reproductive isolation could take a vastly longer amount of time than what might be reflected in ecological, genetic, and morphological characters that are very relevant for realistically determining whether species interactions occur (Abbott *et al.* 2013). Definitions such as Darwin's (1859) concept described above; Mallet's (1995) suggestion of defining a species as forming genotypic clusters; the Phylogenetic

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Species Concept (PSC; see Wheeler & Meier 2000); or Avise's (2000) suggestion of identifying phylogeographic barriers may be more intuitive than the BSC for some taxa.

There are many examples of interspecific gene flow between animals in sympatry, for which the BSC is not an entirely appropriate description of a species, and such gene flow is increasingly being considered a more common occurrence than previously believed. Examples include birds, such as many of the birds of paradise, grouse, or ducks; insects such as butterflies or *Drosophila*; mammals such as grizzly bear X polar bear hybrids; several whale, dolphin and porpoise species (for sources and a review, see Mallet 2008); reptiles such as the watersnakes *Nerodia fasciata* X *N. sipedon* (Mebert 2008); and the American box turtles *Terrapene carolina* X *T. ornata* (Dodd 2002; Lutterschmidt *et al.* 2007; Cureton II *et al.* 2011).

Regarding *Terrapene* specifically, there have been several studies assessing interspecific hybridization and introgression between *T. ornata* and *T. carolina*. Lutterschmidt *et al.* (2007) performed morphometric analyses on shell morphology and concluded that ~14% of the 177 turtles analyzed in the study were putative hybrids. Cureton II *et al.* (2011) conducted molecular analyses with eight microsatellite loci and one mtDNA gene (*Cytb*) to assess hybridization and introgression, and found evidence for parental crosses and introgression of hybrids with parental types. Several other case studies have documented hybrids between *T. carolina* and *T. ornata* (e.g., Clark 1935; Blaney 1968; Ward 1968), and there is not much, if any, disagreement in the literature that *T. carolina* and *T. ornata* form distinct species. Thus, it is evident that using the BSC to delimit species of *Terrapene* is not an ideal method. Given the tendency of this group to hybridize and introgress, criteria other than reproductive isolation and the occurrence of hybridization might be better suited in this particular case (e.g., phylogenetic and molecular genetic data and analyses).

Because the *Terrapene* are of conservation concern throughout their range (Dodd 2002), and because many conservation efforts are species-based and tend to ignore subspecies, it is imperative that their classification be correctly resolved. The molecular evidence of Martin *et al.* (2013) suggest the *T. mexicana* clade forms a distinct species and we believe that the genetic evidence provided to support this newly recommended classification via the PSC outweighs concerns regarding hybridization utilizing a BSC framework.

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