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## Genetic Relatedness of Populations of the Mud Turtle, *Kinosternon baurii*, from Mainland Florida and the Upper and Lower Florida Keys. Linnaeus Fund Research Report

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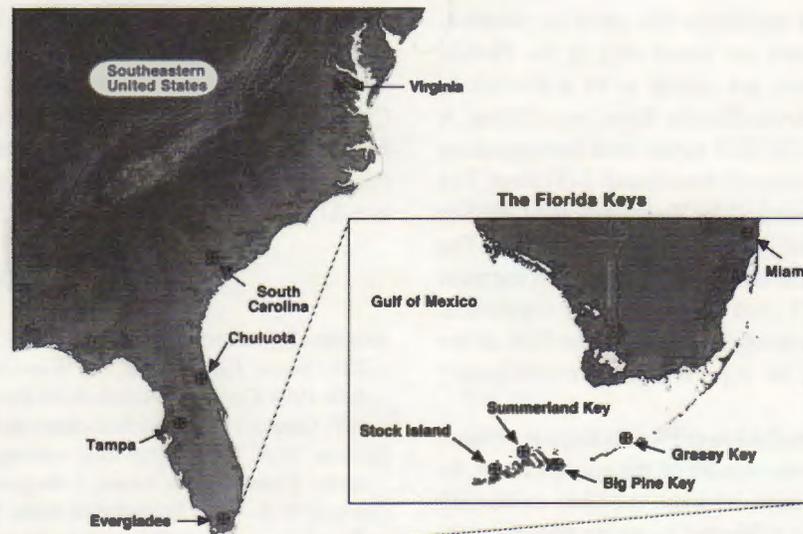
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The striped mud turtle, *Kinosternon baurii* Garman, is a small, aquatic turtle that ranges from the lower Florida Keys north to Virginia (Iverson, 1992). Throughout most of their range, striped mud turtles are found in freshwater habitats, however, in the lower Florida Keys they can be found in both freshwater and brackish water habitats (Dunson, 1981). The lower Florida Keys populations are listed as endangered by the state of Florida and may warrant special attention.

A potential exists for restricted gene flow between populations in the lower Florida Keys and those in the remainder of the species' range because they are separated by a seven-mile expanse of open ocean. Historically, the lower Florida Keys populations were considered a separate subspecies (*K. baurii baurii*) distinct from the mainland *K. baurii palmarum* (Stejneger, 1925). Both morphological and physiological differences have been proposed as supporting this subspecific designation (Uzzell and Schwartz, 1955; Dunson, 1981). Although no subspecies currently are recognized, taxonomic controversy continues regarding the genetic subdivision among striped mud turtle populations (see Dunson, 1981; Lazell, 1989).

The objective of this study was to assess the degree of genetic isolation that exists between mainland, upper, and lower Florida Keys populations of the striped mud turtle. Mitochondrial control region DNA sequence data were examined to determine levels and patterns of variation in 36 *Kinosternon baurii* individuals from 10 different geographic locations from throughout the species' range.

*Methods.* — Each captured turtle was marked and measured, and a small blood sample was withdrawn before the turtle was released at its site of capture. Sampling locations are illustrated in Fig. 1 and are: Stock Island, Dade Co., Florida ( $n = 7$ ), Summerland Key, Dade Co., Florida (3), Big Pine Key, Dade Co., Florida (2), Grassy Key, Dade Co., Florida (3), Snapper Creek Canal, Miami, Dade Co., Florida (3), Nine Mile Pond and Royal Palm Hammock, Everglades National Park, Dade Co., Florida



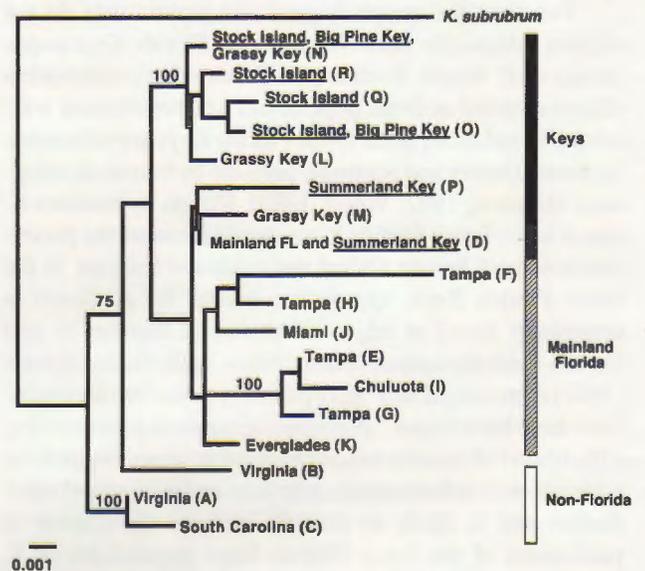
**Figure 1.** Topographic map of the southeastern United States and the southern tip of Florida showing location of *Kinosternon baurii* collected for this study. Map images are from the NOAA National Data Centers GLOB Project and can be found on the internet at <http://www.ngdc.noaa.gov:80/seg/topo/globe.shtml>.

(3), Ecological Research Area, USF, Tampa, Hillsborough Co., Florida (6), Picketts Cove, Chuluota, Seminole Co., Florida (3), Savannah River Ecology Laboratory, Aiken Co., South Carolina (3), and Cohoke Mill Creek, King William Co., Virginia (3).

Total cell DNA was extracted from the lysed blood using a standard phenol/chloroform protocol (Ausubel et al., 1993). Approximately 450 base pairs of the 5' end of the mtDNA control region (near the tRNA<sup>PRO</sup>) were amplified using primers from Walker et al. (1995). The purified and concentrated DNA was sequenced using an ABI automated sequencer (DNA Sequencing Core, University of Florida). All sequences were aligned manually using the program SeqEd (Applied Biosystems). Phylogenetic analyses of aligned sequences were done using maximum parsimony, maximum likelihood distance estimates using the Hasegawa-Kishino-Yano model (HKY; Hasegawa et al., 1985), and minimum evolution (Rzhetsky and Nei, 1992) options in PAUP\* (version 40b4a; Swofford, 1998). All trees were rooted with *Kinosternon subrubrum*, the sister taxon of *K. baurii* (Iverson, 1998; D. Walker, *pers. comm.*).

**Results and Discussion.** — Out of a total of 415 bases sequenced, there were 20 variable sites (4.8%) defining 18 haplotypes. Of the variable sites, nine (45%) were parsimoniously informative. All haplotypes except three were found in only a single sampling location. One common haplotype was found in all but three of the Florida sites. Even though sample sizes generally were small, all locations except one had more than one haplotype. The Tampa, Florida, sample was the most diverse with five haplotypes found in six individuals, and the South Carolina sample was the least diverse with a single haplotype found in all three individuals sampled. HKY genetic distances between haplotypes generally were small and ranged from 0.24% to 2.26%.

With 18 operational taxonomic units and only 20 variable characters, any phylogenetic estimate is likely to be only weakly supported. Nonetheless, there does appear to be some systematic information available. Three general groupings can be seen in the neighbor-joining tree of HYK distances (Fig. 2): 1) a non-Florida group, 2) a mainland Florida group, and 3) a cluster of haplotypes between the non-Florida and mainland Florida clades. With the excep-



**Figure 2.** Phylogenetic tree of *K. baurii* control region haplotypes estimated using maximum-likelihood distances and neighbor-joining clustering. Haplotype designations are indicated in parentheses. Lower Florida Keys population names are underlined. Branch lengths are proportional to the number of changes and the scale bar represents the number of expected changes per site. Numbers above branches indicate the frequency at which that node was found among the 1451 equal most parsimonious trees. Only nodes found in 75% or more of the trees are indicated. The solid bar indicates the Florida Keys samples, the light hatched bar indicates the mainland Florida samples, and the dark hatched bar is the widespread haplotype (D).

tion of the most common haplotype (D), all of the intermediate clustering haplotypes are found only in the Florida Keys, however, there does not appear to be a distinction between the upper and lower Florida Keys populations. A heuristic search resulted in 1451 equal most parsimonious trees of 36 steps. A few clades are found in all 1451 trees. The upper and lower Keys clade and the Virginia/South Carolina clade are found in 100% of the parsimony trees (Fig. 2). The mainland Florida clade also is found in a majority of the most parsimonious trees (54%) but is not strongly supported. Most other associations are found in less than 50% of the trees and are unlikely to represent true evolutionary patterns.

*Kinosternon baurii* in the lower Florida Keys is isolated geographically from the remainder of the species range by open ocean. This connection between the Gulf of Mexico and the Atlantic Ocean is subjected to strong tidal currents and may present a physical barrier to dispersal for *K. baurii*. In addition to potential geographic isolation, the lower Florida Keys populations appear to be better adapted to saline water and, unlike the mainland populations, can be found in brackish ponds (below 15 ppt salt; Dunson, 1992). The lower Florida Keys populations previously have been described as morphologically differentiated from the upper Florida Keys and mainland (Uzzell and Schwartz, 1955), however, individuals with similar pigmentation patterns to those of the lower Florida Keys have been found in mainland populations (Iverson, 1978; Lamb and Lovich, 1990).

Together, the morphological and genetic data do not support subspecific status for the lower Florida Keys populations of *K. baurii*. Even so, we believe that conservation efforts directed at these populations are nonetheless warranted. Populations in the lower Florida Keys are vulnerable because of heavy and continual pressure by human development (Dunson, 1992; Wood, 1992). Efforts to preserve *K. baurii* in the lower Florida Keys should focus on the preservation of both human altered and unaltered habitats. In the lower Florida Keys, appropriate habitat for *K. baurii* is commonly found at edges of hardwood hammocks and human-made mosquito control ditches. In the latter, Dunson (1992) commented that "exceptionally dense turtle populations have been found." A conservation strategy possessing a likelihood of success would be aimed at preserving natural ecotones such as hardwood hammocks and mosquito control ditches and is likely to directly increase the chance of persistence of the lower Florida Keys populations of *K. baurii*.

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