The yellow pond turtle (Mauremys mutica) and Annam leaf turtle (Mauremys annamensis) are both listed under Convention on International Trade in Endangered Species (CITES) Appendix II, and are threatened mainly by the food and medicine trade in Asia (van Dijk 2000). Mauremys mutica is considered endangered (International Union for Conservation of Nature [IUCN] 2006) but is common in farms and markets; whereas, Mauremys annamensis is considered critically endangered (IUCN 2006) and is even a member of “The World’s Top 25 Most Endangered Turtles” (Turtle Conservation Fund [TCF] 2003). It has been seen in the wild by scientists only twice since 1939 (Asian Turtle Conservation Network [ATCN] 2006; Parham et al. 2006). Whereas Mauremys mutica is widely distributed throughout Asia (spanning over 2600 km in Japan, China, and Vietnam), the entire distribution of Mauremys annamensis is restricted to a small area of central Vietnam (Iverson 1992). These species are closely related (Feldman and Parham 2004; Spinks et al. 2004) but are easily distinguished by the striping on the head; Mauremys mutica has a single broad temporal stripe that ends behind the eye; whereas, Mauremys annamensis has several thin stripes that meet at the tip of the nose (Iverson and McCord 1994; Yasukawa et al. 1996). Although Mauremys mutica (Fig. 1) and Mauremys annamensis (Fig. 2) are easily distinguished morphologically, Feldman and Parham (2004) used DNA to hint at a more complicated interrelationship and undescribed diversity within these 2 species.

The Chelonian Research Foundation provided funding through the Linnaeus fund to perform a more detailed genetic study, sampling more individuals and discerning an ad-
ditional genetic marker to clarify the evolutionary relationships within and between *M. mutica* and *M. annamensis*.

The purpose of this study was to use DNA to explore the inter- and intraspecific relationships and diversity of *M. mutica* and *M. annamensis*. In addition, my collaborators and I used these data to evaluate and make recommendations on conservation approaches.

We sequenced 32 individuals of *M. mutica* and *M. annamensis* for a mitochondrial DNA marker (ND4 and adjacent tRNAs) and a subset of these individuals for a nuclear DNA marker (R35). Our sampling included specimens from the wild, markets, turtle farms, and trade seizures. Although a majority of these samples did not have locality data, we included them to get general ideas of genetic diversity as well as evaluate the use of trade specimens (markets, farms, trade seizures) for conservation.

For the mitochondrial DNA, we find a species-level break that corresponds to geography instead of the current taxonomy; 1 group corresponds to eastern China *M. mutica*, while the second group corresponds to *M. annamensis* and *M. mutica* from Hainan and Vietnam. Not only are *M. mutica* and *M. annamensis* paraphyletic, but *M. annamensis* also exhibits surprisingly high genetic diversity for being highly restricted to central Vietnam, appearing in 2 distinct clades.

Nuclear DNA shows similar results in that our current delineation of species does not capture patterns of genetic diversity. Three well-supported clades appear, corresponding to *M. annamensis*, *M. mutica* from Hainan Province, and *M. mutica* from eastern China. One difference of the nuclear data compared to the mitochondrial data is that nuclear data places eastern China *M. mutica* closer to *M. annamensis*, where mitochondrial data show the converse pattern. This discordance between mitochondrial and nuclear DNA may be a result of incomplete lineage sorting and/or hybridization, alternatives between which we cannot distinguish with our current data set.

Because *M. mutica* and *M. annamensis* are rare in the wild, current conservation plans have been to use trade specimens for captive breeding, with the goal of eventually releasing them into the wild. Not only are there strict guidelines on the reintroduction of animals into the wild (IUCN 2002), our genetic results show that species designations based on morphology do not represent the diversity found in *M. mutica* and *M. annamensis*. Therefore, the creation of breeding groups based on morphology has the potential to produce hybrids. Releasing such genetically polluted or misidentified animals into the inappropriate geographic area would disrupt natural biological processes. Given the uncertain provenance of trade animals, my collaborators and I feel that additional systematic research on such samples would yield diminishing returns. Instead, we strongly recommend more fieldwork to locate turtles in the wild. These individuals can be studied in their natural habitat to elucidate their distribution and ecology and sampled nonlethally for DNA studies. Moreover, given the problems associated with well-intended but misguided captive breeding efforts based on trade samples, we feel that more emphasis should be placed on in situ conservation efforts. As the natural habitat in Asia is quickly being converted for human use, it is imperative to protect the remaining wild areas. Protecting these areas and understanding what lineages naturally occur there is necessary before we can begin to think about reintroduction of animals into the wild.

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Literature Cited


Linnaeus Fund Research Report: Assessing the Home Range and Oviposition Site Selection in Freshwater Turtles from Pristine and Oil-Polluted Habitats in the Niger Delta, Nigeria

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The River Niger’s Delta region in southern Nigeria (West Africa) is the major oil-producing area of sub-Saharan Africa and has been environmentally altered in the last 30 years by the oil-industry boom (De Montclos 1994; Fasakin et al. 1995; Niger Delta Environmental Survey [NDES] 1998). It is calculated that over 5800 km of oil pipelines have been built throughout the delta since 1958, and many of them have repeatedly malfunctioned, resulting in catastrophic oil-spillage events (Carbone 2002). For instance, between 1986 and 1996 there were 2796 oil spills in this region, resulting in a total of about 2,500,000 barrels of oil released into the natural environment (Carbone 2002).

Obviously, the study of the effects of habitat contamination due to oil spills has become a prominent issue for the scientists working in the Niger Delta region, and indeed some studies have been published in the recent years with reference to effects of oil pollution on amphibians (Akani et al. 2004; Luiselli and Lea, in press), and crocodilians (Ekpubeni and Ekundaio 2002).

The 2001 Linnaeus Fund Research Award supported field research on the comparative ecology and habitat utilization of 2 turtle communities of the Niger Delta area, 1 occurring at a stream surrounded by pristine forest and another at a stream polluted by a catastrophic event of oil spillage approximately 10 years before (Luiselli and Akani 2002; Luiselli et al. 2004). This research study revealed both direct and indirect effects of oil pollution on the complexity and habitat use of communities of Nigerian freshwater turtles, despite the fact that over 10 years had elapsed between the pollution event and the actual study. The main direct effect was a considerable reduction of the turtle specific diversity, with 50% of the species being lost after oil spillage, and also a very strong decline in the numbers of turtle specimens for those species that were able to survive the catastrophic pollution event (i.e., *Pelusios niger* and *Pelusios castaneus*). Another direct consequence of the oil-spillage event was that habitat use changed considerably in 1 species, i.e., *P. niger*, which shifted from an intensive use of swamps into the rainforest before spillage to an almost complete abandonment of this habitat type after the spillage event. This was obviously a consequence of the fact that the swamps became strongly polluted after the oil