De Montclos, M.A. 1994. Le Nigéria. Kurthala, Paris. Federal Republic of Nigeria. 2000. Annual Business Report. 2000. Abuja, Nigeria

Gibbons, J.W., Lovich, J.E., Tucker, A.D., FitzSimmons, N.N., and Greene, J.L. 2001. Demographic and ecological factors affecting conservation and management of the diamondback terrapin (*Malaclemys terrapin*) in South Carolina. Chelonian Conservation and Biology 4:66–74.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Luiselli, L., and Akani, G.C. 2002. An investigation into the composition, complexity and functioning of snake communities in the mangroves of south-eastern Nigeria. African Journal of Ecology 40:220–227.

Luiselli, L., and Akani, G.C. 2003. An indirect assessment of the effects of oil pollution on the diversity and functioning of turtle communities in the Niger Delta, Nigeria. Animal Biodiversity and Conservation 26:57–65.

Luiselli, L., Akani, G.C., and Capizzi, D. 1998. Food resource partitioning of a community of snakes in a swamp rainforest of south-eastern Nigeria. Journal of Zoology, London 246:125–133.

Luiselli, L., Akani, G.C., and Capizzi, D. 1999. Is there any interspecific competition between dwarf crocodiles (*Osteolaemus tetraspis*) and Nile monitors (*Varanus niloticus ornatus*) in the swamps of central Africa? A study from south-eastern Nigeria. Journal of Zoology, London 247:127–131.

Luiselli, L., Akani, G.C., and Politano, E. 2006. Effects of habitat alteration caused by petrochemical activities and oil spill on the habitat use and interspecific relationships among four species of Afrotropical freshwater turtles. Biodiversity and Conservation 15:3751–3767.

Luiselli, L., Politano, E., and Angelici, F.M. 2000. Ecological correlates of the distribution of terrestrial and freshwater chelonians in the Niger Delta, Nigeria: A biodiversity assessment with conservation implications. Revue d'Ecologie (Terre et Vie) 55:3–23.

Mathur, D., and Silver, C.A. 1980. Statistical problems in studies of temperature preferences of fishes. Canadian Journal of Fisheries and Aquatic Sciences 37:733–737.

Niger Delta Environmental Survey. 1998. Environment and Socio-Economic Characteristics, Vol. 1. Port Harcourt, Nigeria: Niger Delta Environmental Survey, p. 272.

Odu, C.T.I., Nwboshi, L.C., Fagade, S.O., and Awani, P.E. 1989. Final report on post-impact Study of SPDC "8" Nun River delivery line spillage. Report to Shell Petroleum Developmental Company, Port Harcourt, Nigeria.

Politano, E. (Ed.). 1998. A study of the fauna of the Niger Delta and environmental impact assessment of the construction of two natural gas pipelines in the Rivers State. Port Harcourt, Nigeria: Japan Gas Company Corporation—Aquater Reports, pp. 532.

Singh, J., Moffat, D., and Linden, O. 1995. Defining an environmental development strategy for the Niger Delta (two volumes). Lagos, Nigeria: World Bank (Industry and Energy Operations Division, West Central Africa Department).

Statsoft Inc. 1996. STATISTICA for Windows, release 5.0. Tulsa, OK: Statsoft, Inc.

## Linnaeus Fund Research Report: Conservation Genetics of the Yellow Pond Turtle (*Mauremys mutica*) and the Annam Leaf Turtle (*Mauremys annamensis*)

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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, *M. 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 *M. mutica* is widely distributed throughout Asia (spanning over 2600 km in Japan, China, and Vietnam), the entire distribution of *M. 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; *M. mutica* has a single broad temporal stripe that ends behind the eye; whereas, *M. annamensis* has several thin stripes that meet at the tip of the nose (Iverson and McCord 1994; Yasukawa et al. 1996). Although *M. mutica* (Fig. 1) and *M. 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 additional 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.

**Figure 1.** Mr. Chen, of the Tunchang Turtle Farm in Hainan Province, showing and describing the darker "Vietnam/Hainan" color morph of Mauremys mutica (photo by J. Fong).

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 market (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.

**Figure 2.** *Mauremys annamensis* (photo by R. Schaffer).

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### Literature Cited

ATCN. 2006. *Mauremys annamensis* recorded in natural habitat after 65 years. http://www.asianturtlenetwork.org/library/ATCN_news/2006_articles/Mauremys_annamensis_recorded_in_natural_habitat_after_65_years.html. (19 December 2006).

Feldman, C.R., and Parham, J.F. 2004. Molecular systematics of old world stripe-necked turtles (Testudines:Mauremys). Asiatic Herpetological Research 10:28–37.

Fong, J.J., Parham, J.F., Shi, H., Stuart, B.L., and Carter, R.L. 2007. A genetic survey of heavily exploited, endangered turtles: caveats on the conservation value of trade animals. Animal Conservation 10(4): 452–460.

IUCN. 2002. Guidelines for the placement of confiscated animals. Prepared by the IUCN/SSC Re-introduction Specialist Group. Gland, Switzerland/Abu Dhabi, United Arab Emirates: IUCN/ERWDA.

IUCN. 2006. 2006 IUCN red list of threatened species. www.iucnredlist.org (4 May 2006).

Iverson, J.B. 1992. A Revised Checklist with Distribution Maps of the Turtles of the World. Richmond, VA: Privately Printed, 363 pp.

Iverson, J.B., and McCord, W.P. 1994. Variation in East Asian turtles of the Genus *Mauremys* (Batagurdiae; Testudines). Journal of Herpetology 28:178–187.

Parham, J.F., Stuart, B.L., and Orlov, N.L. 2006. *Mauremys annamensis*: distribution record. Herpetological Review 37:239.

Spinks, P.Q., Shaffer, H.B., Iverson, J.B., and McCord, W.P. 2004. Phylogenetic hypotheses for the turtle family Geoemydidae. Evolution 32:164–182.

Turtle Conservation Fund. 2003. The world's top 25 most endangered turtles. http://www.turtlesurvival.org/Top_25_Endangered.htm (20 September 2006).

Van Dijk, P.P. 2000. The status of turtles in Asia. Chelonian Research Monographs 2:15–23.

Yasukawa, Y., Ota, H., and Iverson, J.B. 1996. Geographic variation and sexual size dimorphism in *Mauremys mutica* (Cantor, 1842) (Reptilia: Bataguridae), with description of a new subspecies from the Southern Ryukyus, Japan. Zoological Science (Tokyo) 13:303–317.

## 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