mum carapace width, 375 mm; midline plastron length, 439 mm; maximum plastron width, 261 mm; maximum shell depth, 217 mm. Both plastron and carapace are extremely worn and deeply pitted, annuli are no longer discernible, and plastral scute seams barely so. The turtle was reportedly collected on 10 February 2001 as it crawled across a sandbar in the upper Dokhtawady River. The turtle was later killed and eaten, but said to contain no oviductal eggs or noticeably enlarged follicles. The fisherman, a lifelong resident of the area, stated this was the only specimen of *Kachuga* that he had ever captured.

The shell had characteristics of both *K. trivittata* and *K. dhongoka*. According to Ernst and Barbour (1989), both sexes of *K. dhongoka* exhibit a median vertebral stripe and two poorly defined lateral stripes on the carapace, while in *K. trivittata* these stripes are present in males only; females have a uniformly brown carapace. Our specimen appears to have both a median vertebral stripe and lateral stripes, and is larger than the carapace length reported for male *K. trivittata* (46 cm). Moreover, the posterior border of the second vertebral is pointed posteriorly in *K. dhongoka*, a characteristic obvious in our specimen (Table 3), but not reported for *K. trivittata* (Ernst and Barbour, 1989). Also, the length of the second vertebral is greater than the width, and the third vertebral is wider than long in *K. dhongoka*, but these measurements are approximately equal or the scute is slightly wider than long in *K. trivittata* (Ernst and Barbour, 1989). Interestingly, the second vertebral of the specimen is longer than wide, and the third vertebral is wider than long (Table 3), a description consistent with *K. dhongoka*. However, the specimen has an obvious median keel with prominent projections on the second and third vertebrals and a reduced projection on the fourth vertebral (Table 3) as reported for *K. trivittata* (Ernst and Barbour, 1989). *Kachuga dhongoka* has a median keel, but this is reduced to a posterior projection on the second and third vertebral scutes of adults (Ernst and Barbour, 1989). Finally, the plastral formula of the specimen (abd > fem > hum > pect > an > gul) agrees with that reported for *K. trivittata*, rather than *K. dhongoka* (abd > fem > pect > hum > an >< gul) (Smith, 1931; Ernst and Barbour, 1989).

Despite the inconsistent morphological characteristics, the specimen is most likely *K. trivittata*, the only species of *Kachuga* confirmed from Myanmar (Iverson, 1992). *Kachuga dhongoka* occurs only in the Ganges and Brahmaputra drainage of Nepal, Bangladesh, and northeastern India (Ernst and Barbour, 1989; Iverson, 1992) and is unlikely to be found in Myanmar. Myint Maung (1976) reportedly obtained a single specimen of *K. dhongoka* in the early 1970s near Mandalay; however, this specimen has since been lost (Myint Maung, *pers. comm.*) and may have been misidentified.

Table 3. Measurements and description of vertebral scutes from a *Kachuga* shell (possibly *K. trivittata*) obtained at Yee Village along the Dokthawady River, Myanmar, on 18 March 2001.

Vertebral	Midline length (mm)	Maximum width (mm)	Vertebral projection
1	70.0	82.0	Absent
2	99.1	88.6	Prominent
3	61.1	80.4	Prominent
4	110.6	76.5	Projection present, but considerably worn
5	85.0	113.5	Absent

Results of the current and previous investigations (Thorbjarnarson et al., 2000; van Dijk, in press) indicate that viable populations of *K. trivittata* no longer occur in much of the Ayeyarwady River. Likewise, *K. trivittata* is probably extirpated from the lower Chindwin River. A remnant population may occur in the upper Dokhtawady River, and the situation in the headwaters warrants investigation. The possible occurrence of *K. trivittata* in the upper Chindwin, Sittang, and Salween rivers has yet to be investigated. However, with the exception of the latter, these rivers have been extensively degraded by a variety of ecological insults including gold mining, deforestation, agriculture, over-fishing, and siltation (Scott, 1989; Saw Tun Khaing, *pers. comm.*), and are unlikely to support significant numbers of *K. trivittata*. Consequently, we regard *K. trivittata* as Critically Endangered in Myanmar.

Melanochelys trijuga edeniana. — The distribution of this endemic subspecies is poorly known. Locality records are available from lower Myanmar, including Rakhine and Karen States, and Bago and Magwe Divisions (Theobald, 1868; Iverson, 1992; Platt et al., 2001a). We examined shells of locally collected turtles at Hti Chiang Town, Kathar, and Shwegu during March 2001.

Morenia ocellata. — This endemic species is generally thought to be restricted to the Ayeyarwady Delta, lower Sittang River, and coastal regions of the country (Ernst and Barbour, 1989; Iverson, 1992). However, Kuchling (1995) noted market specimens in southern China that appeared to have been collected nearby and speculated that *M. ocellata* may occur much farther north than suggested by previous records. On 7 March 2001 we examined a *M. ocellata* shell in Singkaing Village, approximately 20 km upstream from Mandalay. According to villagers, the turtle was captured in late December 2000 or early January 2001 in floodplain grassland near the village and deposited two eggs shortly thereafter. Villagers regarded *M. ocellata* as rare, and long-term residents stated they had encountered only one other specimen. This record extends the distribution of *M. ocellata* approximately 700 km upstream from previously reported populations in the Ayeyarwady Delta. Furthermore, fishermen at Wacheck Village and Pakokku claimed to occasionally catch *M. ocellata* in the Ayeyarwady River, but specimens were unavailable for our examination.

In November 2000 we also examined a large (800+) group of *M. ocellata* at Yadanabon Zoological Garden in Mandalay that had recently been confiscated from illegal wildlife traders. We selected several of the largest turtles for measurement; the midline carapace length (CL) of four females (CL = 222, 226, 235, 239 mm) exceeded the previously reported size maxima of 220 mm (Ernst and Barbour, 1989). The age of these individuals could not be estimated as *M. ocellata* lack conspicuous annuli.

We recommend that more field studies are needed to better define the distribution and natural history of *L. yuwonoi*, especially for conservation applications. Captive observations of the reproductive biology of this species note that only one large (48–52 g) egg, occasionally two, is laid per clutch, with up to 3 clutches per year (Innis, 2003; B. Bonner, *pers. comm.*; J. Vaughan, *pers. comm.*). This reproductive strategy should be considered when determining protective status for this species. If *L. yuwonoi* generally lays one egg per clutch then any sustainable harvest of adults would be considered impossible (Congdon et al., 1993, 1994). *Leucocephalon yuwonoi* is endemic to Sulawesi and relatively accessible to local turtle collectors. We agree with Platt et al. (2001) that this species should be afforded the highest level of protection under Indonesian law.

Acknowledgements. — We would like to thank the following for helping us find and observe this species in the wild: Robert J. Lee, Steven G. Platt, Faudin Taula, and the turtle collectors of Santigi. For setting up local contacts, we thank Kamuran Tepedelen. We also thank W.P. McCord, Barbara B. Bonner, Indraneil Das, Djoko T. Iskandar, Frank Yuwono, Damien C. Schneider, Jeanie Vaughan, and Jonathan Riley for advice, assistance, and technical and moral support. Manuscript preparation support was provided by the University of Georgia's Savannah River Ecology Laboratory and with Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation. Also, we thank W.P. McCord, Steven G. Platt, Kurt A. Buhlmann, Peter C.H. Pritchard, Anders G.J. Rhodin, and Thomas S.B. Akre for reviewing drafts of this report.

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Genetic Analysis of Mitochondrial DNA Variation in Eastern and Western African Spurred Tortoises, *Geochelone sulcata*

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ABSTRACT. – **Genetic analysis of mtDNA in the widespread African tortoise, *Geochelone sulcata*, revealed three closely related haplotypes, two of them specific to animals from, respectively, Sudan in the eastern range and Senegal in the west. One common haplotype was also found in animals from Sudan, Senegal, and Mali.**

The African spurred tortoise, *Geochelone sulcata* (Miller, 1779) is the largest continental tortoise species, weighing up to 100 kg (Lambert, 1993). Its distribution ranges from Senegal in the west to Eritrea (Erythrea) in the east, which corresponds to a belt about 500 km wide across the sub-Saharan African continent (Loveridge and Williams, 1957; Iverson, 1992; Devaux, 2000a). Although considered common and abundant several decades ago, populations have declined since the early 1990s, and they are now highly fragmented (Fig. 1). Causes of decline are mostly related to the explosion of human population and domestic herds, with enhanced desertification triggered by severe droughts in the 1970s (Devaux, 2000a). *Geochelone sulcata* is now included in Appendix II of CITES and benefits from specific conservation program (Stubbs, 1989; Devaux, 1993).

The wide distribution of *G. sulcata* could possibly be associated with morphologic and genetic differences among populations. A study of size and weight relationships between animals from Sudan and Mali, from opposite ends of the

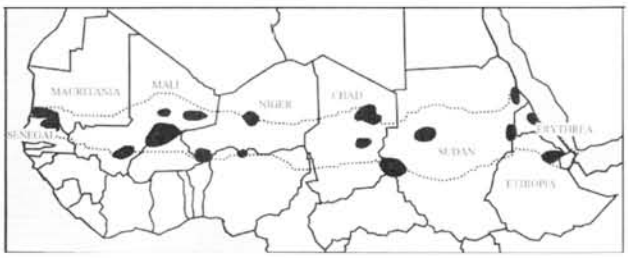

Figure 1. Distribution of *Geochelone sulcata* in Africa (from Devaux, 2000a). Dotted lines indicate the limits at the beginning of the 20th century, while black patches reflect current distribution areas.

range, did not reveal any significant differences (Lambert, 1993), but no analysis of range-wide phenotypic variation has been undertaken so far.

Low morphological diversity does not always correlate to low genetic diversity, as demonstrated in other chelonian species such as *Macroclemmys temminckii* and *Lepidochelys* spp. (Roman et al., 1999; Bowen et al., 1998). Given that conservation programs may require reintroduction or reinforcement actions, it is important to define the genetic status of each population before any release is achieved. We thus performed genetic analyses on wild-caught *G. sulcata* from eastern and western extremes of the distribution area, Sudan and Senegal. To enhance our chances to detect variation, this study focused on the mitochondrial DNA control region, a noncoding region that accumulates mutations more rapidly than functional genes (Lamb et al., 1994; Encalada et al., 1996). Preliminary studies showed that mtDNA could be amplified from buccal swabs, an easy and non-invasive way to collect samples in the field (van der Kuyl et al., 2002).

Methods.—Samples were collected in Sudan and Senegal (January–February 2000) from wild-caught tortoises kept in captivity by dealers before shipping, or in zoos and conservation centers. Buccal swabs were obtained by scraping the inside of the tortoise mouth with a plastic scraper. A leather strap fixed on a wooden fork allowed easy opening of the mouth of the biggest animals (Devaux, 2000b). We analyzed one sample each from 20 individuals, with 11 from Sudan (Kassala Reserve and Port Sudan), 8 from from Senegal (Dakar area), and one from Mali.

DNA was extracted by using silica and guanidinium thiocyanate (Boom et al., 1990). Amplification of 409 nucleotides of the mitochondrial control region sequence was done with a primer set consisting of upstream primer myt001 (5' GAGAAAGACTTAAACCTTC 3'), and downstream primer myt003 (5' GACAAAACAACCAAAGGCCAG 3'), based upon corresponding sequences from *Geochelone nigra* (Genbank accession numbers AF192942–6). Primer myt001 is located in the mt $tRNA^{Pro}$ gene, primer myt003 is in the D-loop sequence. PCR primers were extended with -21M13 and M13RP sequences, respectively, to facilitate direct sequencing. PCR amplifications were done using the following protocol: denaturation 5 min at 95°C, amplification 35 cycles of 1 min at 95°C, 1 min at 55°C, 2 min at 72°C, followed by an extension of 10 min at 72°C.

Table 1. Genetic differentiation and locations of *Geochelone dentata* haplotypes (GSd) reflecting mutations at two different positions.

Sequence	Locations	Position 1 (nt. 48)	Position 2 (nt. 280)
GSd1	Sudan	C	C
GSd2	Senegal	A	T
GSd3	Sudan, Senegal, Mali	A	C

Direct sequencing of the fragments was performed in both directions with a PE-Applied Biosystems 377 automated sequencer, using the Dyenamic Direct Cycle Sequencing Kit and the Dyenamic Energy Transfer Dye Primer Set (Amersham Int., UK). Sequences used in the analyses were deposited with GenBank (accession numbers AY531607-09). Obtained sequences were aligned manually, together with a D-loop sequence obtained from *Testudo graeca* (which was 411 nt in length).

Results. — Three haplotypes were identified, which differed from each other by single nucleotide substitutions (Table 1). The most frequent haplotype, GSd3, was observed in 6 samples from Sudan, 2 samples from Senegal, and the single sample from Mali (Table 2). Haplotype GSd1 was characterized by a cytosine (C) base instead of an adenine (A) at position 48. It was only observed in 4 samples, all from Sudan. GSd2 differed from GSd3 by a thymine (T) replacing a C at position 280, and appeared in 3 samples from Senegal.

Three samples from Senegal were apparently heteroplasmic for GSd3 and GSd2, as two nucleotides, either C or T, were present at position 280, with a C:T ratio of 60:40. These three samples were removed from subsequent analysis.

Using Arlequin software (Schneider et al., 2000), we obtained a value of gene diversity $H = 0.55 (\pm 0.09)$ for Sudan, and $H = 0.60 (\pm 0.17)$ for the Senegal sample. In spite of the small sample size and little genetic diversity, Sudan and

Table 2. Specimens of *Geochelone dentata* analyzed for mtDNA haplotype. All animals were captured in the field, except those from Kessala Reserve whose exact origin in Sudan was unknown.

Haplotype	Origin	Sex	CL (mm)	Locality
GSd1	Sudan	F	250	Port Sudan
GSd1	Sudan	M	255	Port Sudan
GSd1	Sudan	M	250	Kessala Reserve
GSd1	Sudan	F	480	Kessala Reserve
GSd1	Sudan	F	230	Kessala Reserve
GSd2	Senegal	M	350	Dakar
GSd2	Senegal	M	700	M'Bour, Casamance
GSd2	Senegal	F	600	Richardtown
GSd3	Sudan	F	270	Port Sudan
GSd3	Sudan	M	260	Port Sudan
[illegible]	[illegible]	F	630	Est Kessala
GSd3	Sudan	M	700	Kessala Reserve
GSd3	Sudan	M	185	Port Sudan
GSd3	Sudan	F	245	Port Sudan
GSd3	Senegal	M	260	Dakar
GSd3	Senegal	F	260	Ferlo
GSd3	Mali	M	820	Bamako
GSd2+3	Senegal	F	250	Popenkine petite côte
GSd2+3	Senegal	M	230	Dakar
GSd2+3	Senegal	M	730	Dakar

Senegal were significantly genetically different (Fst = 0.26, p = 0.041).

Discussion. — We found very little genetic variation in mtDNA across the range of *G. sulcata*, with the most common haplotype (GSd3) present in Senegal, Sudan, and Mali. However, some genetic divergence does occur between the eastern and western populations of *G. sulcata* from Senegal and Sudan, with two haplotypes (GSd1 and GSd2) derived from the common GSD3 haplotype by a substitution of single nucleotides at positions 48 and 280. This relative lack of variation is not very surprising as a small number of haplotypes in mtDNA sequences is frequently observed in chelonians, and these animals are noticeable for their slow rate of mtDNA evolution (Avise et al., 1992; Lamb et al., 1994; Starkey et al., 2003).

Two haplotypes (GSd1 and GSd2) were found only in tortoises from Sudan and Senegal, respectively. They represent 45 and 37%, respectively, of our samples from these two localities. If these haplotypes are indeed specific to these populations, then GSd1 and GSd2 could provide identification of some individuals from unknown origins. Yet, given that most samples have the same common haplotype GSd3, only a limited proportion of animals would be clearly identified.

Heteroplasmy (the existence of different mtDNA haplotypes in a single individual) was observed in 3 animals from Senegal. A sequencing artefact is unlikely for several reasons: a double peak was observed only at position 280 and showed only two nucleotides (C or T). Therefore this position could be susceptible to mutations (which could have appeared at different periods, or which may have occurred only once and not yet become fixed in the population, as genetic drift is very slow in chelonians). Heteroplasmy has been described previously in green turtles (Encalada et al. 1996) and in many non-reptilian species, including humans.

This study provides an example of the dilemma in conservation biology of determining what we should protect (Avise, 1989; Rojas, 1992; Lande, 1995; Shrader-Frechette and McCoy, 1999). So far, morphological traits of size have not allowed differentiation between populations of *G. sulcata* (Lambert, 1993). As long as we do not know how morphological differences are related to genetic differences, a common sense solution is to limit reintroduction or reinforcement programs to animals of local origin or known genetic identity. Improving our knowledge of genetic and phenotypic variation of *G. sulcata* is urgent to achieve sustainable and efficient conservation programs.

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Distribution of the Black-Breasted Leaf Turtle (*Geoemyda spengleri*) on Hainan Island, China

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ABSTRACT.–***Geoemyda spengleri* (Geoemydidae) occurs in low numbers in remote regions of Hainan Island, China, where it is threatened by commercial trade. Four specimens were recorded on a recent field survey.**

The current distributional records of the black-breasted leaf turtle (*Geoemyda spengleri*) in China are restricted to Hunan, Guangxi, and Guangdong provinces (Sichuan Institute of Biology, 1976). The Hunan record is often excluded (e.g., Tian and Jiang, 1986; Ernst and Barbour, 1989). Yao and Liu (1995) reported a record of *G. spengleri* in Anhui Province, but this may be based upon an introduced specimen (Zhao, 1997). Some literature may not list Hainan separately from Guangdong because Hainan was declared a separate province only in 1988. Nevertheless, earlier literature that specialized on Hainan Island did not mention *Geoemyda spengleri* (Schmidt, 1927; Gressitt, 1940). Li (1958) noted 11 species of freshwater turtles on Hainan Island, but did not list *G. spengleri*.

The earliest reports of *G. spengleri* occurring on Hainan are Zhao (1986) and Tian and Jiang (1986). There are voucher specimens of *G. spengleri* in the Chengdu Institute of Biology, collected at Shuiman Town, Wuzhishan area, and Dali town, Diaoluoshan area, on Hainan Island, but authoritative works (Iverson, 1992; Zhang et al., 1998) exclude *G. spengleri* from the turtle fauna of Hainan Province. In a recent work on the turtles of Hainan Island, de Bruin and Artner (1999) reported finding no evidence of the occurrence of *G. spengleri* and doubted its presence there. Yet, given its known occurrence in northern Vietnam and southern China, the presence of *G. spengleri* on Hainan could be expected.

An important aspect of understanding the distribution of turtles in China is that the presence of a specimen does not verify the existence of a wild population. Multiple examples of erroneous or introduced turtle localities are known (e.g., see Parham and Li, 1999; Fong et al., 2002) because turtles have been moved around China for hundreds of years. Zhao (1998) noted that *G. spengleri* had been imported to Chinese markets from neighboring countries. It is hard or impossible to determine whether turtles are imported or local when found in markets.

With this in mind, in August 2002, I conducted surveys in several remote villages in Qiongzhong County, Hainan Island, to resolve whether *G. spengleri* occurs there natu-

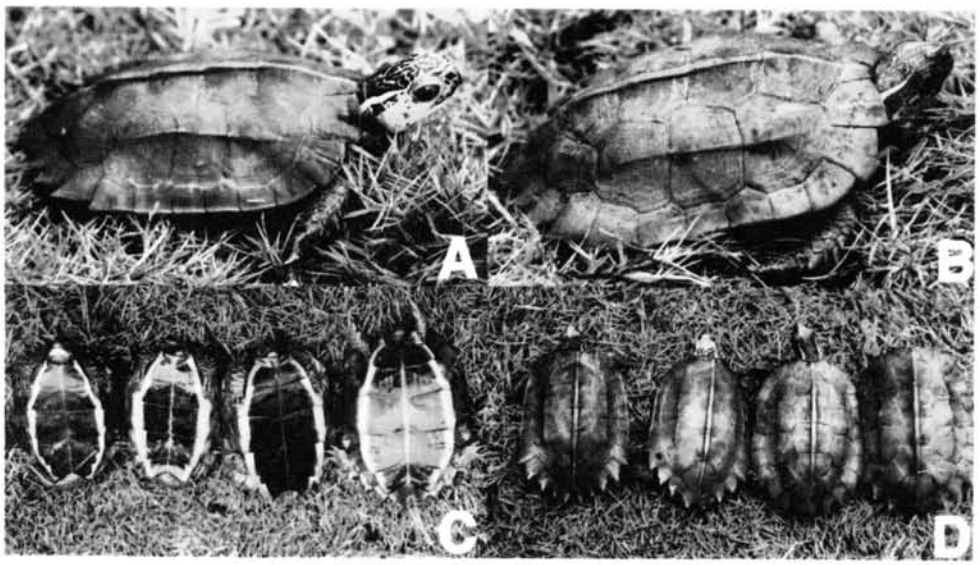

Figure 1. Wild-caught *Geoemyda spengleri* from Hainan Island. **A.** Male from Nanmao; **B.** Female from Xinxiang; **C** and **D.** Ventral and dorsal views of all 4 specimens, from left to right, from Yinggen, Nanmao, Xinxiang, and Xiangtu.