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A COMPARATIVE STUDY OF CROCODILE LIZARDS (*SHINISAURUS CROCODILURUS* AHL, 1930) FROM VIETNAM AND CHINA

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***ABSTRACT.* –** Preliminary morphological comparisons between Chinese and Vietnamese *Shinisaurus* representatives showed no significant differences that would justify a separate taxonomic status of the single known Vietnamese population. Also first mitochondrial DNA sequence comparisons showed very low differentiation therefore, being likely to represent the same taxon. In addition, we provide further information about the habitat, and preliminary data concerning the reproduction and feeding ecology of Vietnamese *S. crocodilurus*. We further stress biogeographical and conservational aspects of the endangered species in Vietnam.

***KEY WORDS.* –** *Shinisaurus crocodilurus*, mitochondrial DNA, morphology, taxonomy, natural history, biogeography.

INTRODUCTION

Until recently, the Chinese crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930 was known only from a restricted area in Guangxi Zhuang Autonomous Region, as well as from the Chinese provinces of Guangdong, Guizhou and Hunan (Fan, 1931; Shen & Li, 1982; Liu et al., 1989; Zhao & Adler, 1993; Mägdefrau & Schildger, 1993; Mägdefrau, 1997; Zhao et al., 1999; Li & Xiao, 2002). More than seventy years after its discovery, Le & Ziegler (2003) reported for the first time on a record of this rare and endangered species outside of China: in May 2002, crocodile lizards were discovered at moderate elevations in the evergreen forest of Yen Tu Nature Reserve, Quang Ninh Province, northeastern Vietnam, thus representing the first record of the species for Vietnam. However, Le & Ziegler (2003) stressed that "further taxonomic work is desirable in the future", as the Vietnamese *Shinisaurus*, although superficially similar to the Chinese populations, were discovered about 500 km from the nearest known population in China.

We now have had the chance to closer investigate the Vietnamese *Shinisaurus* series presented in Le & Ziegler (2003). In addition, some further specimens collected by Vietnamese scientists in Yen Tu were made available from the Zoological Museum of the Vietnam National University Hanoi for morphological and molecular comparisons. We present here the results of our preliminary molecular and morphological comparisons and add further notes on the natural history and biogeography of *S. crocodilurus* in Vietnam.

MATERIALS AND METHODS

Specimens studied are stored in the collections of the Vietnam National University, Hanoi, Zoological Museum (VNUH), the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany and the Zoologisches Museum, Museum für Naturkunde der Humboldt-Universität zu Berlin (ZMB), Germany.

We examined 10 specimens from Vietnam (all from Yen Tu Nature Reserve, Quang Ninh Province, northeastern Vietnam): VNUH 19.5.'02-1 to 19.5.'02-3, VNUH 31.8.03-1 to 31.8.03-4, and ZFMK 83901 to 83903. From China, 39 specimens were available for comparisons, among them the lectotype ZMB 34034 (Fig. 1) from near Loshiang, "Yao Shan" (Mt. Yao) in "Kwangsi Prov." (Guangxi Zhuang Autonomous Region), China, coll. et don. S. S. Shin (Good et al., 1993); the paralectotype ZMB 34035 (an adult female, same data as lectotype) has been dissected and was not available for our study. The remaining 37 specimens derived from the Chinese pet trade and lack precise locality data (ZFMK 39424, 40557, 41332, 42534, 42535 to 42537, 43788 to 43790, 44308, 44691, 46042 to 46044, 47711, 53923 to 53932, 53934 to 53937, 53939, 53940, 57856, 57857, 63573, 73089, 78802 and 78803).

Measurements were taken to the nearest millimetre (mm) using a metre rule or sliding vernier caliper, interpolated to the nearest 1/10 mm. Abbreviations are as follows: SVL = snout-vent length (from tip of head to cloaca); TaL = tail length (from cloaca to tail tip); TL = total length; HL = head length (from snout tip to end of head scales); HW = maximum head width; L4 - lamellae beneath fourth toe ($L4^1$ - to toe insertion; $L4^2$ - enlarged lamellae only).

For comparisons of genital structures, the invertedly fixed, left hemipenis of the adult Vietnamese specimen ZFMK 83901 (SVL 146 mm, TaL 158 mm) was removed from the tail base and then was everted based on the method described by Pesantes (1994) for snakes and for lizards by Ziegler & Böhme (1997, see also Ziegler et al., 2004). For genital morphology terminology, we refer to Böhme (1988) and Ziegler & Böhme (1997).

Genomic DNA was extracted from the blood of an adult pet trade specimen putatively from China kept in the Aquarium of the Zoological Garden Cologne (Cologne Zoo SC1), from the liver of the adult female specimens ZFMK 83902 (field no. YT 05) and ZFMK 83903 (field no. YT 06), as well as of the juvenile specimen VNUH 31.8.03-4 using Chelex-extraction. A fragment of the mitochondrial 16S rRNA gene of each specimen was amplified by using primers 16SA-L (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SB-H (5'-CCG GTC TGA ACT CAG ATC ACG T-3'), see Palumbi et al. (1991), and by 35 cycles of PCR: initial denaturation, 120 sec. 95°C, 34 cycles of 30 sec. at 95°C, 60 sec. at 55°C and 60 sec. at 72°C, annealing temperature 55°C. Double-stranded DNA was sequenced on an ABI 3770 capillary sequencer. We obtained sequences of the zoo specimen as well as of specimens ZFMK 83902, ZFMK 83903, and VNUH 31.8.03-4, which have been deposited in Genbank under the accession numbers EF061309 to EF061312. These sequences were compared among each other with the phylogenetic data editor program, PhyDe, Version 0.98 (www.PhyDe.de). Additionally, we included the sequences of another specimen putatively from China in our study, that is deposited in the Nagoya University Museum, Japan, under NUM-Az 0370 (see Kumazawa, 2004: Genbank accession number AB080274).

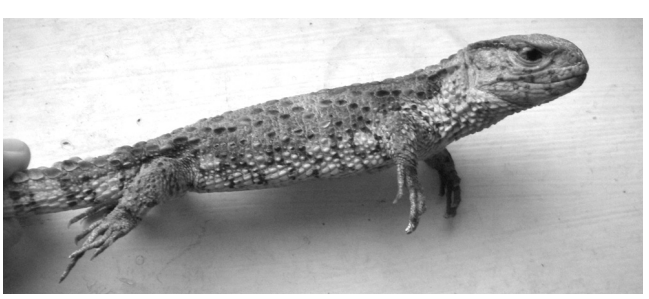

Fig. 1. Male lectotype of *Shinisaurus crocodilurus* (ZMB 34034) from Guangxi Zhuang Autonomous Region, China. Photograph by T. Ziegler.

RESULTS AND DISCUSSION

***Mitochondrial DNA sequence data.* –** The comparison of the obtained fragments which were amplified to 479 base pairs (bp) of the same haplotype has shown 100% (479/479 bp) congruence of the three Vietnamese specimens, VNUH 31.8.03-4, ZFMK 83902 and ZFMK 83903 whereas the specimens putatively originating from China (Cologne Zoo SC1; NUM-Az 0370, see Kumazawa, 2004) showed only a divergence of 1 bp to the other samples. This very low genetic differentiation of 0.2% uncorrected pairwise sequence divergence is clearly within the range of intraspecific differentiation in squamates, as revealed by multiple studies in the past years (e.g., Vences et al., 2004; Poulakakis et al., 2005; Carranza et al., 2006).

***Morphology.* –** We conducted a careful external morphological comparison of the Vietnamese voucher specimens with the original familiar, generic and specific description by Ahl (1930), with the extended descriptions by Fan (1931) and Zhao et al. (1999) as well as with museum vouchers from China. Because comprehensive comparisons between the different *Shinisaurus* populations from China are still lacking to our knowledge, and as museum vouchers mostly lack precise locality data (see, e. g., Macey et al. 1999; Kumazawa, 2004), we could not consider possible differences between Chinese populations in this study. However, Zhao et al. (1999) state that the banding patterns of Chinese *S. crocodilurus* "vary with biogeographical distribution".

Concerning colour pattern, we could not find major differences between the Chinese and Vietnamese *Shinisaurus* examined. We also observed the slight sexual dimorphism stated by Ahl (1930) for Chinese *S. crocodilurus*, viz. showing somewhat stronger developed head crests and enlarged neck scales in the males. However, Ahl's (1930) assumption that

Table 1. Selected scale counts (minima, maxima, mean and standard deviation; measurements in mm) of seven Vietnamese (including four dissected embryos of ZFMK 83902) and 34 Chinese *Shinisaurus crocodilurus* deposited in the ZFMK collection, in comparison with pholidosis data of Chinese specimens as presented in the original description by Ahl (1930) and in the extended description by Fan (1931).

	***S. crocodilurus* from Vietnam (n = 7)**	***S. crocodilurus* from China (n = 34)**	**after Ahl (1930)**	**after Fan (1931)**
Supralabials	18–21 19.6 ± 1.0	16–21 19.0 ± 1.3	—	19
Adjacent rows of supralabials	1–2 1.9 ± 0.4	1–2 1.9 ± 0.2	1	1
Enlarged Infralabials	11–15 12.5 ± 1.2	10–16 12.9 ± 1.6	—	—
Adjacent infralabial rows (counted below eye)	3–4 3.9 ± 0.4	3–5 4.0 ± 0.3	3	3
Enlarged collar scales	9–12 10.9 ± 1.1	10–12 11.1 ± 0.8	10–12	—
Longitudinal ventral rows	12–15 14.0 ± 1.0	13–15 13.9 ± 0.6	13	12–13
Transversal ventral rows	36–40 37.9 ± 1.5	31–42 36.0 ± 2.2	26–27	28
Enlarged scales before cloaca	5–8 6.6 ± 1.1	4–7 5.6 ± 1.0	6–7	6
Tail whorls	40–43 41.3 ± 1.2	37–45 41.2 ± 1.9	36–40	40
Lamellae below fourth toe	24–27 25.2 ± 1.1	17–26 21.7 ± 1.8	—	20

the Chinese males differ from the females in having a red (versus a grey) ground coloration with an anteriorly reddish brown venter (versus a cream to light red venter in females), was already falsified as individual colour variation by Fan (1931). Mägdefrau & Schildger (1993) summarized that Chinese males rarely have a completely red venter, but more often red, orange and yellow blotches on throat, belly and flanks; in contrast, Chinese females bear a beige to light yellow ventral and flank pattern. However, as some females also may show a conspicuous red pattern, a colour-dependent sexing seems rather unreliable. We observed the same situation in the Vietnamese specimens. From live photographs of the adult and subsequently dissected male specimen VNUH 31.8.03-3 (Fig. 2) the distinct orange to red flank, belly, throat and head area was well discernible, whereas the adult male VNUH 31.8.03-2 differed by showing only grey head sides. Whereas belly, throat and head sides of four adult Vietnamese females was cream to light brown or yellow (Fig. 3), one of the three females depicted in Le & Ziegler (2003) in contrast had a red throat area and distinct, large red blotches that were framed by dark at the lower flanks as well as on the lower tail base.

Also the scalation comparisons revealed no significant differences between the Chinese and Vietnamese *Shinisaurus* populations examined (Table 1). The shape and structures of the subsequently everted left hemipenis of the adult

Fig. 2. Male *Shinisaurus crocodilurus* (VNUH 31.8.03-3) from Yen Tu Nature Reserve, Vietnam. Photograph by Le Khac Quyet.

Fig. 3. Female *Shinisaurus crocodilurus* (ZFMK 83903) from Yen Tu Nature Reserve, Vietnam. Photograph by Le Khac Quyet.

Vietnamese specimens ZFMK 83901, ca 16 mm in length (Fig. 4), do not differ from the hemipenis descriptions of Chinese specimens by Ahl (1930) and Böhme (1988) as well as from the hemipenis drawings of Chinese *Shinisaurus* depicted in F. Zhang (1986), Böhme (1988), and Ziegler & Böhme (1997). The only slight morphological difference we were able to detect concerned the number of scales below the fourth toe. Whereas the 39 Chinese vouchers (including the lectotype ZMB 34034) had 17-26 (21.8 ± 1.7) lamellae beneath the fourth toe to toe insertion (and only 17-25 [21.1 ± 1.6] enlarged lamellae beneath the fourth toe), the ten Vietnamese specimens showed 24-28 (25.5 ± 1.4) lamellae to toe insertion (and only 19-25 [22.0 ± 2.1] enlarged lamellae, respectively, see also Tables 1-2).

Fig. 4. Oblique sulcal view of the left hemipenis of an adult *Shinisaurus crocodilurus* from Vietnam (ZFMK 83901); due to the eversion after fixation the organ appears elongated and both terminal lobes are not upwards directed due to a preparation artefact. Photograph by T. Ziegler.

Although the Vietnamese *Shinisaurus* specimens showed somewhat higher lamellar counts below the fourth toe compared with the Chinese populations, such partial overlapping scalation features do not provide significant data for a proper separation of the different geographical populations. In view of the strong molecular sequence similarity, we choose to interpret the somewhat different lamellar counts between the Chinese and Vietnamese populations examined by us as variation that does not warrant taxonomic separation.

***Habitat and Ecology.* –** The *Shinisaurus crocodilurus* from Yen Tu in northeastern Vietnam are still the only known population outside of China. Yen Tu Nature Reserve comprises more than 16,000 ha (160 km^2; www.birdlifeindochina.org) and lies within Bac Giang and Quang Ninh provinces (21°09'–21°23'N 106°38'–107°02'E), about 80 km from the border with China (compare the map depicted in Le & Ziegler, 2003). Yen Tu is part of one of the largest remaining evergreen forest areas in north-eastern Vietnam (Fig. 5) and may host a unique biodiversity that is no longer found elsewhere in the country (www.birdlifeindochina.org). *Shinisaurus* specimens were found at altitudes of 400 to 800 m a.s.l., with Mount Yen Tu being with up to 1,068 m the highest point in the nature reserve. Chinese *S. crocodilurus* are known to occur from 200 to 1,500 m a.s.l., where they dwell along densely vegetated and slow running mountain streams or ponds (Zhao et al., 1999; Le & Ziegler, 2003). In the core area of Yen Tu, they have been found active at daytime, with a provisionally estimated number of 10 to 15 individuals per square kilometre. The lizards were resting on branches at slow running stream banks (Fig. 6) or on stones near stream ponds, and escaped into the water when disturbed, as already stated by Fan (1931) for Chinese specimens (see also Zhao et al., 1999). For further ecological observations of the Vietnam population see Le & Ziegler (2003).

The adult females collected during summer season (mid-May) 2002 (VNUH 19.5.'02-1 to 19.5.'02-3, depicted in Le & Ziegler, 2003) had each five to seven eggs of 9.9-11 (10.6 ± 0.6) mm maximum size (VNUH 19.5.'02-1: left side three,

Fig. 5. Habitat of *Shinisaurus crocodilurus* in north-eastern Vietnam: the evergreen lowland forest of Yen Tu Nature Reserve, Quang Ninh province. Photograph by Le Khac Quyet.

Table 2. Selected measurements and enlarged scales beneath the fourth toe of the Vietnamese representatives of *Shinisaurus crocodilurus* (minima, maxima, mean and standard deviation; measurements in mm); for abbreviations see materials and methods.

	males (n = 4)	**females (n = 5)**
SVL	146.0–154.0 150.5 ± 3.4	147.0–162.0 154.4 ± 6.6
TaL	141.0–191.0 170.3 ± 25.0	171.0–216.0 198.2 ± 17.3
TL	293.0–345.0 320.8 ± 26.1	321.0–378.0 352.6 ± 22.6
HL	30.7–35.0 33.2 ± 1.8	29.4–33.5 31.5 ± 1.5
HW	23.1–25.0 24.0 ± 0.8	20.4–23.8 22.4 ± 1.3
$L4^1$	25–28 26.8 ± 1.0	24–26 24.8 ± 1.0
$L4^2$	22–25 23.3 ± 1.3	19–25 21.4 ± 2.3

right side four eggs; VNUH 19.5.'02-2: left side four, right side three eggs; VNUH 19.5.'02-3: left side two, right side three eggs). The maximum size of oviduct eggs was 2.2-3.1 (2.8 ± 0.5) mm in diameter. Adult females that were collected in mid-September (autumn) most probably were ready to give birth or already had given birth to their offspring: ZFMK 83902 had in addition to small eggs with a maximum diameter of 3.2 mm four large eggs with well developed embryos on the right side (SVL 34.8-38, TL 83.3-84.5 mm), and three eggs with embryos on the left side; the diameter of the yolk mass measured up to 11.2 mm. The adult female ZFMK 83903 had in addition to small eggs of up to 3.5 mm width dilated and thus folded oviducts, indicating that giving birth must already have taken place. The adult males that were collected during autumn, at the end of August 2003 (VNUH 31.8.03-1 to 31.8.03-3), had testes lengths of 10.6 mm and testes widths from 6.6 to 8.0 (7.4 ± 0.7) mm. The adult male that was collected in mid-September 2005 had a 10.1 long and a 9.2 mm wide testis.

Fig. 6. Such slow-running streams are inhabited by *Shinisaurus crocodilurus* in Yen Tu Nature Reserve. Photograph by Le Khac Quyet.

Concerning the natural diet, we found remains of aquatic and terrestrial arthropods (undetermined insects, one spider, and one crustacean) in the gastro-intestinal tracts of four specimens; remains of ground particles and earth in the guts most probably came from already digested earthworms (observations on feeding on earthworm-like invertebrates was reported by Le & Ziegler, 2003). In the guts of two adult male specimens we found crustacean claw remains of 7.4 and 10.9 mm length (in the specimen VNUH 31.8.03-2) and a katydid ovipositor of 5 mm width and 20 mm length (in the specimen ZFMK 83901). The gut of the adult female specimen ZFMK 83903 showed fragmented remains of winged insects (Pterygota) besides numerous endoparasitic worms as well as ground particles (up to 5 mm large pebble). Lastly, we found remains of an insect and a spider in the gut of the juvenile specimen VNUH 31.8.03-4. Chinese *Shinisaurus* are known to ingest worms, cockroaches, beetles, small fishes, tadpoles and frogs (Fan, 1931; Zhao et al., 1999).

The herpetofauna of Yen Tu is still imperfectly known (see also Nguyen et al., 2000). Recently, Darevsky et al. (2004) described two new scincid species from Mount Yen Tu: *Sphenomorphus cryptotis* and *S. devorator*. During field studies in Yen Tu in Mid-September 2005 two of us (LKQ, VNT) found the former species (ZFMK 83899, 83900) occurring syntopically with *Shinisaurus crocodilurus*. A second stream-dwelling lizard species that was found by us together with *S. crocodilurus* was the water skink *Tropidophorus hainanus* (ZFMK 83898). Like *Shinisaurus crocodilurus*, *Tropidophorus hainanus* and *Sphenomorphus cryptotis*, with its superficial ear opening and the laterally depressed tail, are all highly aquatic lizard species that are adapted to swim and dive in forest streams.

Zoogeographical and conservation aspects. **–** In China, *Shinisaurus crocodilurus* has been recorded so far only from limestone areas in Guangxi Zhuang Autonomous Region, from Guizhou and Hunan provinces, as well as most recently

also from Luokeng Nature Reserve in Guangdong province (e.g. Shen & Li, 1982; Mägdefrau & Schildger, 1993; Zhao et al., 1999; Li & Xiao, 2002). As limestone in tropical China occurs mainly in Guangxi and Yunnan provinces of southern and central China (Zhu et al., 2003) the discovery of further populations can be expected, as was already stated by Mägdefrau (1987). Furthermore, Zhu et al. (2003) stressed that the limestone flora in southern Yunnan shares the most genera with the limestone flora of northern Vietnam among the floras compared in their study (e.g. from subtropical China, and northern Thailand). Thus, it could be expected from vegetational similarities, that the corridor between northeastern Vietnam and southern China shares more common faunal elements than previously thought. Recently, Ohler (2005) stressed the problem of traditionally separated taxonomies on both sides of the Chinese-Vietnamese border, although the Chinese tropical and subtropical fauna must be included in the faunal analysis of the Oriental region. Several examples of parallel taxonomies in anurans were highlighted by Ohler (2005) who demonstrated that only "rare" species show a country-specific distribution but most of the species being found to occur on both border sides. In addition, Bobrov (1997) has provided a critical evaluation of former, traditional zoogeographical concepts, that erected the boundary along the China-Vietnam frontier, but which did not correspond to his recent lizard fauna analyses.

Furthermore, the poorly known crotaline viper species *Protobothrops cornutus*, formerly only known from few localities in central and northern Vietnam (Ziegler & Herrmann, 2002; Herrmann et al., 2004), was recently recorded from China for the first time, viz. from two regions in Guangxi (David et al., in press). Thus, the recent finding of *Shinisaurus crocodilurus* in north-eastern Vietnam underlines the close zoogeographical affinities between north-eastern Vietnam and adjacent southern China. Because the discovery took place in the lowland evergreen forest, further discoveries of populations even in non-limestone areas in the Vietnam-China border region seem possible. However, as this forest type has been lost from most other areas in north-eastern Vietnam, the importance of Yen Tu Nature Reserve as refuge not only for the endangered Chinese crocodile lizard must be strongly underlined.

Le & Ziegler (2003) already summarized the threats to Vietnamese *Shinisaurus* representatives as well as to the biodiversity of Yen Tu Nature Reserve in general, ranging from illegal timber extraction and collecting non timber forestry products together with habitat disturbance, hunting and pet trade to increasing tourism development. As first measures the authors proposed to include the species, that is listed on CITES Appendix II since 1990, into the Red data book of Vietnam's animals. To guarantee the preservation of *Shinisaurus crocodilurus* in north-eastern Vietnam and possibly in adjacent areas, we furthermore recommend to: 1) proceed with field studies focusing on population size estimates, habitat requirements and natural history; 2) conduct field surveys in adjacent areas to learn more about the current distribution; and 3) render respective conservation measures and habitat management proposals for Yen Tu Nature Reserve.

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