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On the Cover: *Varanus mertensi* and *Crocodylus johnstoni*

The Mertens' water monitor (*Varanus mertensi*) and freshwater crocodile (*Crocodylus johnstoni*) depicted on the cover and inset of this issue were photographed by **Max Jackson** in the Kimberly Region of Western Australia on 6 October 2018.

At around 1600 h, an adult *V. mertensi* (ca. 90-100 cm in total length [TL]) was observed moving along the edge of a sandstone escarpment lining a creek. Shortly thereafter, the lizard discovered a recently deceased *C. johnstoni* (ca. 40-45 cm TL) on the bank of the creek. After briefly investigating the *C. johnstoni* by smell, the *V. mertensi* quickly seized the crocodile by the head and whipped it against a rock several times before it began to swallow it whole.

Although *V. mertensi* has seen extensive declines throughout its range in Queensland and the Northern Territory where it has been impacted by the spread of the invasive cane toad (*Rhinella marina*) and is now listed as Endangered by the IUCN Red List (Shea *et al*. 2018. *Varanus mertensi*. The IUCN Red List of Threatened Species 2018: e.T83778246A101752340), it is reportedly common at the site where this observation took place (M. Jackson, pers. obs.).

BIAWAK

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INTERNATIONAL VARANID INTEREST GROUP
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The International Varanid Interest Group is a volunteer-based organization established to advance varanid research, conservation, and husbandry, and to promote scientific literacy among varanid enthusiasts. Membership to the IVIG is free, and open to anyone with an interest in varanid lizards and the advancement of varanid research. Membership includes subscription to *Biawak,* an international research journal of varanid biology and husbandry, and is available online through the IVIG website.

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Varanus bengalensis. Tamanthi Wildlife Sanctuary, Hkamti District, Sagaing Region, Myanmar. Photographed by **Matt Evans.**

NEWS NOTES

Louisville Zoo Receives Komodo Dragon

A juvenile Komodo dragon (*Varanus komodoensis*) has gone on public display at the Louisville Zoo. The young male, named Romulus after the founder of the city of Rome, was hatched in March at the Fort Worth Zoo. The animal is currently on display in its own space within the larger outdoor dragon enclosure, which will serve as its permanent home when it reaches maturity.

Source: WDRB.com; 31 May 2018

Officials Consider Public Opinion Poll for What to do with Lumpini Park Monitors

Bangkok Metropolitan Administration (BMA) officials have noted that Asian water monitors (*Varanus salvator macromaculatus*) living in Bangkok's urban Lumpini Park have been reproducing quickly and disturbing visitors to the park, and that they may hold a public opinion poll to ask residents whether the lizards should be evicted from the park. A population size of around 400 individuals is estimated in the park, and it is claimed that the species can reproduce two or three times a year in the park. The BMA relocated 150 adult monitors from the park in 2016, and an additional 150 individuals in 2018. The administration has noted that it will consider holding an opinion poll before taking any further action.

Source: The Nation (Thailand); 4 September 2018

Four Arrested in Killing of Bengal Monitor

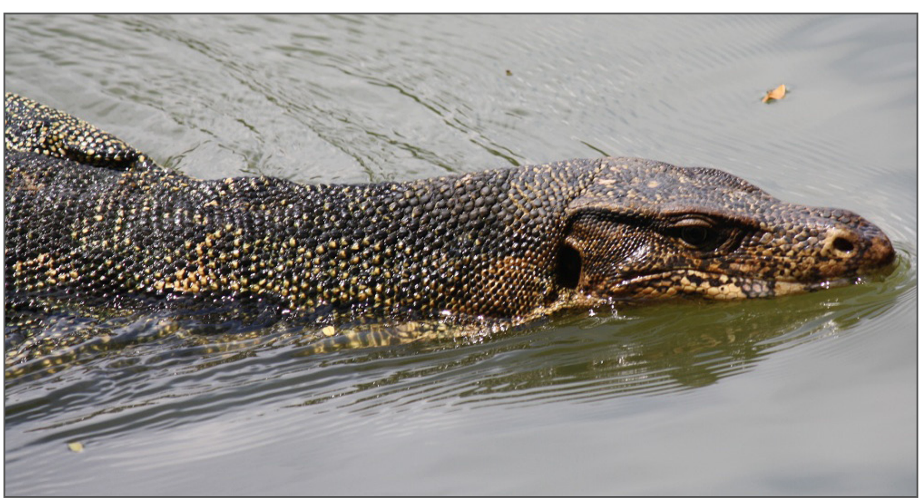
Four residents of Greater Noida, Uttar Pradesh, India have been arrested for the illegal killing of a Bengal monitor (*Varanus bengalensis*) that had entered into their house. Video footage of the killing was posted online, which also led to additional charges of "trophying". Bengal monitors are protected in India under the Wildlife Protection Act of 1972, which calls for punitive action against capturing, killing, poisoning, snaring or trapping protected species.

Source: The Times of India; 24 June 2018

Seizure of 49 Bengal Monitor Hemipenes

A recent raid by forest officials and members of the Wildlife Crime Control Bureau in Chikkamagaluru, India resulted in the seizure of 49 sets of dried Bengal monitor (*Varanus bengalensis*) hemipenes and the arrest of four individuals. The perpetrators were stopped in Chikkamagaluru where they traveled to sell the reproductive organs. Wildlife activists note that this activity had been confined to northern India, and that this seizure represents the first in southern India.

Source: The Deccan Chronicle; 16 December 2018



Varanus salvator macromaculatus. Lumpini Park, Bangkok, Thailand. Photographed by **Robert W. Mendyk**

Komodo Dragon Breeding Facility at Chattanooga Zoo

Chattanooga Zoo has announced the planning of a new facility specifically for the breeding of Komodo dragons (*Varanus komodoensis*). Currently, the zoo is home to two dragons: a male, Kadal, and female, Charlie, both seven years old. Both animals were transferred to the zoo as part of the Species Survival Plan but have yet to be introduced to each other. In addition to work areas and facilities to raise resulting offspring, the area will serve as a connecting space between the zoo's indoor dragon exhibit, a new outdoor exhibit, and an off-display enclosure. The new facility opened to the public in July.

Source: The Chattanoogan; 29 June 2018

Man Arrested in Catalonia with Illegal Komodo Dragon

Officials with the Nature Protection Service (Seprona) of the Civil Guard recently seized an illegally sourced Komodo dragon (*Varanus komodoensis*) from a private property in Catalonia, Spain. The specimen exhibited prior injuries including a missing front foot and partially missing tail, and had been illegally acquired along with 20 specimens of other reptile and invertebrate species. The animals were in the care of a local resident described as a 43-year-old US citizen, who was charged with crimes against the protection of flora and fauna, smuggling, and illicit arms possession. According to Europol, this is the first illegally-acquired Komodo dragon to be seized in Europe. The animals have since been transferred to zoological care centers in the region.

Source: www.elpais.com; 23 July 2018

Four Komodo Dragons Hatch at San Antonio Zoo

Four Komodo dragons (*Varanus komodoensis*) recently hatched at the San Antonio Zoo. These hatchlings represent the second and largest successful clutch of *V. komodoensis* hatched at the zoo. The offspring will be placed on display in the coming months.

Source: News4SA.com; 2 November 2018

Varanus komodoensis. Rinca, Lesser Sundas, Indonesia. Photographed by **Avishai Shuter**.

ARTICLES

Biawak, 12(2), pp. 73–83

Testing the Applicability of ^{15}N Isotopic Marker in Skin Tissue to Distinguish Between Captive and Wild Monitor Lizards

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Abstract - In an effort to meet the demand for the trade and to reduce pressure on wild populations of monitor lizards (*Varanus* spp.), several countries have encouraged the establishment of captive breeding facilities. The fear has been expressed, however, that these facilities are not able to produce the quantity of specimens they claim and therefore supplement their supply with wild specimens. Thus, reliable methods are required for verifying the declaration of origin. Stable isotope analyses have been discussed as a potential method to discriminate wild from captive specimens. We herein designed a controlled feeding study and marked ten specimens of three *Varanus* species (*V. acanthurus, V. macraei,* and *V. melinus*) at the Cologne Zoo in Germany with ^{15}N enriched glycine in order to examine the time lag for the isotopic signal to appear in renewed epidermis. We found that the ^{15}N enriched marker was detected within two to five weeks after exposition and conclude that ^{15}N isotopic signature in keratin skin tissue is likely to change quickly after the introduction of novel diets. Thus, distinguishing captive-bred from wild origin based on $\delta^{15}N$ values in shed skin material might not be effective due to permanent, rapid epidermal renewal and therefore of limited forensic relevance. Thus, the study of isotopic signals from tissue samples including several layers and tissues could be an alternative approach. Furthermore, diet shift studies relating to isotopic incorporation rates as well as reconsidering the examination of site-specific markers are recommended. Additional methods need to be tested for suitability to identify the origin of monitor lizards being declared as captive-bred, which can prevent the laundering of wild-caught individuals through breeding farms.

Introduction

Monitor lizards face a number of potential threats, including habitat destruction, human consumption, traditional medicine, and collection for the global leather industry and pet trade (Schlaepfer *et al*., 2005; Bennett *et al*., 2010). Concerns over high levels of exploitation and trade in an increasing number of *Varanus* species have been reported throughout the last decade (Pernetta, 2009; Koch *et al.*, 2013; Crook & Musing, 2016). Owing to concerns that international trade may have a detrimental impact on species survival, the entire genus *Varanus* was listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1975, apart from *V. bengalensis*, *V. flavescens, V. griseus, V. komodoensis* and *V. nebulosus* which were included in Appendix I. Between 1990 and 2014, nearly 55 million specimens of *Varanus* species were reportedly legally traded internationally of which approximately 20 million specimens were exported and approximately 35 million specimens were re-exported (Crook & Musing, 2016). The principal commodities exported during this period were skins (~90%) and live specimens (6%). For some of the commercially used species CITES export quotas are in place in some countries of origin in order to limit the international trade to a sustainable level. However, in some cases concerns have been expressed that current quotas may not reflect the actual conservation status of respective species or do not sufficiently consider recent taxonomic changes and potential unrecognized lineages (Koch *et al.*, 2010; Setyawatiningsih *et al.*, 2015; Shaney *et al.*, 2017; Welton *et al.*, 2014). Several species which are internationally sold for especially high prices in the pet trade are known to have extremely limited distribution ranges, assuming that even small offtake might be detrimental to wild populations (*e.g.*, Bennett, 2015; Koch *et al.*, 2013).

In an effort to still meet the international demand for leather products and pets by reducing the pressure on wild populations, several countries have encouraged the establishment of captive breeding facilities. Between 2003 and 2012, Indonesia reported exports of over 40,000 captive-hatched (F) and captive-bred (C) *Varanus* specimens (Crook & Musing, 2016). However, severe reservations have been expressed about the conservation impact of these facilities, due to reports that many animals traded as captive-bred have in fact been sourced illegally from the wild (CITES, 2013; Outhwaite *et al.*, 2015; Bennett, 2015). Evidence of breeding farms used to illegally launder green tree pythons (*Morelia viridis*) has been confirmed (Lyons & Natusch, 2011), and investigations of the captive-breeding facilities of tokay geckos (*Gekko gecko*) have raised doubts to the logistical and financial ability of these facilities to produce the quantity of specimens they claim to harvest (Nijman & Shepherd, 2009, 2015).

Fraudulent claims of captive-breeding or ranching undermine trade regulations in place to protect species. Parties to CITES have recognized the importance of developing court-proof mechanisms to help combat this phenomenon, which include tools to help law enforcement authorities to accurately identify cases of fraudulent source declarations (CITES, 2013; TRAFFIC, 2013). Furthermore, a new CITES resolution (Res. Conf. 17.7) to review trade in animal specimens reported as produced in captivity for species-country-combinations of concern has been recently established (CITES, 2017).

Forensic analytical methods can play an important role in conserving and managing wild populations as well as in the investigation of wildlife crime through identification and profiling of tissue samples (Voigt *et al.*, 2012). Stable isotope analysis has been proposed as a potential tool to differentiate between wild and captive-sourced animals (Natusch *et al*., 2017). The quantitative measurement of stable isotope ratios in metabolically inert tissue samples has potential for accurately determining the origin of respective organisms due to the fact that the isotopic composition of certain elements, such as carbon and nitrogen, of a consumer reflects its diet and contains information on its respective local food web and its ecosystem (Ehleringer & Matheson, 2007). Wild specimens generally feed on a variety of available prey organisms, which in turn may have consumed numerous different taxa. These diverse isotopic sources, together, can indicate the presence of a specific complex food web or certain geographic region (Fry, 2006). By contrast, captive animals are usually kept under a controlled feeding regime of a few selected food sources, which generally have been in contact with less variable isotopic sources (Peterson & Fry, 1987; Ewersen *et al*., 2018).

Isotopic analyses of carbon and nitrogen have already successfully been applied to distinguish wild caught from captive-bred crocodile lizards (*Shinisaurus crocodilurus*) (van Schingen *et al*., 2016), farmed and wild salmon (Dempson & Power, 2004), Australian prawns (Carter *et al*., 2015) and bream (Rojas *et al*., 2007). Isotopic analyses have also been used to distinguish captive and wild mammals such as wolves (Kays & Feranec, 2011), and farm-bred vs. wild mink (Hammershøj *et al*., 2007). Dittrich *et al.* (2017) indicated isotopic composition to be a useful tool to distinguish between intensively farmed and naturally grown frogs. They assumed that the little isotopic variation in frog legs from the same supplier indicates specimens to have been farmed, while stronger isotopic variability and higher $\delta^{15}N$ composition might be an indication that frogs have been caught in the wild.

Here, we present the results of a controlled feeding study in three different small- to medium-sized *Varanus* species kept at the Cologne Zoo: the arid-adapted ridge-tailed monitor (*V. acanthurus*) which reaches about 70 cm in total length (TL) and is distributed over the tropical and subtropical portions of Western Australia, the Northern Territory, northwest Queensland, and associated offshore islands (Dryden, 2004); the blue-speckled tree monitor (*V. macraei*), an arboreal species with a prehensile tail that can grow up to 110 cm TL, from Batanta, a small island close to the northwest coast of the Bird's head Peninsula, Irian Jaya, New Guinea (Böhme & Jacobs, 2004; Ziegler *et al.*, 2010); and the quince monitor (*V. melinus*), a tropical lowland forest dweller reaching up to 128 cm TL, from the Sula Archipelago, Moluccas, Indonesia (Ziegler & Böhme, 2004; Ziegler *et al.*, 2010). The study was designed in order to examine the time lag for isotopic signals to appear in cyclically renewed *Varanus* scales. This information should help to assess the loophole through which wild-caught individuals could potentially be laundered in breeding farms.

Methods

Feeding study

Ten specimens of three monitor lizard species (*Varanus acanthurus,* n = 4; *V. macraei,* n = 2; *V. melinus,* n = 4), weighing between 50 and 250 g, participated in a controlled feeding study. The animals were kept separately in different enclosures in the Terrarium Section of the Cologne Zoo (see Figs. 1-3 and Table 1 for details on the specimens). In June and August 2015, samples of shed skin fragments were collected from all specimens (Fig. 4). The specimens were fed twice with 1-3 dead new born mice, depending on the target specimen's weight, consecutively on 22 and 29 September 2015. The dead mice had each been injected with 50 μl of liquid ^{15}N-enriched isotopic marker (glycine). After the specimens were fed the injected mice, a normal feeding regime was resumed: all monitor lizards were fed two times a week with invertebrates (*V. acanthurus* mainly with crickets, *V. melinus* and *V. macraei* with crickets, locusts and occasionally earthworms and *Zophobas* larvae). At maximum once a week, all species were provided with vertebrates of adequate sizes (*V. acanthurus* mainly newborn mice, *V. macraei* 1-3 week-old mice, *V. melinus* young to adult mice or small rats, and occasionally chicks or fish). In addition to the test group (n = 10), two specimens of *V. acanthurus* composed the control group (Table 1). After the isotopic marker was applied, the enclosures of all individuals were inspected and screened for shed skin fragments on a daily basis. To facilitate the collection of skin samples, the specimens were kept on relatively uniform ground substrate during the sampling period, such as sand for *V. acanthurus*, and pine bark or a mixture of coconut fibre and plant soil for *V. macraei* and *V. melinus*, respectively. Other furnishings that provided hiding and climbing opportunities and water bowls remained inside the terraria. The skin fragments (Fig. 5) were removed and stored in labelled polyethylene bags until analysis. If detected, pieces of loose shed skin which were still attached to the animals were carefully removed with tweezers. Samples were collected between October 2015 and March 2016.

Isotope analysis

All samples were analysed at the accredited (DIN EN ISO/IEC 17025:2005) Agroisolab Facility for

Fig. 1. Adult *Varanus acanthurus* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 2. Adult *V. macraei* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 3. Subadult *V. melinus* in an off-exhibit enclosure at the Terrarium Section of the Cologne Zoo. Photographed by **Anna Rauhaus**.

Fig. 4. Samples of shed skin fragments were collected from all studied specimens, either directly as in this *V. acanthurus*, or from the ground of their enclosures. Photographed by **Anna Rauhaus**.

Stable Isotope Research in Jülich, Germany. Samples were dried and cut into small aliquots with a scalpel. Sub-samples of 1–4.5 mg were loaded into 4 x 6 mm tin capsules for carbon and nitrogen isotopic measurements by a Nu HorizonR continuous flow isotope ratio mass spectrometer. Results were reported relative to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N2 ($\delta^{15}N$), respectively, and measured isotopic ratios (R) were expressed in δ units in the conventional permil notation, where:

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x\ 1000$$

After every tenth sample the calibrated laboratory standard (leucine) was also measured. The laboratory standard was calibrated against a set of international standards (carbon: IAEA-CH-6, IAEA-CH-7; nitrogen: IAEA-N-1, IAEA-N-2). In order to assess the precision of the analyses, at least two replicate measurements for each sample were performed, when sufficient material was available. Analytical uncertainties, based on these replicate analyses were typically in the range of 0.1 ‰ ($\delta^{13}C$, $\delta^{15}N$) and corresponding relative errors were 0.4 % ($\delta^{13}C$) and 1.4 % ($\delta^{15}N$). Mean weekly isotope values (the first week began at 4 October 2015) were calculated in order to avoid the risk of point estimates (Jackson *et al.*, 2011). Variation of the normal feeding regime was controlled by measuring $\delta^{13}C$ of the collected skin fragments and showed no significant differences over time.

Results

It was found that the $\delta^{13}C$ value of all specimens varied slightly during the study period but the standard deviation never exceeded 0.4‰ (Fig. 6, Tables 2–4). The $\delta^{15}N$ value of the two control specimens varied more throughout the study period, with a standard deviation

Fig. 5. Shed skin fragments of *V. acanthurus* (left), *V. melinus* (middle), and *V. macraei* (right). Photographed by **Anna Rauhaus**.

Table 1. List of specimens included in the controlled feeding study. D = Dosage of liquid ^{15}N isotopic marker. *= received in 2007 as adult. Specimens were weighed in September 2015.

ID	Species	Sex	Hatch date	Origin	Weight	D 22.9.	D 29.9.
R193	*Varanus acanthurus*	f	unknown*	Berlin Zoo	ca. 130 g	control	
R689	*Varanus acanthurus*	m	07/2014	Cologne Zoo	ca. 80 g	control	
R700	*Varanus acanthurus*	m	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R692	*Varanus acanthurus*	f	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R694	*Varanus acanthurus*	f	07/2014	Cologne Zoo	ca. 80 g	2 x 50 µl	2 x 50 µl
R195	*Varanus acanthurus*	m	06/2008	Cologne Zoo	ca. 180 g	3 x 50 µl	3 x 50 µl
R790	*Varanus macraei*	f	07/2014	Cologne Zoo	50-90 g	2 x 50 µl	2 x 50 µl
R773	*Varanus macraei*	f	11/2014	Cologne Zoo	50-90 g	1 x 50 µl	2 x 50 µl
R661	*Varanus melinus*	m	06/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R759	*Varanus melinus*	?	05/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R757	*Varanus melinus*	m	08/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl
R758	*Varanus melinus*	f	08/2014	private	ca. 250 g	2 x 50 µl	2 x 50 µl

of 2.17‰, and variation was particularly prominent in R193 (Table 2).

The maximum $\delta^{15}N$ value detected amongst the control specimens (R193, R689) was 15.2‰ (Table 2), therefore a $\delta^{15}N$ value < 20‰ was assumed as "glycine marker not visible" for *Varanus acanthurus* and the two other tested *Varanus* species. In case the $\delta^{15}N$ value exceeded this threshold, we expected the "glycine marker being detected", correspondingly. In seven of the ten specimens taking part in the feeding study, the ^{15}N enriched signal was detected in shed skin fragments within two weeks after the onset of the study (Fig. 7, Tables 2–4). In all individuals the signal was visible at the latest five weeks after the application of the isotope marker. One specimen (R195) did exceed the isotopic marker detection threshold, but only once (Table 2).

The peak was particularly prominent in juvenile and sub-adult specimens, weighing between 50 and 90 g, for which shedding usually takes place more frequently (Arnold, 1986). However, no statistical significance (t-test; $t = -2.12$, d.f. = 4.07, $p = 0.1003$) could be detected for the mean weekly $\delta^{15}N$ values of the two weight classes: (i) 80–90g; (ii) >180g. In three specimens (R661, R692, R790), the isotopic signal was detected within the first week after the start of the feeding study (Fig. 7). From three individuals (R661, R700, R758), samples were collected over a period of 25 weeks. Even after this timespan, the signal could still be detected in *V. acanthurus* (R700), whereas two specimens of *V. melinus* (R661, R758) evidently showed shed skin fragments without glycine marker after 14 weeks (Fig. 7). The low values were followed by peak $\delta^{15}N$ values in week 16 in all three specimens (R661, R700, R758). Specimen R661 developed a skin disease sometime between week 16 and 25, which may have altered the moulting cycle to some extent.

Discussion

This study was a pilot project to offer a basis for additional studies to distinguish between captive- and wild-bred monitor lizards. We could show that the carbon source of the general feeding regime was relatively constant over time (standard deviation: < 0.4‰ for $\delta^{13}C$, Fig. 6, Table 2). The standard variation of the nitrogen source in the control specimens, however, varied between 0.4‰ and 2.17‰ for $\delta^{15}N$ (Table 2). Such $\delta^{15}N$ variation in zoo specimens is possibly linked to the trophic position and reflects prey taxa (beetle larvae, crickets), which are fed, amongst others, on industrially produced powder and pellets, such as fish flakes and wheat germ but also on fresh apples and carrots. However, this variation of the ^{15}N source potentially masks isotopic differences between captive and wild diets, which lie in the range of 2‰ to 3‰, as it has been established for the crocodile lizard (*Shinisaurus crocodilurus*) (van Schingen *et al.*, 2016). In *S. crocodilurus* the tail tip was analyzed, which includes several skin layers and tissues and might offer a broader overview over the diet than only the outermost layer as was applied in this study.

In contrast to humans, which have a continuous production of skin cells, squamates produce new skin cyclically (Alibardi, 2000). Thus, we assumed that enriched $\delta^{15}N$ values from the feeding study would

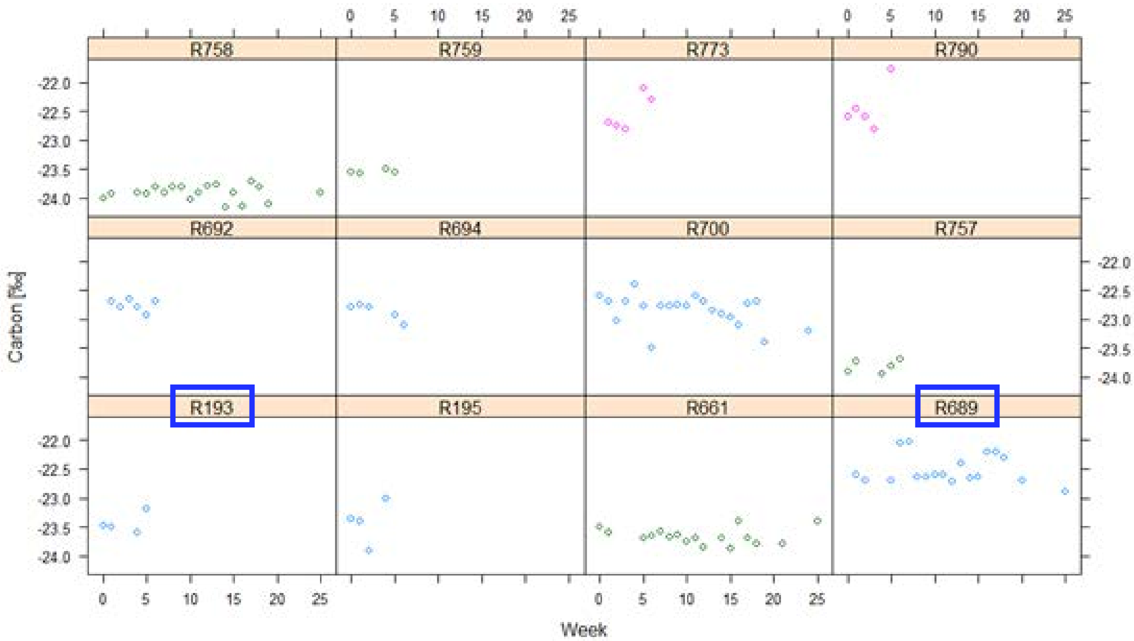

Fig. 6. δ[13]C values over time of specimens, including the control group (R193 and R689), separated by species (blue *V. acanthurus*, pink *V. macraei* and green *V. melinus*). The y-axis shows the δ[13]C values in permille. The x-axis gives the time in weeks. Each box represents one individual. The IDs of the individuals are shown on the rose x-axis (for details see Table 1). The control specimens (R193 and R689) are blue-rimmed.

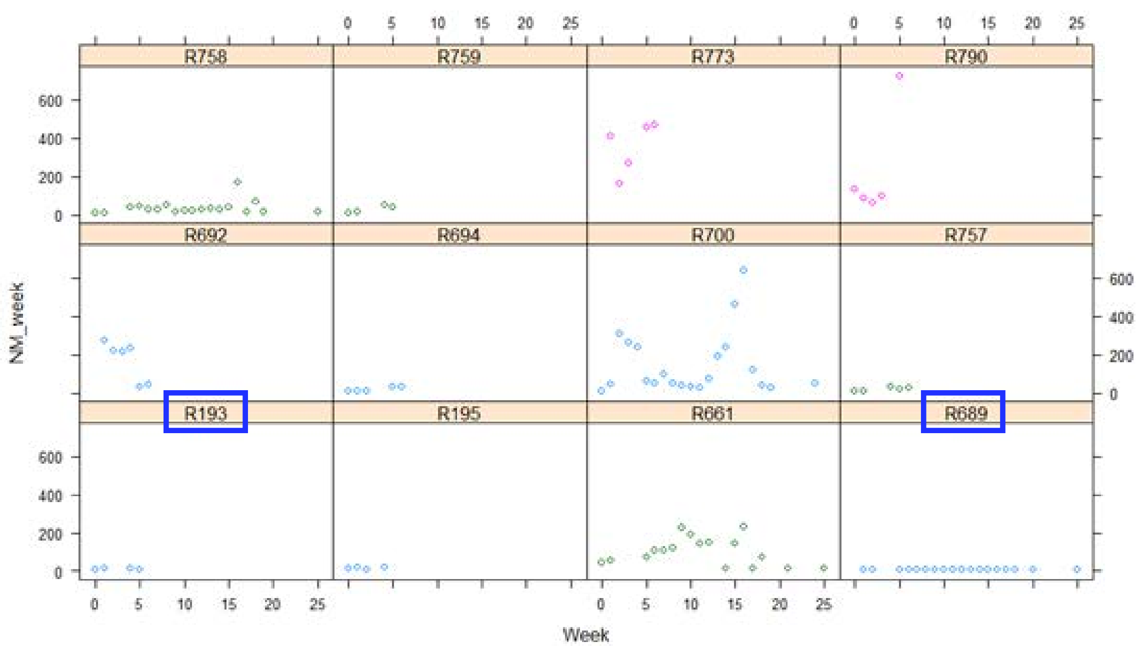

Fig. 7. δ[15]N values over time of specimens, including the control group (R193 and R689), separated by species (blue *V. acanthurus*, pink *V. macraei* and green *V. melinus*). The y-axis shows the δ[15]N values in permille. The x-axis gives the time in weeks. Each box represents one individual. The IDs of the individuals are shown on the rose x-axis (for details see Table 1). The control specimens (R193 and R689) are blue-rimmed.

Table 2. $\boldsymbol{\delta}^{13}$C and $\boldsymbol{\delta}^{15}$N isotope values for all *Varanus acanthurus* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015. * = control specimens.

	*R193		R195		*R689		R692		R694		R700	
Week	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
0	-23.47	10.53	-23.37	12.47					-22.8	8.85	-22.6	8.9
1	-23.5	11.85	-23.4	21.3	-22.6	8.1	-22.7	274.9	-22.75	9.05	-22.7	44.9
2			-23.9	11.6	-22.7	8.5	-22.8	221.7	-22.8	8.9	-23.03	309.2
3							-22.65	213.8			-22.7	264.25
4	-23.6	15.2	-23	18.2			-22.8	231.5			-22.4	240.5
5	-23.18	10.7			-22.7	8.05	-22.93	32.83	-22.93	33.03	-22.78	59.63
6					-22.05	8.15	-22.7	45.75	-23.1	33.9	-23.5	51.3
7					-22.03	7.27					-22.78	98.08
8					-22.63	7.23					-22.78	50.3
9					-22.63	7.47					-22.75	38.4
10					-22.6	7.2					-22.77	35.03
11					-22.6	6.95					-22.6	25.67
12					-22.72	7.12					-22.7	72.2
13					-22.4	7.5					-22.85	194.35
14					-22.65	7.45					-22.9	237.5
15					-22.63	7.23					-22.97	462.28
16					-22.2	7.65					-23.1	642.65
17					-22.2	7.35					-22.73	120.85
18					-22.3	7.35					-22.7	38.9
19											-23.4	27
20					-22.7	7.2						
24											-23.2	48.1
25					-22.9	7.4						
Mean	-23.44	12.07	-23.42	15.89	-22.51	7.51	-22.76	170.08	-22.88	18.75	-22.85	146.19
SD	0.18	2.17	0.37	4.64	0.25	0.42	0.1	103.57	0.14	13.44	0.27	164.85

only be detectable until the start of the next moulting process. However, we found that the ^{15}N enriched marker was detectable in all specimens within a few weeks of, or even days after exposition to the isotope marker. This indicates that the *Varanus* species tested appear to shed at least some parts of the skin constantly as it has already been demonstrated for the genital skin of lacertid lizards (in den Bosch, 2001). Most of the specimens which showed traces of the isotopic marker in their skin were still relatively young, implying that they are still shedding their skin more frequently. We also observed that *V. acanthurus* shed rather large skin pieces at a time over a period of a few days, whereas *V. melinus* and especially *V. macraei* mostly shed much smaller epidermal pieces or individual scales without a dedicated onset of a shedding cycle.

Depending on the amount of time skin fragments are laying in the enclosures, decomposition processes are assumed to alter the isotopic composition of the skin, which may contribute to a broader isotopic variation in the samples. Since animals shed their skin in pieces and some old skin parts may remain attached to the animals longer than others, one cannot assure that the chronological order in which single skin pieces were collected correspond to the relative age of the skin per se. Thus, the temporal time axis has to be interpreted with caution. This phenomenon also has forensic relevance: distinguishing captive-bred from wild origin based on δ^{15}N values might not be effective due to the rapid epidermal renewal cycle, with the consequence that new signals are detectable in the epidermis within

Table 3. $\boldsymbol{\delta}^{13}$C and $\boldsymbol{\delta}^{15}$N isotope values for all *V. macraei* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015.

	R773		R790	
Week	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
0			-22.6	131.6
1	-22.7	411.8	-22.45	84.95
2	-22.75	164.7	-22.6	62.6
3	-22.8	269.5	-22.8	98.4
5	-22.1	456.73	-21.77	723.67
6	-22.3	472.1		
Mean	-22.53	354.97	-22.44	220.24
SD	0.31	133.05	0.4	282.53

Table 4. **δ**^{13}C and **δ**^{15}N isotope values for all *Varanus melinus* specimens expressed as mean per week. SD = Standard deviation. Isotope marker (^{15}N enriched glycine) was applied on 22 and 29 September 2015.

	R661		**R757**		**R758**		**R759**	
Week	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**
0	-23.5	44	-23.9	9	-24	10.07	-23.55	10
1	-23.6	55.5	-23.73	12.03	-23.93	10.83	-23.57	15.6
4			-23.95	31.3	-23.9	37	-23.5	53.5
5	-23.7	70.83	-23.8	23.2	-23.93	44.6	-23.55	37.4
6	-23.65	110.85	-23.7	29.3	-23.8	28.4		
7	-23.57	111.63			-23.9	27.5		
8	-23.67	121.57			-23.8	51.45		
9	-23.63	226.37			-23.8	18.05		
10	-23.75	191.2			-24.03	20.3		
11	-23.7	141.57			-23.9	24.4		
12	-23.85	152.05			-23.78	25.53		
13					-23.77	32.3		
14	-23.7	14.6			-24.15	26.55		
15	-23.87	144.9			-23.9	37.03		
16	-23.4	234.7			-24.13	168.57		
17	-23.7	13.3			-23.7	14		
18	-23.8	75.5			-23.8	71.1		
19					-24.1	17.5		
21	-23.8	15.2						
25	-23.4	16.2			-23.9	17.3		
Mean	-23.66	102.35	-23.82	20.97	-23.91	35.92	-23.54	29.13
SD	0.14	72.93	0.11	10.05	0.13	35.47	0.03	20.09

two to three weeks after exposition to new dietary regimes and food webs. ^{15}N isotopes are incorporated directly from the diet into the tissues of the lizards (Urey, 1947). Therefore, wild-caught individuals could readily be laundered undetected through breeding farms at least if cyclically renewed skin material is investigated by isotope testing.

We also found a δ^{15}N peak in sampled skin fragments from week 16 (Fig. 7), suggesting that shed skin material was formed and deposited immediately after the application of ^{15}N enriched glycine in September 2015. This would translate into a moulting cycle of approximately four months for the *Varanus* specimens tested in this study. Our results confirm that ^{15}N isotopic signature in keratin skin tissue is likely to change quickly after the introduction of novel diets. Thus, breeders could theoretically manipulate isotope ratios within *Varanus* spp. collected from the wild to create signatures indicative of captive provenance, thus masking their "wild-type" isotopic label (Natusch *et al.*, 2017). However, our results also demonstrated that through an intentional addition of "isotopically labelled" materials (*e.g.*, glycine) to the diet of captive specimens, the creation of site-specific (or farm-specific) signatures can be created (Ziegler, 2016). Our results have shown that the ^{15}N pool in *Varanus* sp. only gradually declines over time and can still be detected with confidence after 16 weeks. This result is particularly promising for developing a reference framework of breeding farms against which particularly valuable specimens can be cross-checked along the trade chain.

However, isotopic markers were shown to rapidly produce great variance and fluctuations in isotopic signatures of varanid skin over short time periods. Due to the great variance it can be assumed that it would be impossible to differentiate wild and captive specimens that had recently been fed with isotopic markers. Thus, we assume that isotopic markers as a label for captive specimens in certain breeding facilities are not suitable to differentiate between captive and wild animals unless the markers are used to track specific specimens. Furthermore, although ^{15}N markers are not very expensive and stable isotopic analysis of $\delta^{13}C$ and $\delta^{15}N$ can be obtained for less than $100 USD, the application of isotopic markers in large breeding farms would be labour-intensive and might pose implementation difficulties.

However, the variance of isotopic signatures has shown to be much smaller in captive specimens than in wild lizards, snakes and frogs (Dittrich *et al*., 2017; Natusch *et al*., 2017; van Schingen *et al*., 2016). This approach, however, requires sufficient and georeferenced samples of both wild and captive specimens in order to form a sound judgement on the specimens' source. Adult and slow-growing ectotherms reportedly have lower incorporation rates than similarly sized adult endotherms (Cloyed *et al*., 2015). The rate of isotopic incorporation differs among tissues within a species as well as among species with different metabolic demands, body sizes, growth rates and protein turnover (Cloyed *et al*., 2015). While skin and blood plasma have a relatively fast isotopic incorporation rate and thus provide diet information integrated over short timescales (days or weeks) before collection, other tissues such as bone collagen incorporate isotopes very slowly and thus provide diet information integrated over much longer timescales (years). Warne *et al*. (2010) conducted a diet switch study (from C^3 based to C^4 based diet) in small prairie lizards (*Sceloporus undulatus*) and large collared lizards (*Crotaphytus collaris*) showing that carbon retention times in skin tissue last around 94 and 311 days, respectively. Lattanzio & Miles (2015) showed an isotopic turnover occurring after around 15.5 days in claw tissue of *Urosaurus ornatus* according to a diet shift study. Isotopic incorporation rates were shown to depend on the temperature, age and size of individuals (Warne *et al*., 2010), namely that isotopic incorporation rates were shown to decline with body mass, while growth was found to substantially contribute to isotopic incorporation. A similar diet shift study for adult *Varanus* specimens of different sizes is suggested in order to get an estimate on the time span needed until such a change is visible in the skin tissue under normal conditions. Furthermore it could be investigated if, and to what extent isotopic signatures of specimens with different origins that are kept together in a group, (*i.e*., in a zoo collection) differ from each other.

Our results have shown that there are limitations to this technique. In addition to stable isotope work, molecular approaches to track the provenance of certain species of reptiles have been developed recently and have demonstrated interesting insight into the broad-scale geographic structure of genetic diversity (Murray-Dickson *et al*., 2017). It might be worthwhile to test the combination of both relatedness and habitat signatures. Although the overall accuracy of such a procedure remains to be determined, it is foreseeable that error probabilities can be decreased to levels acceptable for decision makers and law enforcement.

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Valuable Varanoids: Surveys of Reptile Traders in Japan Reveal Monitor Lizards Without Import Records

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Abstract - Reptiles are one of the most heavily harvested terrestrial faunas in the world, with varanoid lizards (*Varanus* and *Lanthanotus*) as some of the most sought-after reptiles. Where previous studies have looked solely at official import and export records, such records provide little information on illegal trade in varanoid lizards. In the present study, covert surveys were used to detect varanoid lizards with a likely illegal origin and compare the species observed with legal import records. Data on the availability of live *Varanus* and *Lanthanotus* in Japanese reptile shops were gathered during surveys of 16 reptile shops in the districts of Tokyo, Kanagawa and Osaka and at the winter edition of Reptile Fever, a reptile fair in Osaka. A total of 221 live varanoid lizards, of 40 taxa, which included four subspecies, were documented. Prices were obtained for 26 taxa and ranged from $149 USD for *V. gilleni* to $53,100 USD for *V. giganteus*. For five of the observed taxa (*V. giganteus, V. marmoratus, V. nuchalis, V. obor* and *V. semiremex*) no legal import records could be found, suggesting these specimens entered Japan illegally. Trade in violation of national or international legislation is often carried out covertly without trade records, and creates a challenge for monitoring species' conservation status. The lack of trade records may allow overexploitation of species to go unnoticed, resulting in underestimations of threats.

Introduction

Reptiles are among the most heavily harvested terrestrial faunas in the world, with 152 million reptiles traded between 1975 and 2014 (Harfoot *et al*., 2018). Varanoid lizards, which include the monitors (*Varanus*) and earless monitor (*Lanthanotus borneensis*) are some of the most sought-after reptiles (Weissgold, 2000; Pernetta, 2009; Nijman, 2010; Koch, 2013) and are traded both legally and illegally for a wide range of purposes including for their skins (Shine *et al*., 1996), meat (Bifarin *et al*., 2008), traditional medicine (da Nóbrega Alves et al., 2008) and as pets (Pernetta, 2009; Bennett, 2015).

Varanoid lizards are popular pets because of their often-bright colors, unique behavioral complexity and intelligence, and often have a high value attached (Koch *et al*., 2013; Bennett, 2015). Smaller-sized varanoids such as *Varanus indicus, V. prasinus* and *V. macraei* are of particular interest to wildlife traders, as they require less space to maintain in captivity and are in high demand by the international pet trade (Auliya, 2003; Bennett, 2015). Many of these species are endemic to remote areas or small islands. Access to these areas created for other purposes (*e.g.*, logging) can allow traders to more easily obtain rare varanoids (Wilkie *et al*., 2000; Suarez *et al*., 2009), increasing the number of species available in the global pet trade.

Although the volume of commercial trade in *Varanus* skins far outnumbers the trade in varanoids for pets, far more species are represented in the global pet trade (Auliya, 2003; Koch, 2013). Yet, the impact of the global trade in live *Varanus* and *Lanthanotus* remains poorly understood due to a lack of basic biological information and status in the wild for many species (Koch *et al*., 2013). For several species of range-restricted monitors, their harvest from the wild in combination with their restricted distributions, were found to be of conservation concern, leading the European Union to impose import restrictions (*i.e., V.d umerilii, V. jobiensis, V. beccari* and *V. salvadorii*; Engler & Parry-Jones, 2007). Since 2017, with the addition of *Lanthanotus borneensis*, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists all varanoid lizards, with the majority of species listed in Appendix II. CITES Appendix II includes species that are not necessarily threatened with extinction but may become so if the trade in these species is not regulated. Trade in these species is only allowed with CITES documents from participating export countries.

Even though all varanoid lizards are now listed on the CITES Appendices, there is a growing body of evidence that these animals are illegally traded across international borders (Welton *et al*., 2013; Bennett, 2014; Jimenez-Bustamante & Rentería, 2018). Previous studies on the trade in live *Varanus* (*e.g.*, Pernetta, 2009; Bennett, 2015) mainly focused on official import and export records, allowing only for the detection of discrepancies in transactions with legal paperwork. Utilizing a different methodology, the present study used physical surveys of Japanese reptile traders to detect specimens with a likely illegal origin, including those not identified in the transactions recorded by CITES. In order to assess the legality of the observed specimens, observed species were compared with import records for Japan.

Methods

Data on the availability of varanoid lizards in Japanese reptile shops were gathered during covert surveys of 16 reptile shops in the districts of Tokyo, Kanagawa and Osaka as part of a wider TRAFFIC (wildlife trade monitoring network) market survey in February 2017 (Wakao *et al.,* 2018). Each reptile shop was visited either once or twice, and a full inventory of all reptiles available was made. Shops were revisited if the first survey was unsuccessful (*e.g.*, shop was closed) or did not result in a full inventory. A full inventory was defined as when all enclosures in the shop could be observed. Besides specialized reptile shops, a second covert market survey was conducted at the winter edition of Reptile Fever, a reptile fair in Osaka. All varanoid lizards present were recorded and vendors were opportunistically questioned about the origin of their specimens to compare with import data and relevant legislation. Price data were gathered opportunistically and converted to USD using the average conversion rate for February 2017, (100 JPY = 0.88514 USD). Species observed were compared with Japanese import records obtained from the CITES Trade Database (available at http://www. cites. org/eng/resources/trade.shtml). The database was searched for any live *Varanus* imported by Japan (importer-reported quantity) for commercial purposes (code 'T'), including both range states and non-range states. As *Lanthanothus* was only added to the CITES Appendices in February 2017; subsequently, no trade records were available for this species.

Results

A total of 221 live varanoid lizards of 40 taxa were recorded, which included four subspecies (Table 1). The three most common species encountered were *V. exanthematicus* (n = 59), *V. melinus* (n = 18), and *V. salvator* (n = 17). Single specimens were observed for 14 out of 40 taxa, and only seven taxa averaged more than two specimens per shop. Prices were obtained for 26 taxa, ranging from $149 USD for *V. gilleni* to $53,100 USD for *V. giganteus*. For four species, price tags of over $10,000 USD were observed, with the total combined quoted value for all varanoids observed in the surveys reaching $231,274 USD.

Origins were obtained for only six individuals, comprising five taxa. Only for *Varanus salvator bivittatus* was a captive-bred origin reported. Two observed *V. melinus* were reportedly born in captivity, while a wild-caught origin was reported for single specimens of *V. macraei*, *V. niloticus* and *V. salvator salvator*.

CITES Trade Data

Between 2000 and 2017, Japan reported the commercial import (purpose code 'T') of 35 *Varanus* taxa, comprising a total of 41,702 live specimens. The most common taxa were *V. exanthematicus* (n = 15,849) and *V. salvator* (n = 12,382) as well as 155 specimens imported as *Varanus* spp. Based upon the CITES Trade Data, the number of *Varanus* imported was relatively stable until 2016, when imports suddenly increased to 8,432 live specimens, four times the annual average between 2000 and 2015 (Fig. 1). In particular, in 2016, the number of wild caught (source code 'W') *Varanus* (n = 6271) increased by almost fivefold when compared to the previous year (n = 1485). Between 2000 and 2017, wild caught *Varanus* comprised on average 63% (range 46%-83%) of *Varanus* imported by Japan. Indonesia was the most important exporter of *Varanus* to Japan, with 16,725 live *Varanus* reportedly imported from Indonesia between 2000 and 2017 (Fig. 2). Ghana (n = 8,562) and Togo (n = 7,698) were also important exporters of live *Varanus* to Japan (Fig. 2).

The CITES Trade Database does not contain import records for 5 species observed in Japanese reptile shops and at the reptile fair. No import records

Table 1. Observed number of live varanoid lizards in the districts of Tokyo, Kanagawa and Osaka in February 2017. Price in USD is average price converted using the exchange rate of 100 JPY = 0.88514 USD. Minimum and maximum price and number of specimens per shop are presented in brackets. Quantity imported refers to the importer reported quantity of live varanoids with purpose code 'T', as mentioned in the UNEP-WCMC CITES Trade Database over the period 2000-2017.

Genus	Subgenus	Species	Quantity Imported	Shops	Quantity	Avg. Number per Shop	Average Price in USD (range)	Number of Individuals with Quoted Prices
Varanus	*Empagusia*	*Varanus rudicollis*	977	1	1	1	-	-
	Euprepiosaurus	*Varanus beccarii*	33	2	3	1.5 (1-2)	-	-
		Varanus doreanus	535	3	4	1.3 (1-2)	$873 (618-1133)	4
		Varanus indicus	793	5	6	1.2 (1-2)	$264	2
		Varanus jobiensis	521	2	3	1.5 (1-2)	$381	1
		Varanus macraei	236	2	5	2.5 (1-4)	$1,328	4
		Varanus melinus	480	11	18	1.6 (1-4)	$800 (425 - 1133)	8
		Varanus obor	-	2	2	1		-
		Varanus prasinus	325	8	9	1.1 (1-2)	$471 (441-529)	4
		Varanus spinulosus	152	4	9	1.8 (1-3)	$2,637	4
		Varanus yuwonoi	23	1	1	1	$2,301	1
	Odatria	*Varanus acanthurus*	697	4	8	2 (1-5)	$602 (425-779)	7
		Varanus auffenbergi	9	6	9	1.5 (1-2)	$876 (248 -2,478)	5
		Varanus gilleni	53	1	1	1	$149	1
		Varanus glauerti	146	2	2	1	$3,522	1
		Varanus kingorum	30	1	1	1	-	-
		Varanus pilbarensis	76	1	1	1	-	-
		Varanus primordius	13	1	1	1	$1,133	1
		Varanus semiremex	-	1	1	1	$20,355	1
		*Varanus t. tristis**	123	1	3	3	-	-
		*Varanus t. orientalis**	-	2	4	2	$867	2
	Papuasaurus	*Varanus salvadorii*	73	1	1	1	$3,983	1
	Polydaedalus	*Varanus albigularis*	1,190	3	3	1	$529	1
		Varanus exanthematicus	15,849	18	59	3.1 (1-12)	$867	2
		Varanus niloticus	4,601	2	3	1.5 (1-2)	-	-
	Soterosaurus	*Varanus cumingi*	-	3	5	1.7 (1-2)	-	-
		Varanus marmoratus	-	2	2	1	-	-
		Varanus nuchalis	-	1	1	1	$2,637	1
		Varanus salvator	12,389	10	17	1.5 (1-3)	$2,810 (1,752 - 6,018)	7
		*Varanus s. bivittatus**	-	3	3	1		-
		*Varanus s. macromaculatus**	-	2	2	1	$2,213	1
		*Varanus s. salvator**	-	1	1	1	$175	1
	Varanus	*Varanus giganteus*	-	1	1	1	$53,100	1
		Varanus gouldii	36	1	5	5	-	-
		Varanus mertensi	53	1	1	1	-	-
		*Varanus panoptes horni**	79	4	6	1.5 (1-2)	-	-
		Varanus spenceri	3	1	1	1	$10,620	1
		Varanus varius	2	5	6	1.2 (1-2)	$13,558 (1,133 - 23,010)	4
Lanthanotus	-	*Lanthanotus borneensis*	-	5	12	2.4 (2-4)	$8,842 (1,752 -26,550**)	10

*CITES Trade Data are not available to subspecies level; ** Price per pair.

were found for *V. giganteus* (Fig. 3), *V. marmoratus*, *V. nuchalis*, *V. obor* and *V. semiremex*. Increasing the search parameters to before the year 2000 did not reveal any import records of these species. For the Australian endemic *V. giganteus* (Pianka & King, 2004), only the export of four captive bred specimens from Australia to the United States was reported in 2008. The trader revealed that the specimen was obtained via a trader that acquired the specimen in Southeast Asia, raising concerns about how this specimen entered Japan. Southeast Asia is also mentioned in the CITES Trade Data when it comes to the Australian endemic *V. semiremex*; this species was observed in Japan, but the only trade records reported were the export of 35 wild-caught specimens from Indonesia to the United States in 1997 (3) and 2002 (32). However, this Australian endemic (Pianka & King, 2004) is not known to occur in Indonesia, and there are no records of Indonesia legally importing this species from Australia. Another interesting observation was the presence of six sub-adult and adult *V. varius*, whereas import records only reveal the importation of two specimens between

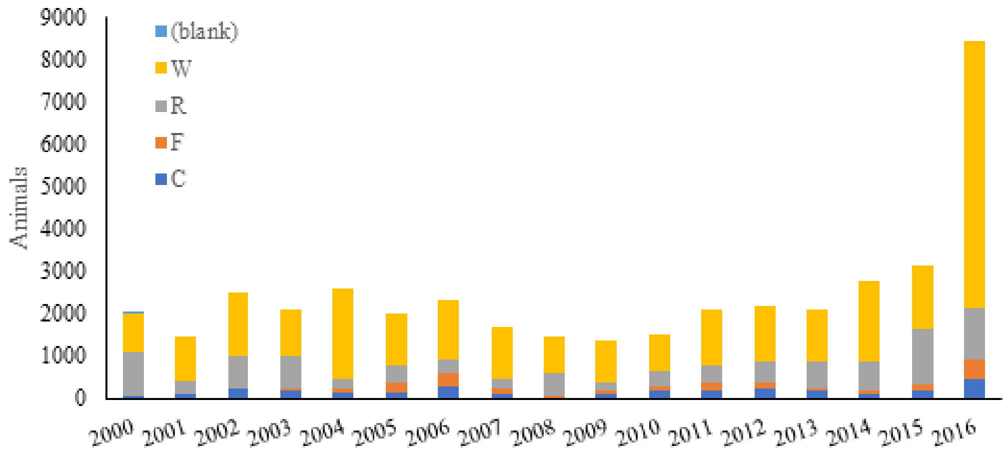

Fig. 1. Number of varanid lizards imported by Japan between 2000 and 2017, with the corresponding source codes. CITES Source Codes: C= Captive-bred, F = Born in captivity, R = Ranched, W = Wild-caught, (blank) = no source code attached. Source: UNEP-WCMC CITES Trade Database.

2000 and 2017. Similar observations were made for the two Philippine endemic species *V. marmoratus* and *V. nuchalis*, for which only a few specimens have been exported for commercial purposes to the United States (six and three, respectively), but none were ever exported to Japan.

Discussion

Japan is an important market for live varanoid lizards. Although import records were found for most species which suggests that most lizards were likely of legal origin, the lack of legal import records for five species suggests that those specimens entered Japan illegally. For a number of taxa, the asking prices were extraordinarily high (Table 1), suggesting that Japanese reptile keepers are willing to pay considerable money for exclusive specimens. This was further acknowledged when the seller of the *V. giganteus* told the investigators that the animal had been "spoken for" for $53,100 USD. However, some of the average prices reported here, particularly for *V. exanthematicus*, the most commonly traded species, may not be accurate representations of the species

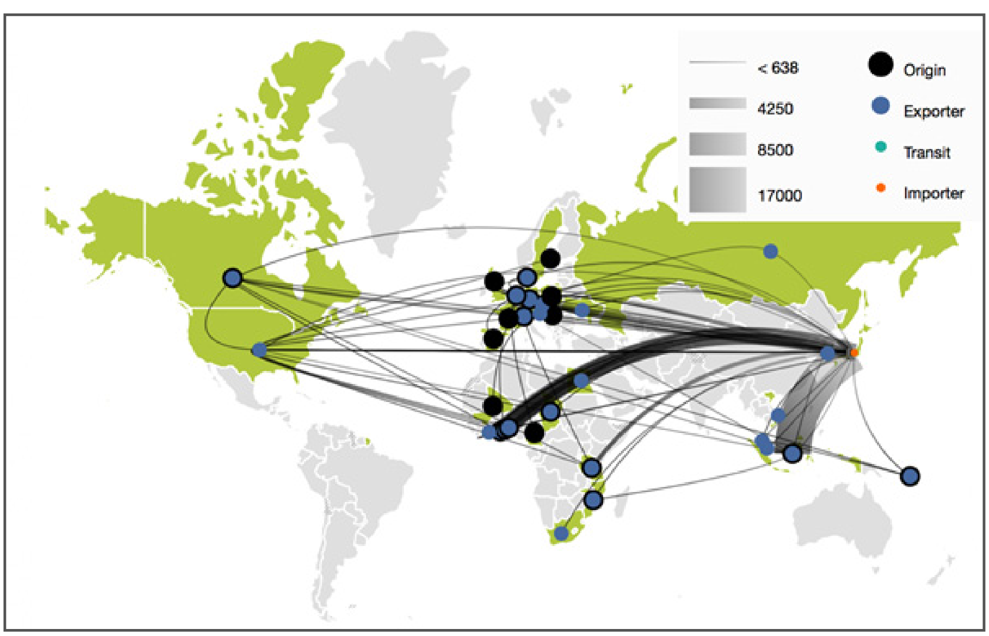

Fig. 2. Live varanid lizards imported by Japan for commercial purposes in the period 2000 – 2017. Source: UNEP-WCMC CITES Trade Database. Developed using Trade Mapper (https://trademapper.aptivate.org).

Fig. 3 Perentie (*Varanus giganteus*) for sale in a Japanese reptile shop for $53,100 USD. Photograph courtesy of TRAFFIC.

given the limited number of individuals encountered with advertised prices (Table 1).

Where in a previous study it was observed that the source of varanid lizards is changing from wild-caught to ranched or animals born in captivity (*i.e.*, CITES Source Codes 'C' and 'F') (Pernetta, 2009), import data for Japan showed a consistently large proportion of wild-caught specimens, with a major increase observed in 2016. Although the source of these animals differs strongly between taxa and export country, it is unclear what caused this spike in the import of wild-caught specimens. The overall lack of information relating to the origins of specimens posted on their enclosures in the present study made it impossible to compare these with the reported origins of imported specimens.

The willingness of Japanese reptile keepers to pay high prices for species that are uncommon in captivity increases the incentive to smuggle species with export restrictions into the country (Courchamp *et al*., 2006). The prices mentioned in this report are high and caution is required when publishing any information that might stimulate trade (Courchamp *et al*., 2006). Nevertheless, for many of these species, prices were also displayed on the websites of these reptile shops (Wakao *et al*., 2018). The desirability of varanoid lizards by Japanese reptile keepers seems to be reflected in the high number of taxa observed, including taxa that have no import records or barely any commercial trade records at all. Southeast Asia plays a significant role in this trade, both as region of origin (*e.g.*, taxa from the Philippines) and transit (*e.g.*, taxa from Australia).

The lack of import records for five species suggests that these were illegally imported into Japan. As all varanoid lizards are now listed in CITES Appendices, all international trade must be documented. The high number of Australian endemic species is particularly interesting and of concern as only three species (*V. gilleni* [n=4], *V. varius* [n=2] and *V. giganteus* [n=4]) were legally exported from Australia for commercial trade between 1975 and 2017, with a combined total of 10 specimens (CITES Trade Database, 2018). Commercial exports of live native wildlife is prohibited in Australia (Alacs & Georges, 2008), which makes Australian monitor lizards highly desirable for the global pet trade (Alacs & Georges, 2008; Wilson-Wilde, 2010). While many Australian monitors are now widely kept by collectors and are genuinely bred in captivity (Horn & Visser, 1989, 1997; Retes & Bennett, 2001), there are still many species that enter the global pet trade illegally. Besides Australian endemics, several other species observed are subjected to trade restrictions with either a total trade ban (*e.g.*, *V. s. salvator* – Sri Lanka), or limitations to specimens bred in captivity, such as *V. cumingi*, *V. nuchalis* and *V. marmoratus* in the Phillipines (E. Sy, pers. comm., 2018), and *V. indicus, V. prasinus, and Lanthanotus borneensis* in Indonesia (Government Regulation (GR) 7/1999 and Nr. P.20 1/6/20180). Especially for species that can only be traded when bred in captivity, there is a growing body of literature that suggests wild-caught specimens are likely to be laundered to circumvent such trade restrictions (Lyons & Natusch, 2011; Bennett, 2014; Janssen & Chng, 2018; Jimenez-Bustamante & Rentería, 2018).

Trade in violation of national legislation in range states is often carried out covertly, without trade records, and creates a challenge for monitoring species' conservation status. Without this knowledge, a species might be at risk of overexploitation, but this might go unnoticed as trade records are lacking. This is of particular concern for range-restricted taxa (Kuchling, 2007; Kiester *et al*., 2013; Meiri *et al*., 2018), for which even low levels of harvesting might have significant impacts (Pernetta, 2009). It is therefore imperative that studies are conducted that look at the legal import and export records, in combination with physical market surveys to detect illegal trade. Documenting illegal trade in species will assist in accurately assessing the conservation status of a species and any impacts that trade might have.

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Successful Hatching of a Patched *Varanus beccarii* (Doria, 1874) Egg at the Saint Louis Zoo

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Abstract - Paraffin wax and a section of latex medical glove were used to patch a black tree monitor (*Varanus beccarii)* egg that ruptured after 118 days of artificial incubation. The embryo continued to develop following the procedure and the egg hatched successfully 39 days later. To date, the juvenile appears healthy with no ill effects from either the rupture or repair of the eggshell.

Introduction

The shell of a reptile egg has several vital functions, including protection of the embryo and regulation of the internal environment of the egg (Packard & DeMarco, 1991). As with most other squamates, monitor lizards (genus *Varanus*) lay eggs with flexible parchment-like eggshells. Pliability of the eggshell accommodates changes in the size and shape of these eggs (Packard *et al*., 1982). However, there are constraints to this flexibility and damage to artificially incubated eggs may arise from improper nesting, handling during excavation or transport, and environmental conditions during incubation (Packard & Phillips, 1994; Köhler, 2005; Eidenmüller, 2007).

When reptile eggs are damaged during artificial incubation, manipulations of the incubation environment, such as reducing the level of moisture, may be useful in stopping further damage (Packard & Phillips, 1994). Sometimes, damaged eggs can hatch successfully without any additional interventions (Ackerman *et al*., 2002). However, when severe cracking or rupturing has occurred, repairs to the shell can be attempted. Repairs should serve to stabilize the eggshell, preventing more structural damage, loss of contents from the egg, or infiltration by other organisms, while still permitting gas and water exchange.

Flexible-shelled eggs are particularly problematic to repair as the eggshell typically remains more elastic than the sealant (Ackerman *et al*., 2002). Many materials have been used for reptile egg repair (Ackerman *et al*., 2002; Augustine & Saunders, 2015); published accounts of repairs on ruptured flexible-shelled eggs have utilized pieces of shell from infertile or previously hatched eggs (Maxwell, 2005; Adragna & Madden, 2009), cyanoacrylate adhesives (Ackerman *et al*., 2002; Davis, 2014), transparent medical dressings (Augustine & Saunders, 2015), and waxes (Maxwell, 2005; Fischer, 2012).

Several cases of *Varanus* eggs rupturing during artificial incubation have been described, involving species from the subgenera *Odatria* (pygmy monitors) and *Euprepiosaurus* (tree monitors). For the repair of split *Varanus* eggs, multiple techniques have been detailed. In one report involving the subgenus *Odatria*, a ruptured *V. acanthurus* egg was mended with a section of shell from a previously hatched egg of another species (*V. tristis*), continued development, and eventually hatched successfully (Adragna & Madden, 2009). Published attempts at fixing eggs of species in the subgenus *Euprepiosaurus* have produced mixed results. One *V. prasinus* egg was successfully repaired using tissue (cyanoacrylate) glue (Davis, 2014). However, the embryo of a damaged *V. beccarii* egg sealed with wax died prior to hatching (Fischer, 2012). Herein, an additional account describing the patching of a ruptured *V. beccarii* egg is provided. This method of utilizing paraffin wax and a piece of latex glove resulted in a successful hatching.

General Husbandry

A pair of black tree monitors (*V. beccarii*) has been housed at the Saint Louis Zoo since 2007. The female hatched at the Milwaukee Zoo in January 2007; the male was imported from Indonesia in 2001 and is of unknown age. On 30 June 2017, the adult pair was moved into a 2.8 x 1.7 x 4 m (L x W x H) exhibit outfitted with live plants, concrete rockwork, fake vines, natural wood branches, and two artificial dead trees (Fig. 1). The enclosure received filtered sunlight through a skylight and two basking lights (160W PowerSun UV, Zoo Med Laboratories, Inc., San Luis Obispo, California, USA) provided supplemental heat and ultraviolet light. The thermal gradient ranged from 25 °C to approximately 40°C directly under the basking lights. A varied diet was offered, consisting of gut-loaded and supplement-dusted live invertebrates (*e.g.*, crickets, roaches, mealworms) once or twice a week and either neonate mice, hard-boiled egg, or quail chicks approximately once a week.

The artificial trees (approximately 1.5 and 2 m high) were constructed from fiberglass and wireframes covered by epoxy modeling compound (Apoxy Sculpt, Aves Studio, LLC, Hudson, Wisconsin, USA). A 5-gallon plastic bucket (26 x 33 cm) was concealed within the upper end of each tree. A removable lid was fastened to each bucket to provide keeper access, and a mixture of moistened coir, sphagnum moss, and hardwood mulch was added to the interior. Water was periodically added to keep the substrate inside the buckets slightly wet, but not overly saturated. Slightly below the top of each bucket, a roughly 8 cm diameter hole was cut into the side, creating an opening which allowed the *V. beccarii* entry into the cavity for nesting and retreat.

Oviposition and Early Incubation

On 16 September 2017, the female buried a clutch of three eggs inside one of the nest chamber buckets. Two of the eggs were misshapen, deflated, and nonviable. The remaining egg (45.6 x 21.1 mm, 13 g) looked viable externally and was transferred to a clear plastic box (16.5 x 16.5 x 5 cm) for artificial incubation (Fig. 2). A single 2 mm diameter hole in the box lid provided air exchange. The egg was partially buried in medium coarse horticultural vermiculite that had been hydrated with aged tap water at a ratio of 1:0.9 by weight. The total mass of the box with substrate and the egg was recorded before the box was placed inside an incubator (Nature's Spirit, LLC, Vicksburg, Michigan, USA) controlled by a thermostat (Herpstat 1, Spyder Robotics, Rochelle, Illinois, USA) set at a constant temperature of 29 °C.

During incubation, the egg was frequently observed through the transparent incubator window and lid of the box. The box was periodically removed from the incubator and weighed to determine the amount of moisture lost through evaporation. Aged tap water was added to the vermiculite farthest from the egg until the box matched its initial mass. On occasion, while the box was out of the incubator, development of the egg was assessed using a candler (Powerlux SL-PL, Olba B.V., Coevorden, The Netherlands), typically without handling of the actual egg. The last addition of water to the substrate took place over a week prior to the date of the rupture, and the incubator was not opened nor was

Fig. 1. Left: A section of the adult *Varanus beccarii* enclosure showing live plants, artificial trees and other features. Right: The female emerging from a nest cavity in one of the artificial trees.

Fig. 2. The viable *V. beccarii* egg at the start of incubation.

the box moved during the two days before the incident.

Egg Rupture and Patching

On 12 January 2018, 118 days into incubation, the egg was visually inspected and a rupture was observed on the upper surface of the eggshell. Approximately 0.5 ml of albumen was protruding from a 3-4 mm hole in the egg. The albumen was slightly yellow, clear, and did not display any signs of decay. While the egg remained on the substrate, it was carefully candled. Movements of the embryo and an abundance of blood vessels were discernable.

For the repair, the egg was left in place within the box. A 10 x 10 mm section was cut from a disposable medical glove (Ambitex Powder Free Latex Glove, Tradex International, Inc., Cleveland, Ohio, USA). By the time patching of the eggshell was attempted, the surface of the protruding albumen had become slightly dry and viscous. Due to concern that wiping might apply too much pressure to the egg, small sterilized scissors were used to help separate the albumen from the egg. The area was blotted with tissue paper to absorb any remaining fluid. The latex patch was then quickly placed over the hole before more albumen could leak from the egg and carefully held in place with the tip of the scissors. Paraffin wax from a lit candle was then dripped on and around the latex patch to seal it in place. Once the repair was complete, the box was closed and returned to the incubator.

Late Incubation and Hatching

Following the repair, the egg was still occasionally candled without being handled directly. No further movements were seen during candling, but healthy blood vessels continued to be observed. Water lost from the box through evaporation was no longer replenished and the substrate was allowed to dry slowly. Approximately two weeks prior to hatching, the egg began to dimple slightly. At this time, the egg also became more difficult to assess via candling. Deflation of the egg continued until it began to hatch on 20 February 2018, 39 days after the repair. The hatchling did not emerge from the site of the patched hole but rather at one end of the egg. During hatching, movements of the exiting lizard caused the patch to detach from the egg (Fig. 3). The egg was transferred to a box with moist paper towels and returned to the incubator until the hatchling had completely left the egg. Mass of the hatchling was 9 g on 24 February 2018.

The hatchling was set up in a small enclosure containing cork bark, a live plant, and natural wood branches. A basking spot (100W PowerSun UV, Zoo Med Laboratories, Inc., San Luis Obispo, California, USA) provided ultraviolet light and temperatures similar to the thermal gradient in the adult enclosure. Humidity was kept high (> 60%) through daily misting. Within a few days, the neonate began feeding on live invertebrates. Subsequently, the juvenile was offered food several times per week and readily accepted crickets, fresh newborn mice, mealworms, roaches, and other small prey.

Discussion

Many factors, including an inadequate oviposition site, handling during excavation or transport, and environmental conditions during incubation, can be responsible for damage to artificially incubated eggs (Packard & Phillips, 1994; Köhler, 2005; Eidenmüller, 2007). For the rupture reported here, the proximate cause is not immediately obvious. The nesting cavity appeared to be suitable and the female buried the egg normally in the middle of the chamber. During excavation, the egg was handled with great care. Once incubation began, the egg was rarely picked up directly. For candling, the egg was typically kept on the substrate and the light source gently placed along one side. Furthermore, during the two days prior to the incident, the incubator had not been opened nor had the box been moved.

Since neither nesting nor handling appears to be a likely cause, it seems probable that incubation conditions might have played a role in prompting the rupture. The external environment is capable of inducing substantial

Fig. 3. The *V. beccarii* emerging from the egg. During the hatching process, the patch of wax and latex fell off the eggshell and can be seen to the left of the egg.

changes in flexible-shelled reptile eggs, such as those of *Varanus*. In one instance, a rapid change in air pressure, produced by opening an incubator door, was presented as the possible cause of a *Varanus* egg rupture (Adragna & Madden, 2009). In the current paper, unlike the setup used by Adragna & Madden (2009), neither the incubator nor the box containing the egg was sealed tightly. Therefore, it is doubtful that an air pressure differential led to the rupture.

Most suggested causes of *Varanus* egg ruptures relate to hydric conditions during incubation; monitor eggs appear to be particularly sensitive to moisture levels (Moldovan, 2008). Availability of water is a key environmental condition for flexible-shelled eggs, as they are capable of taking in moisture from the surrounding substrate and will expand during this process (Packard et al., 1982). However, damage may occur if too much water is absorbed (Packard & Phillips, 1994). Bursting of *Varanus* eggs has been attributed to the use of an excessively wet substrate (Fischer, 2012) and to the rapid rehydration of partially desiccated eggs (Davis, 2014).

For the rupture documented here, sudden uptake of water is unlikely. The same level of moisture had been maintained throughout incubation until the rupture, and no water had been added to the substrate for at least a week prior to the incident. It is possible that too much water was initially added to the incubation substrate and the water potential (a quantification of the tendency of water to move from one area to another) of the substrate subsequently remained too high, as during additions of water to replace evaporation, no compensation was made for the amount absorbed by the eggs. High water content in the substrate could have caused the egg to swell to the point where the eggshell weakened and was no longer able to contain the hydrostatic pressure, resulting in rupture. The ratio of substrate to water used here (1 part vermiculite to 0.9 part water) was slightly drier than the equal proportions generally recommended for most reptile eggs (Packard & Phillips, 1994). However, while water potential initially increases rapidly as small amounts of water are added to dry coarse vermiculite, it is comparatively less affected by additional water above a ratio of approximately 1:0.5 (Packard & Phillips, 1994).

Therefore, the substrate in this account likely had a water potential only slightly below those of substrates implicated in previous ruptures of *Varanus* eggs, which had been hydrated at ratios of 1:2 and 1:1 (Fischer, 2012; Davis, 2014). This suggests that, if excess water absorption was the cause of the rupture, it may be desirable for the incubation of tree monitor eggs to either use vermiculite with a further reduction of water or a substrate-less technique as suggested by Fischer (2012). However, there are two caveats to this approach: first, water could be drawn from the eggs under dry conditions, requiring careful monitoring for dehydration and possibly increasing the risk of damage from frequent handling; and second, *Varanus* eggs have been reported to rupture even when not in contact with moist substrate (Adragna & Madden, 2009; Fischer, 2012).

Another possible factor is that the ultrastructure of the eggshell could have been inherently weak due to a physiological issue with the female. The misshapen and nonviable nature of the other two eggs in the clutch supports this idea. Although flexible, the parchment-like eggs of most squamates typically possess a thin calcareous layer (Packard & DeMarco, 1991). Shelling of eggs occurs within the glandular uterus region of the oviducts (Siegel *et al*., 2014). Dysfunction of the oviducts can cause abnormalities in eggshells; associations between defects in eggs and environmental stress, oviductal infections, or genetics of the mother are well established in poultry (Reynard & Savory, 1999; Feberwee *et al*., 2009; Wolc *et al*., 2012). Egg quality is also affected by nutrition of the female (Köhler, 2005). If calcium or another maternal contribution to the egg was insufficient in the diet of the female, the integrity of the eggshell could potentially have been impacted.

In this case, however, the female seemed to acclimate quickly to the exhibit and be in good overall health. The diet provided to the pair of *V. beccarii* appeared to be nutritionally complete and similar in composition to what is typically offered this species (Eidenmüller & Wicker, 1993; Mendyk & Horn, 2011; Fischer, 2012). No noticeable defect in the eggshell was apparent upon initial recovery of the egg from the nest site or throughout the incubation period before the rupture; yet, a weakness in the eggshell ultrastructure cannot be definitively excluded. If a flaw was present, environmental conditions could have still contributed to the rupture. However, if the eggshell was sufficiently weak, the rupture could have occurred even under incubation conditions that would have typically been ideal for the species.

When reptile eggs break during artificial incubation, modifying the incubation environment can prevent further damage (Packard & Phillips, 1994). For incubation of the egg here, the substrate was allowed to slowly dry following the repair. Regardless of the cause of the initial rupture, lowering of the water potential in the substrate would have reduced pressure within the egg, decreasing stress on the eggshell, and possibly keeping other ruptures from occurring. However, the wax portion of the patch was less yielding compared to the shell; this is a shortcoming with many sealants used in the repair of flexible-shelled eggs (Ackerman *et al.*, 2002). A sudden decrease in moisture could have caused deflation to occur too quickly, leading to the repair material falling off prematurely. Due to the responsiveness of *Varanus* eggs to water, it seems that any changes in hydric conditions during incubation should be gradual, avoiding rapid fluctuations which may damage eggs (Davis, 2014). Additionally, drier conditions at the end of incubation may prove helpful in preventing full-term embryos from dying within the eggs just prior to hatching (Fischer, 2012).

Damaged eggs will sometimes hatch successfully without major intervention (Ackerman *et al*., 2002). However, in this case, repair was deemed necessary given the severity and timing of the break. The *V. beccarii* egg reported here ruptured three-quarters of the way through incubation but still successfully hatched after a total incubation length of 157 days. The timing of hatching was slightly less than the period of 172-203 days reported by Eidenmüller and Wicker (1993). However, it was within the range of 157-162 days given by Wanner (1991) and variation in incubation length is frequently observed in monitor lizards (Eidenmüller, 2007). The neonate appeared normal and was active and feeding soon after hatching. To date, it has appeared to be healthy and vigorous (Fig. 4). Despite the rupture and subsequent repair of the egg, there do not seem to have been any ill effects on the juvenile.

Further refinements in the husbandry and artificial incubation of *V. beccarii* are needed, including investigations on appropriate nutrition, the water dynamics of eggs, and the ideal level of moisture in the incubation substrate. Special attention should be given to the cause and prevention of egg rupture during

Fig. 4. The juvenile *V. beccarii* at one month of age.

artificial incubation. However, when ruptures occur in the flexible-shelled eggs of *V. beccarii*, and other monitor lizards, the wax and latex method presented in this account offers another viable method of successfully repairing the eggshell.

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Hatha Jodi: An Illegal Trade of Misused Scientific Facts or Blindfolded Myths and Beliefs?

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Abstract - We provide a short overview on the recently emerged illegal trade in the reproductive organs of monitor lizards (*Varanus* spp.) in India. There, they are intentionally sold as an alleged plant root locally known as Hatha Jodi. Hatha Jodi is the root of tiger's claw (*Martynia annua*), an herbaceous medicinal plant which has a variety of traditional applications in Ayurveda medicine. The objective of this short report is to increase awareness of this ongoing wildlife crime, in the hopes that this will help restrict and terminate the illegal exploitation of South Asian monitor lizards for their body parts.

The utilization of wild plants and animals can be regarded as one of the most commonly adapted strategies for the survival of human beings (Piper & Rabett, 2009). Prehistoric cave paintings depicting the hunting of wild animals including monitor lizards have been found in Central India and Australia (Wakankar & Brooks, 1976; Carr, 1963). They are considered to express the hope of these early humans for the continued survival of their prey species (Das, 1989). Today, however, the exploitation of wildlife for the sake of traditional beliefs and medicines is one of the major causes for the decline and extermination of numerous species worldwide (Byard, 2016). One such example is the western black rhino (*Diceros bicornis longipes*) from Africa, which was declared extinct by the International Union for the Conservation of Nature (IUCN) in 2011 due to excessive illegal poaching for the demand of Chinese traditional medicines (Biggs *et al*., 2013).

In recent years, a similar situation has emerged in several parts of India with the illegal trade of an alleged plant root locally known as Hatha Jodi (Figs. 1 & 2), meaning "pair of arms" in the Hindi language (D'Cruze *et al*., 2018). However, it was recently revealed that these roots actually represent the dried and partly stained copulatory organs of monitor lizards (Sharma *et al*., in press). As with all male squamate reptiles, monitor lizards exhibit paired copulatory organs (Figs. 3 & 4) known as hemipenes (Böhme, 1988; Zeigler & Böhme, 1997). There are four species of monitor lizards known from India, namely *Varanus bengalensis*, *V. flavescens*, *V. griseus* and *V. salvator* (Koch *et al*., 2013, Chatterjee & Bhattacharyya, 2015). According to the findings of Sharma *et al*. (in press), the Hatha Jodi trade primarily affects *V. bengalensis*, followed by *V. flavescens* and *V. salvator*. The online prices of Hatha Jodi may reach more than $50 USD per set of paired hemipenes (Figs. 1 & 2).

In traditional Indian Tantra religion, Hatha Jodi is used for diverse superstitious beliefs, witchcraft and black magic (Sharma *et al*., in press). Hatha Jodi is originally known to be the plant root of tiger's claw (*Martynia annua*), also commonly known in India as "baghnakhi" (Sharma *et al*., in press). While the roots resemble human arms with clenched fists, the ripened black fruits exhibit curved claw-like tips responsible for the plant's vernacular English name. *Martynia annua* is an herbaceous medicinal plant distributed along the tropical regions of wastelands throughout India (Kenwat *et al*., 2013). In Ayurveda, as one of the most ancient traditional Indian medicines (Patwardhan *et al*,

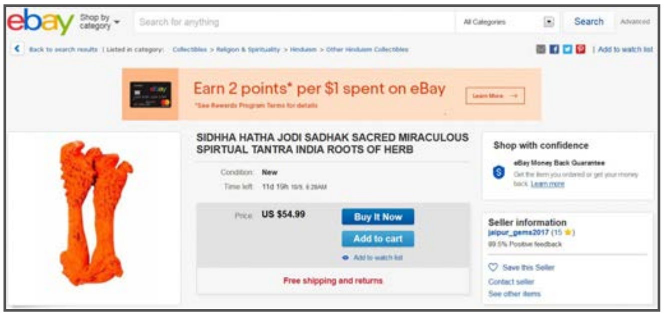

Fig.1. Online advertisement offering monitor lizard genitalia as "Hatha Jodi" on Ebay.com, accessed on 26 September 2018.

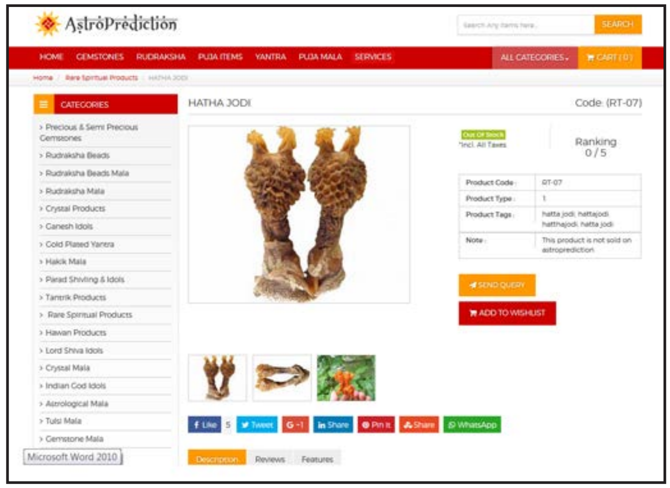

Fig. 2. Online trade of monitor lizard genitalia as "Hatha Jodi" on a Tantra homepage, accessed 13 October, 2018.

2005), *M. annua* is known as "kakanashika" and has a variety of medicinal applications; some of them include its use in the treatment of epilepsy, inflammation, tuberculosis and sore throat. Additionally, it has several applications for wound healing, and antibacterial and antioxidant activities (Kenwat *et al*., 2013). *Martynia annua* has not yet been assessed by the IUCN Red List, but is unofficially considered "Vulnerable" as a rare, endangered plant species (Das *et al*., 2012). Thus, the plant's rarity and the resemblance of its roots to monitor lizards' genitalia are probably responsible for the newly emerged illegal exploitation of varanids in India.

On the other hand, the early utilization of monitor lizards dates back to at least 10,000 years ago (Das, 1989), when monitor lizard body parts had several applications in traditional Indian medicines. Some of these applications included the use of oil obtained from the body fat for the treatment of failing eyesight, thorn pricks, or bites from venomous animals (Das, 1989). It also had applications for treating tuberculosis, skin problems, and sexual debility (Das, 1989). According to Auffenberg (1989), the consumption of monitor lizard flesh is an ancient practice in the Indus Valley (see also Auffenberg's [1994] study on *V. bengalensis* and references therein on the various uses of monitor flesh in Asia). The remains of monitor lizard bones were found at the ancient Harappa site dated to 2500 BC. Auffenberg (1989) listed the following uses of monitor lizards in Pakistan: powder made from the dried hemipenes of *V. bengalensis* mixed with its oil, was used to mark the foreskin of male babies, as this belief was thought to bring wealth and fortune to the child in the future. Additionally, some tribal groups like the Nath caste in Rajasthan, India, used the hemipenes of monitor lizards, probably including *V. bengalensis*, in tantric medicines and black magic (Auffenberg 1994). Evidence was also found for the use of monitor lizard oil on temples as a symbolic gesture of desired fertility.

Fig. 3. Confiscated hemipenes of *V. bengalensis* showing the typical cone-like terminal extensions (hemibacula) and frills (paryphasman rows). Photo: **Thomas Ziegler**.

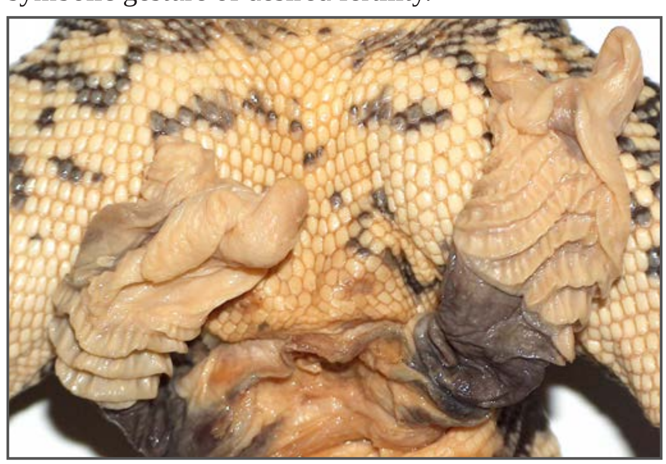

Fig. 4. In situ asulcal view of the everted paired hemipenes of ZFMK 64713, the holotype of *V. cumingi samarensis*. Photographed by **André Koch**.

Thus, the wide use of monitor lizard parts in traditional and superstitious beliefs has ancient roots in South Asia. One question that arises from these facts is whether the trade and application of Hatha Jodi really is a newly emergent threat or whether it was prevalent long ago. In other words, is global commercialization through online websites responsible for turning local utilization into a worldwide illegal trade?

In several parts of India today, monitor lizards are still widely consumed locally, with the body oil being sold for thousands of Indian rupees (INR) to people residing in metropolitan cities for the treatment of rheumatism (SB, personal observation). Therefore, to curb the illegal trade and exploitation of monitor lizards in India, will it be enough to focus solely on the Hatha Jodi trade? In any case, recently recognized illegal sales of Hatha Jodi are a major setback for Indian monitor lizard populations. It is hoped that this report will create widespread awareness that can be used to help restrict and terminate the illegal use of monitor lizard body parts in the near future. In this regard, recent measures taken by the Indian Government along with their execution through the Wildlife Crime Control Bureau to curb the illegal trade of Hatha Jodi are appreciable efforts (Sharma *et al*., in press).

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An Observational Note on Mating Behavior and Male-Male Combat of the Bengal Monitor *Varanus bengalensis* (Daudin, 1802) in the National Botanical Garden, Dhaka

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Abstract – A single observation of courtship, mating, and ritualized combat behavior is reported for *Varanus bengalensis* in Bangladesh. Some peculiar behaviors such as basking between copulations and post-mating combat behavior are also described.

Introduction

The Bengal monitor or common Indian monitor (*Varanus bengalensis*) has a broad distribution that ranges from Afghanistan to Java, including southeastern Iraq, Iran, and Afghanistan, Pakistan and India, Southern Nepal, Bhutan and China, Vietnam, Laos, and islands in the Strait of Malacca and the Greater Sunda Archipelago (Auffenberg, 1994; Pianka, 1995), and is widely distributed in Bangladesh (Hasan *et al*., 2014). It is distinguished from *V. flavescens* and *V. salvator* which also occur in Bangladesh by its nostril being nearer to the eye than the tip of the snout (Hasan *et al*., 2014). In Bangladesh, *V. bengalensis* reaches a maximum length of 100 cm (Khan, 1988). Courtship in *V. bengalensis* on the Indian subcontinent occurs from April to July, and female recrudescence (or true vitellogenesis) lasts from April to June when the ovarian follicles mature (Auffenberg, 1994). Ritualized male-male combat and courtship begins in late April when day length is increasing (Auffenberg, 1994).

This article reports on an observation of courtship, copulation, and ritualized combat in *V. bengalensis* in Bangladesh, as well as basking behavior between copulation attempts.

Study Site and Methods

Observations took place at the National Botanical Garden, in Dhaka, Bangladesh on 4 August 2015. The National Botanical Garden of Bangladesh and the Bangladesh National Herbarium make up the largest plant conservation center in the country, comprising a total area of around 84 ha. It is located at Mirpur in Dhaka, adjacent to the Dhaka Zoo (23°49′21.0324″ N, 90°20′52.5084″E; Fig. 1).

Observations were recorded with photographs and video using multiple cameras. We used a temperature and humidity sensor meter for recording temperature (29.3 °C) and relative humidity (86%) data.

Observations

While walking along a narrow trail through a partially shaded area with tall grasses, ferns, and tall trees at around 1200 h on 4 August 2015, we observed two *V. bengalensis* cross the trail, with one individual in close pursuit of the other (both measured > 90 cm, but one was noticeably heavier than the other, which, based on copulatory behaviors seen later, we suspect was male). At first, the male was seen chasing the female from behind, and then managed to pin the female down with its forelimb on the female's neck. During this time, the female was whipping its tail and jerking much of its body in an effort to escape from the male. The male attempted to keep the female from fleeing by using its forelimb to encircle and wrap around the forelimb of the female. In most cases, the female was able to escape from under the male, which often escalated into a fast chase. The male was able to stop the female once

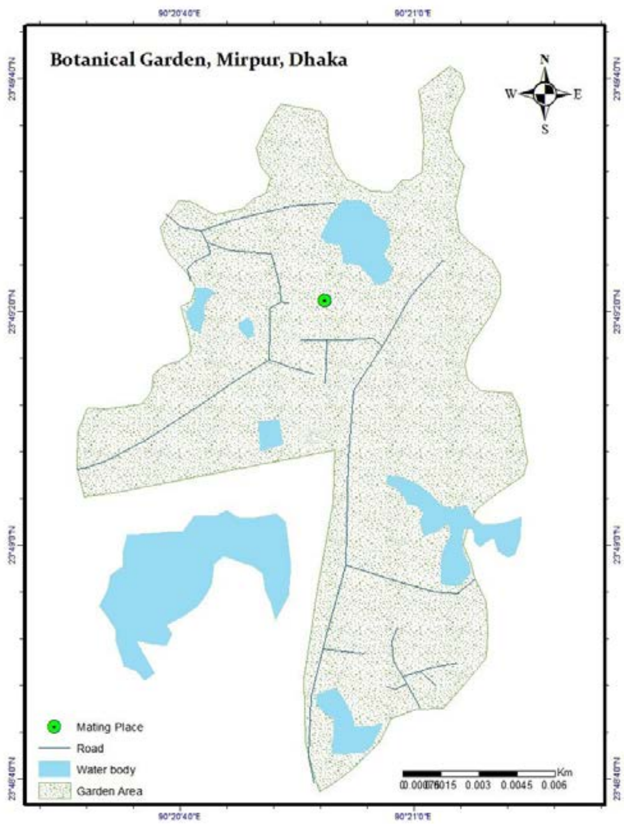

Fig. 1. National Botanical Garden, Dhaka, Bangladesh

again, approximately one meter from where she was first pinned down. These observations are consistent with the copulatory behavior described elsewhere for *V. bengalensis* by Auffenberg (1994) and by Cota (2011) for *V. salvator.*

We observed a total of five copulations between the pair over the course of 26 min (1304 to 1330 h). Copulation took place under a secluded bush (Fig. 2). In between observed copulations, the pair left the bush to enter into a patch of sunlight to bask. Following the first copulation, the female entered into a patch of sunlight while the male remained mounted to her back and remained in this position for the entire span of her basking activity. Each copulation lasted 46, 106, 94, 164 and 59 seconds, respectively (mean = 84 sec), and the time spent basking between each copulation was 113, 72, 112 and 127 seconds, respectively (mean = 106 sec).

Fig. 2. Copulation in *Varanus bengalensis*

Following the last observed copulation, a suspected male of similar size approached the male that had just copulated with the female. The original male approached the rival hissing, and the female left the area. Standing on their hind legs and grasping each other firmly about the neck and shoulders with their forelimbs (Fig. 3), they hissed and used sharp sideways jerking movements of the head to knock their opponent down, sometimes completely falling over. This struggle was interspersed with biting to the neck behind the ear, which did not appear to produce any signs of blood or injury. The combatants took turns pressing each other to the ground, swirling around each other on the ground, and were breathing heavily and visibly exhausted as previously described by Horn (1994). After having its head pressed to the ground with the original male's forelimb, the rival male jerked its body loose and fled the area. In total, the combat lasted for 456 sec.

Discussion

In India, male-male combat in *V. bengalensis* usually occurs during the breeding season, beginning in mid-April (Auffenberg, 1994). Ritualized combat has been documented in several varanid species (Sterling, 1912;

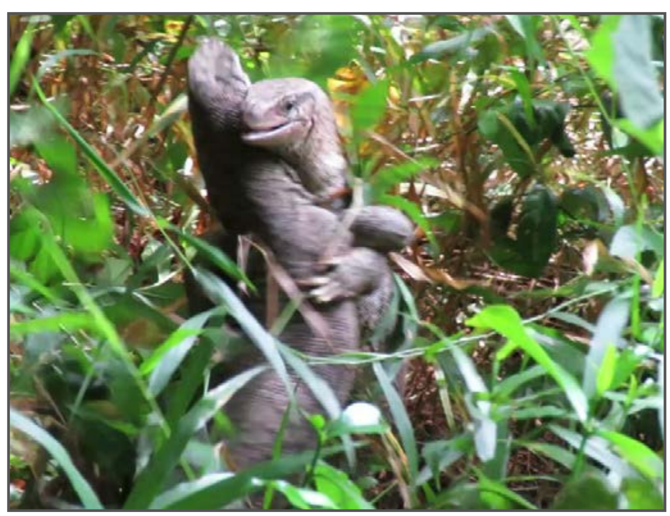

Fig. 3. Post-copulatory ritualized combat in *V. bengalensis*.

Lederer, 1929; Waite, 1929; Ali, 1944; Deraniyagala, 1958; Murphy & Mitchell, 1974; Vogel, 1979; Auffenberg 1981; 1988; 1994; Thompson *et al.*, 1992) and is a significant aspect of their reproductive biology because it represents a test that determines which males are available to copulate with females (Auffenberg, 1983). Auffenberg (1994) postulated that male-male combat usually occurs prior to mating, rather than after copulation as described in this report. Ritualized combat occurring after copulation has taken place has not previously been reported for *V. bengalensis*.

Although basking is a common thermoregulatory behavior in monitor lizards, basking in between copulations has not been documented in *V. bengalensis* before, nor has the duration of basking activity.

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Preface to "Translation of Some of Daudin's (1802, 1803) Original French Descriptions of African Monitors of the *Varanus* (*Polydaedalus*) *niloticus* Group"

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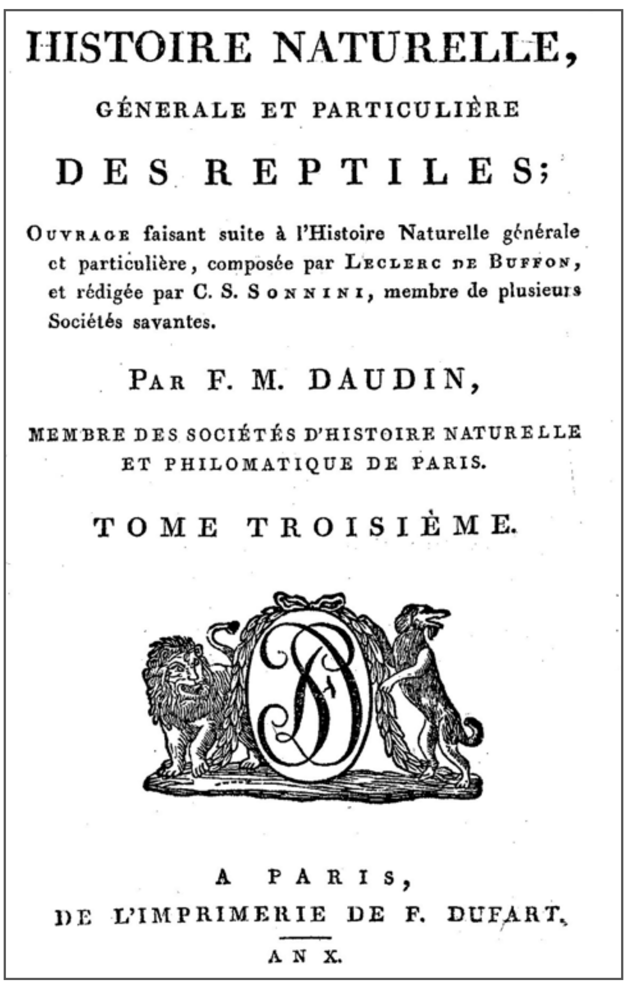
In many ways, Francois Marie Daudin was a tragic character. Crippled in early childhood, his mother died when he was 14 and he died of consumption, a few weeks after his wife, aged just 27, and leaving a one year old son. But Daudin had the passion, drive and financial means to pursue his interest in zoology and over just five years published a huge amount of books and papers on the natural history of birds, shells, reptiles and amphibians, many of which are key taxonomic references today. For example, the eight volume *Histoire naturelle, générale et particulière des reptiles* (Natural History of Reptiles) published between 1802–1803 contains over 500 species descriptions, including three species of monitor lizard currently recognized: *Varanus bengalensis*, *V. indicus* and *V. griseus*, and others that may be resurrected in the future. A recent biography (Bour, 2011) did not attempt to define how many of Daudin's descriptions are still recognized, but provides a comprehensive bibliography of his work.


HISTOIRE NATURELLE,

GÉNERALE ET PARTICULIÈRE

DES REPTILES;

OUVRAGE faisant suite à l'Histoire Naturelle générale et particulière, composée par LECLERC DE BUFFON, et rédigée par C. S. SONNINI, membre de plusieurs Sociétés savantes.

PAR F. M. DAUDIN,

MEMBRE DES SOCIÉTÉS D'HISTOIRE NATURELLE ET PHILOMATIQUE DE PARIS.

TOME TROISIÈME.

A PARIS,
DE L'IMPRIMERIE DE F. DUFART.
AN X.

We now know that all three of Daudin's monitor lizards are representatives of species complexes whose taxonomy is, at best, rudimentary and whose distributions are very large. The deep and cryptic speciation revealed in *V. niloticus* (Dowell *et al*., 2015) is likely present in all of Daudin's monitor lizards and molecular assessments of the groups are long overdue. If Daudin were alive today, maybe it would have already been done.

The color plates reproduced here were provided courtesy of Josef Schmidtler.

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Biawak, 12(2), pp. 105–110

Translation of Some of Daudin's (1802, 1803) Original French Descriptions of African Monitors of the *Varanus* (*Polydaedalus) niloticus* Group

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Daudin, F.M. 1802. Histoire Naturelle, Générale et Particulière, des Reptiles. De L'Imprimiere de F. Dufart, Paris. Pp. 51-57.

THE NILE TUPINAMBIS, or THE EGYPTIAN MONITOR (1).

The naturalist Geoffroy, professor of zoology at the natural history museum of Paris, collected this species of Nile tupinambis from Egypt. It is very similar to the starry tupinambis of Senegal, and seems to differ from it only by the irregular disposition of its spots and by the lack of double keels on its tail. I describe it here under the name *tupinambis du Nil* or *varan d'*Égypte.

(1) *Tupinambis niloticus* ; *suprá sub-viridis fuscescente maculatus, subtùs pallidior, squamis sub-hexagonis margine granulatis, caudá sub-cylindricá non carinatá, compressá et longitudine corporis*.

Lacerta nilotica. Linnæus, Syst. nat. – *Idem*, Gmelin, Syst. nat. pag. 1075, n° 37. – *Idem*, Hasselquist, Iter. ægypt. palest. pag. 361. – *Idem*, Forskœl, Descript. anim. ægypt. pag. 13, n° 2. – *Le triangulaire*. Daubenton, Dict. erpét. encyclop. méthod. – *Idem*, Lacépède, Hist. des quadr. ovip. in-12, tom. II, p. 129. – *Le lézard du Nil*. Hist. amph. fasc. secundus, pag. 195 et suiv.

Until now, it has not been correctly observed by any author; at least I did not find its complete description in any publication.

This Daudin; Hist. des rept. par Latreille, tom. I, pag. 246. – *Scincus niloticus*. Schneider, reptile is similar to the starry and picketed tupinambis of Bengal, by the shape of its different parts, and by the configuration of its nearly hexagonal scales, granulated on their border, and surmounted by a small keel at their center; and it only differs from them by a small number of characters, being however enough to show that it should be considered as a distinct species.

Its tail is nearly cylindrical, verticillated, without any crest or keel above; and it occupied half of the total length which is about two and a half feet in the specimen I have observed. Its ventral scales are a little bit more rugous in contact than those of the other tupinambis, because their borders are nearly as granulated as by those of the back.

Its color is dirty gray to light yellowish, without any spots and slightly paler below.

Hasselquist, disciple of Linnæus and author of a travelogue to the Levant and Egypt, is the only one among all naturalists who has described this animal from nature; however, its description is not sufficient, not even accurate enough, to allow proper classification of this animal in the saurian order; this is why, until now, all the naturalists proposed a wrong assignment for the correct position of this lizard.

Linnæus and Gmelin placed it in the ninth section of the lizard genus, between the five and two striped skinks, under the name *Lacerta nilotica*; and Schneider also placed it recently among the skinks. However, if we examine the description of this saurian carefully, it becomes obvious that it should belong to the genus *Tupinambis*, because of its body scales transversally arranged in rings, and because of its long tail, verticillated and compressed especially towards its end. I consequently first placed this saurian among the lizards themselves in Latreille's reptile book; and now I classify it in the genus of the *Tupinambis*.

This animal is the *lézard triangulaire* of Daubenton and Lacépède.

The Nile tupinambis, named *varan* or *varar* by the Arabs according to Forskœl, is about three feet and two inches long.

Hasselquist, in the original edition of his travelogue, compared the Nile tupinambis to the common skink; which has certainly induced error by naturalists until now.

The head is oblong, depressed, and a little bit higher than the neck in the cranium area; snout is oblong, triangular, depressed, flattened, marked on its center by a longitudinal angle, obtuse at its end which is slightly elongated and covered with rounded, raised and smooth scales; the mouth is wide and very split; teeth are small, very acute, convex in front and few in number.

Neck size is two times head length.

Body is not depressed, slightly projecting at its middle, on the flanks. Back is covered by oblong nested scales placed by rings and marked by a keel which is slightly prolonged as a small spine at its posterior part; on each side, against those keels, scale border is marked by whitish dots. Moreover, Hasselquist noted that the middle of the back possesses a wide longitudinal line formed by four scale rows distinct from the others by their shape, and only similar to those of the venter. The abdominal scales, or rather those of the venter, are prismatic because of the presence of an oblong tubercle, which is a keel, and their border is covered with projecting points. The four scale rows of the back and those of the venter are thus all covered with a keel which is more extended and slightly pointed on the back scales.

Tail is twice as long as the body, and even longer; it has slightly more than its anterior half verticillated, cylindrical, while the other tail part is triangular, provided with a longitudinal angle on each of its both sides: its scales, like those of the feet, are slightly smooth and similar to those of the back; however, those of the lower part of the feet are roundish.

The internal lateral finger, or the thumb of the front feet, is two times shorter than the others; the second and fifth digits are of equal length; the third and fourth are also of equal size between them, and longer than the others. The thumb and the little finger of the feet are shorter than the three others; the second digit is longer than the thumb; the third and fourth are of equal size but longer than others.

Color of this saurian is shiny brown above and whitish below.

Hasselquist found the tupinambis that I here describe in marshy places close to the Nile in Egypt. Forskœl also observed this animal there and he described it by the following characters:

"Its tail is long, verticillated, covered with blunt and truncated scales; back scales possess a slightly acute keel; and the lower thigh is without warts."

All naturalists reporting on the saurian after Hasselquist, among them Linnæus, have so reduced and truncated the description that the author has given, that I was first induced to error about the place the reptile has to occupy in the order of saurians; and I wrongly presumed, in the reptile book recently published by my colleague Latreille, that the Nile lizard could be a young, perhaps abnormal crocodile: the suspicion was partly founded on the specific character assigned by Linnæus (1), and on the ridiculous opinion of some inhabitants of Egypt, thinking that this reptile originated from crocodile eggs laid in the sand, and that crocodiles only hatch from those deposited in the water.

(1) *Lacerta nilotica* ; *caudá longá, extimo triquetrá, corpore glabro, dorso squamarum lineis quatuor*. Gmelin and Linnæus, Syst. nat. p. 1077, n° 5.

This animal is carved on some ancient Egyptian monuments, and it was venerated by them because it is very fond of crocodile eggs and it hunts juveniles on the bed of the river, as do the ferocious turtles of the Euphrates and the Caroline.

Daudin, F.M. 1802. Histoire Naturelle, Générale et Particulière, des Reptiles. De L'Imprimiere de F. Dufart, Paris Pp. 59-66.

SECOND SECTION. TUPINAMBIS

With a tail topped by a small double keel and slightly saw serrated

Their tail is compressed on the sides, and topped by a double longitudinal fold or keel and slightly saw serrated. The section comprises five species inhabiting Africa and India.

THE STARRY TUPINAMBIS OF AFRICA. (1)

See plate XXXI in this volume.

This species, very elegant in the arrangement of small circles of white scales that are arranged in black transverse bands on its dorsum, was indeed illustrated several times by Seba under different names and as an inhabitant of Amboina, in New Spain, or from Brazil. It seems rather to live in Africa, only from Senegal where it is named *galtabé*, to the Cape of Good Hope.

(1) *Tupinambis stellatus* ; *suprà fusco-niger, fasciis transversis dorsalibus albido ocellatis; caudá longâ, suprà tenuiter carinato serratâ*.

Lézard magnifique d'Amboine (Magnificent Lizard of Amboina). Seba, t. I, pl. XCIV, fig. 1, 2 and 3 – *Lézard téjuguaeu de Ceilan (Tejuguaeu Lizard of Ceylon), slightly discolored*. Seba, tom. I, pl. CLIX, fig. 2; tom. II, pl. CV, fig. 1. – *Large tilcuetzpallin Lizard from New Spain, to the report of Hernandez*. Seba, tom. I, planche LCVII, figure 2. – *Lizard named taraguico ayguraba in Brazil*. Seba, tom. I, planche XCVIII, figure 3. – *Lizard from America, called cordyle or spiny tailed*. Seba, tom. I, pl. CI. This is without any doubt an old tupinambis, totally brownish, on which the starry spots disappeared, and on which only some yellowish spots are subsisting, mostly above members. See what I write about it in pages 423 and 424 in the present tome. – *Le tupinambis (The tupinambis)*. Lacép. Hist. nat. des quadrup. ovip. in-12. Tom. I, pag. 305 and next pl. X. – *Lacerta capensis*. Sparrman ; Voyage au cap de Bonne –Espérance et autour du monde (Travel to the Cape of Good Hope and around the World), French translation, in-8°, tom. III, pag. 259 and follow. – *Stellio salvaguardia*. Laurenti, Synops. Rept. pag. 57, n° 92. It is the spiny tailed lizard of Seba which is cited in the synonymy. – *Galtabé*, in Senegal. – *Lacerta monitor*. George Shaw, Natur. Miscel. in-8°, n° 7, pl. 21.

The head of this magnificent tupinambis is flat on top of the elongated skull, slightly obtuse, and similar to a four-sided pyramid, like that of other tupinambis: it is a bit more compressed on its sides, in front of the eyes and the top of the anterior third is rather inclined; the top and the sides are entirely covered with small smooth scales, hexagons or pentagons; while those same parts are covered with fairly large and numerous scaly plates by the tupinambis sauvegarde of America. Its nostrils resemble those of the tupinambis from the new continent, that is to say, they are elongated, arranged in length on the sides of the upper jaw and slightly apart from the tip of the snout, which is not swollen above like the one from the Bengal tupinambis. It has twelve short and conical teeth on each side of the upper jaw, and only ten on each side of the lower jaw.

This Tupinambis is relatively black and dark above, and whitish or rather ashen below. It has pale yellowish straight lines transversally across the top of the head; its cheeks are quite pale, intermixed with small blackish spots: above the neck, one can see several yellowish white lines which are very angular, with their angle pointing back. On the dorsum and cylindrical base of the tail, one can see ten transverse dark black bands, each accentuated by five to nine spots or whitish circles, which gives the animal an ocellated appearance. Between these bands, whose number varies more or less depending on the individual, there are a number of scattered and whitish spots that extend over most of the tail length. Limbs are covered with a certain amount of speckling and small, rounded whitish spots. On the ventral surface there are eight narrow blackish cross bands, set aside.

All scales located on the neck, body, limbs and tail are very small, rounded, smooth and slightly raised in their center, and a little granulated on their edges. On the ventral surface and tail, the scales are slightly larger, squarish, oblong, disposed in transverse lines, and also similar to those of the back. They are regular hexagons in the chest, under the limbs, and around the tail. There are approximately twelve transverse rows on the chest between the arms, and seventy-two on the ventral surface.

The tail is about as long as the body, whorled, thick and cylindrical at its base, then laterally compressed and provided above with a small double keel, somewhat similar to a longitudinal fold, and finely saw-serrated above all the rest of its extent; then it tapers. The anus is transverse.

The four legs are sturdy, thick, and each provided with five separate and clawed fingers: the nails are laterally compressed, strong and hooked. The outer finger of the hind foot, which matches our little finger, is inserted on the side and slightly below the base of the others; moreover, the four thumbs are very short.

Dimensions of a large starry tupinambis from my natural history collection

	Feet	Inches	Lines
Total length	4	1	6
Head length		3	6
Head width at nostrils level			7
Head width a eyes level		1	6
Length of neck		4	9
Circumference of neck		7	3
Body length until above anus	1	1	9
Body circumference at its middle	1		
Length of the whole tail	2	5	6
Tail circumference at its base		7	
Length of the unkeeled tail part		4	6
Length of the anterior limbs to the claw		6	
Circumference of anterior limbs at their base		5	
Length of the posterior limbs		7	6
Circumference of posterior limbs at their base		6	3

*Note from the translator: 1 foot = 324 mm; 1 inch = 27 mm; 1 line = 2.25 mm.

Seba placed this beautiful saurian (tom. II, pl. CV, fig. 1) under the name *ocellated tejuguacu lizard* (Lézard téjuguacu ocellé) or *saurus* of Ceylon (*saurus* de Ceilan). According to that author, the Dutch in Ceylon call it the *sea shore defender* (*défenseur du rivage de la mer*) and the French *sea lizard* (*lézard de mer*). It feeds on small dead fishes, either from the sea or from rivers, which were washed ashore by the waves.

We must undoubtedly relate this African tupinambis to the one Sparrman described in his journey, under the name *lizard of the Cape of Good Hope*. This lizard is the largest of all those found in the colony, because its body is two feet long, and its tail three; it is even suspected it can get larger. Its body is covered with very small scales; its color is black and brownish green above, whitish below, with sixteen or eighteen irregular black transverse bands, namely, about eight bands of the yoke, and nine others on the pectoral and abdominal regions: the tail is compressed, keeled above, marked with sixteen to eighteen white areas, and the same number of black stripes, alternately arranged like rings until its end which is black. Its feet each have five separate fingers, and as many elongated sharp nails. According to Sparrman, this lizard has some resemblance to the one Seba figured tom. I, pl. XCIV, fig. 1, under the name of *Ceylon lizard* (*lézard de Ceilan*), by the rings on its body; but the Cape Lizard has more, and did not indeed have the same colors. I must add here that Sparrman noticed that this tupinambis from the Cape of Good Hope, of which he took two juveniles, is amphibious, loves water as much as earth, and moreover, it is extremely lively: this traveler grabbed it by the neck, so it could not bite him, and seeing that the animal struggled to free itself with much violence, he rapidly perforated its heart and head skull with a large needle and several punctures; but the tupinambis still seemed very willing to flee. Then they pressed its body repeatedly with violence; its four legs were tied together, and it was hung in a noose that shook very strongly. After forty-eight hours the animal released itself from the noose, but we soon found it, seeming much enfeebled. Sparrman then plunged it into brandy, and after a quarter of an hour it still lived and struggled. The locals believe, probably rightly, that this large lizard, which is obviously neither bad nor poisonous, might be easily tamed. (Sparrman, Journey to the Cape of Good –Hope and around the World, French transl., in-8°, tom. III, pag. 259 and follow.).

In 1790, George Shaw gave in his The Naturalist's Miscellany, a quite inaccurate figure, and a good description of the starry tupinambis; but he was wrong in claiming that this saurian also lives in the East Indies and South America. Indian and American tupinambis are quite different animals from the species that I have just described.

Daudin, F.M. 1803. Histoire Naturelle, Génerale et Particulière des Reptiles. Tome Huitème. De L'imprimerie de F. Dufart, Paris. Pp. 353.

SECOND SECTION.

Tupinambis with a tail topped with a small, slightly saw-serrated double keel.

7. Starry tupinambis; *tupinambis stellatus*.

Blackish brown above, with transversal bands formed by small whitish ocellated circles and dotted between them with whitish points; tail long, slightly saw-keeled above.

8. Nile crested tail tupinambis; *tupin. niloticus*.

Blackish brown above, with bands, points and small whitish ocellated circles irregularly placed; tail long, slightly keeled in saw above.

9. Bengal tupinambis; *tupinambis bengalensis*.

Ashy above, dotted here and there with black and whitish; black bands on cheeks; throat punctuated with black, whitish below; tail long, slightly saw-keeled above.

10. Ornate tupinambis; *tupinambis ornatus*.

Black, with a white throat striped by nine transversal black bands, seven rows of transversal round white spots on the back, and twelve to eighteen whitish rings around the tail slightly saw-keeled above. Var. A. Ornate tupinambis having eighteen rings around the tail.

11. White throated tupinambis; *tupin. albigularis*.

Below and sides of the head and throat whitish, spotted with brown; two whitish lines going from the eyes to the neck; tail long, slightly saw-keeled above.

12. Variegated tupinambis; *tupinambis variegatus*.

Blackish above, with streaks formed by lines and transversal double rows of small rounded yellow spots; tail two times as long as the rest, slightly saw-keeled above.

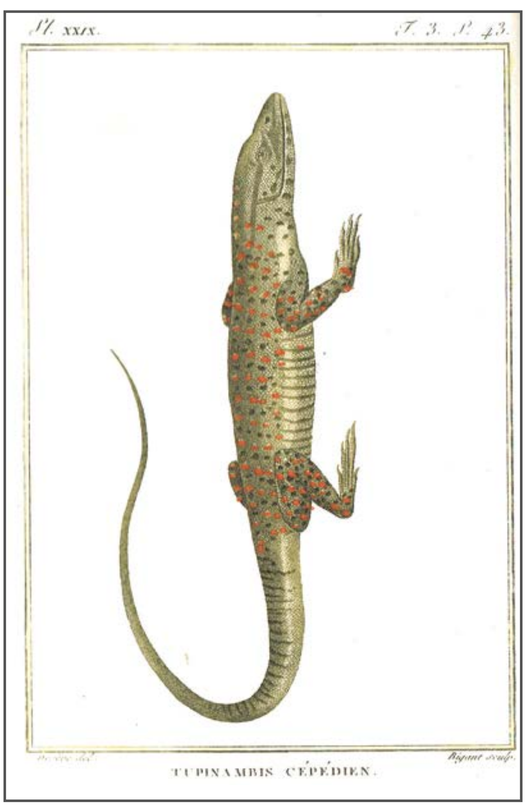

Plate XXIX. Tupinambis cepedien.

Plate XXX. Tupinambis indien.

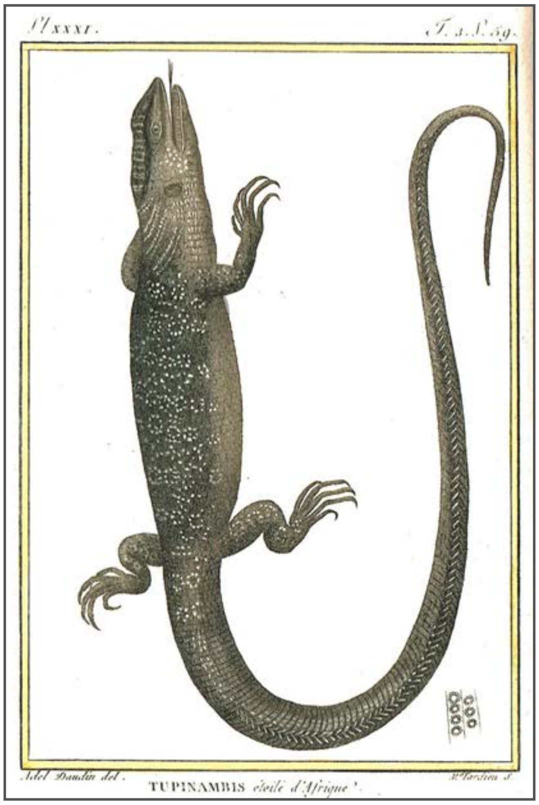

Plate XXXI. Tupinambis étoile d'Afrique.

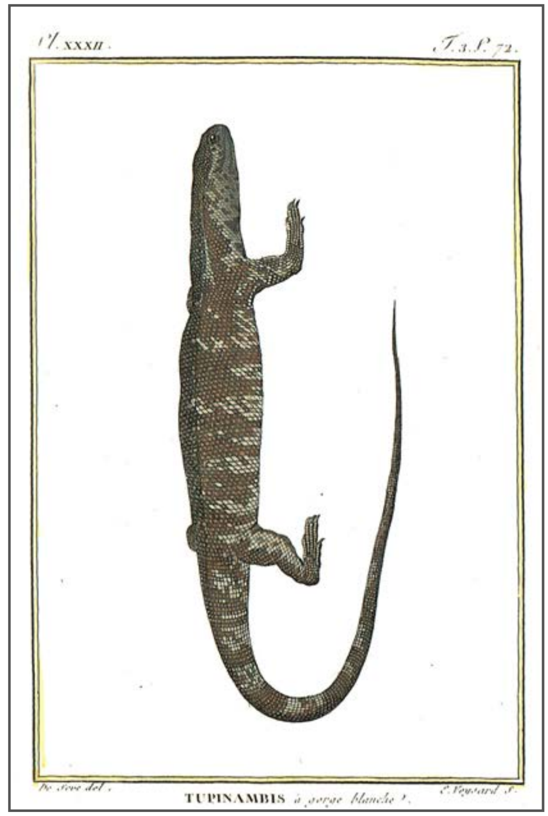

Plate XXXII. Tupinambis a gorge blanche.

**The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views of the U.S. Fish and Wildlife Service.*

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Peacock Monitor
Varanus auffenbergi

2018
Acrylic on canvas
24 x 30”

by KATIE KARL
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