OBSERVATION OF BIOFLUORESCENCE IN THE HORNED ADDER (*BITIS CAUDALIS*) (SQUAMATA: VIPERIDAE) AND COMMON EGG-EATER (*DASYPELTIS SCABRA*) (SQUAMATA: COLUBRIDAE) FROM SOUTHERN AFRICA

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In 1972, Stahnke reported a game changing incident: during a night excursion in 1945 to search for fluorescent rocks with ultraviolet (UV) lights, one participant saw a bright shining rock from a distance, which quickly disappeared as it was approached. It turned out to be a scorpion of the genus *Hadrurus*.
Since then, biofluorescence has been described in many marine animals like fishes (Sparks et al. 2014), cnidarians and crustaceans. In terrestrial animals fluorescens has been described in many arthropods (Lawrence 1954) including insects (Lloyd 1983; Johnsen et al. 2006; Olofsson et al. 2010), scorpions (Kloock et al. 2010; Gaffin et al. 2012) and solifugids (Cloudsley-Thompson 1978). Furthermore, numerous studies have been conducted on vertebrates like amphibians (Taboada et al. 2017), reptiles (Odate et al. 1959; Vaz Pinto et al. 2021; Lobon-Rovira et al. 2022), birds (Hausmann et al. 2003) and mammals (Kohler et al. 2019). Biofluorescence has been recorded in reptiles since the early 1950s (Odate et al. 1959), including Gekkota (Sloggett 2018; Top et al. 2020; Prötzel et al. 2021, Vaz Pinto et al. 2021; Lobon-Rovira et al. 2022), Chamaeleonidae (Prötzel et al. 2018) and turtles (Gruber and Sparks 2015).

It is noteworthy that in snakes (suborder Serpentes), the first investigations were already conducted by Odate et al. (1959). He examined the skins of three colubrids (Japanese Ratsnake, *Elaphe climacophora,* Japanese Four-lined Ratsnake, *Elaphe quadrivirgata*, and Japanese Woodsnake, *Euprepiophis conspicillata*) and the viperid Mamushi, *Gloydius blomhoffii*, for fluorescent substances. Hulse (1971) reported the first observation of fluorescence in the Western Blind Snake, *Rena humilis*. Further observations reported fluorescence in the Blue-banded Sea Krait, *Laticauda laticauda* (Seiko and Terai 2019) and in the Colombian Long-tailed Snake, *Enuliophis sclateri* (Fuentes et al. 2021).

The observations reported in this note were conducted with a TATTU U3S LED Ultraviolet (UV) torch which uses a wavelength of 365 nm and emits a brightness of 80 lumens. Photographs were taken using a Sony Cybershot DSC-RX III with Exmore RS 1,0 ´´CMOS Sensor.

OBSERVATIONS

Common Egg-eater, *Dasypeltis scabra* (Linnaeus, 1758)

While searching for scorpions around 22h30 late in December 2022, a bright shining line was seen on the ground of a carport close to Omaruru, Erongo, Namibia. At first this line was considered to be a small piece of rope, but as it was approached the 'rope' tried to escape. The 'rope' was then identified as a juvenile Common Egg-eater, *Dasypeltis scabra*, which reflected a fluorescent blue all over the body with the lighter areas of the pattern blazing brighter when exposed to UV light (Fig. 1).

Figure 1. Left: Juvenile Common Egg-eater, *Dasypeltis scabra*, under UV light (365 nm) after it was caught in a carport in Omaruru, Erongo, Namibia. Right: The same snake photographed under natural light.

The Common Egg-eater is a nocturnal snake which is most abundant in dry thornveld. It is small and very slender and reaches a body length of up to 1160 mm (Marais 2004). The observed specimen had a body length of approximately 300 mm and was therefore considered to be a juvenile.

Horned Adder, *Bitis caudalis* (Smith, 1839)

Inspired by the observation of biofluorescence in the Common Egg-eater, a juvenile female Horned Adder, *Bitis caudalis*, from the same biotope close to Omaruru, Erongo, Namibia was examined with UV light and white light. Again, the little snake showed a bright fluorescent blue all over under the UV light (Fig. 2).

Figure 2. Left: Juvenile female Horned Adder, *Bitis caudalis*, from Omaruru, Erongo, Namibia under UV light (365 nm). Right: The same snake under natural light.

Like with the Common Egg-eater, the lighter areas of the pattern blazed brighter compared to the darker parts. This resulted in a strong accentuation of the overall appearance of the snake. This observation was later confirmed in an adult male Horned Adder, which also fluoresced under UV light (Fig. 3).

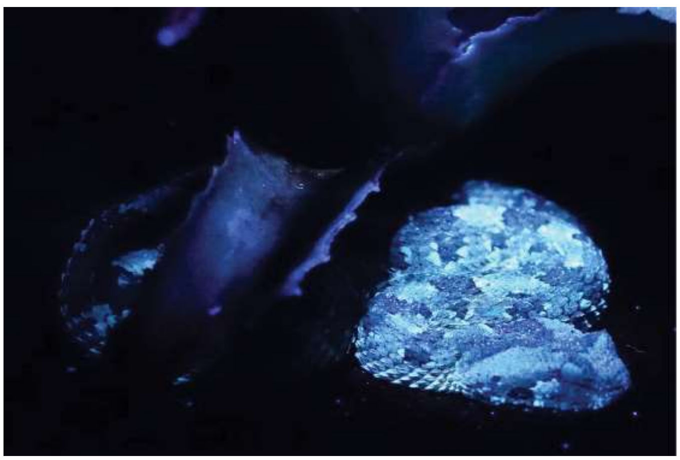

Figure 3. Adult male Horned Adder, *Bitis caudalis*, from Omaruru, Erongo, Namibia under UV light (365 nm).

To determine where the fluorescent substances are located, a one week old moulted skin of the same juvenile Horned Adder was examined. The shed skin was in perfect condition and the strong fluorescent blue light reflection was observed in the shed skin as well (Figs. 4 & 5).

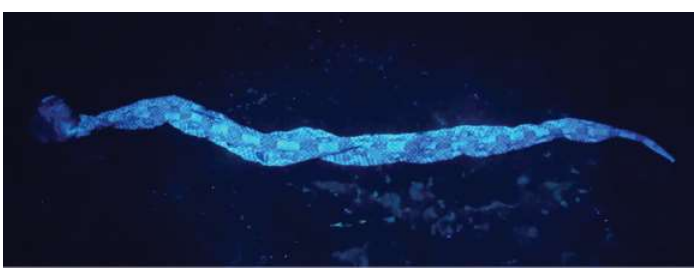

Figure 4. Shed skin of the juvenile female Horned Adder, *Bitis caudalis*, emits a bright blue light under UV light (365 nm).

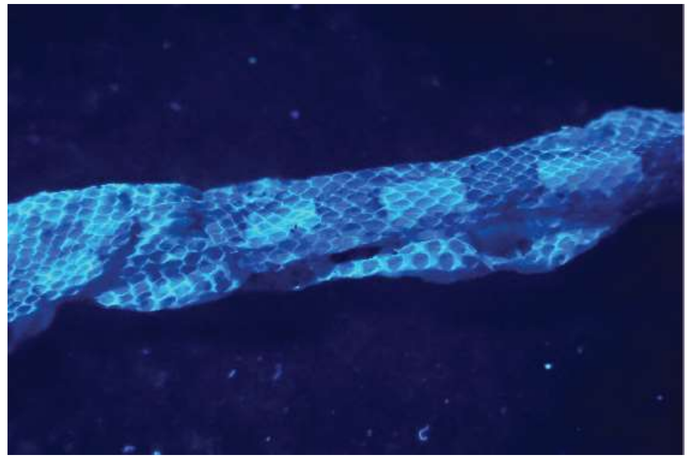

Figure 5. The less pigmentation there is in the shed skin, the brighter it shines under UV light at 365 nm. The less pigmented epidermis between the higher pigmented areas can be clearly seen.

DISCUSSION

Fluorescence is a widespread optical phenomenon that can be observed in aquatic as well as terrestrial habitats. Several mechanisms for biofluorescence have been described to date:

Bone-based fluorescence

It has long been known that bones have fluorescent qualities (Bachman and Ellis 1965). Bone fluorescence can be studied very well in geckos (Prötzel et al. 2021; Vaz Pinto et al. 2021; Lobon-Rovira et al. 2022) as their epidermis stretches thinly over the joints and the skull and the emitted blue light can shine through. In chameleons, tubercles arising from bones of the skull displace all dermal layers other than a thin, transparent layer of epidermis, creating a 'window' onto the bone (Prötzel et al. 2018). Bone-based fluorescence emits bright blue light at wavelengths of 440–500 nm. In contrast to geckos and chameleons, the epidermis of snakes is rather thick and robust so that bones cannot shine through.

Lymph- and gland secretion-based fluorescence

This mechanism of fluorescence has been described for amphibians whose skin is strongly lubricated by serous and mucous glands with pigmentary cells filtering into the skin (Taboada et al. 2017). As the skin of most amphibians is very thin, bone-based fluorescence can also be observed. The robust skin of snakes is not lubricated and possesses only a few glands compared to amphibians so that a lymph- and gland secretion-based fluorescence can be excluded as the likely mechanism for the observed fluorescence in these snakes.

Fluorescence by iridophores

While Prötzel et al. (2021) examined the Namib Web-footed Gecko, *Pachydactylus rangei*, using a UV light, they found a strong green light emission at the side and around the eyes of the gecko at a wavelength of 516 nm. Histological examination of the skin in the emitting parts of the body identified iridophores in the dermis as the source of fluorescence.

Stratum corneum-based fluorescence

In the 1950s, several investigations on reptile skin extracts provided evidence for fluorescent substances. Pteridine-derivates were found in the skins of the colubrid green snakes, *Philothamnus*, Boomslang, *Dispholidus typus* (Blair and Graham 1955), Japanese Ratsnake, Japanese Four-lined Ratsnake and Japanese Woodsnake, and the viperid Mamushi (Odate et al. 1959).

As the observed fluorescence in the Horned Adder could also be observed in the exuviae (the shed skin), the origin of this effect must be located in the stratum corneum of the epidermis or rather in the robust β-layer. McMullen et al. (2012) examined the fluorescence of different keratin tissues like hair, skin, claws, and horns of various species. They identified tryptophan and its metabolite kynurenine as major fluorophores in the epidermis. The maximum wavelength for tryptophan excitation was 290 nm and for emission 330 nm, both in the invisible light spectrum. However, the excitation peak for kynurenine was at 370 nm and the emission peak at 435 nm – in the visible blue light spectrum. These findings correspond to another tryptophan derivative molecule beta-carboline, which is responsible for the fluorescence in the cuticle of scorpions (Stachel et al. 1999).

The emission of kynurenine is strongly influenced by pigmentation. Melanin is the most abundant and widespread pigment in reptile skin yielding black, brown, grey, rufous, and buff color shades (McGraw 2006). The epidermis of snake skin is to some extent also heavily pigmented with melanin as can be seen in the exuviae of the Horned Adder. Melanin suppresses the fluorescence of tryptophan and kynurenine (McMullen et al. 2012), and for this reason the shed skin fluoresces more brightly in the areas of low pigmentation compared to areas with high pigmentation when exposed to UV-light. That is why the typical pattern of the Horned Adder can still be seen under UV light. Furthermore, the microstructure of the dark scales differs significantly from the microstructure of the pale scales. The surface of the dark scales is covered with leaf-like structures and crests which are up to three times higher than on the pale scales

(Spinner et al. 2013; Singh and Alexander 2017). Therefore, the dark scales demonstrate much lower reflectance and higher absorbance than other scales in the UV-near infrared spectral range.

To date, observations of biofluorescence in snakes are quite rare and further investigations, especially regarding the origin of biofluorescence in snakes, must be done. Furthermore, the role of fluorescence in snake biology remains to be clarified.

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