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3D reconstruction of fang replacement in the venomous snakes *Dendroaspis jamesoni* (Elapidae) and *Bitis arietans* (Viperidae)

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Venomous snakes use highly specialized teeth, so-called fangs, to kill living prey items. The evolution of these fangs and that of the associated venom-producing and -delivery system has been the subject of continuous research (e.g., KOCHVA 1978, KARDONG 1982, KNIGHT & MINDELL 1994, JACKSON 2003, FRY et al. 2008, VONK et al. 2008). In addition to the evolutionary origin of tubular fangs, their ontogenetic formation has been studied as well (e.g., TOMES 1874, BOGERT 1943, KLAUBER 1972, LAKE & TREVOR-JONES 1987, LAKE & TREVOR-JONES 1995, JACKSON 2002). While our knowledge has significantly increased thanks to the integrated evaluation of palaeontological, morphological, physiological, molecular and other data, some details still remain unresolved. One such remaining problem concerns the timing and regulation of fang replacement, i.e., when and how a functional fang is replaced by a new fang. This topic has been briefly touched by JACKSON (2007). Snakes replace their teeth, including the fangs, regularly and continuously, therefore there is always a number of replacement fangs posterior to the functional one. The maxilla typically has sockets for two fangs in lateral versus medial positions. At any given time, one would expect one fang to be solidly fused (ankylosed) to the socket and the other more or less loose and in the process of either attaching or being shed. However, as a functional safety factor, the phase when both fangs are ankylosed should overlap to some degree, avoiding a time window where both the replacement fang and the one in the process of being shed would not be fixed to the upper jaw. According to KLAUBER (1972), this period of overlap must be short,

since two functional fangs on one side (i.e., both are firmly set in neighbouring sockets) are observed very infrequently.

Here we report for the first time on the fang configuration at the stage around fang replacement, using CT-data with 3D visualisation. During our field expeditions in the Democratic Republic of the Congo, two specimens of venomous snakes were found where 'double fangs' were visible on one side of the maxilla. We use this term to describe the stage during fang replacement when both fangs in both sockets are 'out' and clearly visible. However, we could not test whether the new fangs were fully functional, since the soft tissues and presence of venom in the venom canals were not examined.

An adult specimen of Jameson's mamba, *Dendroaspis jamesoni* (TRAILL, 1843) (Serpentes: Elapidae), was found at Bomane on the Aruwimi River on 24 May 2010. It was collected and preserved, and is currently deposited in the Royal Belgian Institute of Natural Sciences in Brussels (voucher specimen RBINS:ZTN:CRT4055). Furthermore, an adult specimen of puff adder, *Bitis arietans* (MERREM, 1820) (Serpentes: Viperidae), was found in the Kundelungu National Park around 25 km southwards to Katwe on 22 November 2011. The snake is now voucher specimen RBINS:ZTN:UP391.

The micro-CT scans of the heads of both snake specimens were performed at the Centre for X-ray Tomography of the Ghent University (<http://www.ugct.ugent.be>; MASSCHAELE et al. 2007) using the transmission head of a dual head X-ray tube (Feinfocus FXE160.51) and an a-Si

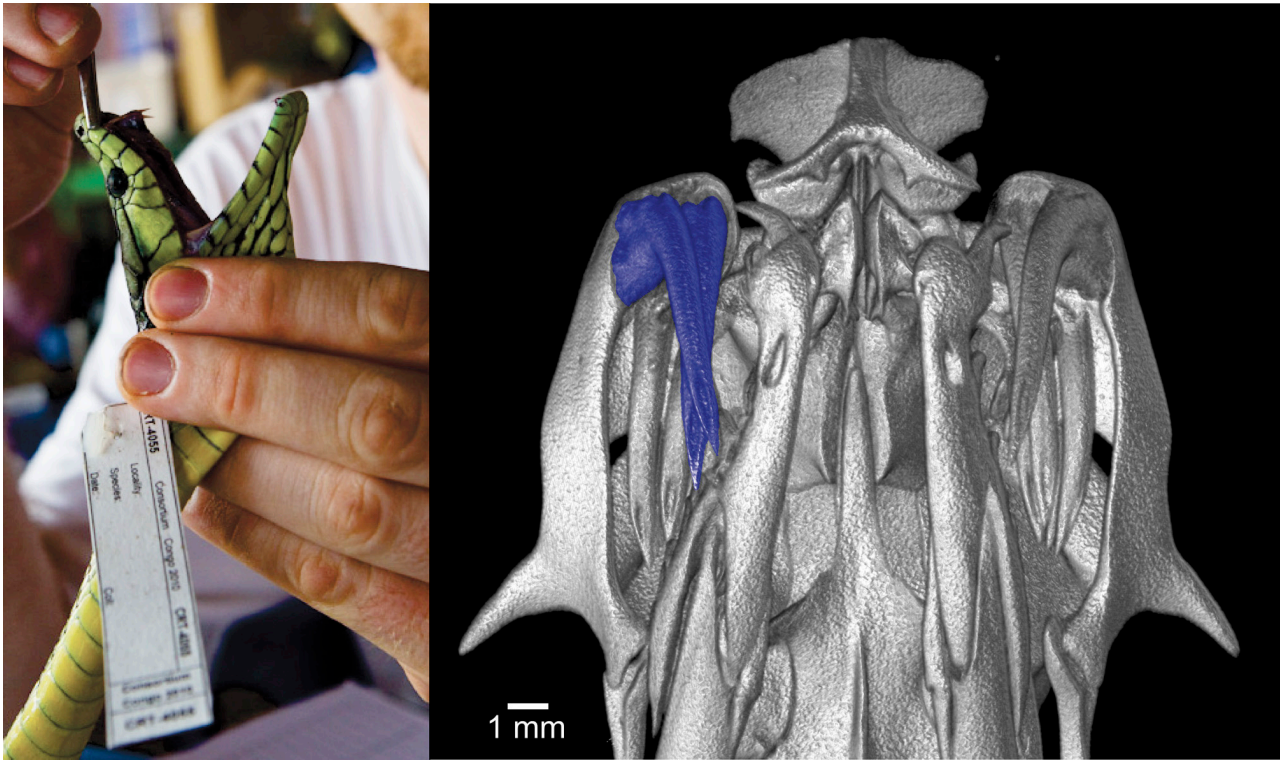


Figure 1. Head of an adult *Dendroaspis jamesoni* (Serpentes: Elapidae). Left: Head of the preserved specimen with fangs visible on the right-hand side. Right: Frontal ventral view of the skull.

flat panel detector (PerkinElmer XRD 1620 CN3 CS). The tube voltage was selected to be between 120 and 130 kV. For the Jameson's mamba, 1801 projections were recorded, covering 360°, with an exposure time of 2 s per projection, resulting in a voxel size of 36 µm. For the puff adder, 1201 projections with an exposure time of 2 s per projection were recorded, resulting in a voxel size of 70 µm. Reconstruction of the tomographic projection data was done using the in-house-developed Octopus-package (<http://www.octopus-reconstruction.com>; VLASSEN BROECK et al. 2007). Volume and surface rendering was performed using Amira 5.4.3 (VSG).

These two species allow an interesting comparison. Mambas, like other elapid snakes, have proteroglyph dentition with relatively short fangs ankylosed to a less mobile and longer maxilla, whereas viperids have solenoglyph dentition with large fangs attached to a mobile and significantly reduced maxilla. In both cases, however, to different grades, the maxillae rotate relative to the ectopterygoid and other cranial bones during a strike. In viperids, the greatly elongated fangs are folded backwards in the resting position, “with base and point at about the same level, and with the bulge of the fang-curve fitting into a hollow in the lower jaw” (KLAUBER, 1972), whereas in elapids, they are not.

Mambas (genus *Dendroaspis*) are large snakes with a maximum total length of > 2 m. They possess comparatively large fangs, with their maxillae being rather mobile

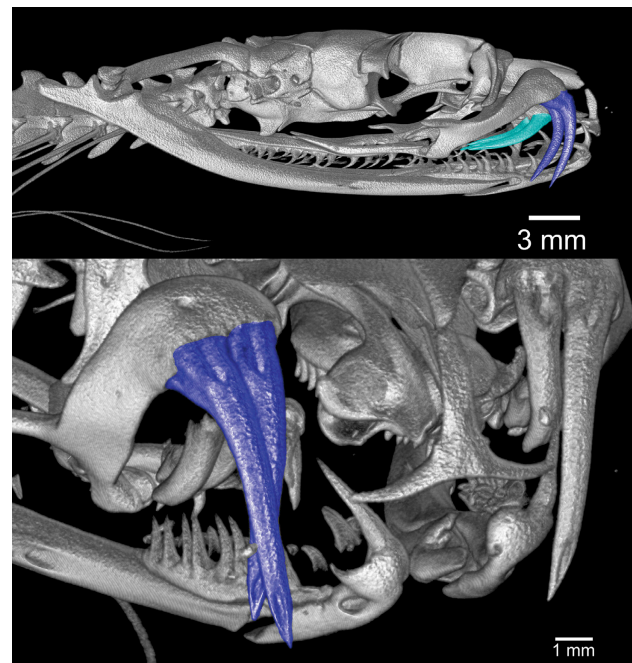


Figure 2. Top: Lateral view of the complete head of *Dendroaspis jamesoni*. Functional and first replacement fangs are marked in dark blue, further replacement fangs in light blue on the right-hand side. Bottom: Detailed frontolateral view of the fangs.

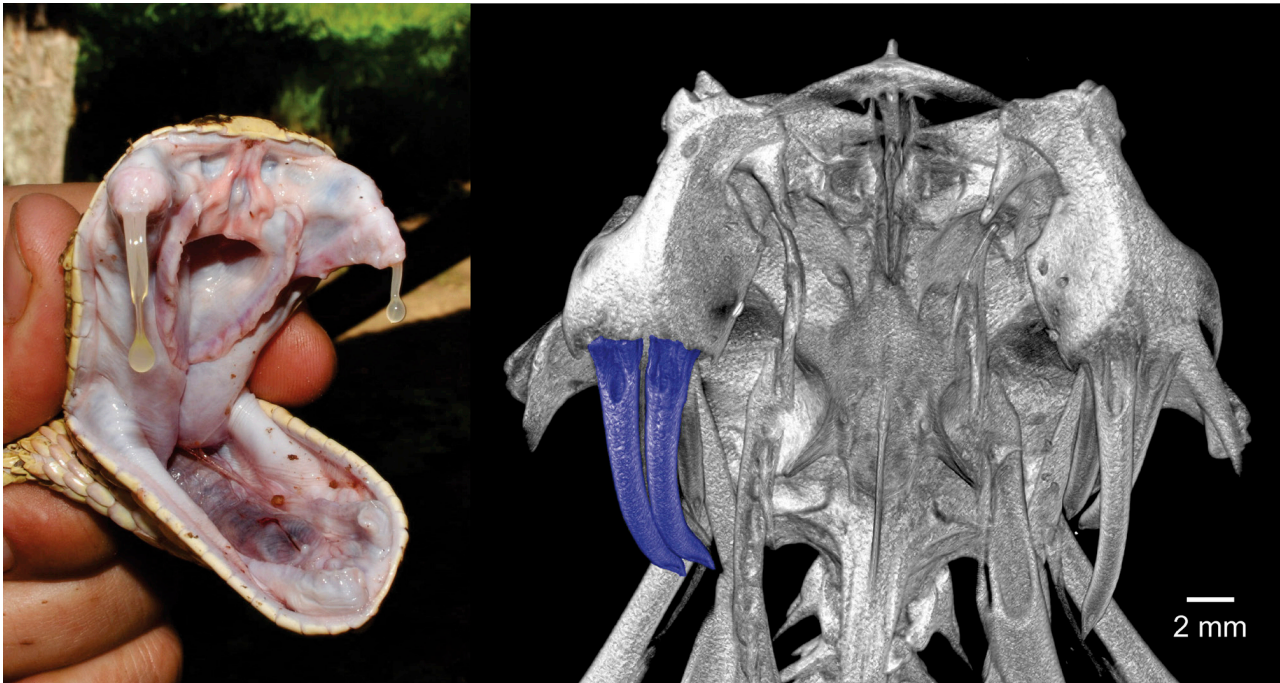


Figure 3. Head of an adult *Bitis arietans* (Serpentes: Viperidae). Left: Head of the specimen with all fangs visible. Right: Frontal ventral view of the skull.

compared to other elapids. Fangs of adult *Dendroaspis jamesoni* typically range between 6.4 and 8.0 mm in length (BOGERT 1943). A reconstruction of the Jameson's mamba skull (Figures 1–2) shows that on the right side, the functional fang in lateral position abuts against a medial replacement fang, with both being similar in length ('double fangs' are marked in dark blue in the reconstructions). The first replacement fang appears to be fully developed and almost completely ankylosed to the maxilla (Fig. 2); further replacement fangs on the right-hand side are marked in light blue in the lateral view. This is different for the medial replacement fang on the left-hand side, which is clearly not yet ankylosed. The distal end of that fang points posteriorly, and the fang is positioned more horizontally than vertically. Furthermore, both fangs on the right-hand side are visible from the outside, suggesting that in the case of a strike, both fangs would be functional in penetrating the prey. However, the strike angles of these fangs differ slightly, with the distal end of the replacement fang still pointing more caudally. Having two fangs in these unequal positions may not be very efficient during a strike, as there will always be one fang that will not penetrate the prey axially, and thus experience bending forces at the tip. To evaluate this aspect, however, kinematic simulations of the strike would be necessary.

Puff adders (*Bitis arietans*) are heavy ambush predators in Africa, and they have very large fangs that may exceed 30 mm in length, fused to short but wide maxillae. The

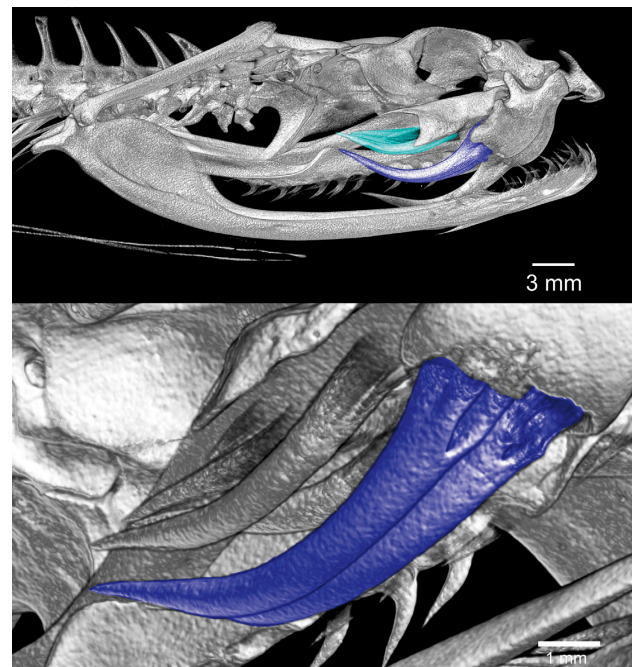


Figure 4. Top: Lateral view of the complete head of *Bitis arietans*. All fangs are in resting position, 'double fangs' are marked in dark blue, replacement fangs in light blue on the right-hand side. Bottom: Detailed frontolateral view of the fangs on the right side of the skull.

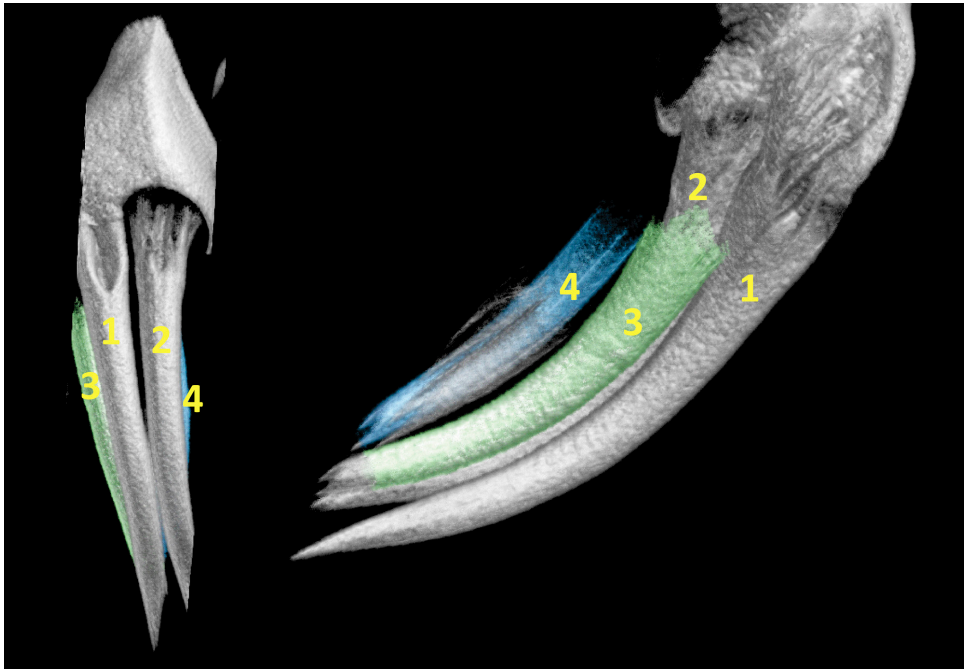


Figure 5. Frontal (left) and lateral views (right) of the fangs of *Bitis arietans*. Numbers indicate the sequence of fangs in the replacement series.

anatomy of the skull and the mass of attached muscles facilitate a powerful strike, and the amount of injected venom can be very high compared to that in other venomous snakes (see also Fig. 3, left). Our specimen was fixed and preserved with the mouth closed, and therefore its fangs remained in a resting position during the reconstruction. On the right-hand side, two fangs are visible in a parallel position, resulting in equal striking angles (Figures 3–4). From a mechanical point of view, both fangs would be fully efficient in penetrating a prey animal. At first sight, both of these fangs seem to be fully functional. They are roughly equal in size and positioned very close to each other. To have a better view, pieces of bones were digitally cut away around the fangs to make close-up views possible (Fig. 5). These show that fang No. 1, fixed in a lateral socket of the maxilla is the functional fang, while the one less firmly ankylosed in the medial socket is the next fang in the replacement series. The alternate possible interpretation would have been that the medial fang is older than the lateral one and in the process of loosening prior to being shed. However, based on a comparison of the angles of the shafts of fangs 1 and 2 (# 1 is in a closer-to-vertical position, and # 2 lies at an angle intermediate between 1 and replacement fang 3), we interpret fang # 2 as being in the process of attaching to the maxilla rather than in the process of detaching. According to KLAUBER's (1972) explanation of the sequence of fang replacement, fang No. 3 will replace whichever in-place fang (lateral or medial) is shed first.

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