

Research



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Biomechanics

Has snake fang evolution lost its bite?

New insights from a structural mechanics viewpoint

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Venomous snakes—the pinnacle of snake evolution—are characterized by their possession of venom-conducting fangs ranging from grooved phenotypes characterizing multiple lineages of rear-fanged taxa to tubular phenotypes present in elapids, viperids and atractaspidines. Despite extensive research, controversy still exists on the selective pressures involved in fang phenotype diversification. Here, we test the hypothesis that larger fangs and consequently a shift to an anterior position in the maxilla evolved to compensate for the costs of structural changes, i.e. higher stress upon impact in tubular fangs compared to grooved fangs. Direct voxel-based stress simulations conducted on high-resolution μ CT scans, analysed within a phylogenetic framework, showed no differences in stress distribution between the three fang phenotypes, despite differences in (relative) fang length. These findings suggest that additional compensatory mechanisms are responsible for the biomechanical optimization and that fang length might instead be related to differential striking behaviour strategies.

1. Introduction

Venomous snakes have fascinated scientists and society since ancient times [1,2]. Despite their reputation as frightening animals, venomous snakes have been studied for centuries with a significant amount of work focused on the evolutionary origin, development and diversification of the venom-delivery system, including fangs, glands and venom toxins [1–11]. Surprisingly, important questions still remain unresolved, particularly those concerning the driving forces underlying the diversification of the venom-delivery system in snakes. Given that snakes are responsible for a significant amount of human envenomation cases, understanding the evolution of the venom apparatus and its constituents could contribute significantly to our understanding of the biological role of venom and play a vital role in facing challenges related to snake bite cases [2].

In snakes, three types of modified venom-delivery systems have been described: (i) enlarged grooved fangs (i.e. ‘grooved phenotype’) located at the posterior end of the maxilla [5–9], evolved convergently in various lineages of advanced snakes (figures 1 and 2); (ii) fangs with enclosed venom-conducting canals and visible suture line connecting the orifices (i.e. ‘closed, non-fused phenotype’) associated with an anterior fixed position in the maxilla [5–9], evolved convergently in elapids including cobras, mambas and sea snakes, and in the atractaspidine *Homoroselaps* (figures 1 and 2); (iii) fangs with enclosed venom-conducting canals and smooth surface between the orifices (i.e. ‘closed, fused phenotype’) associated with an anterior mobile position in the maxilla [5–10], evolved convergently in viperids, including vipers and rattlesnakes, and in the atractaspidine *Atractaspis* (figures 1 and 2). Anteriorly located fangs develop

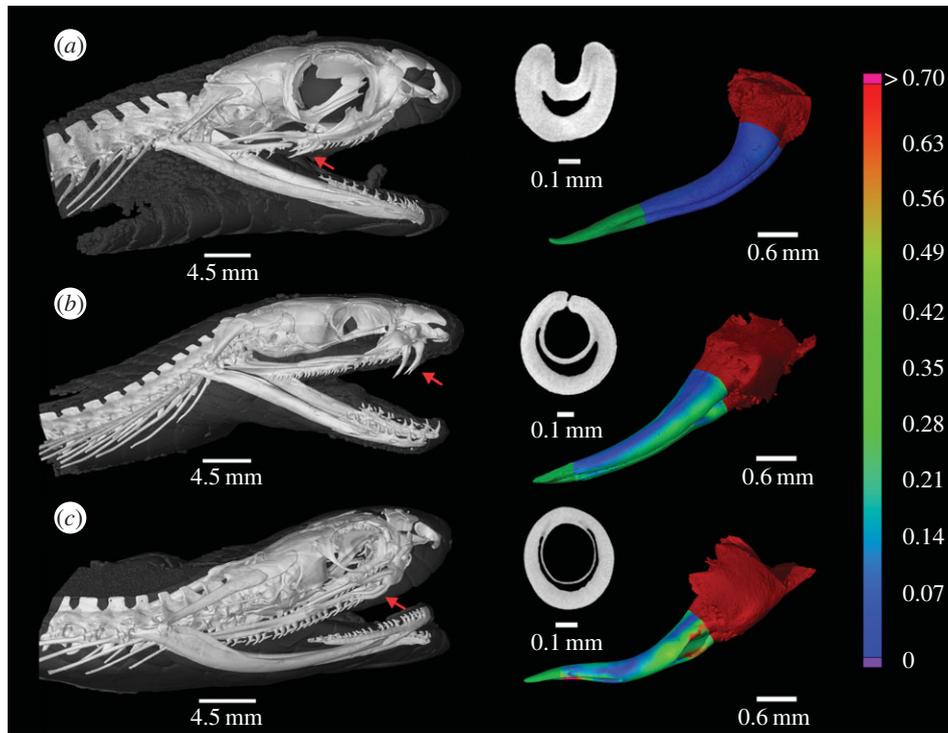


Figure 1. Three-dimensionally rendered μ CT images illustrating the morphological variation in snake fang phenotypes. (a) Grooved phenotype of a boomslang (*Dispholidus typus*) located at the posterior end of the maxilla; (b) closed, non-fused phenotype of a Cape cobra (*Naja nivea*) associated with an anterior fixed position in the maxilla; (c) closed, fused phenotype of a night adder (*Causus rhombeatus*) associated with an anterior mobile position in the maxilla. The red arrow indicates the position of the fang with respect to the skull. Stress distributions are shown for a representative of each fang phenotype with the gradient showing the maximum Von Mises stress (in GPa). A force was applied to the tip of the fang (indicated in green), whereas the base of the fang (indicated in red) was considered a region of immobility.

from the posterior end of the maxilla through the loss of the anterior dental lamina, indicated by a lack in expression of the sonic hedgehog (*shh*) gene, and displacement of a developing fang from a posterior to an anterior position [2,3,5]. A study on the viperid *Trimeresurus albolabris* showed the venom-conducting canal itself develops through a combination of two mechanisms. First, an infolding event occurs early in the development due to the invagination of epithelial cells independently of *shh* signalling [11]. Contact is made between the sides of the invaginating wall, thereby forming an enclosed canal. The canal is enlarged through proliferation of epithelial cells, coinciding with increased *shh* signalling, followed by apoptosis, which results in an empty venom-conducting tube. Second, gradual accretion of dentine occurs, thereby building the fang up from the tip to the base [11].

While the majority of studies have emphasized the evolutionary development of venom-conducting fangs [1–10], the selective pressures underlying the independent origins of tubular fangs remain elusive. The evolution of venom-delivery systems has been repeatedly linked to dietary and ecological specialization [4], yet remarkably little information on the mechanical behaviour of venom-delivery systems is available in this regard [14]. Based on the aforementioned findings, we ask the following questions: (i) Do fangs with a venom-conducting canal experience higher stress under load than grooved phenotypes? (ii) If so, are mechanisms present to compensate for the higher stress distributions? More specifically, we are interested in testing whether an increase in (relative) fang length and consequently shift from a posterior to an anterior position (to accommodate larger fangs) might have evolved to compensate for the increased stress concentrations. Using micro-computed

tomography (μ CT) and voxel-based stress simulations we compare the morphology and mechanical behaviour of fangs between the three fang phenotypes and ultimately aim to provide more insight into the driving forces underlying fang diversification.

2. Material and methods

High resolution X-ray computed tomography was applied to provide full 3D image datasets of the skulls and fangs of 20 snake species (grooved: $n = 7$; closed, fused: $n = 6$; closed, non-fused: $n = 7$) represented by adult preserved specimens belonging to the Ellerman Collection at Stellenbosch University. First, the head of each specimen was scanned using a GE Phoenix v|tome|x L240 dual tube CT instrument (General Electric, Wunstorf, Germany) located at the CT-scanning facility, Stellenbosch University [15]. The scanning settings were as follows: 160 kV, 100 μ A and a spatial resolution of 100 μ m. Acquired datasets were reconstructed with the system-supplied software Phoenix datos|x 2, producing 3D datasets, which were subsequently analysed using VGStudioMax v. 3.0 (Volume Graphics GmbH, Heidelberg, Germany). For each scan we measured the skull length from the occipital condyles to the nasal bone. Second, a single fang was excised from each specimen and scanned using a Nanotom S μ CT system (General Electric, Wunstorf, Germany) at 60 kV, 300 μ A, and a spatial resolution of 2–10 μ m. The length of each fang was measured from the edge of the basal orifice to the tip of the fang along the curve (see electronic supplementary material, S1).

Mechanical loading simulations were performed in VGStudioMax v. 3.0 using the function STRESS ANALYSIS, which makes use of direct voxel-based static loading simulation rather than the traditional meshing followed by simulation (see e.g.

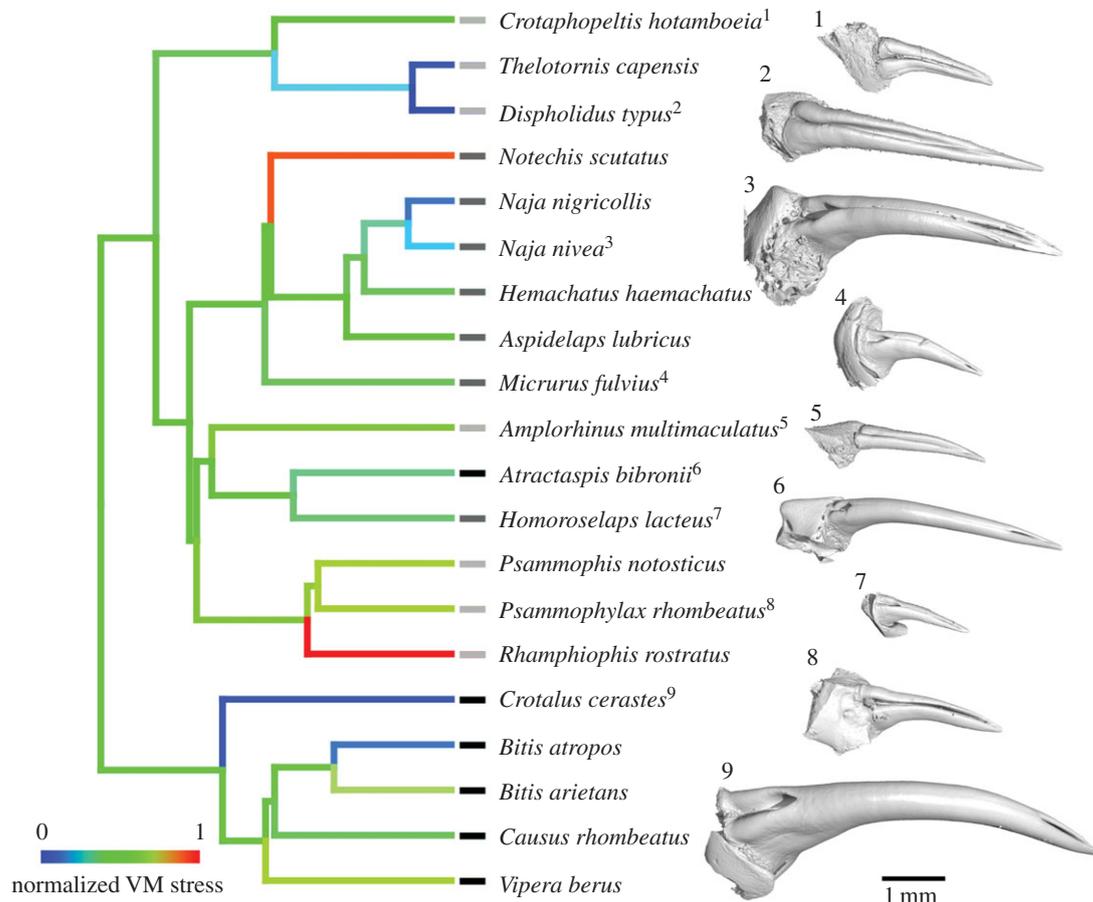


Figure 2. Phylogeny based on [12] showing the relationships among the species studied by high resolution μ CT imaging and accompanied by scaled 3D-rendered images representing the three fang phenotypes. Fangs with closed, fused venom-conducting canals, indicated in black, evolved convergently in the ancestor of the Viperidae and *Atractaspis*, whereas fangs with closed, non-fused venom-conducting canals, indicated in dark grey, evolved convergently in the ancestor of the Elapidae and *Homoroselaps*. Grooved fangs, indicated in light grey, show little phylogenetic affinity and are present in various clades. Normalized maximum von Mises (VM) stress values (parallel force) are mapped onto the snake phylogeny using the 'plotBranchbyTrait' function implemented in the R package phytools [13].

[16]). A direct 3D stress analysis was performed by applying a load of 5 N both parallel and perpendicular to the tip of the fang and we examined the stress distributions by means of the maximum von Mises (VM) stress. Material properties of the fangs were as follows: a Young's modulus of 20 GPa, a Poisson's ratio of 0.3 and assuming no anisotropy. Although these parameter settings might deviate slightly from actual values [14], we were merely interested in examining potential differences in stress distribution between fang phenotypes, all factors being equal. Likewise, it must be noted that the absolute magnitude of maximum VM stress might not represent a relevant physical parameter, especially because fangs do not appear to be able to withstand the applied load (see electronic supplementary material, S2).

A phylogenetic ANOVA using the function 'phylANOVA' implemented in the R package phytools [13] was employed to test for differences in VM stress between the three phenotypes. We used a recent time-calibrated phylogenetic tree by Zheng & Wiens [12], pruned to include the 20 taxa for which data were available (figure 2), as input for the phylogenetic comparative analyses. Secondly, we used a phylogenetic generalized least square regression (function 'pGLS' in phytools [13]) to test for a relationship between VM stress and fang length. Lastly, we tested whether relative fang length (i.e. corrected for skull length) differed between the fang phenotypes. We first regressed fang length against skull length using the function 'phyl.resid' in phytools [13] and subsequently used a phylogenetic ANOVA to compare the residuals between the three fang phenotypes. Data are available from the Dryad Digital Repository [17].

3. Results and discussion

Taking into account the phylogenetic relationships among venomous snakes, we found that maximum VM stress under load was similar between the three fang phenotypes (phylANOVA; parallel: $F_{1,19} = 0.28$, $p = 0.92$; perpendicular: $F_{1,19} = 1.07$, $p = 0.72$). The highest VM stress was in most cases located near the tip of the fang (see *Causus rhombeatus*, figure 1), a finding consistent with that observed in other studies conducted on spider fangs [18] and scorpion stingers [19]. Von Mises stress was negatively correlated with fang length (pGLS; parallel: $r = -0.97$, $p = 0.04$; perpendicular: $r = -0.96$; $p < 0.001$). However, even after correcting for interspecific differences in fang length, maximum VM stress did not differ between the fang types (phylANOVA on residual stress values; parallel: $F_{1,19} = 0.41$, $p = 0.89$; perpendicular: $F_{1,19} = 1.28$, $p = 0.68$). Selection towards larger anteriorly positioned fangs is therefore unlikely to have occurred to compensate for the costs of structural changes. Instead, our results suggest that fangs, like scorpion stingers [20], may be biomechanically optimized and that additional compensatory mechanisms are present besides an increase in length. Firstly, high curvature, as seen in the fused fangs of viperids (see electronic supplementary material, S1), could serve as protection against fracture [21]. Secondly, a recent study by Jansen van Vuuren *et al.* [14] showed that the fangs of *Bitis arietans* had higher hardness and

Table 1. Summary of morphological measurements and results from stress simulations (mean \pm s.d.) for each fang phenotype. Relative fang length is represented by the residuals of a phylogenetic regression of fang length on skull length.

variable	grooved phenotype (<i>n</i> = 7)	closed, non-fused phenotype (<i>n</i> = 7)	closed, fused phenotype (<i>n</i> = 6)
fang length—absolute (in mm)	2.33 \pm 1.00	3.20 \pm 1.23	7.27 \pm 5.08
fang length—relative	-0.16 \pm 0.10	-0.10 \pm 0.10	0.19 \pm 0.17
max. VM stress—parallel (in GPa)	2.80 \pm 2.55	1.98 \pm 2.07	1.63 \pm 1.15
max. VM stress—perpendicular (in GPa)	0.60 \pm 0.46	1.49 \pm 1.65	1.61 \pm 0.56

elastic modulus values than, for example, *Naja nivea*. The difference appears to be related to changes in the structure of hydroxyapatite and the proportion of organic versus inorganic components [14]. Lastly, variation in structural nanofibres might cause differential anisotropy from tip to base thereby improving the stiffness of the fang as is the case in spiders [19]. Unfortunately, this information cannot be obtained through μ CT alone and further mechanical testing and/or scanning electron microscopy is required to validate this theory. Nevertheless, relative fang length differed between the three fang phenotypes (phylANOVA; $F_{1,19} = 14.65$, $p = 0.02$). The closed, fused fangs of viperids and *Atractaspis* were relatively larger than grooved fangs (Bonferroni *post hoc* test; $p = 0.02$). The relative length of closed, non-fused fangs of elapids and *Homoroselaps* was similar to that of grooved fangs (Bonferroni *post hoc* test; comparison with closed, fused phenotype: $p = 0.08$; comparison with grooved phenotype: $p = 0.57$; table 1). This difference in relative fang length between viperids and the other advanced snakes might be related to strike behaviour rather than increased stress associated with structural changes as initially hypothesized. In venomous snakes, two predatory strategies dominate: rear-fanged snakes and elapids typically display a *bite-and-hold* strategy, whereas most vipers and *Atractaspis* use a *bite-and-release* strategy, especially for larger prey [22,23]. The divergence in striking behaviour would undoubtedly have required an increase in the efficiency of venom delivery, i.e. the evolution of larger fangs with a closed, fused venom-conducting channel to maximize the injection of venom into prey. It must, however, be noted that our statistical test might not be powerful enough to

detect (additional) differences in relative fang length because of the relatively small sample size and known variation in fang length, especially in taxa possessing a grooved phenotype [8].

In conclusion, our results indicate that the structural changes associated with the formation of the venom-conducting canal in tubular fangs do not appear to be associated with an increase in stress under load. Furthermore, despite the negative correlation between fang length and maximum VM stress, the larger fangs present in viperids does not seem to be a compensatory evolutionary response, but might instead be related to differential striking behaviour strategies.

Ethics. This study did not require ethical clearance because the μ CT scanning made use of preserved specimens located at the Ellerman Collection, Stellenbosch University.

Data accessibility. All data underlying the manuscript are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.bv37v> [17].

Authors' contributions. C.B. and A.d.P. conceived and designed the study; A.d.P. performed the μ CT scanning, created the three-dimensional reconstructions and analysed the μ CT images with C.B.; C.B. analysed the data and wrote the manuscript with comments from A.d.P. Both authors approve the final version of the article and agree to be accountable for the content therein.

Competing interests. We declare we have no competing interests.

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