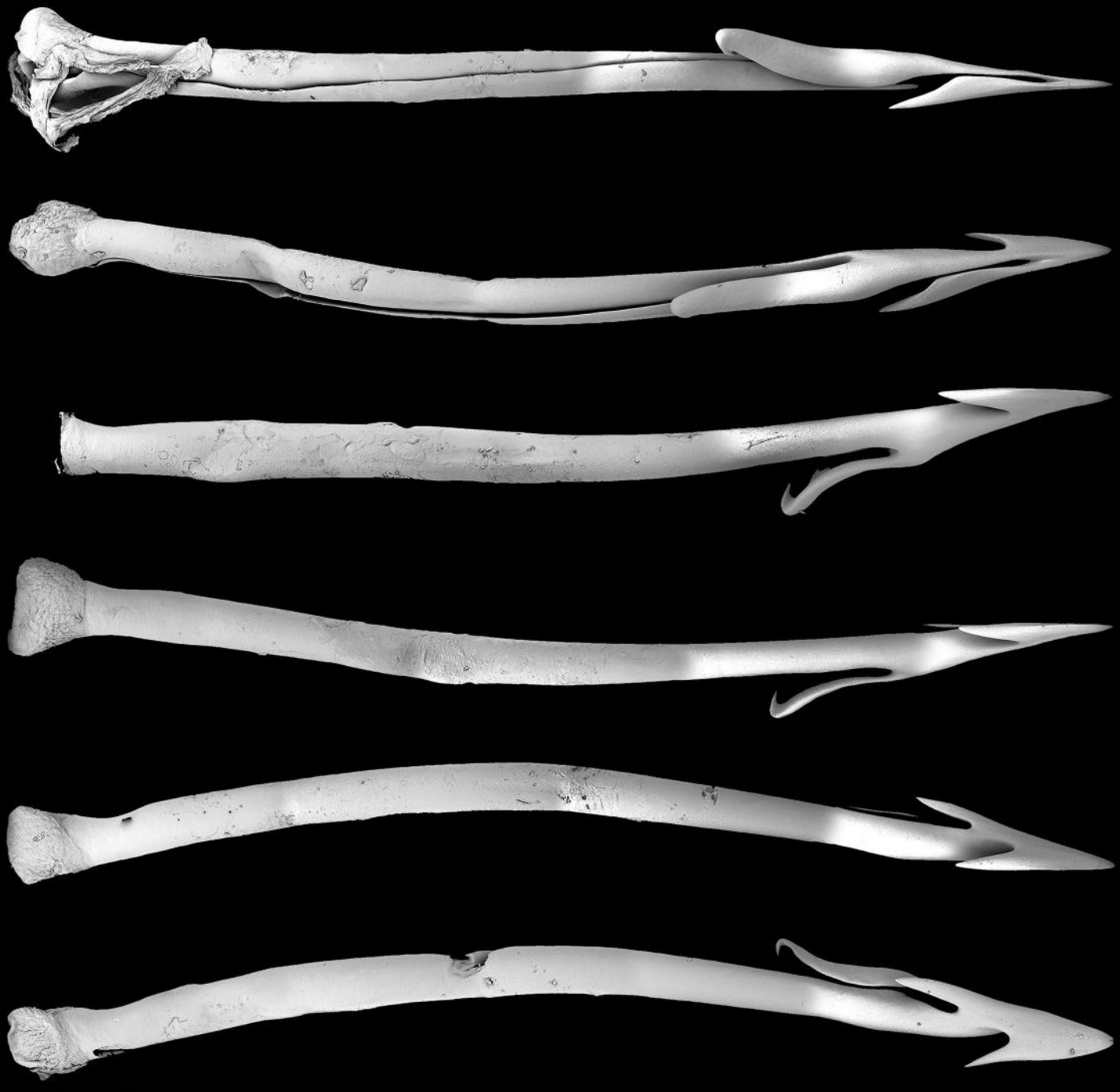
Current Biology

Volume 29
Number 16

August 19, 2019

www.cell.com



Correspondence

The high speed radular prey strike of a fish-hunting cone snail

Joseph R. Schulz[1,*], Ian Jan[1], Gurleen Sangha[1], and Emanuel Azizi[2,*]

Cone snails are venomous marine gastropods that hydraulically propel a hollow, chitinous radular harpoon into prey [1,2]. This radular harpoon serves both as projectile and conduit for venom delivery. In the fish-hunting cone snail *Conus catus*, the radular harpoon is also utilized to tether the snail to its prey, rapidly paralyzed by neuroexcitatory peptides [2,3]. Effective prey capture in *C. catus* requires both fast-acting neurotoxins and a delivery system quick enough to exceed the prey fish's rapid escape responses [4]. We report here that the cone snail's prey strike is one of the fastest in the animal kingdom. A unique cellular latch mechanism prevents harpoon release until sufficient pressure builds and overcomes the forces of the latch, resulting in rapid acceleration into prey [2]. The radular harpoon then rapidly decelerates as its bulbous base reaches the end of the proboscis, a distensible hydrostatic skeleton extended toward the prey [2], with little slowing during prey impalement. The velocities achieved are the fastest movements of any mollusk and exceed previous estimates by over an order of magnitude [1].

In preparing for the prey strike, the cone snail pressurizes the lumen of the proboscis proximal to a muscular sphincter surrounding the inner lumen within the proboscis (*MS* in Figure 1A). Once an appropriate location is found on the prey, the muscular sphincter relaxes, thus propelling venom fluid toward the bulbous base of the harpoon (* in Figure 1A,B). During this priming step, the base of the radular harpoon engages a latch mechanism. Cone snails utilize a unique cellular latch composed of tall epithelial cells rich in microfilaments, which form a constriction that initially resists the advancement of the radular harpoon (*C* in Figure 1A,B). The radular harpoon is held until overcome and released toward the prey. Once at the end of the proboscis, the bulbous base of the radular harpoon is held as venom is delivered (Figure 1A,B, bottom panels).

Previous recordings noted the rapid movement of the radular harpoon during release but this occurred too rapidly to analyze the kinematics of the strike [1,2]. To visualize the trajectory of the radular harpoon during release within the translucent proboscis of *C. catus*, an individual snail was coaxed to extend its proboscis down a narrow seawater-filled chamber toward prey held near the end of the recording chamber (Figure 1A,B; Video S1 in Supplemental Information, published with this article online). Ultra-high

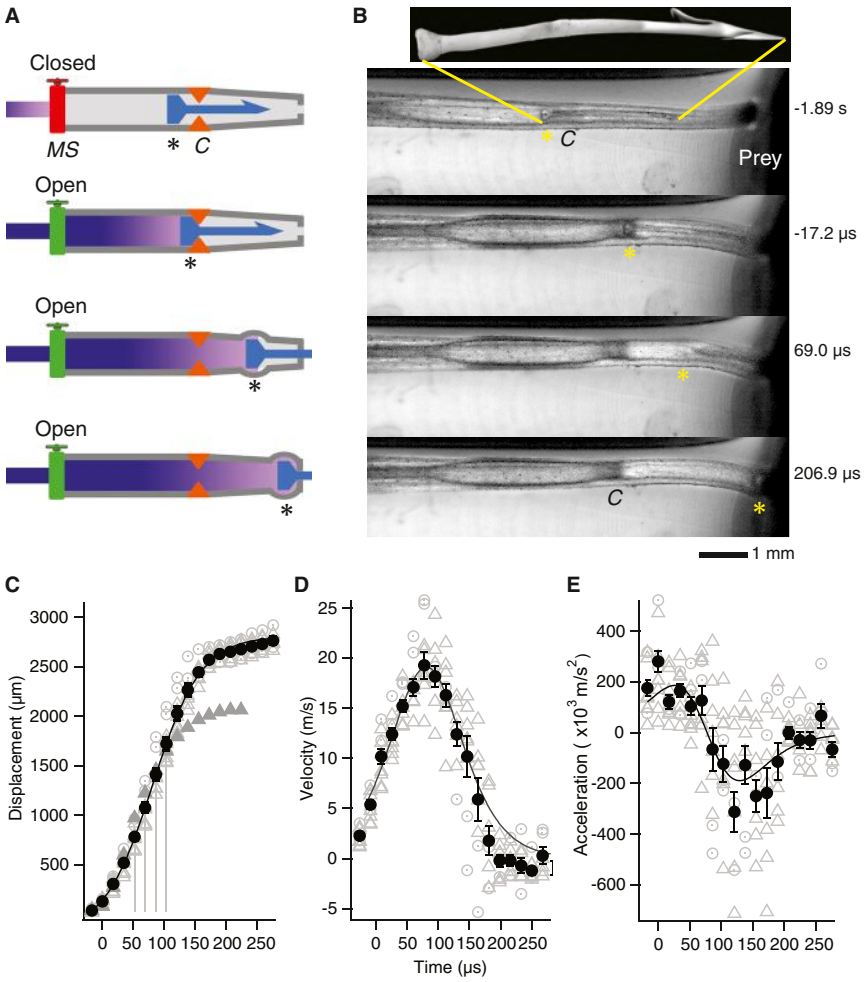


Figure 1. Kinematic analysis of the cone snail prey strike.
(A) A schematic of the cone snail prey strike mechanism depicts the muscular sphincter (*MS*) as a valve and the constriction (*C*) as a narrowing in the distal proboscis. The radular harpoon (bulbous base*) is propelled into the prey after clearing the constriction in the proboscis lumen but does not leave the proboscis. (B) Top panel: Scanning electron composite micrograph of a *C. catus* radular harpoon (2.6 mm). Bottom panels: Ultra-high speed video stills of the cone snail prey strike. Times in microseconds before and after release from the constriction (scale bar = 1 mm; *C* = constriction, * = base of tooth). (C) Average tooth displacements over time during the prey strike (filled circles; ± SE, n = 9) includes strikes to the eye (open circles) and body (open triangles). Grey lines to zero indicate time of impalement when noted. Prey strikes to boney elements reduced radular harpoon displacement (filled triangles), preventing full impalement. (D) Average velocities during the prey strike (filled circles; ± SE, n = 9) includes strikes to the eye (open circles) and body (open triangles). (E) Average accelerations during the prey strike (filled circles; ± SE, n = 9) includes strikes to the eye (open circles) and body (open triangles).

speed videography was used to capture the displacement, velocity and acceleration of the radular harpoon during the strike (see Supplemental Experimental Procedures). The radular harpoon is rapidly propelled into the prey with most of the displacement occurring within 100 microseconds (Figure 1C). No meaningful difference was noted for different sites of impalement, excluding a strike into a boney element that limited overall displacement of the harpoon (Figure 1C, grey triangles). The radular harpoon is accelerated to an average peak velocity of 19.3 m/s, with peak velocities exceeding 25 m/s (Figure 1D). These peak velocities, occurring close to the time of impalement (Figure 1C,D), indicate little slowing during prey contact. Additionally, *C. catus* individuals of different shell lengths had similar time courses and velocities (Figure S1A,B). The radular harpoon is rapidly accelerated into prey with an average peak acceleration exceeding 280,000 m/s^2 and maximal accelerations exceeding 400,000 m/s^2 (Figure 1E). Such extreme values are similar to those for a bullet fired from a pistol [5]. Within 120 microseconds of achieving peak accelerations, the harpoon is rapidly decelerated on average by over 300,000 m/s^2, with maximal decelerations exceeding 700,000 m/s^2 (Figure 1E). These high decelerations could aid venom flow through the narrow apertures of the radular harpoon (https://data.mendeley.com/datasets/355nt9sv73/draft?a=e9390447-0848-46fd-89f7-b3dded7f47b0).

The ultra-high speed videos also allowed us to examine changes within the proboscis preceding the prey strike in much greater detail than in previous studies. During the priming step prior to harpoon release, axial expansion of the proboscis lumen between the muscular sphincter and constriction (*MS* and *C* in Figure 1A) occurs for ~9 ms causing a 14.7% (±0.5; n = 7) average maximal expansion. With the constriction serving as a latch, this slower buildup translates into the rapid release of the harpoon. Once this latch is overcome, the radular harpoon moves through the lumen of the distal proboscis, with the bulbous base of the harpoon (* in Figure 1A,B) distending the lumen walls by over 30% (data not shown).

Although these maximum velocities approach the highest recorded values for the ultra-fast mandibular strikes of trap-jaw ants and termites (67 m/s) as well as the raptorial strikes of stomatopods (mantis shrimp; 31 m/s) [6], key features of the cone snail's prey strike distinguish it from these other mechanisms. During the cone's prey strike, the radular harpoon is hydraulically propelled axially through the lumen of the proboscis. By contrast, these other mechanisms involve the rotation, or swinging, of the lever-like appendages with velocities measured at the free ends. While different in execution, all of these systems do share the common feature of a latch-mediated mechanism to control the timing and rate of energy release [7]. The ultrafast nematocyst discharge of cnidarians is the fastest comparable prey strike in a venomous animal, with stylet eversions reaching final velocities of 18.5–37.1 m/s [8]. These values are similar to the cone snail's; however, the duration of the nematocyst discharge occurs on a sub-microsecond time scale. Furthermore, nematocyst discharge occurs on the cellular level. Conversely, the cone snail's radular strike requires coordination on a multi-cellular, whole animal level.

Similarities in the radular harpoon and the structure of the proboscis among all cone snails, as well as previous high speed studies [2], indicate that the ultra-fast prey strike is not unique to fish-hunting cone snails. Anatomically similar structures representing the latch (tall epithelial constriction) and muscular sphincter have also been noted in several other species of the hyperdiverse Conoidea superfamily [9,10]. These features along with the hollow harpoon-like radular teeth suggest that this prey strike mechanism is not unique to cone snails, but instead may be utilized to varying degrees by thousands of marine gastropod species.

SUPPLEMENTAL INFORMATION

Supplemental Information contains one figure, experimental procedures, and one video, all of which can be found with this article online at https://doi.org/10.1016/j.cub.2019.07.034.

ACKNOWLEDGEMENTS

We acknowledge support from the U.S. Army Research Laboratory and the U.S. Army Research Office under contract/grant number W911NF-15-1-0358 (G.S.) and the Undergraduate Research Center at Occidental College (I.J.).

AUTHOR CONTRIBUTIONS

J.R.S. and E.A. conceived the ideas for the manuscript, J.R.S., I.J., and G.S. collected data, J.R.S. and I.J. conducted data analysis, J.R.S. and I.J. wrote the manuscript, and G.S. and E.A. edited the manuscript.

REFERENCES

1. Schulz, J.R., Norton, A.G., and Gilly, W.F. (2004). The projectile tooth of a fish-hunting cone snail: *Conus catus* injects venom into fish prey using a high-speed ballistic mechanism. Biol. Bull. *207*, 77–79.
2. Salisbury, S.M., Martin, G.G., Kier, W.M., and Schulz, J.R. (2010). Venom kinematics during prey capture in *Conus*: the biomechanics of a rapid injection system. J. Exp. Biol. *213*, 673–682.
3. Kelley, W.P., Schulz, J.R., Jakubowski, J.A., Gilly, W.F., and Sweedler, J.V. (2006). Two toxins from *Conus striatus* that individually induce tetanic paralysis. Biochemistry *45*, 14212–14222.
4. Turesson, H., Satta, A., and Domenici, P. (2009). Preparing for escape: anti-predator posture and fast-start performance in gobies. J. Exp. Biol. *212*, 2925–2933.
5. Tse, M. (2003). Acceleration of a bullet - The Physics Factbook. Acceleration of a Bullet. Available at: https://hypertextbook.com/facts/2003/MichaelTse.shtml [Accessed February 22, 2019].
6. Patek, S. (2015). The most powerful movements in biology. Am. Sci. *103*, 330–337.
7. Ilton, M., Bhamla, M.S., Ma, X., Cox, S.M., Fitchett, L.L., Kim, Y., Koh, J., Krishnamurthy, D., Kuo, C.-Y., Temel, F.Z., *et al.* (2018). The principles of cascading power limits in small, fast biological and engineered systems. Science *360*, eaao1082.
8. Nüchter, T., Benoit, M., Engel, U., Özbek, S., and Holstein, T.W. (2006). Nanosecond-scale kinetics of nematocyst discharge. Curr. Biol. *16*, R316–R318.
9. Medinskaya, A.I. (2002). Foregut anatomy of the Turrinae (Gastropoda, Conoidea, Turridae). Ruthenica *12*, 135–159.
10. Kantor, Y.I., and Puillandre, N. (2012). Evolution of the radular apparatus in Conoidea (Gastropoda: Neogastropoda) as Inferred from a Molecular Phylogeny. Malacologia *55*, 55–90.

[1]Department of Biology, Occidental College, Los Angeles, CA 90041, USA. [2]Ecology and Evolutionary Biology, University of California Irvine, Irvine, CA 92697, USA.
*E-mail: jschulz@oxy.edu (J.R.S.), eazizi@uci.edu (E.A.)

Supplemental Information: The high speed radular prey strike of a fish-hunting cone snail

Joseph R. Schulz, Ian Jan, Gurleen Sangha, and Emanuel Azizi

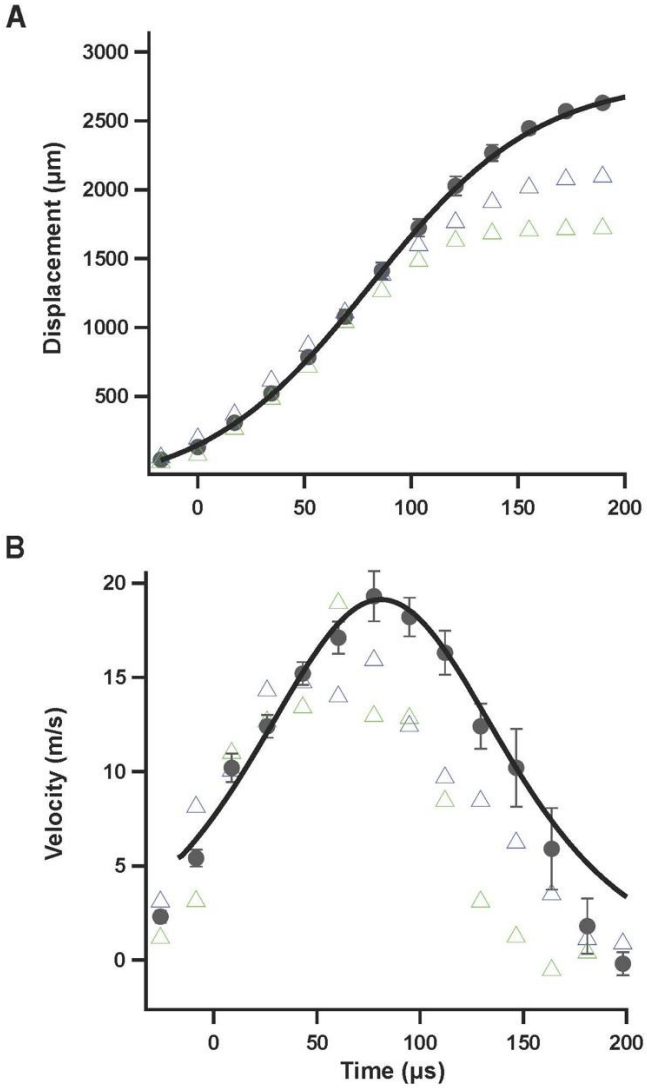


Figure S1- Kinematic analysis of the prey strike
(A) Average tooth displacements and (B) average tooth velocities during the prey strike (filled circles; ±SE, n=9; shell length 35.9 mm) compared to the values of body strikes from two other *C. catus* snails: shell length 32.6 mm (blue open triangles) and shell length 27.2 mm (green open triangles).

Supplemental Experimental Procedures

Feedings

Three *Conus catus* (shell lengths: 35.9 mm, 32.6 mm, and 27.2 mm) were offered fish, mainly *Poecilia reticulata*, euthanized via severing the spinal cord at the hindbrain boundary, on a weekly basis. The snails were fed either directly or after striking into "milking" tubes. To coax the snails to fire into the tubes, severed fish caudal fins were secured on parafilm using a second piece of parafilm with a window exposing the rays of the fins. To reinforce consistent strikes to the milking tubes, the snails were fed only after successful tube strikes. The snails were fed a minimum of once every two weeks. All protocols were approved and performed in accordance with the institutional animal care and use committee (IUCAC) at Occidental College.

High Speed Filming

We acquired high speed videos (Vision Research, Phantom VEO 410L) at 58,000 frames-per-second (fps). Feedings took place in a custom acrylic feeding tray with a narrow trough (3 x 23 x 1.5 mm). The camera was focused at the end of the trough (filming dimensions: 10 x 3 mm). By guiding the constantly moving, flexible proboscis toward the prey held with forceps into filming position, we then evoked the snail to strike by gently tapping the prey to the end of the proboscis. More of the trough and the proboscis were included in the framing for prey strikes than cuvette shots. Both epi-illumination and diascopic lighting were utilized. Epi-illumination consisted of two 150W fiber optic lamps (Schott-Fostec, LLC) with four light pipes downwardly directed at the feeding tray. The improved diascopic illumination via a mirrored microscope base improved the visual clarity and lowered the required ambient lighting. Camera settings and video capture were controlled via Phantom Camera Control (Vision Research).

Image Analysis

Videos of the prey capture were trimmed to specific events surrounding tooth release. ImageJ (Version 1.52a; NIH, Bethesda, MD) was utilized for image adjustments and an unsharp mask filter for improved clarity. For the strike analyses, we manually tracked the center of the bulbous base of the tooth in each frame using the multi-point tool. The tracking started in the two frames prior to the tooth clearing the cellular constriction and ended when either the base of the tooth is out of view or stopped moving. Displacement values were determined between two consecutive position tracking data points. Velocity and acceleration data were calculated using consecutive displacement and velocity values, respectively. The displacement average curve was fitted [displacement (microns)=$2809.01/\ 1+e^{-(t-85.059/39.185)}$; t (microseconds)] and differentiated to obtain average curves for velocity and acceleration in IGOR Pro (Version 8.0.1.2; Wavemetrics, Portland, OR). The time scale for the kinematic graphs of the strike was between -17.24 μs and 275 μs, which is the range of tooth displacement during the strike. Using the line tool, we measured the distance from the muscular sphincter to the constriction during the priming step prior to the strike and the vertical width of the lumen at a point 1000 μm distal to the constriction during the strike.

Radular Tooth Imaging

Radular teeth were extracted from dissected radular sac of *Conus catus*. Cleaned with dilute bleach and dehydrated via an ethanol series, six teeth were imaged at different axial positions via Scanning Electron Microscopy (SEM). Composite images of the teeth were constructed, adjusted

for brightness and scaled proportionally in Photoshop Elements (Version 15; Adobe Systems, San Jose, CA). We determined tooth length from light micrographs and the ImageJ line measurement tool.