



Grooves to Tubes: Evolution of the Venom Delivery System in a Late Triassic “reptile”

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Abstract

Venom delivery systems occur in a wide range of extant and fossil vertebrates and are primarily based on oral adaptations. Teeth range from unmodified (Komodo dragons) to highly specialized fangs similar to hypodermic needles (protero- and solenoglyphous snakes). Developmental biologists have documented evidence for an infolding pathway of fang evolution, where the groove folds over to create the more derived condition. However, the oldest known members of venomous clades retain the same condition as their extant relatives, resulting in no fossil evidence for the transition. Based on a comparison of previously known specimens with newly discovered teeth from North Carolina, we describe a new species of the Late Triassic archosauriform *Uatchitodon* and provide detailed analyses that provide evidence for both venom conduction and document a complete structural series from shallow grooves to fully enclosed tubular canals. While known only from teeth, *Uatchitodon* is highly diagnostic in possessing compound serrations and for having two venom canals on each tooth in the dentition. Further, although not a snake, *Uatchitodon* sheds light on the evolutionary trajectory of venom delivery systems in amniotes and provide solid evidence for venom conduction in archosaur-line diapsids.

Introduction

Venom-conducting teeth are a complex adaptation that has evolved in a wide range of vertebrates, with recent claims providing evidence for venom delivery in conodonts (Szaniawski 2009), the Komodo dragon (Fry et al. 2009), and a sphenodontian (Reynoso 2005) and other studies mapping the complex evolution of venom production in squamates (Fry et al. 2006, 2008) and fish (Smith and Wheeler 2006). Squamates, especially snakes, are the best-known venomous vertebrates and have evolved several tooth morphotypes that can be broadly classified as possessing either an open channel (groove; opisthoglyphous) or an enclosed canal (tube; proteroglyphous and solenoglyphous). Studies on the development of the fangs in extant snakes show that, while there is no folding of tissue, the teeth pass through morphological stages that are suggestive of an evolutionary trajectory in which the grooves deepen and fold over, as early-stage teeth show a basal groove (despite being destined for a position at the aperture), whereas late-stage teeth show a completely enclosed canal (Bogert 1943; Kochva 1987; Jackson 2002). Fossil evidence for the evolution of fangs is lacking, as the oldest fossil snake fangs (Miocene) already possess

enclosed canals (Kuch et al. 2006), helodermatid lizards have retained the “primitive” (opisthoglyphous) configuration since the Late Cretaceous (Nydam 2000), and the serrated teeth of the possibly venomous Komodo dragon lack a groove entirely (Fry et al. 2009). This crucial gap is filled by a new species of the Late Triassic archosauriform reptile *Uatchitodon* from North Carolina, demonstrating that the evolutionary trajectory hypothesized by developmental data has evolved in at least one reptilian lineage.

Although currently known only from isolated teeth, *Uatchitodon* is tentatively assigned to Archosauriformes (Sues et al. 1994; Sues 1996) based on thecodont tooth implantation (inferred from roots) and the presence of serrated carinae (Godefroit and Cuny 1997; Heckert 2004). *Uatchitodon* is readily diagnosed by three apomorphies: very tall, laterally compressed tooth crowns (crown height greater than three times mesiodistal basal length), infoldings on the labial and lingual surfaces, and compound denticles (denticles with subdivided or irregular cutting edges). The infoldings range from open grooves (*Uatchitodon kroehleri*) to fully enclosed canals (*Uatchitodon schneideri* sp. nov.).

Two other archosauriform taxa (*Graoulyodon* (Godefroit and Cuny 1997) and *Sinornithosaurus* (Gong et al. 2010)) have been interpreted as possessing venom-conducting teeth. The sole evidence for venom conduction in *Graoulyodon* is a shallow groove on the only known tooth, and most of the evidence in *Sinornithosaurus*, including a maxillary depression and “enlarged” teeth, can be explained without invoking venom conduction (Gianechini et al. 2010). We argue here that *Uatchitodon* represents both the oldest instance of venom conduction in a diapsid reptile and the only well-documented example among archosauriform diapsids and thus provides us with a window into the evolution of venom-conducting teeth and increases our knowledge of archosauriform disparity.

Institutional Abbreviations: MNA, Museum of Northern Arizona, Flagstaff; NCSM, North Carolina Museum of Natural Sciences, Raleigh; USNM, National Museum of Natural History, Washington, DC.

Geological setting

First described from the Tomahawk locality of Virginia (Vinita Formation, Newark Supergroup; Late Triassic: Carnian; Sues 1991; Sues et al. 1994), *Uatchitodon* has since been discovered in two other Late Triassic (early Norian) sites: the Moncure locality of North Carolina (Cumnock Formation, Newark Supergroup) and the *Placerias* Quarry of Arizona (Bluewater Creek Formation, Chinle Group; Kaye and Padian 1994; Sues 1996). All three localities are notable for yielding extremely diverse faunas (with at least ten tetrapod taxa each), and the sites in

North Carolina and Virginia have yielded abundant cynodont remains, a rarity in Late Triassic Laurasian deposits (Sues and Olsen 1990). All three sites also represent broadly similar palustrine depositional environments with fossiliferous strata dominated by dark mudstones with minor carbonate nodules (Kaye and Padian 1994; Sues et al. 1994). The Tomahawk locality is the stratigraphically oldest, and both the *Placerias* Quarry and Moncure localities are slightly younger (Kaye and Padian 1994; Lucas et al. 1997; Sues 1996; Sues and Hopson 2010). The Tomahawk and Moncure localities are of particular interest as they are in close geographic proximity (~300 km apart), their chronostratigraphic proximity is easily demonstrable (Cumnock-equivalent strata directly overlie the Vinita Formation; Weems and Olsen 1997), and both have yielded more than two dozen measurable teeth or tooth fragments of *Uatchitodon*.

Materials and methods

We examined the apical-most broken surface on teeth from all three localities, capturing images using both a Nikon D100 digital camera attached to a Stenni SV6 binocular dissecting microscope and an FEI Quanta 200 environmental scanning electron microscope (SEM). We then obtained measurements of the maximum height and width of the canal, the distance from the mesial and distal carinae to the canals, and the width of teeth (see [Supplementary Materials](#)) from these images with ImageJ and analyzed these data using nonparametric tests and linear regressions in R v2.11.1. Images were edited in Adobe Photoshop CS5 by adjusting brightness and contrast and eliminating the original background to clearly present the teeth. NCSM 24731 was embedded in epoxy resin and sectioned twice, with the basal-most section being polished with 600–1,200 μm aluminosilicate grit prior to etching in 10% HCl for approximately 20 s and coated with gold-palladium alloy to enhance contrast during SEM imaging, following Sander (1999).

Systematic paleontology

Reptilia

Archosauriformes

Genus *Uatchitodon* Sues 1991

Type species—*U. kroehleri* Sues 1991

Revised diagnosis—very tall (crown height greater than three times fore-aft basal length), labiolingually compressed tooth crowns with compound denticles along at least the posterior carina and enamel-lined infoldings on the lingual and labial surfaces of the crown and most apical portion of the root.

U. kroehleri Sues 1991

Holotype—USNM 448611, isolated tooth crown embedded in matrix exposing the lingual side.

Type locality and horizon—Tomahawk locality, Midlothian, VA, USA; Vinita Formation, Richmond basin, Newark Supergroup.

Revised diagnosis—tooth crowns strongly compressed labiolingually and with median infoldings that are open along >20% of the infolding's height, creating mostly open channels that lack discrete apertures along the lingual and labial surfaces of the crown.

U. schneideri, sp. nov.

Holotype—NCSM 24753, a complete, weakly labiolingually compressed tooth crown with an enclosed canal and faint suture along the external surface.

Type locality and horizon—locality NCPALEO 1906 (Moncure microvertebrate locality), near Raleigh, NC, USA; Cumnock Formation, Sanford subbasin of the Deep River basin, Newark Supergroup.

Referred specimens—from type locality: NCSM 24732, NCSM 24754–NCSM 24757, NCSM 25238–NCSM 25252, the thin sections of NCSM 24731, and numerous uncatalogued fragments. From the *Placerias* Quarry (Bluewater Creek Formation, Chinle Group; Lucas et al. 1997): MNA V3680, a complete tooth crown and a crown with missing tip.

Etymology—named for Vince Schneider (North Carolina Museum of Natural Sciences) in recognition of his many contributions to the paleontology of North Carolina, including the discovery and excavation of the Moncure locality.

Diagnosis—*Uatchitodon* teeth with weaker labiolingual compression and median infoldings that are enclosed along a significant proportion ($\geq 80\%$) of infolding's height, terminating in apical apertures on the lingual and labial surfaces of the crown.

Comments—we designated a specimen from the North Carolina locality as the holotype because the latter has provided a significantly larger sample than the Arizona site and the Arizona material may have a different density of serrations on the carinae.

Isolated tooth crowns of *Uatchitodon* are readily identified, and we analyzed the morphology of the two larger samples to quantitatively analyze variation within and between the two species. One-tailed Wilcoxon tests show that the North Carolina *U. schneideri* have less labiolingually compressed teeth ($N=14$ *U. kroehleri*, $N=26$ *U. schneideri*; p value < 0.01) and canals that are offset more distally ($N=13$ *U. kroehleri*, $N=25$ *U. schneideri*; p value 0.02).

Description and analysis

Inferring venom conduction exclusively from teeth is difficult because many factors can produce grooves on

teeth, especially in mammals (Folinsbee et al. 2007). We evaluated alternative hypotheses to both the origin of the infoldings (wear-induced, ontogenetic/replacement, or positional) and their function (mechanical strength/suction reduction, or grooming) by examining histological sections and by measuring the shape of the canals and size of the teeth (see [Supplementary Materials](#)).

We examined a sectioned tooth under an SEM and found that incremental growth lines in the dentine extend around the canals (Fig. 1). Given that wear would not affect the growth of the dentine, we reject both ontogeny and wear over the course of ontogeny as explanations for the infolding. Previous studies found no evidence of significant ontogenetic change in archosaurian teeth (Currie et al. 1990). We also reject ontogeny as a factor in the canal-shape variation in *Uatchitodon* based on the geographic distribution of the two morphotypes (only opisthognathous teeth are present in the large sample from Tomahawk, the Moncure specimens are all solenognathous) and the lack of correlation in a linear regression between canal shape and fore-aft length of the plane at which the canals were measured ($N=25$, R^2 -adj=0.09, $p=0.14$; with compensation for the estimated distance from the tip, see [Supplementary Materials](#)). The hypothesis that the morphotypes represent different stages of tooth replacement can also be rejected based on the presence of resorption pits at the base of complete teeth representing both morphotypes and by the geographic separation of the two forms. Despite widespread heterodonty in diapsid reptiles, we can rule out positional differences as the source of canal-shape variation, through a regression of the angle of the carinae relative to the center of the tooth (Hungerbühler 2000) against the shape of the lingual canal and found no significant effect ($N=16$, R^2 -adj=0.06, $p=0.72$). Further, no similar teeth lacking

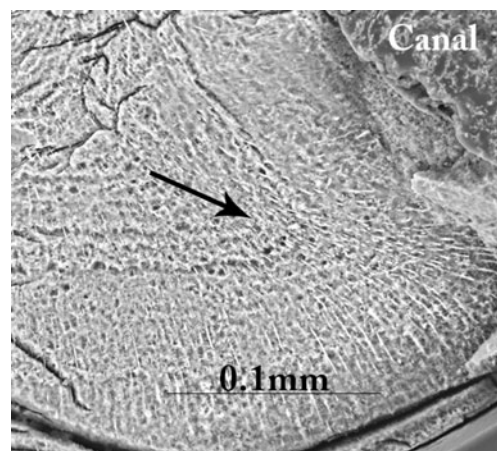


Fig. 1 SEM micrograph of the acid-etched basal transverse section of NCSM 24731 showing incremental growth lines in the dentine around the canal (upper right). The external surface of the tooth crown is on the bottom right. Scale bar=100 μ m

grooves or canals occur with comparable frequency as *Uatchitodon* at either of the well-sampled Moncure or Tomahawk localities. Also, as with ontogeny, the geographic distribution of distinct morphotypes provides strong evidence against positional variation.

Having rejected reasonable alternatives for groove development, the most plausible hypothesis is that the canals had a function and that canal shape likely underwent directional selection. Groove functions in extant non-venomous tetrapods include sharpening other teeth, grooming, and other mechanical purposes (Folinsbee et al. 2007). The extreme crown height, curved dentine growth lines and enclosed canals render these alternative functions untenable and leave venom conduction as the most plausible hypothesis. Furthermore, extant tetrapods (viperid and elapid snakes and the mammal *Solenodon*) use their teeth with enclosed canals for venom conduction (Orr et al. 2007).

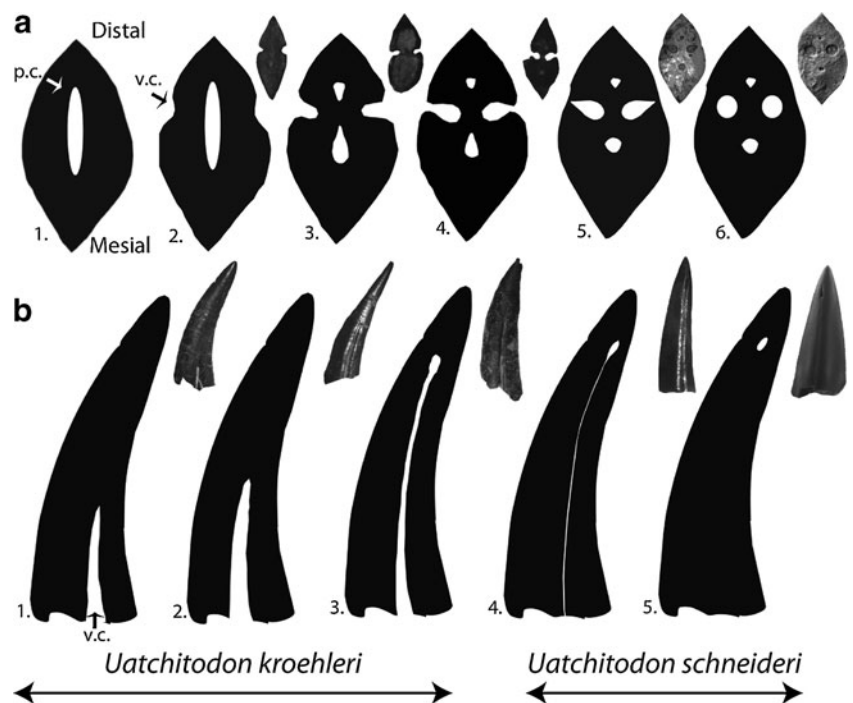
Conclusions and discussion

By establishing the biological origin of the infoldings and rejecting possible functional alternatives, we argue that the teeth of *Uatchitodon* were used in venom conduction and that the variation in these teeth can be used to examine patterns of evolution of venom delivery. Although the combination of serrations and canals in *Uatchitodon* teeth is unlike the teeth of any known squamate, it allows us to document evolutionary changes in tooth structure associated

with venom delivery and note the similarity of these changes to the stages of replacement fangs in snakes (Fig. 2). In particular, the venom canal first evolved as a shallow groove at the base, extending less than a fourth of the way to the apex. As the groove extended apically, it also became narrower and deeper, with a narrower opening to the external surface until just before its apical terminus. Finally, the groove extended almost to the apex, and the infolding became so deep and the opening so narrow that the margins of the groove finally touched, enclosing the channel and creating a tubular canal. A faint seam persists on the external surface of the crowns in some teeth where the two margins of the groove came into contact; this seam has almost disappeared in some teeth from Moncure and in the more complete tooth crown from Arizona. Unfortunately, the tentative phylogenetic position, unique morphology (e.g., two canals on every tooth in the mouth) and the inability to confidently assign teeth to upper or lower jaws preclude a rigorous reconstruction of the soft-tissues associated with these changes.

This structural series is strongly reminiscent of the series seen in snake replacement fangs, where early-stage fangs show a “basal” groove, and late-stage fangs are tubular (although the particular developmental mechanisms are different; Jackson 2002). However, it takes our understanding of the evolution of venom delivery systems a step further by providing empirical evidence that this pathway is advantageous. Previous data suggested that the morphology of venom-conducting teeth was highly conservative in lineages through time, with some clades such as elapids and viperids

Fig. 2 Diagrammatic representation of the transition seen in the teeth of *Uatchitodon* in transverse section (a) and side view (b), with the stratigraphically older, more primitive condition on the left and stratigraphically younger, more advanced condition on the right. Digital images to the upper right of each diagram are of a tooth representative of the canal configuration shown; see [Supplementary Data](#) for the catalog number of individual specimens. p.c. pulp cavity, v.c. venom channel/canal



retaining solenoglyphous teeth for more than 20 Ma (Kuch et al. 2006) and helodermatids retaining opisthoglyphous teeth for more than 70 Ma (Nydam 2000). *Uatchitodon* fills a critical gap in our understanding of venom delivery system evolution by recording the seemingly rapid transition from weakly grooved teeth to those with fully enclosed canals.

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