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INVITED PAPER

Show Me Your Teeth And I Will Tell You What You Eat: Differences in Tooth Enamel in Snakes with Different Diets

Maïtena Dumont[*,1], Joshua Milgram[*], Anthony Herrel[†], Ron Shahar[*], Boaz Shacham[‡], Céline Houssin[§], Arnaud Delapré[§], Raphaël Cornette[§] and Marion Segall[§,¶]

[*]Laboratory of Bone Biomechanics, Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, HUJI, Rehovot, Israel; [†]Mécanismes Adaptatifs et Evolution, UMR 7179, Muséum National d'Histoire Naturelle CNRS, Paris, France; [‡]National Natural History Collections, The Hebrew University of Jerusalem, Jerusalem, Israel; [§]Institut de Systématique Evolution, Biodiversité (ISYEB), UMR 7205, Muséum National d'Histoire naturelle CNRS, SU, EPHE, UA, CP 50, Paris, France; [¶]Department of Life Sciences, The Natural History Museum, London SW7 5BD, UK

[1]E-mail: maitena.dumont@gmail.com

Synopsis Teeth are composed of the hardest tissues in the vertebrate body and have been studied extensively to infer diet in vertebrates. The morphology and structure of enamel is thought to reflect feeding ecology. Snakes have a diversified diet, some species feed on armored lizards, others on soft invertebrates. Yet, little is known about how tooth enamel, and specifically its thickness, is impacted by diet. In this study, we first describe the different patterns of enamel distribution and thickness in snakes. Then, we investigate the link between prey hardness and enamel thickness and morphology by comparing the dentary teeth of 63 species of snakes. We observed that the enamel is deposited asymmetrically at the antero-labial side of the tooth. Both enamel coverage and thickness vary a lot in snakes, from species with thin enamel, only at the tip of the tooth to a full facet covered with enamel. There variations are related with prey hardness: snakes feeding on hard prey have a thicker enamel and a lager enamel coverage while species. Snakes feeding on softer prey have a thin enamel layer confined to the tip of the tooth.

Introduction

Teeth are important tools used by many vertebrates to acquire and process food. Vertebrate teeth have evolved for millions of years and show a myriad of shapes fulfilling various functions (e.g., Teaford et al. 2007; Berkovitz and Shellis 2017). The study of teeth has provided insights into dietary adaptations in mammals given the extensive food processing done by the teeth in this group (e.g., Maas and Dumont 1999; Lucas et al. 2008). Tooth shapes and sizes are highly variable, but in general their structure is highly conserved through evolution. They are made of an external layer of enamel (or enameloid), which protects the teeth as a cap and covers the dentine which, in turn, surrounds a pulp cavity (e.g., Schmidt 1971; Berkovitz and Shellis 2017). The evolution of teeth and enamel (e.g., Teaford et al. 2007; Ungar 2010), their material properties (Rensberger 1997; Cuy et al. 2002; Bechtle et al. 2010, Zhao et al. 2013) and their function have been widely studied in a diversity of vertebrate lineages (e.g., Teaford et al. 2007; Richman and Handrigan 2011; Berkovitz and Shellis 2017).

The enamel comprises the outer layer of the tooth; it is directly in contact with the food or the environment and is therefore exposed to possible damage. It is the hardest and the most durable tissue found in the vertebrate body (e.g., Teaford et al. 1996, Boyde 1997; Rensberger 1997). Its thickness and size are related to diet in some vertebrates. Durophagous primates, for example, develop a relatively thick enamel layer (e.g., Dumont 1995; Scott et al. 2005; Olejniczak et al. 2008). Even if enamel is highly mineralized and more brittle, thicker enamel is thought to make tooth crowns stronger and able to resist large compressive forces (Dumont 1995; Maas and Dumont 1999; Lucas et al. 2008). A thicker enamel coating will also be able to resist wear longer than a thinner enamel coating because more tissue will need to be removed to expose the

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dentine. It was also already shown mostly in mammals (e.g., Kay 1981; Shellis et al. 1998; Lucas et al. 2008; Thiery et al .2017) but also in fish (e.g., Delaunois et al. 2020; Velasco-Hogan et al. 2021) that a thicker enamel/enameloid layer is related to harder diet

Despite the importance of understanding tooth enamel, this tissue remains relatively poorly investigated in reptiles, with mostly quantitative comparisons having been performed for crocodiles and extinct species (e.g., Olejniczak and Grine 2006; Enax et al. 2013; Sellers et al.2019). Compared to mammals, reptiles replace their teeth continuously (Edmund 1969; Zaher and Rieppel 1999; Richman and Handrigan. 2011), a condition called polyphyodonty. Polyphyodonty raises the question of the pertinence of lifelong durability of teeth, teeth will need to be "performant" for few weeks or months instead of decades. Continuous replacement could ensure the replacement of a broken tooth, hypothetically without the need for a tooth that resists failure (e.g., Crofts and Summers 2014). Whether polyphyodont reptile teeth vary in enamel thickness and distribution and why remains to be investigated.

The paucity of comparative studies could be explained by the general thin nature of reptile enamel (e.g., Sander 1999; Jones et al. 2018; Sellers et al. 2019). In extant squamates, most studies have focused on lizards and have described them as having a simple and thin enamel layer (e.g., Owen 1840; Schmidt 1971). There are about 4000 species of snakes with diversified diets ranging from goo-eaters to durophagous species. This dietary diversity is associated with morphological specializations of the teeth (e.g., Berkovitz and Shellis 2017; Segall et al. 2023), but no study to date has demonstrated a link between diet and enamel distribution in snakes. Owen (1840), who first described snake teeth even failed to notice the presence of enamel. Some years later, Tomes (1875) studied 10 genera of snakes and described a thin layer covering their teeth. Levy (1898) observed in *Tropidonotus (Natrix)* and *Pelias (Vipera)* a thin layer of enamel covering the whole tooth. The most detailed description of the enamel in snake teeth can be found in Schmidt (1971). This author observed that the enamel of the large teeth of *Python* is so thin that it can barely be seen under the microscope. To our knowledge there are no other studies looking at the enamel or tooth structure of snakes, especially in a phylogenetic and ecological comparative context.

The aim of this study was to investigate whether enamel thickness and coverage was related to to prey hardness in snakes. We compared the enamel coverage of 61 species using microCT and microscopy images. We then measured enamel thickness on longitudinal sections of the teeth in 63 species of snakes. We chose species to cover the phylogeny of alethinophidian snakes. We chose species with similar diets in different genera, and closely related species with different diets to highlight potential convergence.

Material and Methods

Enamel distribution via CT-scan

We extracted the dentary bone of 63 species of snakes that cover both the phylogenetic and dietary diversity of the group (Table S1). Our sample is composed of specimens from museums (American Museum of Natural History, Muséum National d'Histoire Naturelle, Jerusalem University) and private collections. The dentary teeth were CT scanned using a μCT-scanner, Phoenix Nanotom S (General Electric, Fairfield, CT, USA) at the Institut de Genomique Fonctionelle at the Ecole Normale Superieure (Lyon, France) prior to any invasive techniques. The scans were run with a voltage of 100kV and a current of 70μA, for a voxel size between 0.97–7.50 μm depending on the size of the sample. The 3D reconstruction was performed in the Phoenix datos|x2 software (v2.3.0, General Electric, Fairfield, CT, USA).

Segmentation of the enamel in the teeth was done using Dragonfly (Dragonfly version 3.8.6, Objects Research system, Canada). Enamel can be recognized by its higher absorption level compared to dentine (Fig. 1). The microCT scans did not allow us to quantify the amount of enamel due to a number of factors, including the sample itself and scanning artefacts (e.g., beam hardening). For some species the contrast between enamel and dentine was not high enough, or the enamel was too thin and some samples had preservation artefacts, which did not allow precise quantitative measurements (Fig. S1, S2). For two samples it was not possible to segment the enamel because the teeth had preservation issues: *Stenorrhina degenhardtii* presented a lead (Pb) layer at the surface of its teeth, which resulted in a density artefact (Fig. S1), and *Daboia russelii* presented a "shrinkage" of the teeth, which lead to severely fragmented enamel (Fig. S2). However, for these two samples, microscopic sections allowed us to measure enamel thickness.

The enamel coverage was qualitatively determined using the virtual longitudinal and transverse sections obtained and checked simultaneously via microscopic observations of longitudinal sections, using the methodology explained in Fig. 1 and Fig. S3. We categorized enamel coverage into three groups (Fig. 1, S4) (a) enamel only present at the tip of the tooth (Fig. 1D–F), (b) enamel is present on half of the tooth

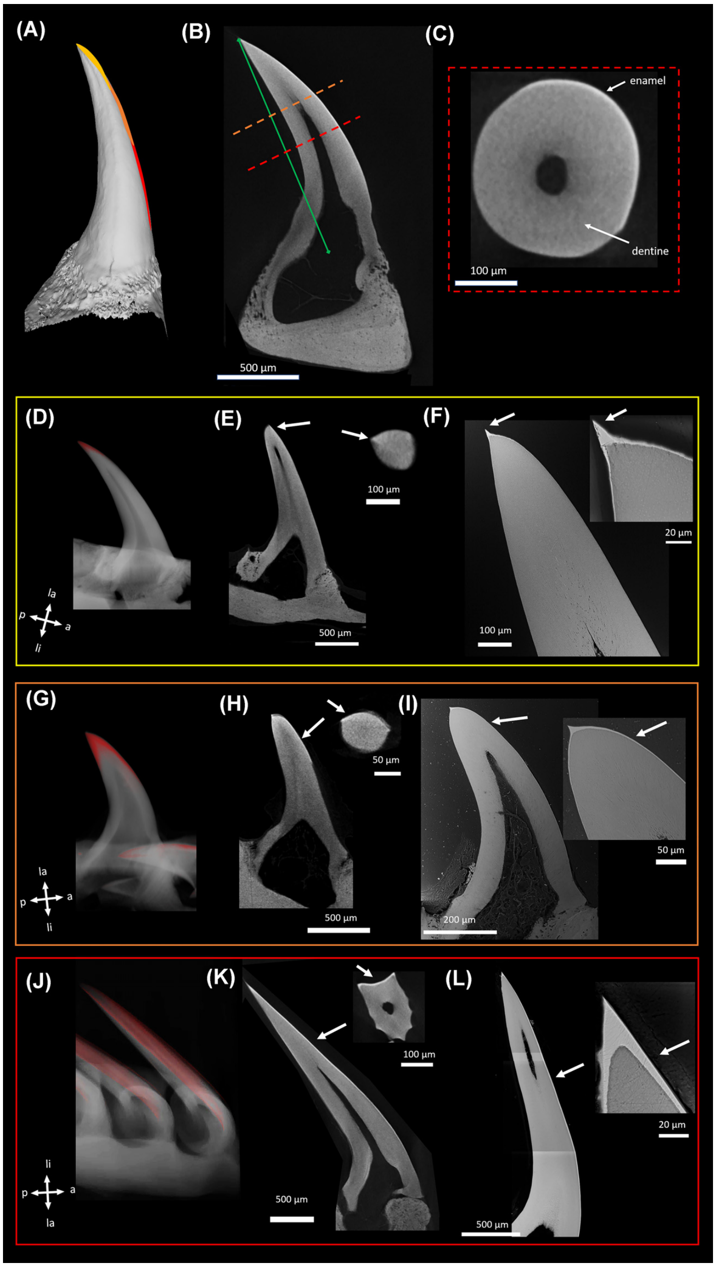

Fig. 1 Gradient of enamel distribution (**A–C**) parameters used for the description of the enamel distribution in snake teeth. Example of a dentary tooth of *Agkistrodon piscivorus*. (**A**) reconstructed tooth illustrating the three different types of enamel coverage in different colors (yellow: tip, orange: half, and red: entire). (**B**) Virtual longitudinal sections obtained in Dragonfly. The green line represents the height of the tooth, from the base and center of the pulp cavity to the tip of the tooth. The red dashed line defines the half of this height. Transverse sections at this level were used to check if enamel was present (see also Figure S3 for more details) (**C**). If enamel was present at this level or even lower, the tooth was described as having the entire tooth surface covered by enamel. If enamel was only present above the red line, the tooth was described as having only half of its surface covered in enamel. The orange dashed line represents one third of the entire height of the tooth. If enamel was only observed above this limit, the tooth was described as having enamel only present at its tip. Enamel only present at the tip of the tooth. (**D–F**) *Corallus annulatus* (**D**) 3D rendering of the teeth and segmented enamel in red. (**E**) Transverse and longitudinal virtual sections of the teeth showing the enamel layer (arrow). (**F**) BSE-SEM images of longitudinal section of one tooth showing the enamel (arrow). Enamel covering half of the antero-labial side of the tooth. (**G–I**) *Cylindrophis ruffus*: (**G**) 3D rendering of the teeth and segmented enamel in red. (**H**) Transverse and longitudinal virtual sections of the teeth showing the enamel layer (arrow). (**I**) BSE-SEM images of longitudinal section of one tooth showing the enamel (arrow). Enamel covering all the antero-labial side of the tooth. (**J–L**) *Homalopsis buccata*: (**J**) 3D rendering of the teeth and segmented enamel in red. (**K**) Transverse and longitudinal virtual sections of the teeth showing the enamel layer (arrow). (**L**) BSE-SEM images of a longitudinal section of one tooth showing the enamel (arrow). The white cross in (**D**, **G**, and **J**) represent the orientation of the dentary tooth in the snake mouth a: anterior, p: posterior, la: labial, and li: lingual.

(Fig. 1G–I), and (c) the enamel is present on the entire anterior face (Fig. 1J–L).

Enamel thickness measurements via microscopy

Snake teeth were extracted from the dentary and embedded in epoxy resin (Epothin, Buehler, Lake Bluff, IL, USA). Longitudinal sections were prepared by sequentially grinding the samples with 800, 2500, and 4000 grit SiC films (Buehler, Lake Bluff, IL, USA), followed by polishing with a nap polishing cloth soaked in diamond suspension (3 and 1 µm; Struers Inc., Cleveland, OH, USA). Grinding and polishing were performed with a polisher (Allied High Tech Techprep Multiprep, USA). Because snake teeth are small (some teeth are below 1 mm in length) and curved, sequentially grinding was carefully checked in order to reach half longitudinal sections of the teeth.

Uncoated polished sections of the snake teeth were imaged on a Phenom XL scanning electron microscope (SEM; Phenom-World BV, Eindhoven, the Netherlands) using a back-scattered electron detector (SEM-BSE) at an accelerating voltage of 15 kV, in a low-vacuum mode.

Enamel thickness was measured using Image J software (ImageJ 1.53q, National Institutes of Health, USA) on all longitudinal images obtained on the SEM (Fig. 2). The measurements were made at the location where enamel is the thickest at the antero-labial side. A minimum of ten measurements were made at different locations along the enamel layer and averages and standard deviations were obtained from these measurements. Due to their small size and their curved and crooked morphology, which could lead to inaccuracy in the measurement, enamel thickness was also measured on few transverse sections. We compared the measures of the enamel thickness on the transverse sections with those on the longitudinal sections to check the accuracy of our measurements (Fig. 2, Table S2). These transverse sections were prepared only for few samples at the level of the tooth where enamel was present according to preceding CTscan observations (cf. Fig. 1); teeth were embedded in epoxy and a line where the enamel layer was observed on the same material in the CTscan was marked on the epoxy resin. We grind carefully from the tip to the base until reaching this mark. A minimum of 10 measurements were made on these sections (Fig. 2) using Image J software (ImageJ 1.53q, National Institutes of Health, USA). Table S2 show that there is no large difference in enamel thickness average in longitudinal and transverse sections.

Tooth length measurement

Tooth length (tooth curvature length) was measured using the FIJI (v1.53q) plugin "Kappa" (Mary and Brouhard 2019), in a previous study by Segall et al. 2023. The ratio of the enamel thickness to tooth length was used to take into account variation in tooth size.

Prey hardness

Prey hardness was divided into three categories: soft (e.g., gastropods, annelids, birds, soft-skinned mammals), medium (e.g., amphibians, fish, thin-scaled lizards such as anoles), and hard (insects, snakes, crustaceans, hard-scaled lizards such as skinks). For generalist species or species with several items in their diet, we considered the most consumed item. The prey hardness for each species was determined through an extensive bibliographical work (see Supplementary Table S1).

Statistics

We tested whether tooth length and enamel thickness were correlated phylogenetic generalized least squares (function *gls* from the "nlme" package (Pinheiro et al. 2023). We then tested whether raw and relative enamel thickness were associated with diet and the type of enamel coverage using the function *phylANOVA* of the package "phytools" using 1000 simulations and a Holm correction for the post hoc pairwise *t*-tests (Revell 2012). To do so, we pruned the tree from Pyron and Burbrink (2014), if species were not present in the tree, we used the closest relative (Fig. S4). Since the raw and relative enamel thickness were not normally distributed, they were Log_{10}-transformed and normality was checked using the *shapiro.test* function of the "stats" package (raw thickness: $W = 0.97854$, $P = 0.36$; relative thickness: $W = 0.97682$, $P = 0.29$). These statistics were performed in R version 4.0.5 (R Core Team 2021). Contingency tests (Fisher's exact test) were used to determine if the enamel distribution was related to prey hardness, using the JUMP software (JMP Pro 16.0.0, USA).

Results

General microstructure and enamel thickness

Snake dentary teeth are composed of dentine and a thin layer of enamel. This layer of enamel is mostly present and observed on the antero-labial facet of their teeth in all snake species examined (Fig. 1, Fig. 2). A very thin layer at the posterior side of the tip and the tooth (Figs. 2, 3A, B, E, G, and S4) can be observed for some species. Some snake teeth present

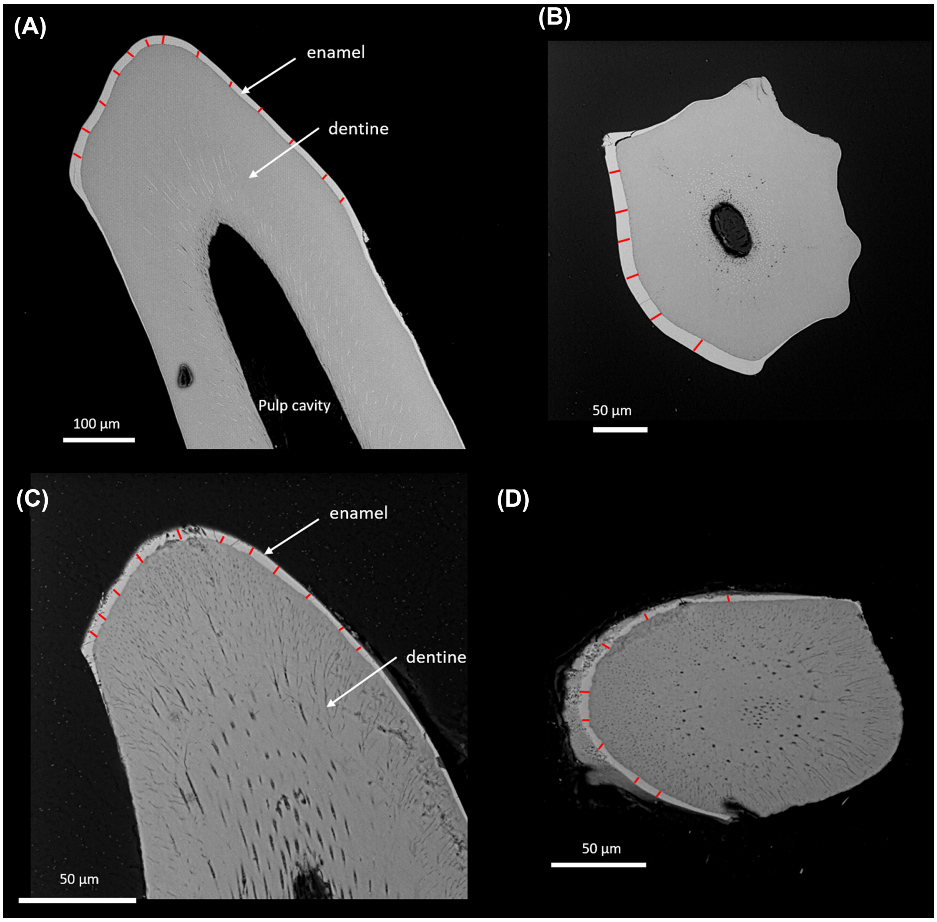

Fig. 2 Enamel thickness measurements on BSE-SEM transversal and longitudinal sections images (**A** and **B**) *Acrochordus javanicus* SEM longitudinal (**A**) and transversal (**B**) sections red lines represent examples where the enamel thickness ware measured (cf. text). (**C** and **D**) *Gerarda prevostiana* SEM longitudinal (**C**) and transversal (**D**) sections red lines represent examples where the enamel thickness was measured in both sections (cf. text).

a thick and distinct enamel layer (e.g., *Liodytes rigida*, *Acrochordus javanicus*, *Grayia ornata*, *Boiga cynodon*, and *Malpolon insignitus)* whereas others have a really thin, barely noticeable enamel layer (e.g., *Anilius scytale*, *Boa constrictor, Corallus annulatus*) (Fig. 3D–G, all species represented in Fig. S4). For these last species, if most of the enamel layer is rather thin, their tip is made up of a thick and pointy enamel layer. Longitudinal sections show variable enamel appearance from corrugated to smooth. A"corrugated" surface (where enamel has fluted surface, but its outer layer is parallel to the DEJ (dentino-enamel junction))can be observed for example in *Acrochordus javanicus* (Fig. 3A), compared to a smooth surface for other species (*cf.* Fig. 3D–F, 3I). Few snakes species *(Anilius scytale, Malpolon insignitus Ophiophagus hannah*, and *Boiga cynodon* (Fig. 3B, S4) showed a worn surface, where outer enamel surface is not smooth and not parallel to the DEJ). On the other hand, *Eirenis decemlineatus,* shows a crenelated surface (Fig. 2C, S4). The crenellation could not be seen on the CTscan (Fig. S4) partly due to their small size (there are 5 µm in height). It is however not clear if the differences in surface, such as the corrugated surface observed in *Acrochordus javanicus* are responsible of the apicobasal ridges observed along the teeth (Fig. 2A and B, Fig. S5). The same phenomenon can be seen in *Homalopsis buccata* (Fig. 1K).

Some snake teeth show fractured enamel, but *Fordonia leucobalia* has a particular enamel morphology where the enamel layer appears to be fractured and detached entirely from the dentine (Fig. 3H). *Atractaspis engaddensis* is the only snake species in our sample, which presents a cone of enamel on its teeth (Fig. 3J); the enamel is not only deposited on the antero-labial side, but present around the entire tooth as confirmed by the virtual transverse sections (insert image on Fig. 3J). This thick enamel ring layer all around the tooth is clearly noticeable, and different from what was observed for all other snake species (cf. Fig. 1, Fig. 2 and Fig. 3A–I, Fig. S4 for microscopic observations), where the enamel

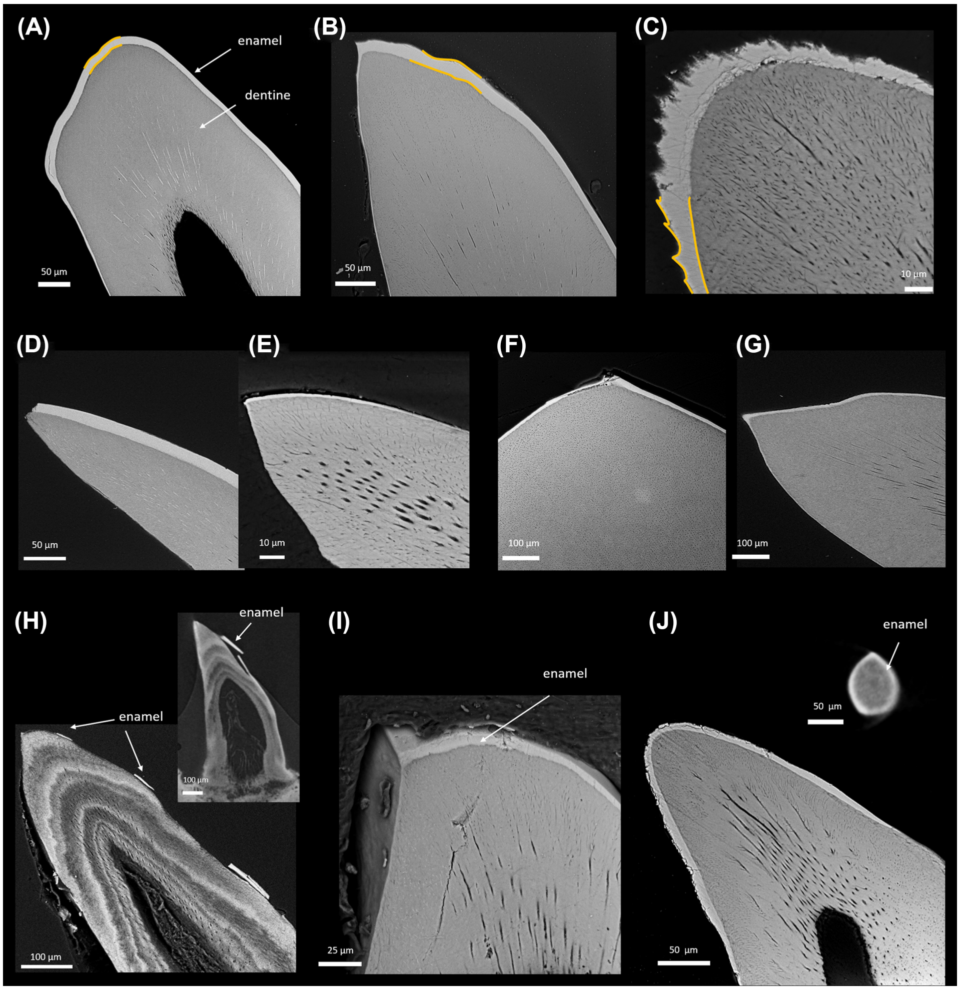

Fig. 3 Variation in enamel general morphology. Differences in enamel surface morphology with (**A**) corrugated enamel surface from Acrochordus javanicus (**B**) worn surface of Boiga cynodon, and crenellated surface of Eirenis decemlineatus (**C**); both enamel outer surface and DEJ (dentino-enamel junction) are highlight in yellow to emphasize the different enamel surface. Different enamel thickness from different snake teeth of different sizes with a small-sized tooth with a thick enamel layer in Liodytes rigida (**D**), a small-sized tooth with a thin enamel layer in Micrurus psyches (**E**), a large-sized tooth with a thin enamel layer in Boa constrictor (**F**), a medium-sized tooth with a medium thick enamel layer in Naja annulata (**G**). Fractured and detached enamel observed in Fordonia leucobalia in SEM and in CT longitudinal sections (**H**), and enamel layer observed on the anterior facet of a Clelia clelia tooth (**I**) compared to the enamel present in all tooth facets in Atractaspis engaddensis in the microscopic image and virtual section

is thick and distinct on the antero-labial side (on the posterior side a really thin almost indiscernible layer of enamel seems to be present). This cone of enamel covered half of the teeth.

The average enamel thickness in the snakes measured ranged from 0.8 µm (*Coluber constrictor*) to 12.8 µm (*Liodytes rigida*) (Fig. 3D–G). There is no correlation between enamel thickness and tooth length ($t = 0.59$, $P = 0.5$; Fig. 4A). However, *Atractaspis engaddensis* is an outlier in our sample because of its unusual enamel morphology and behavior (Fig. 3J, Fig. S6, Discussion). If we remove it from the sample, we obtain a statistical difference of the relative thickness depending on the hardness of the prey ($F = 4.57$, $P = 0.05$), more precisely between hard and soft prey specialists ($t = -2.97$, $P = 0.05$, Fig. 4B). Snakes feeding on hard prey have a thicker relative enamel thickness (average 5.9 ± 5.2 µm) compared to snakes feeding on soft prey (average 3.23 ± 3 µm; Fig. 4B).

We noticed that the range of the enamel thickness varied from one tooth to another suggesting a varied enamel morphology (from smooth to crenelated) as observed in Fig. 3. However, there is no statistical difference of the enamel thickness standard deviation in relation to prey hardness ($F = 0.21$, $P = 0.85$).

Enamel distribution

The distribution of the enamel on the antero-labial part of the teeth of snakes is different between species

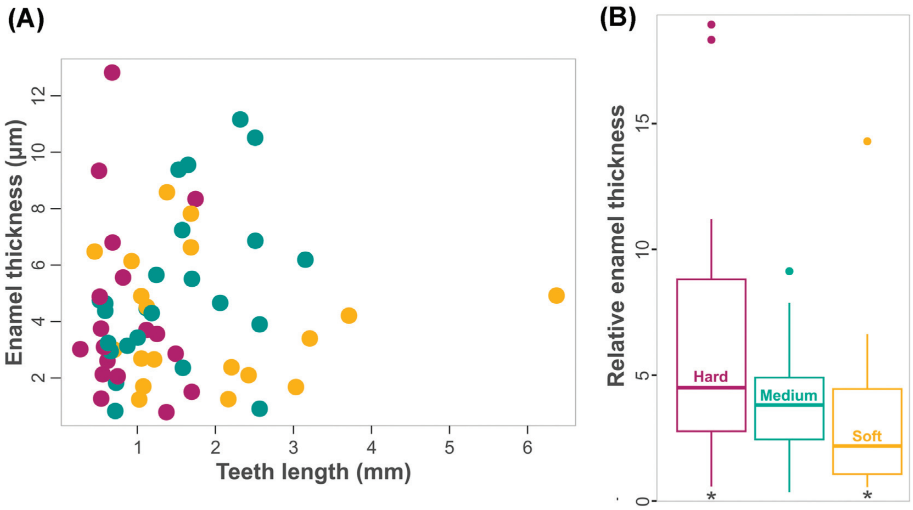

Fig. 4 (**A**) Plot of the enamel thickness versus tooth length. Each dot represents one species, dot color represents prey hardness (dark purple: hard, blue green: medium, and yellow: soft). (**B**) Boxplot representing the differences in relative enamel thickness depending on the hardness of prey. Statistically significant differences are indicated by ∗.

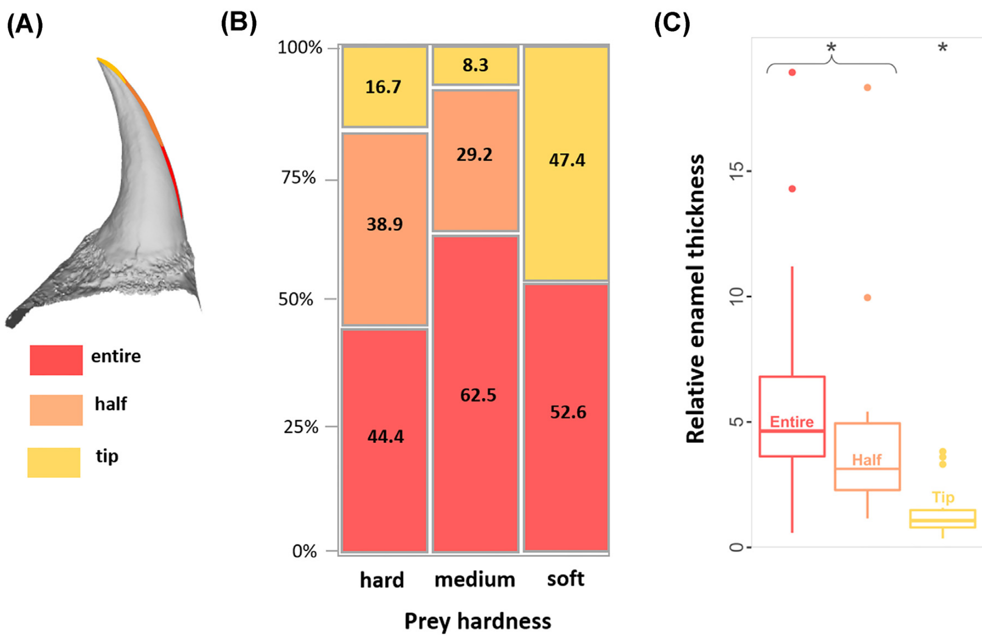

Fig. 5 (**A**) reconstructed tooth illustrating the three different types of enamel coverage in different colors (yellow: tip, orange: half, red: entire). (**B**) Mosaic plot comparing the enamel distribution according to the hardness (**A**) of the prey, labeled by percentage. (**C**) Boxplot of the relative enamel thickness according to the hardness of the prey. Statistically significant differences are indicated by ∗. Groups within the same bracket are not significantly different but are different from groups with the asterisk.

(Fig. 1, Fig. S3, Fig. S4). Among the 61 species analyzed, 33 species have the antero-labial facet of the tooth entirely covered by enamel, whereas enamel covering only half, or only the tip of the tooth represents less than a third (14 species for both groups) of the entire sample. There is a statistical difference in the average enamel thickness depending on enamel distribution ($F = 11.23$, $P = 0.002$), with species presenting enamel only at the tip of the tooth having a thinner enamel than species with the tooth facet entirely covered with enamel ($t = -4.57$, $P = 0.006$, Fig. 5C).

Enamel distribution is also significantly related to prey hardness (Fisher's exact test $P = 0.0026$). Snakes with enamel along the entire tooth eat medium or hard prey, whereas snakes with enamel only at the tip of the tooth eat soft prey (Fig. 5A, 5B, Table S3).

Discussion

Most snakes have an asymmetrical distribution of enamel; an enamel layer is mostly present at the antero-labial side of the teeth and barely discernable on the posterior side. *Atractaspis engaddensis* which has a highly derived feeding apparatus and behavior, was the only species with a distinct enamel layer covering the entire crown of the tooth. Atractaspids, also called stiletto snakes, forage in mammal burrows. Unlike any other species, they have highly mobile maxillae that can move laterally, allowing the snake to envenomate prey without opening the mouth. In addition to this peculiar feature, atractaspids have a highly reduced number of teeth, with no pterygoid teeth, and a limited number of teeth on the palatine and dentary bones. The remaining dentary teeth are located anteriorly to the fangs and do not obstruct their lateral motion. This reduced number of teeth, along with all the peculiarities of *Atractaspis* teeth (short tooth, thick enamel layer and tooth capped entirely with enamel) suggest a highly derived dentition in this species that mostly feeds on small rodents.

The asymmetrical distribution of enamel in snakes is similar to what has been observed in mammals, fish, and other reptiles (e.g., Buchtova et al. 2008; Seidel et al. 2010; Zahradnicek et al. 2012; Zahradnicek et al. 2014). This particular enamel distribution arises from differences between lingual and labial cervical loops, where only the latter contain the stem cells responsible for the deposition of the enamel (e.g., Buchtova et al. 2008; Seidel et al. 2010; Handrigan and Richman 2011). Biomechanically, the asymmetrical distribution of enamel in rodents and primates (aye-aye) results in asymmetrical wear of the teeth and continual sharpening of the occlusal surface (e.g., Tatersall and Schwartz 1974; Druzinsky et al. 2012; Müller et al. 2014). This particular arrangement, in addition to the creation of an epithelial bulge during tooth formation, has been suggested to be a mechanism responsible for the formation of more complex tooth shapes (e.g., multicuspid teeth) or dental ornaments (like enamel ridges and crests) via an increase of the enamel thickness (Buchtova et al. 2008; Zahradnicek et al. 2014). However, previous research showed that snakes lack this epithelial bulge (Buchtova et al. 2008; Handrigan and Richman 2011; Zahradnicek et al. 2014), possibly explaining the simple unicuspid morphology and lack of ornamentation. Most research has been done on Colubridae (corn snake *E. guttata*) and Pythonidae (*Python regius, Python sebae,* and *Python molurus*). The teeth of *Python regius* are almost straight (they present a reduced curvature compared to other teeth, Table S1, previous study by Segall et al. 2023) with a very thin layer of enamel at their tip, yet do not represent the variability we observed. Most snakes in our sample (33/61 species) have the antero-labial facet of the tooth entirely covered by enamel. The differences in thickness, as well as different types of ornamentation (like crenelations, carinae, and apicobasal ridges), observed in different species (e.g., in fish-eaters like *Acrochordus javanicus* and *Homolapsis buccata*) suggests the possible presence of an epithelial bulge or alternatively may point to different developmental processes in snakes.

The enamel of snakes varies in thickness, distribution, and shape. In longitudinal sections of the teeth, some species appear to have a corrugated, crenelated layer, while others have a smooth enamel layer. The enamel on the teeth can be thin (under 2 µm in *Anilius scytale, Calabaria reinhardtiii, Tropiolaemus wagleri, Causus* sp., and *Micrurus psyches*). Yet, other small sized species show a greater enamel thickness (e.g., *Eryx jaculus, Xenodermus javanicus, Gloydius halys, Cantoria violacea, Atractaspis engaddensis*, and *Liodytes rigida*). Snake species that eat slippery prey, such as fish or slugs, show apicobasal-ridges on the posterior side of their teeth (e.g., *Homalopsis buccata* Fig. 1K, *Acrochordus javanicus*, Fig. 2A–B, Fig. S5). We suggest that the ridges may prevent the teeth from getting stuck in the prey by limiting the suction created by the layer of mucus as suggested by McCurry et al. (2019). Alternatively, the ridges could help rigidify the tooth, but since there is almost no enamel along the ridges, the latter hypothesis seems less likely. However, if we consider *stricto sensu* the definition of McCurry et al. 2019, apicobasal ridges are elevated ridges of enamel. From our preliminary observations (Fig. 2A and B, and Fig. S5), in snakes' teeth these ridges are formed of dentine rather than by a localized thickening of enamel. Other teeth surface ornamentations such as carinae (or media-lateral ridges, McCurry et al. 2019) are also observed in few snake samples (cf. Fig. S5) and seem to be due to a thicker enamel edge but should be further explored.

We also observed differences in the enamel distribution on the teeth of snakes ranging from teeth entirely covered by enamel, to teeth being covered only at the upper half, to teeth presenting enamel only at the tip. Enamel distribution in snakes appears to be related to prey hardness. Snakes eating soft prey have teeth with enamel at the tip only. When feeding on soft prey, the enamel at the tip is probably sufficient to allow the tooth to pierce the skin and flesh. The tip of the tooth may also undergo a higher stress for these species and need to be covered by enamel A Finite Element Analysis on fish teeth recently demonstrated that the tip of the tooth of piranha, which purpose is to cut through flesh, undergo higher stress than the tooth of pacu, a durophagous species, in which the stress is distributed along the tooth

(Velasco-Hogan et al. 2021). Enamel thickness is also related to the hardness of the prey, with snakes eating hard prey having a relatively thicker enamel. Enamel thickness has been well studied in mammals, especially in primates and has shown to be, with tooth size, one of the main aspects to control damage resistance according to food hardness (Lucas et al. 2008; Lee et al. 2011; Lawn et al. 2013): an increase of enamel strengthens the teeth (e.g., Dumont 1995; Lucas et al. 2008; Mc Graw et al. 2012). Durophagous snakes present a thicker layer of enamel in both absolute and relative terms, suggesting a possible adaptation to prevent failure while feeding on hard prey. In addition to a thick enamel layer and a large enamel coverage, some durophagous snakes, such as *Liodytes*, have hinged teeth (Savitzky 1981). These results suggest that enamel coverage is related to the functional demand on the teeth and may have been reduced in snakes eating softer prey.

The reduction of the enamel layer observed in snakes raises several questions. Enamel has always been considered as an important protective tissue, crucial for the durability of the teeth. Yet, some mammals do not have enamel on their teeth (e.g., David_Beal et al. 2009; Meredith et al. 2009; Kierdorf et al. 2022). In fish, absence of enamel or enameloid was recently described in one species of Anoplogastridae (Kierdof et al. 2022), but the authors could not find a functional explanation. On the other side, enamel reduction or loss in mammals is presumed to be due to highly specialized feeding habits (Ciancio et al. 2021; Werth 2000; Davit-Beal et al. 2009) and to lead to specific morphological adaptations, such as the hypsodont/hypselodont condition in armadillos (high crown teeth, Davit-Beal et al. 2009) and also possibly differences in the nature of the dentine (Kalthoff 2011, Ciancio et al. 2021). Another explanation for the reduction of enamel in snakes is that their teeth have a different function than those other vertebrates. Snakes use their teeth to capture, manipulate, and transport their prey whole, but they rarely crush or tear apart their food, with only few exceptions (Jayne et al. 2002; Bringsøe et al. 2020). *Fordonia leucobalia* for instance, can tear apart crustaceans, and our specimen shows a fragmented enamel, which could be due to their specific diet/manipulation or can be an artefact of preservation. Thus, except during the strike, it is possible that the load on the teeth is rather low and distributed between all teeth, but this hypothesis remains to be tested.

The high-resolution laboratory CT-scans employed here, show the technical limitations due to the small sizes of the object and the thin aspect of the enamel in some snakes. Propagation phase contrast synchrotron X-ray micro-computed tomography would be helpful in order to characterize more precisely the enamel volume. Our results show that the enamel and the tooth structure of snakes is variable and more complex that what was previously expected. Future detailed investigations on the variability of enamel and tooth surface, for example, may help to better understand the microstructure of the teeth and the differential development of the enamel. Indeed, lateral carinae, and apicobasal ridges are observed to various degrees in different snake species (e.g., Vaeth et al. 1985; Massare 1987; Young and Kardong 1996; Kearney et al. 2006; McCurry et al. 2019). This structural variation is likely important during feeding and further investigations may help us to better understand the evolution and biomechanics of snake teeth.

Conclusion

Snakes present an asymmetric enamel deposition with enamel being present principally at the antero-labial side. Prey hardness is the main ecological factor associated with enamel thickness and distribution. Similar to what has been observed for mammals, snakes feeding on harder prey have a relatively thicker and wider enamel layer. Snakes feeding on soft prey have a thin enamel layer, mostly concentrated at the tip of the tooth. This is the first study showing the variability of enamel in snake teeth and the link with diet. Further work is needed to investigate potential differences in dental microstructure and to understand the biomechanical implications of variation in the enamel distribution.

Authors contribution

M.D. and M.S. conceived and led the research project. M.D. led the writing of the manuscript, collecting the data, and performing the analyses. M.S. helped with the statistical analyses and figures. R.C., R.S., J.M., and A.H. helped in writing the grant proposal. B.S. provided specimens from the herpetological collection of Jerusalem. All authors M.D., J.M., A.H., R.S., B.S., C.H., A.D., R.C., and M.S. contributed to the interpretation and discussion of the results, and to the editing of the manuscript.

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Supplementary data

Supplementary data available at *ICB* online.

Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

Microscopy images will be uploaded in an image data resource.

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