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Show me your teeth and I will tell you what you eat: differences in tooth enamel in snakes with different diets.

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Abstract

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 larger enamel coverage while species. Snakes feeding on softer prey have a thin enamel layer confined to the tip of the tooth

Key words: enamel, diet, snakes, microscopy, CT scanning, teeth

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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 & 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 & 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 & Shellis, 2017). The evolution of teeth and enamel (e.g., Teaford et al., 2007; Ungar, 2010), their material properties (Cuy et al., 2002; Rensberger, 1997; Bechtle et al., 2012, Zhao et al., 2013) and their function have been widely studied in a diversity of vertebrate lineages (e.g., Teaford et al., 2000; Berkovitz & Shellis, 2017; Richman & Handrigan, 2011).

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 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., Olejniczak et al., 2008; Scott et al, 2005; Dumont, 1995). 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 & 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 dentine. It was also already shown mostly in mammals (e.g. Lucas et al. 2008, Kay 1981) but also in fish (e.g.

Delaunoy 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 & Grine, 2006; Enax et al., 2013; Sellers et al., 2019). Compared to mammals, reptiles replace their teeth continuously (Edmund, 1969; Zaher, 1999; Richman & 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 & Summers, 2013). 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 & 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 ten 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 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.

2. 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 μ CT-scanner, Phoenix Nanotom S (General Electric, Fairfield, CT, USA) at the Institut de Genomique Fonctionnelle at the Ecole Normale Supérieure (Lyon, France) prior to any invasive techniques. The scans were run with a voltage of 100kV and a current of 70 μ A, for a voxel size between 0.97-7.50 μ m depending on the size of the sample. The 3D reconstruction was performed in the Phoenix dataview 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 (Figure 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 (Figure 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 (Figure S1), and *Daboia russelii* presented a “shrinkage” of the teeth which lead to severely fragmented enamel (Figure 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 Figure 1 and Figure S3. We categorized enamel coverage into three groups (Figure 1, S4): a) enamel only present at the tip of the tooth (Figure 1D-F), b) enamel is present on half of the tooth (Figure 1G-I), c) the enamel is present on the entire anterior face (Figure 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 (Figure 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 (Figure 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. Figure 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 (Figure 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 & 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 3 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., 2022)). 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 & Burbrink (2014), if species were not present in the tree, we used the closest relative (Figure S4). Since the raw and relative enamel thickness were not normally distributed, they were Log₁₀-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).

3. Results

3.1. *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 (Figure 1, Figure 2). A very thin layer at the posterior side of the tip and the tooth (Figure 2, Figure 3A, 3B, 3E, 3G, S4) can be observed for some species. Some snake teeth present a thick and distinct enamel layer (e.g., *Liodytes rigida*, *Acrochordus javanicus*, *Grayia ornata*, *Boiga cynodon*, *Malpolon insignitus*) whereas others have a really thin, barely noticeable enamel layer

(e.g., *Anilius scytale*, *Boa constrictor*, *Corallus annulatus*) (Figure 3D-G, all species represented in Figure 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* (Figure 3A), compared to a smooth surface for other species (cf. Figure 3D-F, 3I). Few snakes species (*Anilius scytale*, *Malpolon insignitus*, *Ophiophagus hannah*, and *Boiga cynodon*) (Figure 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 (Figure 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 (Figure 2A-B, Figure S5). The same phenomenon can be seen in *Homalopsis buccata* (Figure 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” (Figure 3H). *Atractaspis engaddensis* is the only snake species in our sample which presents a cone of enamel on its teeth (Figure 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 Figure 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. Figure 1, Figure 2 and Figure 3A-I, Figure S4 for microscopic observations), where the enamel 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*) (Figure 3D-G). There is no correlation between enamel thickness and tooth length ($t = 0.59$, $P = 0.5$; Figure 4A). ~~The absolute and relative enamel average thickness do not significantly differ depending on the tested ecological factors (all $P > 0.1$, Figure S6).~~ However, *Atractaspis engaddensis* is an outlier in our sample because of its unusual enamel morphology and behavior (Figure 3J, Figure 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 ($t = -2.97$, $P = 0.05$, Figure 4B). Snakes feeding on hard prey have a thicker relative enamel thickness (average $5.9 \pm 5.2\mu\text{m}$) compared to snakes feeding on soft prey (average $3.23 \pm 3\mu\text{m}$; Figure 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 Figure 3. However, there is no statistical difference of the enamel thickness standard deviation in relation to prey hardness (; $F = 0.21$, $P = 0.85$)

3.2 Enamel distribution.

The distribution of the enamel on the antero-labial part of the teeth of snakes is different between species (Figure 1, Figure S3, Figure 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 ($t = -4.57$, $P = 0.006$, Figure 4B).

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 (Figure 4A, Table S3).

4. 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., Seidel et al., 2010; Buchtova et al., 2008; Zahradnick 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., Seidel et al., 2010; Buchtova et al., 2008; Handrigan & 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 & 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 (Zahradnicek et al., 2014; Buchtova et al., 2008). However, previous research showed that snakes lack this epithelial bulge (Zahradnicek et al., 2014; Buchtova et al., 2008, Handrigan & Richman, 2011), 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*, *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 crenulations, carinae and apicobasal ridges), observed in different species (e.g., in fish-eaters like *Acrochordus javanicus*, *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 2µm in *Anilius scytale*, *Calabaria reinhardtii*, *Tropiolaemus wagleri*, *Causus sp.*, *Micrurus psyches*). Yet, other small sized species show a greater enamel thickness (e.g., *Eryx jaculus*, *Xenodermus javanicus*, *Gloydus halys*, *Cantoria violacea*, *Atractaspis engaddensis*, *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* Figure 1K, *Acrochordus javanicus*, Figure 2A-B, Figure 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 and colleagues (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 (Figure 2A-B and Figure 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. Figure 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, Kierdorf et al., 2022). In fish, absence of enamel or enameloid was recently described in one species of Anoplogastridae (Kierdorf 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 (David-Beal et al., 2009; Werth, 2000; Ciancio et al.; 2021) and to lead to specific morphological adaptations, such as the hypsodont/hypselodont condition in armadillos (high crown teeth, David-Beal et al., 2009) and also possibly differences in the nature of the dentine (Kalthoff et al. 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 (Bringsøe et al., 2020; Jayne et al., 2002). *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 than 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; Kearny, 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.

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Availability of data and material: Microscopy images will be uploaded in an image data resource.

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Figures

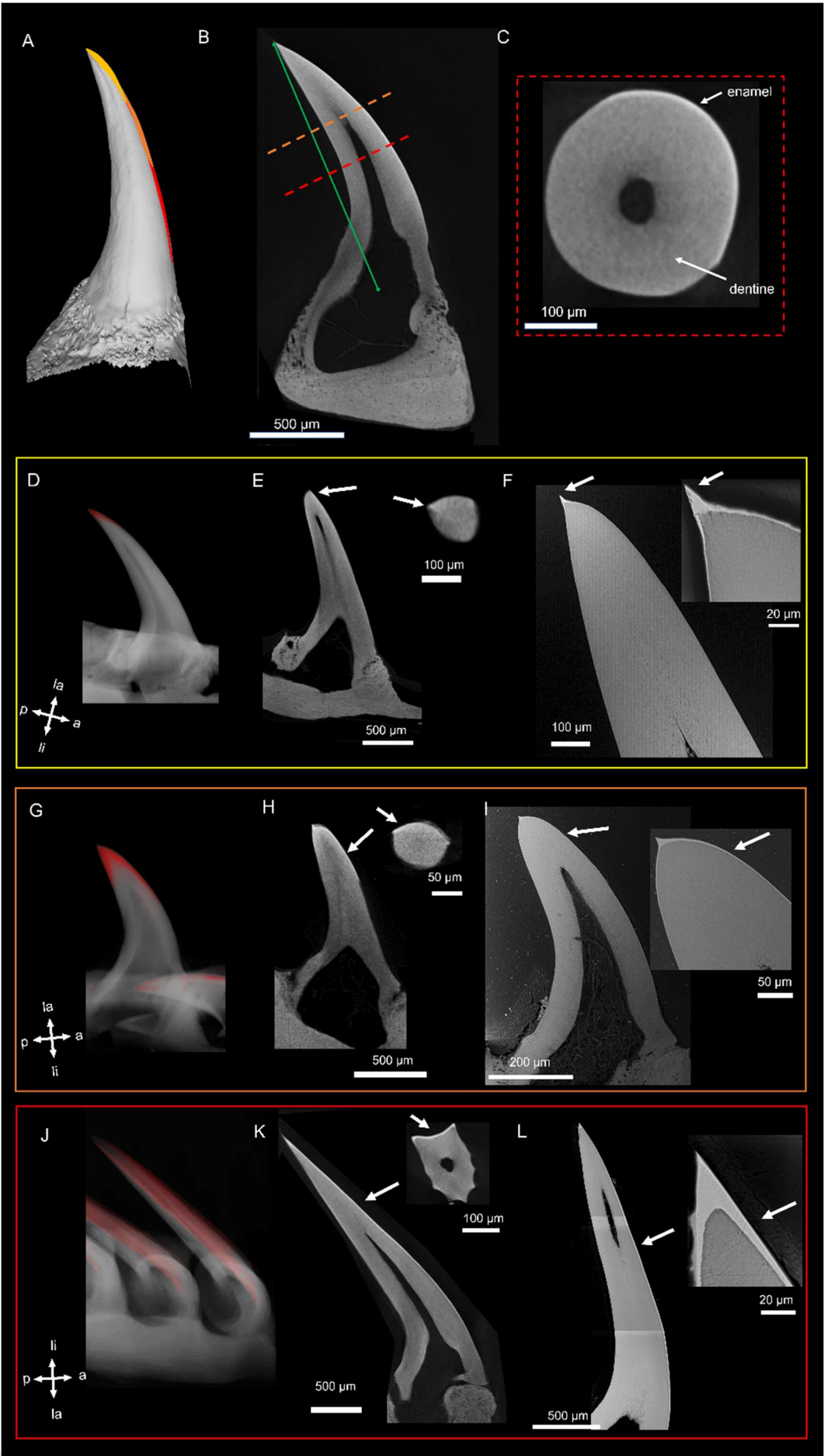


Figure 1 Gradient of enamel distribution A-C: Parameters used for the description of the enamel distribution in snake teeth. Example of a dentary tooth of *Agkistrodon piscivorus*. **A.** reconstructed tooth illustrating the three different types of enamel coverage in different colors (yellow: tip, orange: half, 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 the tooth. Transverse sections at this level were used to check if enamel was present (see also Fig SX 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 skull a:anterior, p: posterior, la:labial and li:lingual.

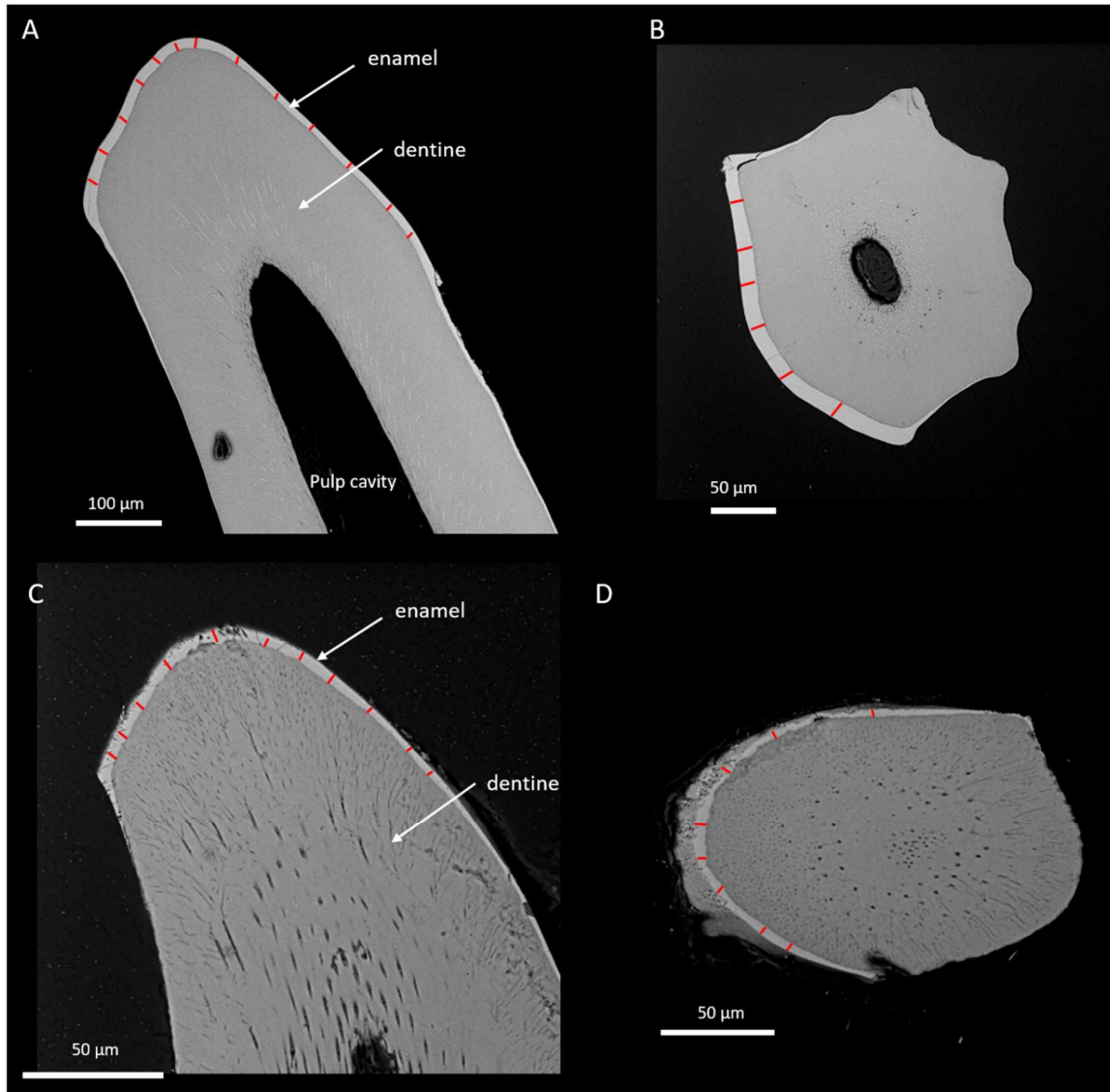


Figure 2: Enamel thickness measurements on BSE-SEM transversal and longitudinal sections images. A-B *Acrochordus javanicus* SEM longitudinal (A) and transversal (B) sections red lines represent examples where the enamel thickness was measured, the (cf text). C-D *Gerarda prevostiana* SEM longitudinal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

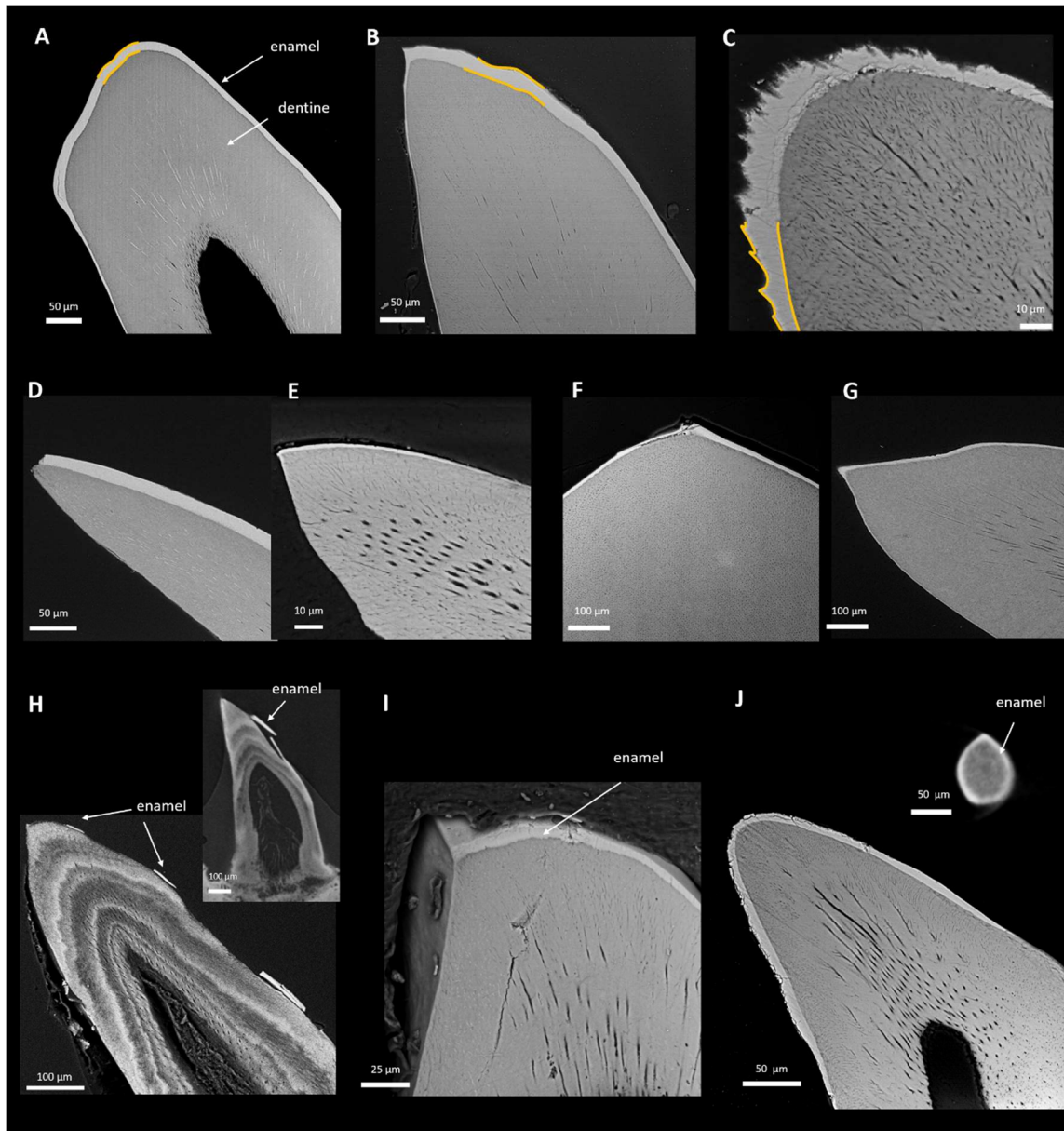


Figure 3: Variation in enamel general morphology. Differences in enamel surface morphology with (A) “corrugated” enamel surface from *Acrochordus javanicus* (B) worn surface of *Boiga cynodon*, and crenellated surface of *Eirenis decemlineatus* both enamel outer surface and DEJ (dentino-enamel junction) are highlight in yellow to emphasize the different enamel surface (C). **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 psuches* (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), 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 (J).

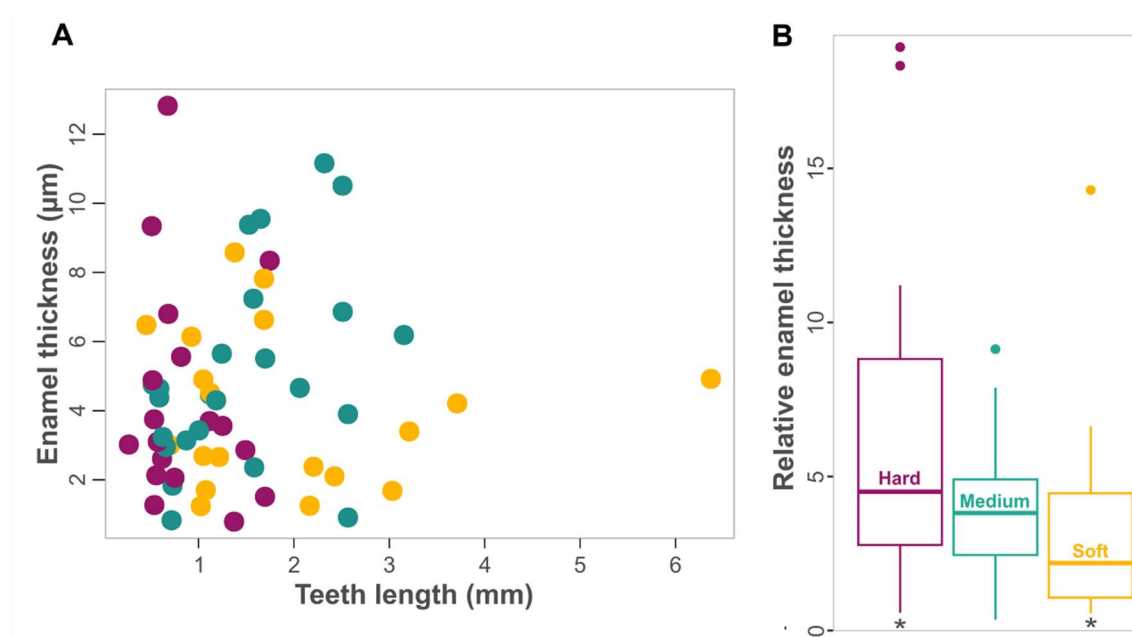


Figure 4: A. Plot of the enamel thickness versus tooth length. Each dot represents one species, dot color represents prey hardness (dark purple: hard, blue green: medium, yellow: soft). **B. Boxplot representing the differences in relative enamel thickness depending on the hardness of prey.** Statistically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

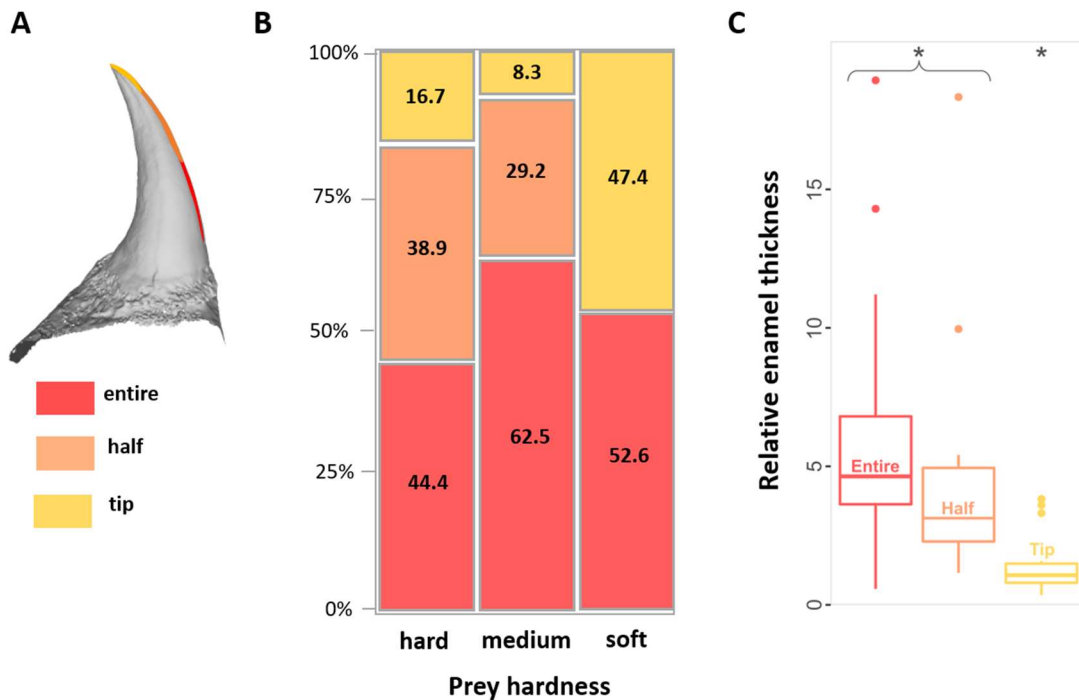


Figure 5: **A.** reconstructed tooth illustrating the three different types of enamel coverage in different colors (yellow: tip, orange: half, red: entire). **B.** Mosaic plot comparing the enamel distribution according to the hardness of the prey, labeled by percentage. **C.** Boxplot of the relative 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.

Supplementary Figures

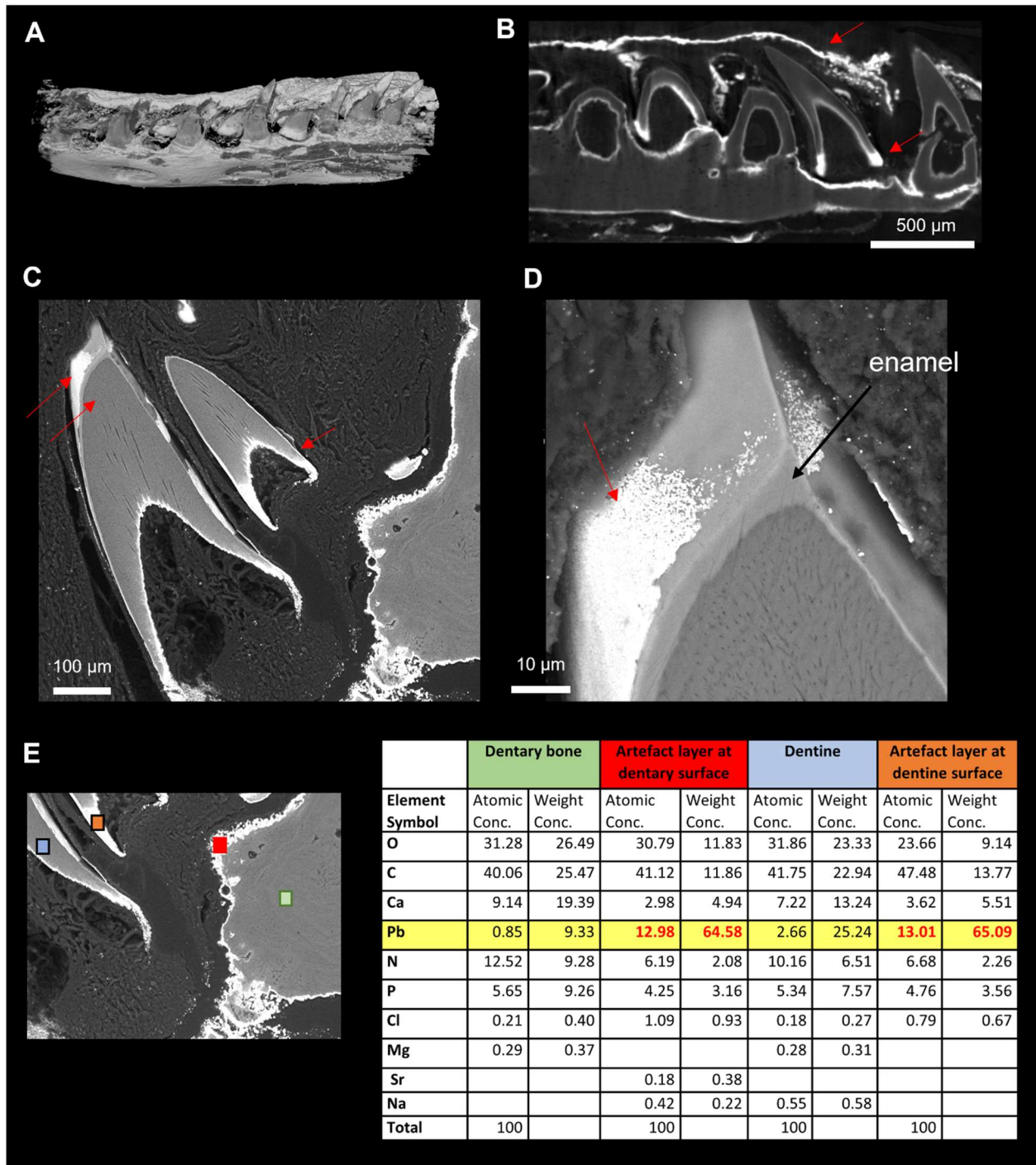


Figure S1: Artefact present on the *Stenorrhina degenhardtii* dentary. CT scans (A, B) of the dentary of this snake display high density layer surrounding the dentary bone and the teeth (red arrows in B). The BSE-SEM microscopic images (C, D) obtained also show the same artefact at the surface of the enamel with bright spots clearly observed on the surface of the teeth (red arrow in D). EDX analysis obtained shows that the artefact is due to lead (Pb) inclusion. (EDX analyses were performed on the Phenom (cf. Material part). Area analyses were made for both dentary, dentine and artefact areas, at 15kV, square area represent 2048 points analyses).

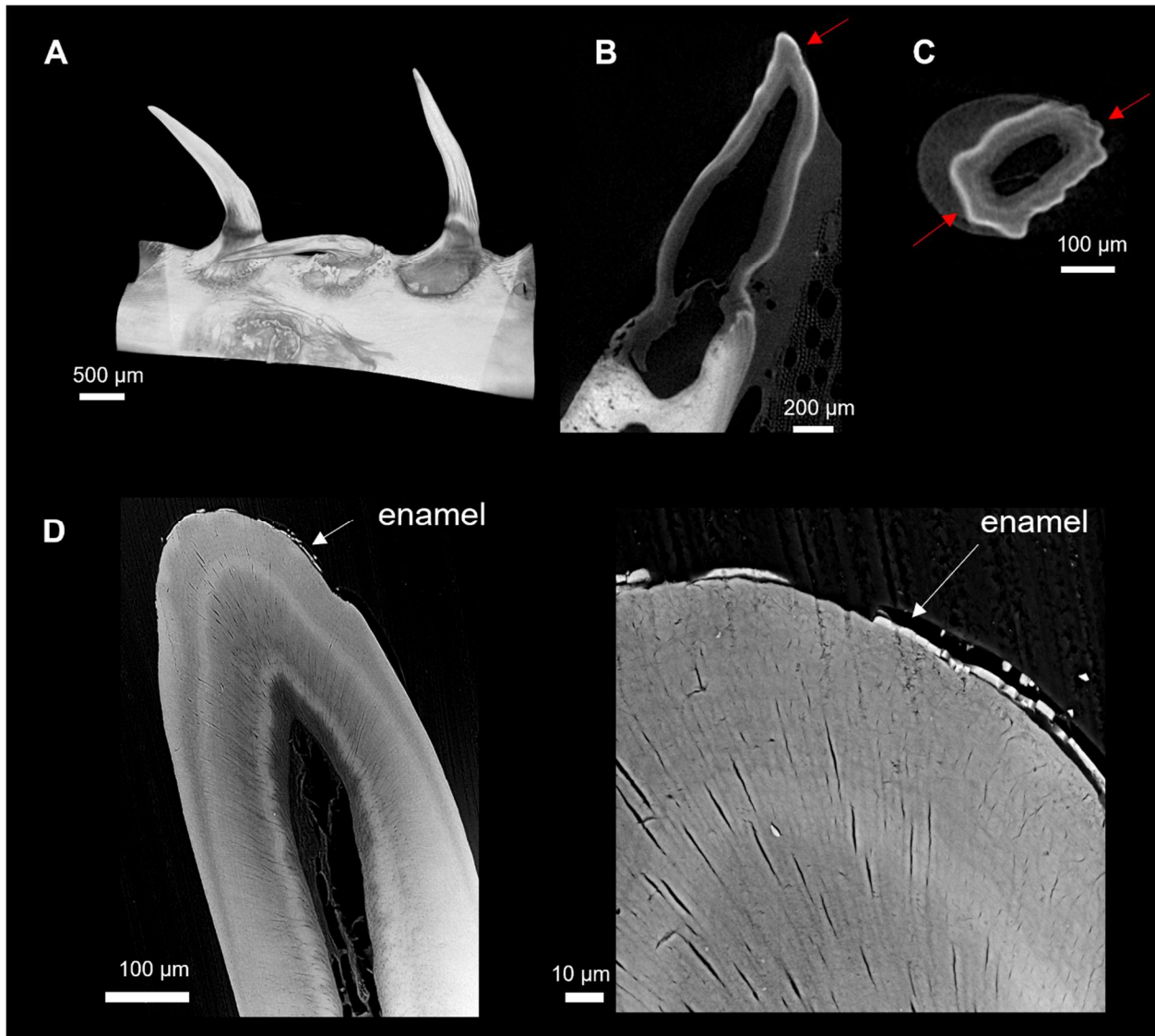


Figure S2: Artefact present on the teeth of *Daboia russelii*. Tooth of *Daboia russelii* look unusual and seem to have suffered from shrinking as observed in the CTscan (A). Virtual sections of the CTscan shown on both longitudinal and transverse sections (B, C) some density lines are present in the teeth. One high dense one at the surface of the teeth seems to be an artefact rather than enamel (red arrow in B and C). Microscopic images (D) of the section prepared in one of the teeth of this snake show the dentine observed in the scan (which could be caused by infiltration of chemicals). The enamel is rather thin and broken and missing in a few parts of the tooth.

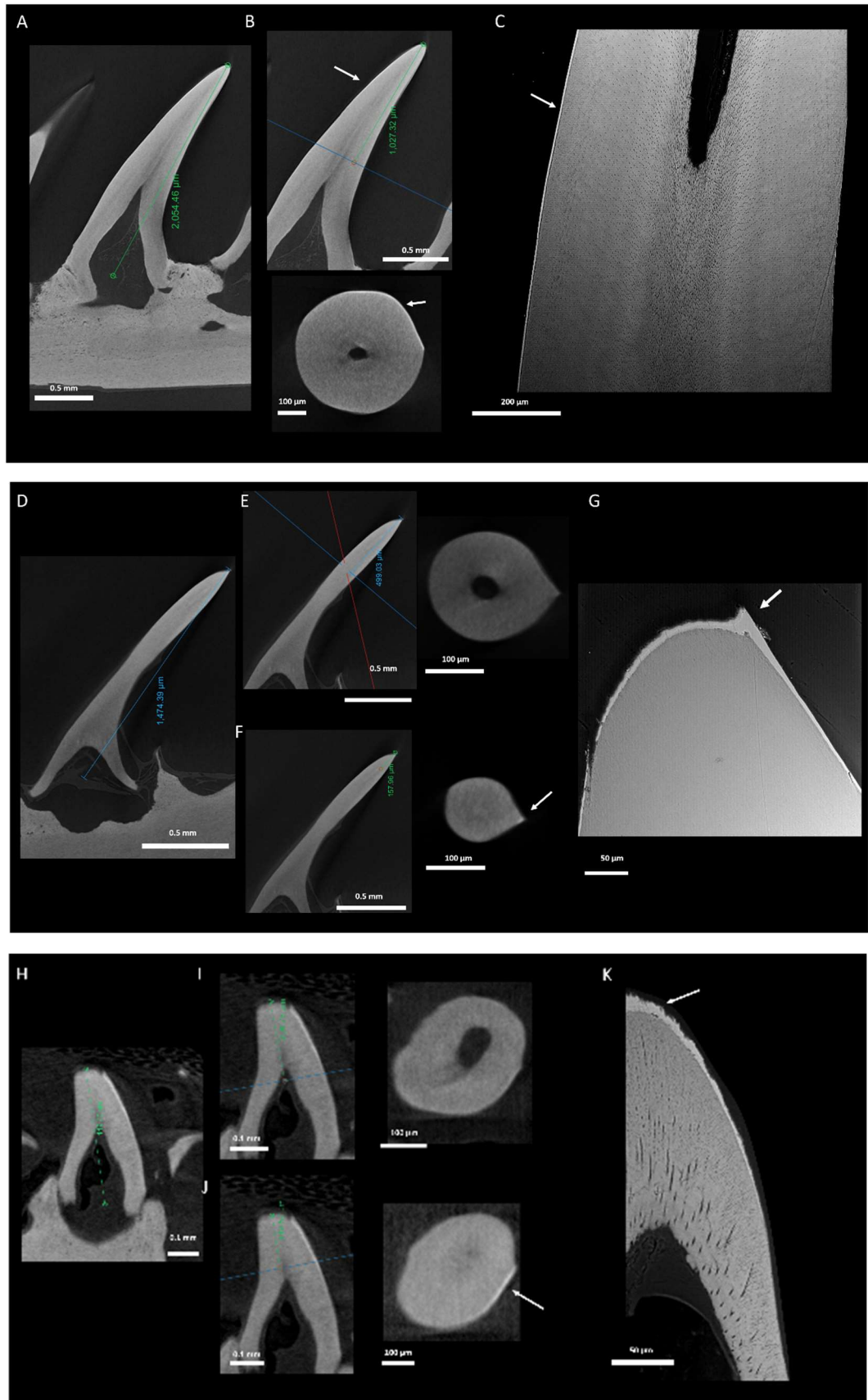


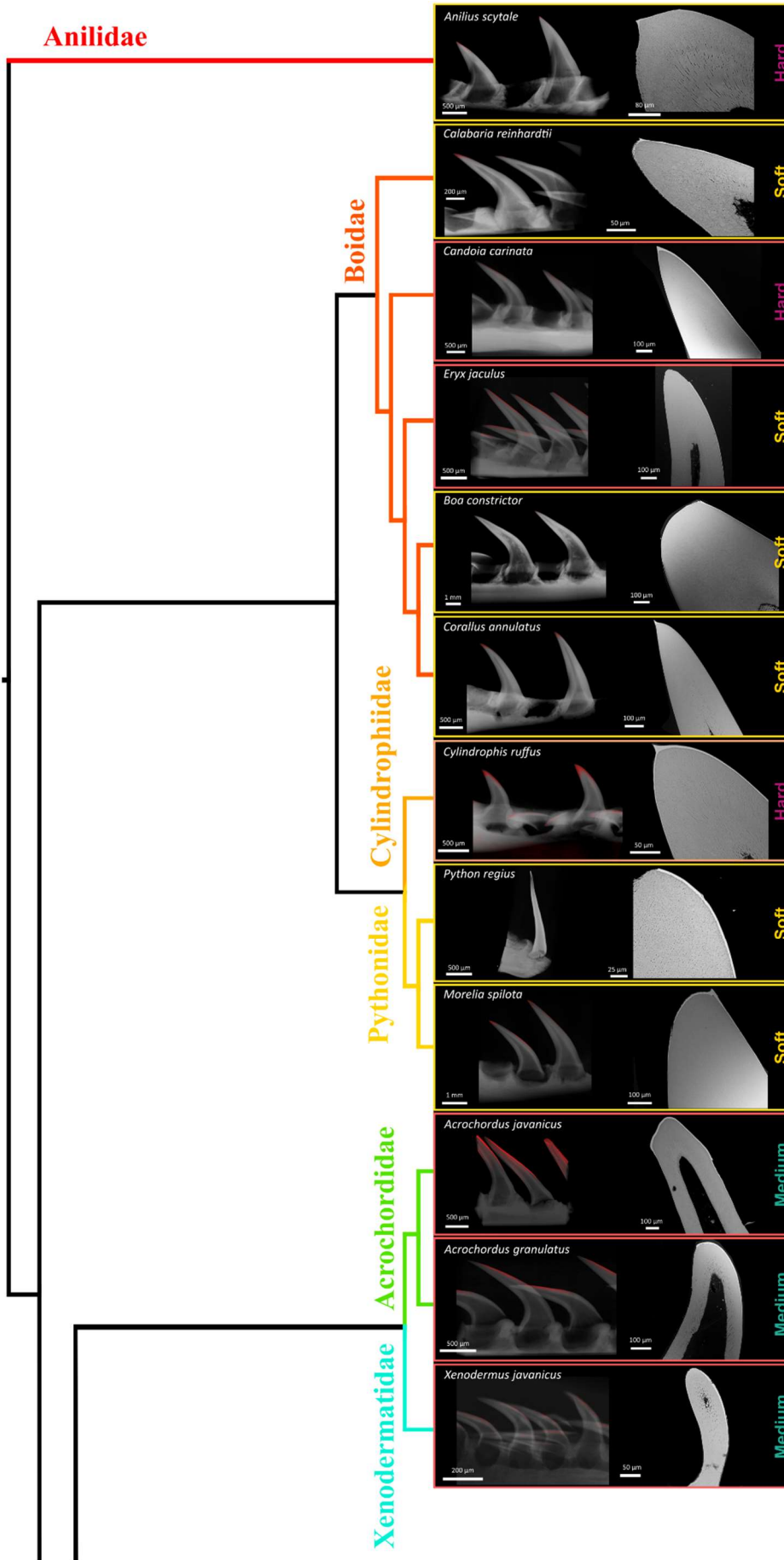
Figure S3: Enamel distribution obtained by CT-scan and electron microscopy. A-C *Boiga dendrophila*, enamel covering the entire anterior face A. Virtual longitudinal sections of the

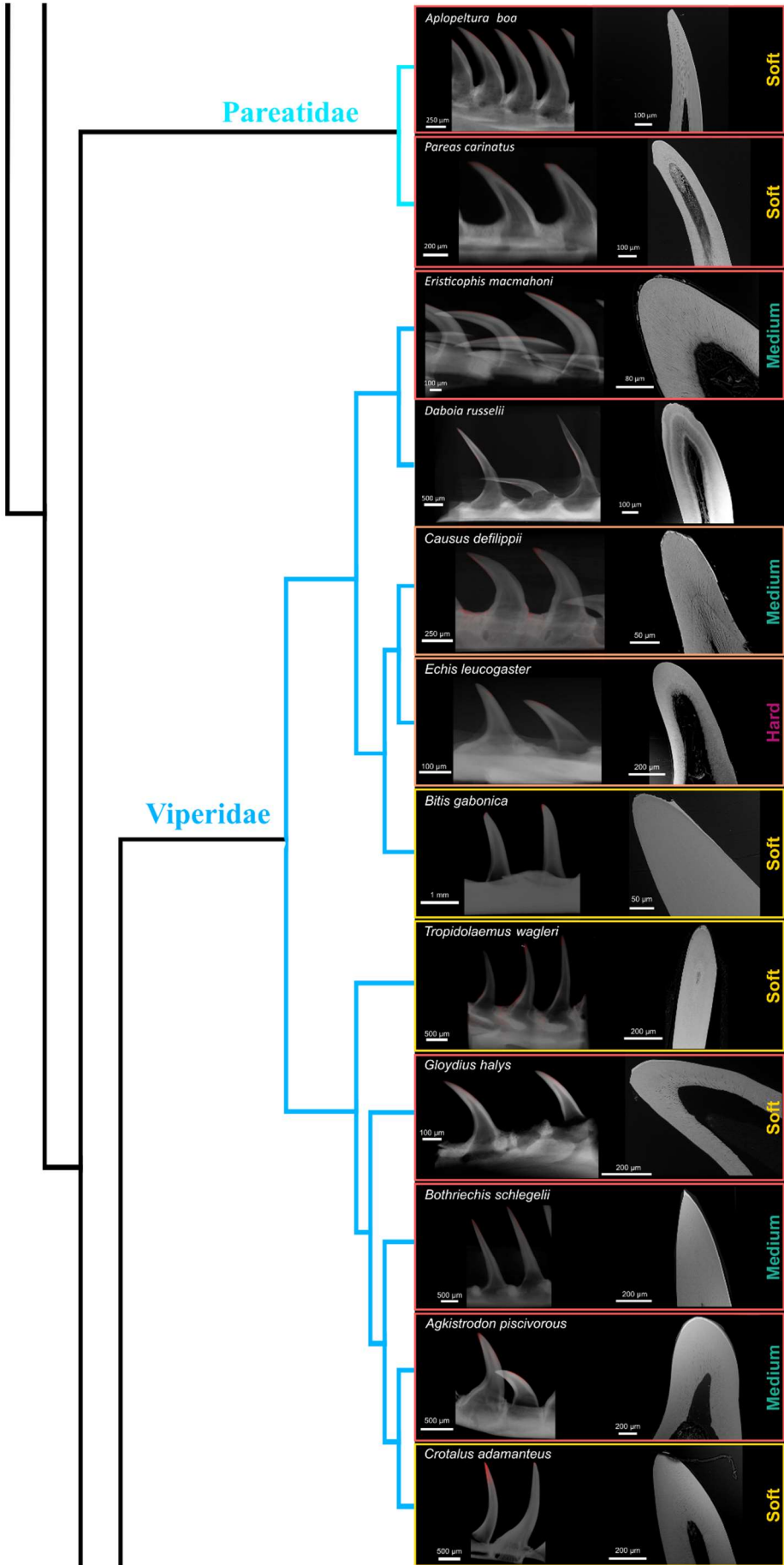
entire tooth with the height of the tooth represented by the green line. B. Virtual longitudinal and transverse sections at mid-height (blue line). At this level of the tooth, enamel is distinct (white arrow). C. SEM-BSE microscopy image of a tooth from the same species with a clearly visible enamel layer present along the entire tooth.

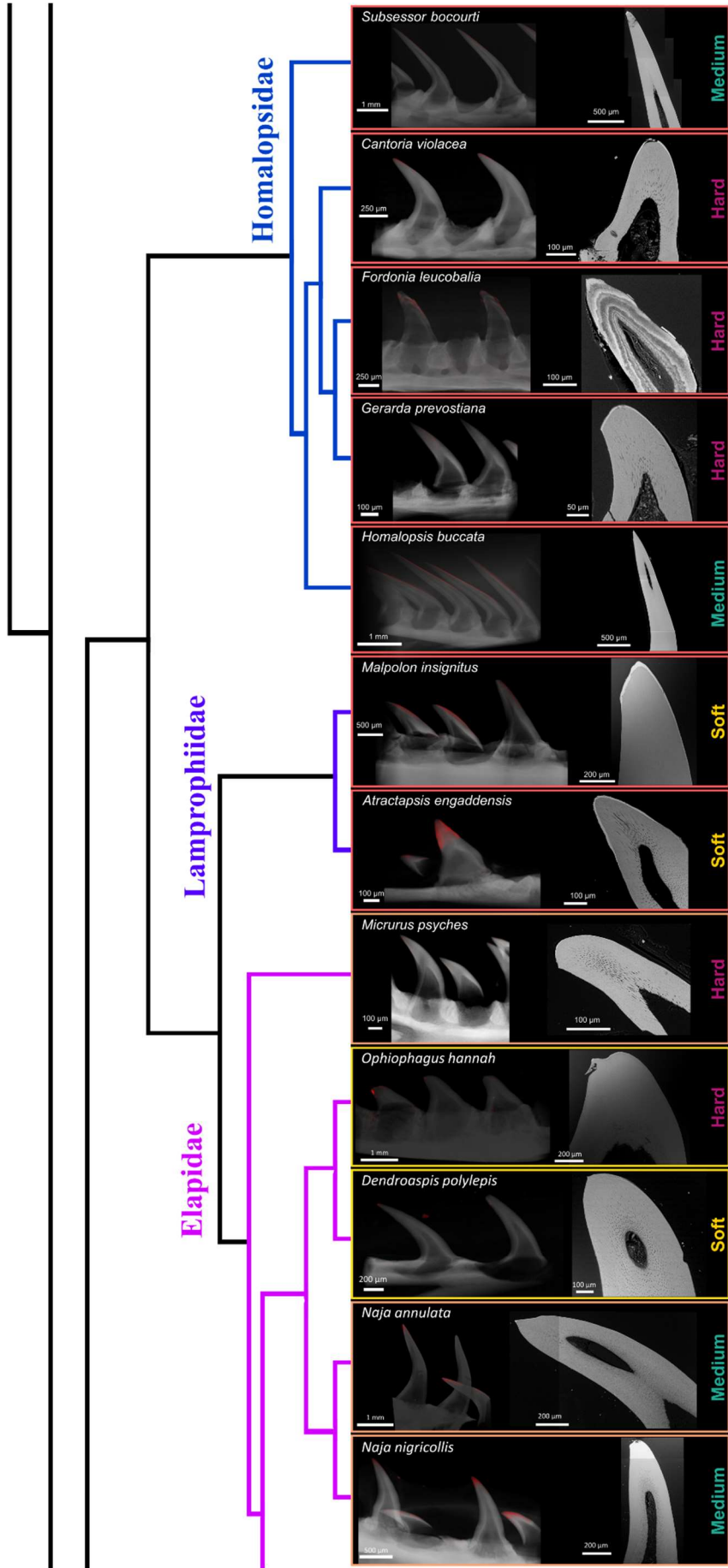
D-G *Morelia spilota*, enamel present only at the tip of the tooth D. Virtual longitudinal section of the entire tooth with the height of the tooth represented by the blue line. E. Virtual longitudinal and transverse sections at mid-height (blue line). At this level of the tooth, enamel cannot be observed. F. Virtual longitudinal and transverse sections of the tooth where the enamel can be distinguished. This corresponds to a section at roughly 10% of the tooth height. G. SEM-BSE microscopy image of a tooth from the same species confirming the enamel appearing only at the tip of the tooth.

H-K *Eirenis decemlineatus* with enamel covering half of the tooth H. Virtual longitudinal sections of the entire tooth with the height of the tooth represented by the green line. I. Virtual longitudinal and transverse sections at mid-height (blue line). At this level of the tooth, enamel cannot be observed (white arrow). J. Virtual longitudinal and transverse sections of the tooth across the upper half of the tooth (less than 30% of its height) showing the distinctive layer of enamel. K. SEM-BSE microscopy image of a tooth from the same species with distinct enamel layer present.

Anilidae

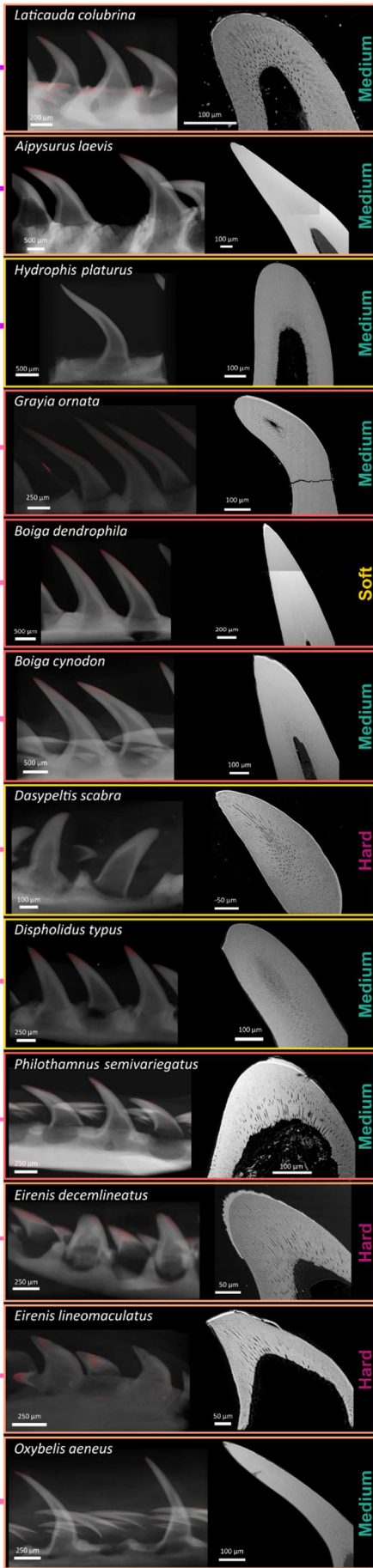




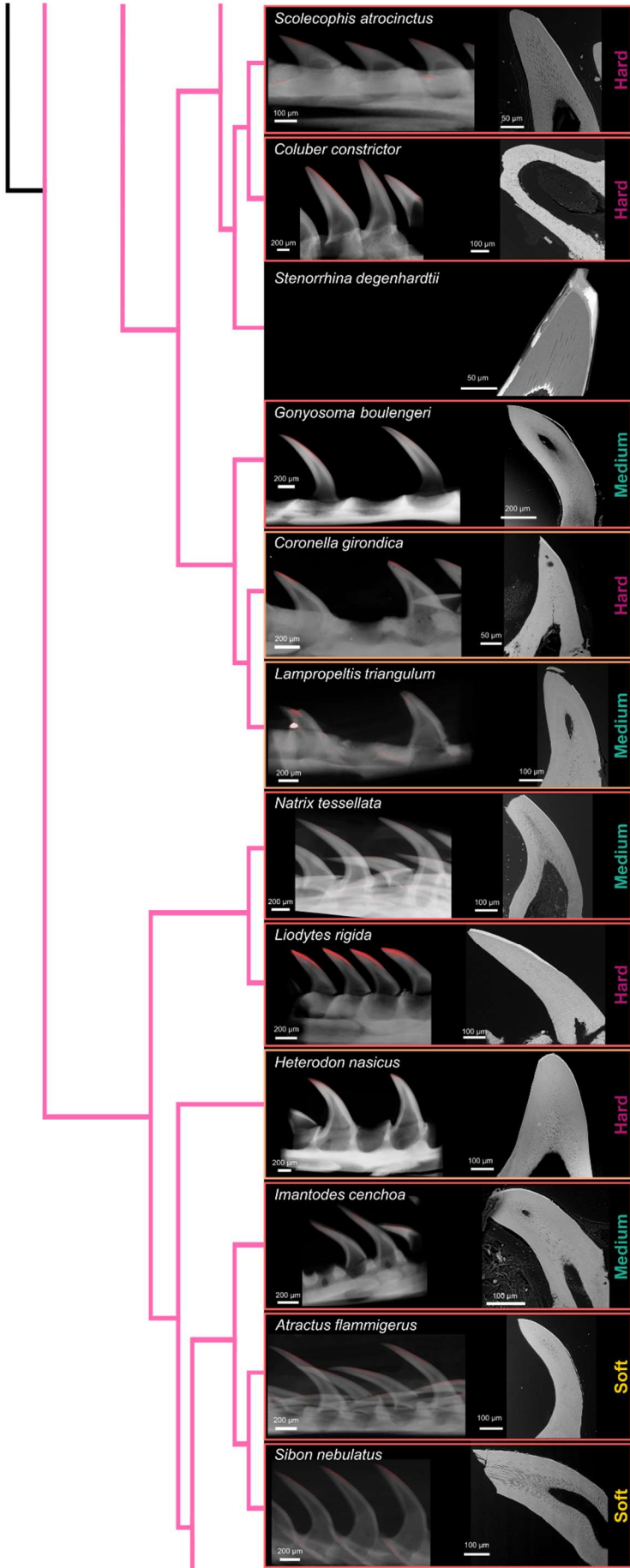


Colubridae

Elapidae



Colubridae



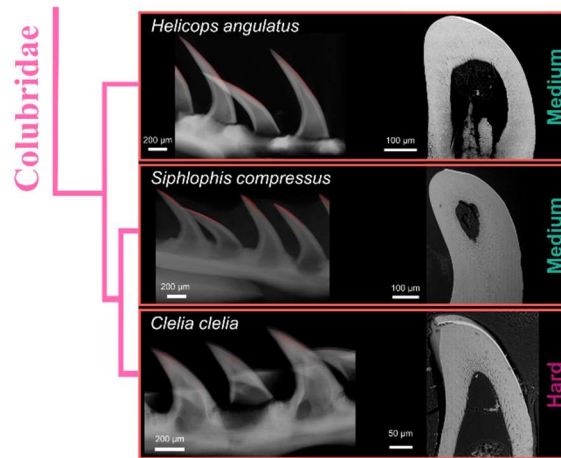


Figure S4: Phylogenetic tree of the snake species included in the study with a 3D view of the different teeth, illustrating the distribution of their enamel (semi-transparent rendering of the teeth and enamel segmented in red) on the left. On the right are represented the different longitudinal sections obtained to confirm the enamel distribution along the teeth. Frame color represents the enamel distribution on the teeth: entire in red, half in orange, and the tip in yellow. Hard, medium, and soft represent the hardness of the dominant prey eaten by each snake species. Only the sample of *Stenorrhina degenhardtii* could not be segmented due to a preservation artifact (cf. Figure S1).

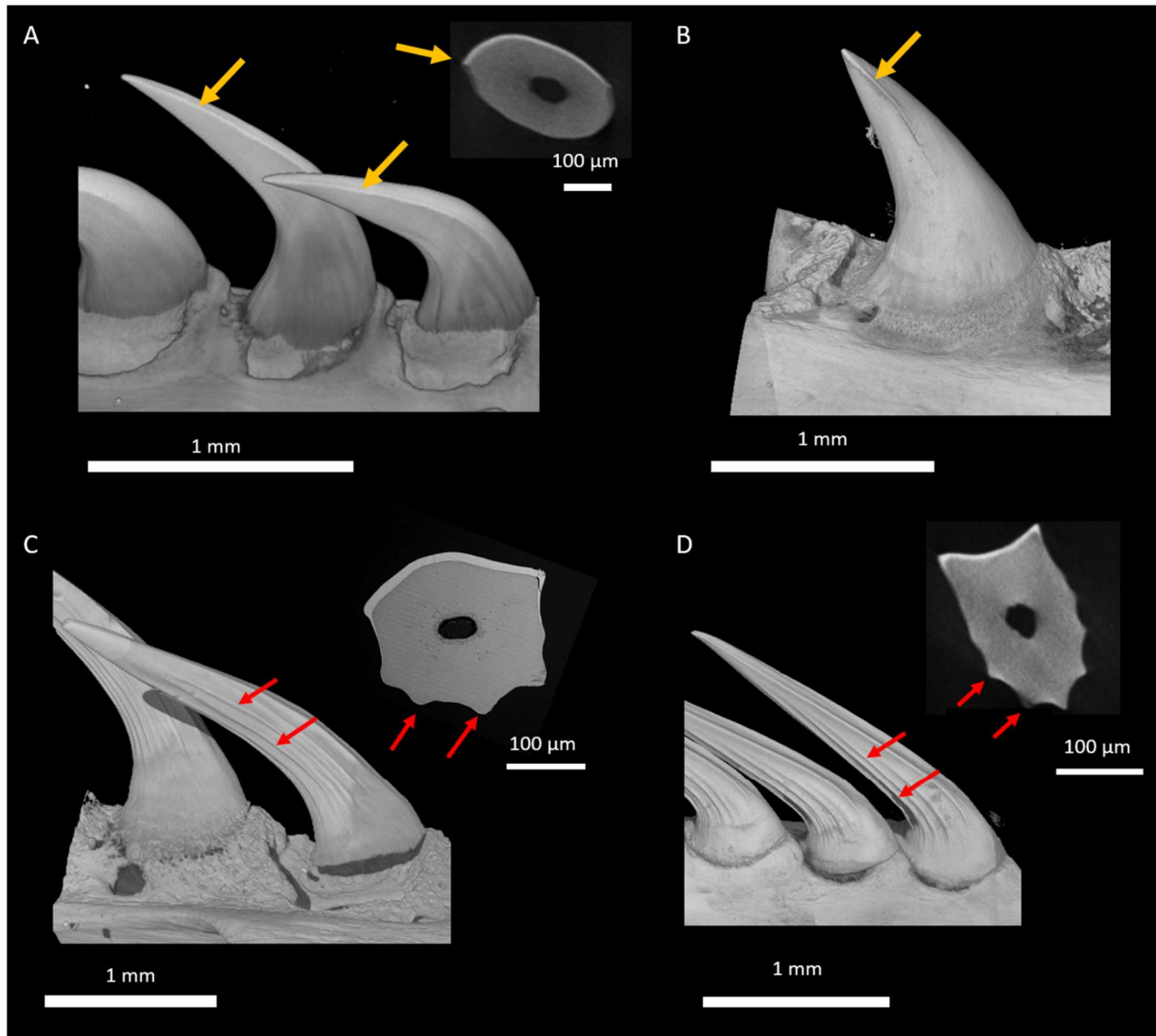


Figure S5: Snake teeth ornamentation. **A.** 3D rendering of *Acrochordus granulatus* teeth showing carinae present in the side of their teeth (yellow arrows). Insert CT transverse section showing the carinae possibly due to a thicker enamel edge. **B.** 3D rendering of *Anilius scytale* showing a deep carinae stopping halfway on the side of one tooth. **C.** 3D rendering of *Acrochordus javanicus* showing apicobasal ridges in the posterior side of their teeth (red arrows). Insert corresponding SEM microscopic transverse section of one tooth showing the presence of these apicobasal ridges which seem to be formed by a thicker amount of dentine. **D.** 3D rendering of *Homalopsis buccata* showing apicobasal ridges in the posterior side of their teeth (red arrows). Insert corresponding CT transverse sections showing the presence of these apicobasal ridges, similar to *Acrochordus javanicus*.

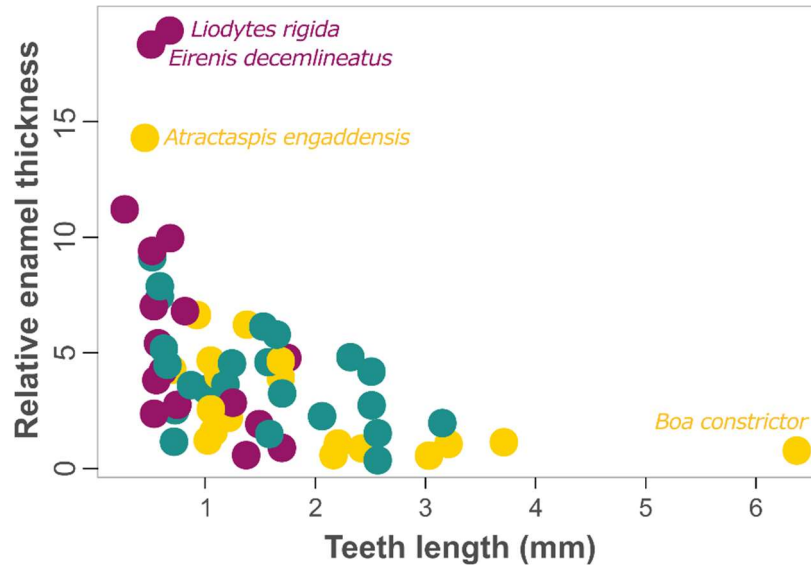


Figure S6: Plot of the relative enamel thickness versus tooth length. Each dot represents one species, dot color represents prey hardness (dark purple: hard, blue green: medium, yellow: soft). *Atractaspis engaddensis* appears to be the only species specializing on soft prey presenting a thick enamel layer, as thick as durophagous species.

Supplementary Tables

Table S1: List of specimens used in this study along with information about the collection, scans, diet, prey hardness, and tooth and enamel measurements and references for diet. + indicates the major prey item.

Species	Collection	Diet	Scan res. (μm)	Hardness	Teeth length (mm)	Mean angle ($^{\circ}$)	Max angle ($^{\circ}$)	Enamel thickness (μm)	Enamel coverage	References [1]
<i>Anilius scytale</i>	A. Herrel	amphisbaenians (+), caecilians, snakes	1.87	hard	1.926	50.6	244.1	1.51 (± 0.47)	Tip	[2,3]
<i>Calabaria reinhardtii</i>	A. Herrel	rodents	1.42	soft	1.278	73.4	194.0	1.7 (± 0.87)	Tip	[4]
<i>Candoia carinata</i>	A. Herrel	skinks (+), geckos, frogs, mammals	2.62	hard	1.800	67.2	169.3	8.34 (± 3.1)	Entire	[5]
<i>Eryx jaculus</i>	HUJI 3634	mammals	2.00	soft	1.847	84.2	226.5	6.63 (± 0.61)	Entire	[6,7]
<i>Boa constrictor</i>	Yoan Eynac	mammals, birds	7.50	soft	6.678	92.7	291.4	4.92 (± 2.42)	Tip	[8]
<i>Corallus annulatus</i>	A. Herrel	mammals, birds	2.75	soft	1.824	66.1	170.6	2.1 (± 2.95)	Tip	[8,9]
<i>Cylindrophis ruffus</i>	Ludovic Faure	caecilians (+), snakes, eels	1.75	hard	1.369	67.0	143.0	2.86 (± 0.93)	Half	[10–15]
<i>Python regius</i>	A. Herrel	mammals, birds	2.15	soft	2.288	49.3	160.2	2.38 (± 0.39)	Tip	[16]
<i>Morelia spilota</i>	A. Herrel	generalist but mainly mammals (+)	3.37	soft	3.813	72.0	201.5	4.21 (± 2.52)	Tip	[17]
<i>Acrochordus javanicus</i>	A. Herrel	eleotrid fish	2.27	medium	2.628	71.8	166.9	10.51 (± 1.34)	Entire	[18]
<i>Acrochordus granulatus</i>	A. Herrel	eleotrid fish, goby-like fish	1.59	medium	1.659	71.3	238.7	5.51 (± 1.53)	Entire	[18–21]
<i>Xenodermus javanicus</i>	A. Herrel	frog	1.00	medium	0.695	75.8	269.8	4.38 (± 0.55)	Entire	[22]

<i>Aplopeltura boa</i>	Karine Falco	snails	1.47	soft	1.248	65.9	243.9	2.66(±0.21)	Entire	[23–25]
<i>Pareas carinatus</i>	Anthony Herrel	snails	1.22	soft	0.551	89.8	243.1	3(±0.74)	Entire	[26–29]
<i>Eristicophis macmahoni</i>	Latoxan	generalist	2.00	medium	1.638	73.7	250.2	2.36(±0.48)	Entire	[30–33]
<i>Daboia russelii</i>	Venomworld	mammals	2.12	medium	2.559	79.7	270.0	2.09(±0.23)	-	[30,33,34]
<i>Causus sp.</i>	Ludovic Faure	amphibians	1.59	medium	0.757	86.1	223.0	1.83(±0.39)	Half	[33,35,36]
<i>Echis leucogaster</i>	Latoxan	centipedes, scorpions, lizards, mammals	1.75	hard	0.593	57.1	153.5	2.61(±0.54)	Half	[30,33,37,38]
<i>Bitis gabonica</i>	Latoxan	mammals (+), birds	3.77	soft	3.156	87.1	279.1	3.4(±1.37)	Tip	[30,33,39]
<i>Tropidolaemus wagleri</i>	AMNH R50991	mammals	1.64	soft	1.966	58.5	120.6	1.25(±0.4)	Tip	[40]
<i>Gloydus halys</i>	Ludovic Faure	mammals	1.18	soft	0.889	66.7	201.6	6.14(±0.76)	Entire	[41]
<i>Bothriechis schlegelii</i>	Venomworld	generalist	2.57	medium	2.575	51.6	164.0	6.86(±1.63)	Entire	[1,42]
<i>Agkistrodon piscivorus</i>	Venomworld	ectotherm generalist	2.00	medium	1.656	69.5	158.8	7.24(±1.62)	Entire	[43–47]
<i>Crotalus sp.</i>	A. Herrel	mammals	4.25	soft	3.259	68.8	154.8	1.68(±0.3)	Tip	[48]
<i>Subessor bocourti</i>	A. Herrel	fish: Osphronemidae (+), Pangasidae, Siluridae, Synbranchidae	2.75	medium	3.051	81.8	331.8	6.19(±1.31)	Entire	[10,49]
<i>Cantoria violacea</i>	LKC-ZRC 2.3317	crustaceans (mostly shrimps)	1.00	hard	0.969	72.0	239.2	5.56(±1.72)	Entire	[49,50]
<i>Fordonia leucobalia</i>	LKC-ZRC 2.3294	hard-shelled crustaceans (crabs, lobster)	1.59	hard	0.827	57.3	108.5	2.06(±0.28)	Entire	[50–52]
<i>Gerarda prevostiana</i>	LKC-ZRC 2.3292	crabs	1.00	hard	0.625	64.1	153.4	3.75(±0.64)	Entire	[49–51]

<i>Homalopsis buccata</i>	A. Herrel	small or long fish (<i>Lebistes reticulatus</i> , <i>Mystus</i> , <i>Monopterus albus</i> , <i>Clarias</i> sp, <i>Puntius binotatus</i>)	1.70	medium	2.263	87.1	295.9	11.16(±1.21)	Entire	[10,49,53]
<i>Malpolon insignitus</i>	HUJI 16560	generalist (mostly mammals)	1.81	soft	1.570	57.3	130.5	8.58(±6.54)	Entire	[54–56]
<i>Atractaspis engaddensis</i>	HUJI 16567	mammals (+), lizards, snakes	1.75	soft	0.430	60.1	181.8	6.48(±1.03)	Entire	[57]
<i>Micrurus psypes</i>	A. Herrel	snakes	0.97	hard	0.545	68.9	112.8	1.27(±0.2)	Half	[58]
<i>Ophiophagus hannah</i>	Latoxan	snakes	2.27	hard	1.203	64.0	149.7	3.7(±1.52)	Tip	[59,60]
<i>Dendroaspis viridis</i>	Latoxan	mammals, birds	1.50	soft	0.987	71.4	281.2	1.24(±1.61)	Tip	[61]
<i>Naja annulata</i>	Latoxan	fish	2.50	medium	2.682	80.3	211.4	3.9(±0.5)	Half	[62]
<i>Naja nigricollis</i>	Latoxan	generalist	1.50	medium	1.199	62.4	132.7	4.47(±1.4)	Half	[63]
<i>Laticauda colubrina</i>	AMNH R38111	eels	1.06	medium	0.819	112.9	77.3	0.83(±0.15)	Half	[52,64–69]
<i>Aipysurus laevis</i>	Latoxan	small or long fish (catfish, small snappers)	2.37	medium	2.244	90.8	200.0	4.66(±0.82)	Half	[52,66,67,70,71]
<i>Hydrophis platurus</i>	A. Herrel	small fish	1.92	medium	2.347	99.6	280.2	0.91(±0.18)	Tip	[52,70,72–79]
<i>Grayia ornata</i>	A. Herrel	long fish (siluriform)	1.29	medium	1.559	91.5	276.7	9.38(±0.91)	Entire	[62,80]
<i>Boiga dendrophila</i>	Latoxan	mammals, birds	1.83	soft	2.134	63.9	167.1	7.82(±2.07)	Entire	[81]
<i>Boiga cynodon</i>	Latoxan	generalist	2.00	medium	2.034	73.4	129.9	9.55(±2.45)	Entire	[81]
<i>Dasypeltis scabra</i>	A. Herrel	eggs	1.04	hard	0.572	67.5	248.8	2.13(±0.8)	Tip	[82]

<i>Dispholidus typus</i>	Latoxan	birds, chameleons, agama, toad	1.75	medium	1.062	57.7	154.1	3.14(±1.22)	Tip	[83]
<i>Philothamnus semivariiegatus</i>	Latoxan	lizards (gecko)	2.00	medium	0.736	74.0	176.0	2.95(±1.29)	Entire	[61,80,84]
<i>Eirenis decemlineatus</i>	HUJI 4780	arthropods (orthopterans, insects, spiders, scorpions, centipedes)	1.50	hard	0.495	52.6	98.9	9.34(±4.1)	Half	[85,86]
<i>Eirenis lineomaculatus</i>	HUJI 16485	arthropods (orthopterans, centipedes, spiders, scorpions)	1.75	hard	0.475	75.6	158.1	3. (±0.32)1	Half	[85,86]
<i>Oxybelis aeneus</i>	A. Herrel	generalist (mostly elongated lizards)	1.12	medium	0.998	82.0	209.2	3.43(±0.76)	Half	[87,88]
<i>Scolecophis atrocinctus</i>	A. Herrel	centipedes	1.01	hard	0.368	64.6	181.8	3.02(±0.49)	Entire	[89]
<i>Coluber constrictor</i>	AMNH R27367	generalist (snakes (+), elongated lizards...)	1.25	hard	1.449	64.0	157.1	0.79(±0.13)	Entire	[90–92]
<i>Stenorrhina degenhardtii</i>	AMNH R38087	arthropods (mostly spiders)	1.59	hard	0.567	56.2	128.0	1.7(±0.2)	-	[93–95]
<i>Gonyosoma boulengeri</i>	A. Herrel	endotherm generalist	1.50	soft	0.776	83.1	284.0	4.52(±0.73)	Entire	[96]
<i>Coronella girondica</i>	A. Herrel	elongated lizards (skinks, lacertids, <i>Anguis</i>)	1.40	hard	0.786	63.8	166.7	6.8(±2.07)	Half	[97]
<i>Lampropeltis triangulum</i>	AMNH R177123	elongated lizards (+, skinks, <i>Sceloporus</i>), snakes, mammals	1.22	hard	0.668	46.3	165.7	3.24(±0.19)	Half	[98]
<i>Natrix tessellata</i>	HUJI 16537	mostly elongated fish (goby, Cyprinidae...)	1.50	medium	1.165	74.5	228.5	4.3(±0.87)	Entire	[99–101]
<i>Liodytes rigida</i>	AMNH R177031	crayfish	1.00	hard	0.672	49.9	147.2	12.82(±1.38)	Entire	[102–105]
<i>Heterodon nasicus</i>	Yoan Eynac	reptiles, toads, eggs, small turtles	1.30	hard	1.255	82.3	267.4	3.56(±0.42)	Half	[106–109]
<i>Imantodes cenchoa</i>	Vincent Prémel	elongated thin scaled lizards (mostly anoles, +,	1.00	medium	0.521	87.3	299.3	4.75(±0.8)	Entire	[3,110]

		<i>Gonatodes sp.</i>), amphibians								
<i>Atractus flammigerus</i>	MNHN 80-24	earthworms	1.25	soft	1.107	70.8	193.7	4.9(±0.4)	Entire	[3,111,112]
<i>Sibon sp.</i>	A. Herrel	earthworms (+), slugs, amphibian eggs	1.02	soft	1.058	83.9	236.1	2.69(±0.41)	Entire	[113,114]
<i>Helicops sp.</i>	A. Herrel	mostly fish (Perciformes, Characiformes, Cyprinodontiformes...)	1.20	medium	1.252	70.6	248.5	5.65(±1.09)	Entire	[3,115–117]
<i>Siphlophis compressus</i>	Vincent Prémel	mostly elongated thin- scales lizards (anoles, <i>Gonatodes</i>)	1.59	medium	0.663	62.5	192.4	4.64(±1.24)	Entire	[1,3]
<i>Clelia clelia</i>	MNHN 79-47	reptiles (mostly snakes +)	1.40	hard	0.529	52.0	189.5	4.88(±1.48)	Entire	[1]

Differences in tooth enamel in snakes with different diets

1 **Table S2:** Measurements of average enamel thickness (in μm) and standard deviation of both
2 longitudinal and transverse tooth sections for some species.

3

Species	Longitudinal section	Transverse section
<i>Boiga dendrophila</i>	7.82 \pm 2.07	5.44 \pm 0.87
<i>Boa constrictor</i>	4.92 \pm 2.42	3.48 \pm 0.51
<i>Crotalus adamanteus</i>	1.67 \pm 0.30	1.61 \pm 0.35
<i>Pareas carinatus</i>	3.03 \pm 0.74	3.0 \pm 0.34
<i>Heterodon nasicus</i>	3.56 \pm 0.41	3.66 \pm 0.46
<i>Gerarda prevostiana</i>	3.75 \pm 0.63	3.33 \pm 1.00
<i>Micrurus psyches</i>	1.27 \pm 0.20	1.28 \pm 0.26
<i>Acrochordus javanicus</i>	10.16 \pm 2.02	10.51 \pm 1.34

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