nature portfolio

Peer Review File

The Origin of Vertebrate Teeth and Evolution of Sensory Exoskeletons

Corresponding Author: Dr Yara Haridy

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
The question of the evolutionary origin of teeth is ongoing, and in particular the relationship of teeth to external odontodes. The authors investigate this question in a very novel manner, considering whether these structures share an ancient sensory function. They begin by re-examining the Cambrian taxon Anatolepis, a putative vertebrate. Using modern imaging techniques, they demonstrate greater similarity to the histology of the fossil invertebrate Aglaspis, particularly between 'dentine' tubules in Anatolepis and sensory tubules in Aglaspis. The authors then compare a range of invertebrate and vertebrate odontodes and associated innervation to support their assertions that 1) Anatolepis is an invertebrate; 2) odontodes, both internal and external, shared a sensory function early in their history. The manuscript is well-written and well-illustrated. I have some comments I'd like them to consider before acceptance:

lines 39-40: 'remain controversial because of challenges of identifying it in fossil forms'
What are these challenges? Aren't the centripetally oriented tubules (toward the pulp cavity) actually easy to recognize?

line 42: 'uniqueness of the mineralized tissues in their pharyngeal elements'
It would be good to say here what this internal tissue has been identified as.

lines 50-51: 'and a tissue dubbed ‘lamelline’ (acellular dentine)'
Do you mean the lamellar basal tissue in line 47 (you refer to it as such in Figure 1)? Can you be clearer about this because Andreev et al. have used the term lamellin for acellular dentine in mongolepid scales. The lamellar basal tissue occurs alongside the tubercles in Anatolepis.

line 51: enamel or enameloid?

line 64: 'we first deployed high-resolution synchrotron tomography…'
I think it would be worthwhile, somewhere in the Results section, to more closely compare your results with the SEM images from Smith et al. For example, can you see peripheral tubules here, or is that something that only came out in your synchrotron scans?

line 66: 'homogenous lamellar basal layer (Fig.1)'
Smith et al had this layer alongside the tubercules- is this what you saw as well? Hard to tell in Figure 1.

line 78: 'Aglaspidid cuticles have a homogenous lamellar structure'
Again, is this continuous beneath, or alongside?

lines 81-82: 'However, this feature was not seen in all scanned aglaspidid cuticle samples'
Can you be more specific- what percentage of specimens were the canals and organs absent from? Did this depend on what part of the body the cuticle was taken from (you mention you took samples from complete specimens).

line 94: 'but most parsimoniously identified as an aglaspidid.'
It would be worth saying earlier that you took the sample from a claw because my thought here was, why isn't the aglaspid material vertebrate?

lines 107-108: 'histological microanatomy of the porcelain crab sensilla is most like that of aglaspidid and Anatolepis tubercles'
Did Anatolepis show the tube-in-tube morphology?

lines 122-123: 'remarkably large dentine tubules stemming from a pulp cavity which repeatedly branch and attenuate to the surface as pores'
Also in Ordovician chondrichthyan scales illustrated by Johanson et al. 2007 and Sansom et al. 1996?

lines 131-132: 'crucial role in the development and function…'
I think the developmental aspect could be discussed further with respect to the sensory function, see comments below.

line 133: 'tail odontodes'
Why did you chose these tail odontodes? Would odontodes on the head (particularly on the rostral region of the head, this being a particularly sensory part of the body) be more appropriate to test? Same goes for the odotontode sampled for Leucoraja.

Line 137-138: 'nerves surrounding the base of odontodes in chondrichthyan'
Interesting that these nerves don't seem to invade the tail odontodes- Johanson et al. 2007 showed these had wide tubules, similar to the Ord. chondrichthyan scale I mentioned above (lines 122-123).

line 137-138: 'surrounding the base of odontodes in chondrichthyans and invading the pulp cavity in the pectoral fin in the catfish'
but not running to surficial pores, so this would be different from the taxa above, (a criterion you used to say these were sensory)

line 139-140: 'innervation associated with the dentine of odontodes is a primitive trait among extant gnathostomes'
but it doesn't enter the chondrichthyan odontode, nor to surface pores?

line 168-169: 'Therefore, we hypothesize that Eriptychius odontodes were more suited to sensory capability than those of Astraspis'
But in the younger tubules of Astraspis, the cavity was more open suggesting a potential sensory functon(line 167)? How are the tubules added/how does the bone grow?

line 170: 'sensory function…'
Could there also be a link between the innervation and tissue development, separate from the sensory function? This is well known in bone? Which came first- development or sensory function?

line 176: 'An ancestral sensory function of dentine…'
Again, could a tissue development role have predated a sensory function?

Figure 2g, h: what does the color blue indicate?

Referee #2

(Remarks to the Author)
This manuscript raises the question of the sensitivity to the surrounding environment in the early vertebrates whose body was entirely covered with a dermal skeleton. The authors propose that the odontodes covering the dermal skeleton acted as sense organs, in the same way as we can feel a 'toothache'. This hypothesis had been proposed by Halstead in 1974, but soon left aside because of alleged presence of anamel cap that would prevent any sensory function of the odontocytes of the dentine. Orvig almost revived it by suggesting that, even in hypermineralised dentine, some dentine tubules could be prolonged to the surface of the enameloid, and thus possibly act as sensory receptors. The question became revisited by new findings of the earliest known vertebrate (or presumed so) odontodes. These come from the Ordovician astraspids and associated scale and armor remains from the Harding Sandstone of USA. Then early vertebrate palaeontologists tried and find even earlier evidence for vertebrate hard tissues, knowing that the living sister group of mineralizing vertebrates, that is, Cyclostomes, produces no mineralized skeleton. Before the discovery of the Cambrian myllokunmingiids and Metaspriggina, which are clearly non-mineralizing jawless vertebrates, the only hint was the conodonts, long disputed, but whose denticles are now recognized as potentially convergent at odontodes, and functionally comparable to the horny 'teeth' of modern cyclostomes. In addition, a number of phosphated 'scraps' turned up in the Ordovician and Cambrian of several sites of the Northern hemisphere and Australia and were assigned to possible vertebrate carapace fragments covered with odontodes, such as in Anatolepis. The interpretation of these remains raised vivid discussions, and some became regarded as arthropod remains, and thus dismissed, in spite of the advice of courageous supporters. Thanks to modern imaging techniques, these remains have been re-examined by the authors, who demonstrate here the lack of evidence for dentine tubules, and thus the probable support for the odonte interpretation. The authors provide strong support for the arthropod (aglapid) interpretation. The authors provide hereby a remarkable review of this question, and this may suggest that the rise of the duly recognized vertebrate odontodes may well have started like neurogenic placodes or taste

buds. In addition this review provides invaluable data on the structure and sensitivity potential of arthropod cuticle.

Referee #3

(Remarks to the Author)
This is an interesting paper. The title promises insights on the origin of teeth and evolution of sensory systems in vertebrates but almost the entire paper is devoted to demonstrating why Anatolepis is NOT a vertebrate. Eliminating Anatolepis from the vertebrate record is important in terms of determining the origin and nature of tissues, particularly dentine, in the earliest odontodes and the authors provide a wealth of invaluable information on skeletal tissues from a wide range of taxa. However, only in the third paragraph from the end of the paper does the focus change to the sensory capability of early vertebrates such as Astraspis and Eriptychius and the authors might have taken more advantage of their new impressive illustrations of same.

There's obviously no argument about an extensive Cambrian record of arthropods, including aglaspidids and the like. The authors selected material of Aglaspis from the Cambrian of Wisconsin, a source of well preserved examples. In contrast, the interpretation of mineralized dermal fragments of Cambrian vertebrates is problematic but, given evidence of soft-bodied antecedents in exceptional preservations, the occurrence of dermal armor in the Cambrian is perhaps not unreasonable. Tomographs of Anatolepis cuticle from the Wilberns Formation of Texas are illustrated for detailed comparison with samples of Aglaspis treated in the same way (Figure 1). The similarities are obvious but the method does not reveal the thin laminae that are a feature of arthropod cuticle in either Anatolepis or Aglaspis. In addition the thickness of the cuticle of Anatolepis is about 4x that of Aglaspis (measured adjacent to the tubercles) and there is the possibility that similarities may be a result of convergence.

The late Cambrian Wilberns Formation material may be arthropodan but I'd be wary of 'definitively aglaspidid' (line 23). Perhaps 'most parsimoniously identified as an aglaspid' is more accurate (line 94). Note, however, that Peel (1979, Ref. 8, p. 114), who ousted Anatolepis from the vertebrates 45 years ago (based on external morphology, not histology), was not prepared to assume that 'all the material in question is congeneric'. Some of the Cow Head specimens (Fortey et al. 1982), which the authors examined but do not figure, are somewhat similar morphologically to Bockelie and Fortey's (1976) original specimens from Spitsbergen where the 'tubercles', at least in some cases, are diamond-shaped blade-like structures unlike those on the Texas specimens. It would be interesting to know whether such specimens show similar internal features. But even if the affinities of Bockelie and Fortey's Anatolepis heintzi could be confirmed as vertebrate, it's early Ordovician in age, not Cambrian, so less of a problem for the odontode story.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have carefully considered and responded to my original comments, including making new synchrotron scans in order to improve image resolution, in part to compare to previous published SEM images of the material. This is an original and significant piece of work and I have no further comments to make.

Referee #2

(Remarks to the Author)
To me, this manuscript looks excellent, and I look forward to seeing it publieshed, as I need to cite it in a chapter I'm currently preparing.

Referee #3

(Remarks to the Author)
The general reader would be helped by a simple statement in the abstract along the lines of: 'The first vertebrates in the fossil record are early Cambrian, odontodes were previously interpreted as appearing in the late Cambrian but these are shown here to be aglaspidid arthropods, hence the earliest such vertebrate tissues are Middle Ordovician.'

I also recommend that you check the paper and supplemental discussion (in particular) carefully for minor errors and take advantage of opportunities for clearer expression.

I haven't checked the text as carefully as a copy editor might, but here are a few examples where changes/corrections might be made (numbers refer to lines in the revised version).

28 'dentin'

265 and in other legends: 'Scale bars' rather than 'Scale bar'. There are inconsistencies in the figures where some scale bars are labeled with lengths and others are not (e.g. none in Figs 2,3 some in Fig 4, all in the Supplementary figures except Fig S6). 'sp.' is italicized occasionally (e.g. Fig 4a) but it should not be.

529 'tubricles'

612 ff Aren’t these GSC specimens? If so they should be listed as 'GSC 65596' for example.

597 ‘aglaspidid’ not 'aglaspidids'. Specimen numbers need to be added to the legend.

The Supplemental Discussion is not as well written as the main text.

Examples of less elegant phrases include:

648 ‘There is a scarcity in the literature regarding …’

654 ‘This further supports that..’

659 ‘The exact means as to how ..’

665 ‘Have major criticisms against them ..’

667 ‘data which support’ (not supports)

690 ‘Therefor not withstanding’ Presumably you mean ‘excluding’ (as presently worded it can be interpreted to mean the opposite).

720 'Survey' (upper case S).

722 'Anatolepis' should be in italics.

724 ‘which is deposition’ ??

763 ‘local naturalist’ This doesn't identify him - local to what?

771 'Ancistrus' should be in italics.

Generic names are not everywhere italicized. 'sp.' is sometimes italicized whereas it should not be.

You might consider citing
Smith, M. P., Sansom, I. J. & Cochrane, K. D. 2001. The Cambrian origin of vertebrates. In Major Events in Early vertebrate Evolution – Palaeontology, phylogeny, genetics, and development (ed. Ahlberg, P. E.), pp. 67–84. The Systematics Association Special Volume 61.

Response to referees:
"The Origin of Vertebrate Teeth and Evolution of Sensory Exoskeletons"

Dear Henry and Referees,

Thank you for your thoughtful reviews that ultimately made this paper stronger. We are also grateful for the opportunity to resubmit our manuscript with new and improved analyses.

We are grateful that all three referees found the paper to be 'interesting' and 'novel' and provides a 'wealth of invaluable information'. To address Referee #1's comments, we made the requested changes to clarify terminology, context, and figure captions. To address Referee #3's comments, we performed new scans at Argonne National Lab (ANL) taking advantage of the recently significant beamline upgrades, allowing us to achieve sharper images. Additionally, we refined the reconstructed images from previous scans using manual alignment with the expertise of Dr. Phillip Vargas. Together, the new scans and manual alignment allowed for clearer visualization of the laminar tissue in both '*Anatolepis'* and aglaspidids, which are currently shown in the Supplementary **Figure 5.** Following Referee#3's insightful advice, we also included imaging of the Ordovician 'vertebrates', also called *'Anatolepis'*, from the Cow's Head formation in Canada, which were scanned at ANL and the new results presented in Supplementary **Fig 6).**

These new data and images, together with improved contrast and refined reconstruction and their images, resolve minute structures such as the lamellae of the basal mineralized tissue. They enable comparison among the original SEM histological interpretations of *Anatolepis* by Smith et al. 1996, the Cambrian *Anatolepis* specimens from Texas, the Cambrian Aglaspidid material from Wisconsin, and finally, the well-known structure of arthropod cuticle. This lamellar structure, among other parallels presented in this paper, makes a strong argument for the absence of mineralized vertebrate tissues in the Cambrian.

We thank the referees for their time, efforts and thoughtful comments that have strengthened this paper. We now present a point-by-point discussion to the referees' comments.

Referee #1 (Remarks to the Author):

The question of the evolutionary origin of teeth is ongoing, and in particular the relationship of teeth to external odontodes. The authors investigate this question in a very novel manner, considering whether these structures share an ancient sensory function. They begin by re-examining the Cambrian taxon Anatolepis, a putative vertebrate. Using modern imaging techniques, they demonstrate greater similarity to the histology of the fossil invertebrate Aglaspis, particularly between 'dentine' tubules in Anatolepis and sensory tubules in Aglaspis. The authors then compare a range of invertebrate and vertebrate odontodes and associated innervation to support their assertions that 1) Anatolepis is an invertebrate; 2) odontodes, both internal and external, shared a sensory function early in their history. The manuscript is well-written and well-illustrated. I have some comments I'd like them to consider before acceptance:

We thank Referee #1 for their kind comments regarding the writing, illustrations and recognition of our novel approach to this controversial topic of odontode origins. We hope that we have addressed their concerns below and in the amended manuscript.

lines 39-40: 'remain controversial because of challenges of identifying it in fossil forms'
What are these challenges? Aren't the centripetally oriented tubules (toward the pulp cavity) actually easy to recognize?

Thank you for this note. We have clarified that the challenges come from the variation in the tissue in Ln51-52. While dentine is easy to recognize due to its distinct nature, as stated, but it comes in diverse forms that can include or exclude cell space. Additionally, dentine tubules are not always associated with a distinct pulp cavity, such as in many scales. Most importantly, the presence of tubules emanating from a central cavity does not define a mineralized tissue as dentine, because arthropod tissues have the same microstructural pattern, as we have now shown in this study.

line 42: 'uniqueness of the mineralized tissues in their pharyngeal elements'
It would be good to say here what this internal tissue has been identified as.

Terms such as "Crown tissue" and "Basal tissues" have been used to describe tissues in conodonts (Murdock et al., 2013; Sansom et al., 1992). There is no agreed-on terminology or homology to these tissues. Since we did not specifically study their histology, we refrain from being more specific and instead cite previous histological work.

lines 50-51: 'and a tissue dubbed 'lamelline' (acellular dentine)'
Do you mean the lamellar basal tissue in line 47 (you refer to it as such in Figure 1)? Can you be clearer about this because Andreev et al. have used the term lamellin for acellular dentine in mongolepid scales. The lamellar basal tissue occurs alongside the tubercles in Anatolepis.

Thank you for this note. Yes, we refer to the same lamellar tissue in line 47 and figure 1. As presented in the introduction, we present the case that if Anatolepis was indeed a vertebrate it would then entangle, that both dentine and lamelline (bone-like tissue) originated in the Cambrian. To satisfy this concern, we removed the term lamelline from the sentence and replaced it with "bone" for clarity in line 63.

line 51: enamel or enameloid?

This line was changed to "Enameloid and enamel evolved later"

line 64: 'we first deployed high-resolution synchrotron tomography…'
I think it would be worthwhile, somewhere in the Results section, to more closely compare your results with the SEM images from Smith et al. For example, can you see peripheral tubules here, or is that something that only came out in your synchrotron scans?
Following this insightful comment, we reanalyzed the images from Smith et al.1996 comparing them to our data. What was noted as a 'concave edge' between the laminae and the tubercle in Smith et al. 1996 is now re-interpreted as a peripheral tubule in cross-section.

Two changes were made in the main text to reflect this novel comparison: 1. on Ln 68, we clarify that "These features were previously interpreted as a pulp cavity and dentine tubules, respectively in SEM images by Smith et al. 1996 (Smith et al., 1996)" and 2. Ln 76 "Notably, the peripheral tubules had been observed previously in SEM images but interpreted as the natural odontode edge."

line 66: 'homogenous lamellar basal layer (Fig.1)'
Smith et al had this layer alongside the tubercules- is this what you saw as well? Hard to tell in Figure 1.
Thank you for this important note. Yes, we recovered the lamellar layer alongside the tubercles, but never below. To better clarify this observation in our manuscript following this and Referee#3's comment, we included a supplemental figure (Figure S5) showing the laminae highlighting the basal layer in multiple specimens of *Aglaspid* and *Anatolepis.*

line 78: 'Aglaspidid cuticles have a homogenous lamellar structure'
Again, is this continuous beneath, or alongside?
This layer is placed alongside and between the tubercles. This was made clearer in both the results section and the additional supplementary figure (Figure S5)

For clarity, we have amended line 78 to "The tubercles vary subtly in morphology and are situated within the thin basal tissue, the tubercles extend beyond the basal tissue that is marked externally by several pore openings."

lines 81-82: 'However, this feature was not seen in all scanned aglaspidid cuticle samples'
Can you be more specific- what percentage of specimens were the canals and organs absent from? Did this depend on what part of the body the cuticle was taken from (you mention you took samples from complete specimens).
Thank you for this observation. We clarified and determined that cuticles from the telson (tail) regions where the horizontal canals seem to be absent, at least from our samples.

The line now reads, "However, this feature was not seen in all scanned aglaspidid cuticle samples and is notably absent from thicker cuticular regions such as the tailspine (Fig 1S, Fig S6)."

line 94: 'but most parsimoniously identified as an aglaspidid.'
It would be worth saying earlier that you took the sample from a claw because my thought here was, why isn't the aglaspid material vertebrate?
Thank you for this comment. We agree with the referee and have moved a mention earlier up in the paper.

Now line 81 reads "High-resolution phase contrast synchrotron scans of coeval aquatic aglaspidid arthropod cuticles**-- extracted from complete specimens--** were used as comparators to *Anatolepis*."

lines 107-108: 'histological microanatomy of the porcelain crab sensilla is most like that of aglaspidid and Anatolepis tubercles'
Did Anatolepis show the tube-in-tube morphology?
Unfortunately, the acid preparation of the *Anatolepis* specimens compromised much of the internal structure of the tubercles and we were unable to resolve the tube-in-tube morphology in the samples labeled as '*Anatolepis'*.

lines 122-123: 'remarkably large dentine tubules stemming from a pulp cavity which repeatedly branch and attenuate to the surface as pores'
Also in Ordovician chondrichthyan scales illustrated by Johanson et al. 2007 and Sansom et al. 1996?
Thank you for this insight. These references have now been included. The sentence was modified as follows "Notably, *Eriptychius* odontodes have remarkably large dentine tubules stemming from a pulp cavity which repeatedly branch and attenuate to the surface as pores as seen also in another Ordovician vertebrate from the Harding sandstone (Johanson et al., 2007; Sansom et al., 1996), similar to aglaspidids."

lines 131-132: 'crucial role in the development and function…'
I think the developmental aspect could be discussed further with respect to the sensory function, see comments below.
For clarification, this line was changed to "Enameloid and enamel evolved later"

line 133: 'tail odontodes'
Why did you chose these tail odontodes? Would odontodes on the head (particularly on the rostral region of the head, this being a particularly sensory part of the body) be more appropriate to test? Same goes for the odotontode sampled for Leucoraja.
Thank you for this comment. The tail odontodes are the first to develop in both cat shark and little skate. Therefore, for whole-mount scanning, using the smallest sample possible was optimal.

Line 137-138: 'nerves surrounding the base of odontodes in chondrichthyan'
Interesting that these nerves don't seem to invade the tail odontodes- Johanson et al. 2007 showed these had wide tubules, similar to the Ord. chondrichthyan scale I mentioned above (lines 122-123).
We thank the referee for this observation. We also thought that they would enter the odontodes, particularly in the 'dentine canals' as they are labeled in Johanson et al. 2007. However, our results did not show the nerves entering deep into the pulp cavity, potentially because those canals serve another purpose. However, determining that other purpose is beyond the scope of this project.

line 137-138: 'surrounding the base of odontodes in chondrichthyans and invading the pulp cavity in the pectoral fin in the catfish' but not running to surficial pores, so this would be different from the taxa above, (a criterion you used to say these were sensory).
Thank you for this point. Yes, we do not image nerves passing through to superficial pores. However, it is important to note that our own dentition is highly sensitive, but nerves do not run to superficial pores. Instead, there are intermediate TRP receptors that play a role in signal transduction from the odontoblast (which has a process to the surficial pore) to the nerve in or surrounding the pulp cavity. Additionally, dentine has the ability to be pain-sensitive and mechanosensitive with an enamel capping (Anderson, 1968; Hossain et al., 2019), but exposed dentine is much more sensitive (as we experience with cavities etc.). In

the main body of the paper, we relied on the cited works for this explanation, however we have now provided a small supplemental discussion section to discuss the state of the art on dentine sensitivity.

line 139-140: 'innervation associated with the dentine of odontodes is a primitive trait among extant gnathostomes' but it doesn't enter the chondrichthyan odontode, nor to surface pores?
Thank you for this note. The current cited literature documents that sensory nerve fibers can be located in the periodontal area outside the tooth proper and they mediate touch and pressure sensations as well as pain. We presume that the same is occurring in chondrichthyan odontodes. This is now explained more clearly in the supplementary discussion.

line 168-169: 'Therefore, we hypothesize that Eriptychius odontodes were more suited to sensory capability than those of Astraspis' But in the younger tubules of Astraspis, the cavity was more open suggesting a potential sensory function (line 167)? How are the tubules added/how does the bone grow?
Potentially, the *Astraspis* odontodes had some sensory function while younger, but regardless, it would not be as sensitive as *Eriptychius* due to the presence of a thick enameloid cap in the latter. The tubules are added inside the odontode as the odontode infills with dentine. This is typical for dentine and general tooth development. Bone growth in *Astraspis* and *Eriptychius* is an interesting and yet unsolved question due to the paucity of specimens. But it seems to grow centripetally as previously described in heterostracans. Bone and skeletal growth in these early vertebrates is the topic of a forthcoming study.

line 170: 'sensory function…'Could there also be a link between the innervation and tissue development, separate from the sensory function? This is well known in bone? Which came first- development or sensory function?
line 176: 'An ancestral sensory function of dentine…' Again, could a tissue development role have predated a sensory function?
We have added an extension to the discussion section in the supplement to better flesh out this argument more fully.

A link between dental tissue development and innervation has been shown in mouse models. Where the tooth germ initiates the proliferation of sensory nerves to the pulp cavity, not that sensory nerves have to be present as a prerequisite for tooth initiation. Additionally, there is substantial evidence that innervation does not influence tooth initiation and growth in mouse models, while in polyphyodonty fishes, the ablation of the trigeminal nerve causes the cessation of tooth replacement.

Figure 2g, h: what does the color blue indicate?
Thank you for this query. We have amended this figure to include a label for the top pores that were in blue.

Referee #2 (Remarks to the Author):

This manuscript raises the question of the sensitivity to the surrounding environment in the early vertebrates whose body was entirely covered with a dermal skeleton. The authors propose that the odontodes covering the dermal skeleton acted as sense organs, in the same way as we can feel a 'toothache'. This hypothesis had been proposed by Halstead in 1974, but soon left aside because of alleged presence of anamel cap that would prevent any sensory function of the odontocytes of the dentine. Orvig almost revived it by suggesting that, even in hypermineralised dentine, some dentine tubules could be prolonged to the surface of the enameloid, and thus possibly act as sensory receptors. The question became revisited by new findings of the earliest known vertebrate (or presumed so) odontodes. These come from the Ordovician astraspids and associated scale and armor remains from the Harding Sandstone of USA. Then early vertebrate palaeontologists tried and find even earlier evidence for vertebrate hard tissues, knowing that the living sister group of mineralizing vertebrates, that is, Cyclostomes, produces no mineralized skeleton. Before the discovery of the Cambrian myllokunmingiids and Metaspriggina, which are clearly non-mineralizing jawless vertebrates, the only hint was the conodonts, long disputed, but whose denticles are now recognized as potentially convergent at odontodes, and functionally comparable to the horny 'teeth' of modern cyclostomes. In addition, a number of phosphated 'scraps' turned up in the Ordovician and Cambrian of several sites of the Northern hemisphere and Australia and were assigned to possible vertebrate carapace fragments covered with odontodes, such as in Anatolepis. The interpretation of these remains raised vivid discussions, and some became regarded as arthropod remains, and thus dismissed, in spite of the advice of courageous supporters. Thanks to modern imaging techniques, these remains have been re-examined by the authors, who demonstrate here the lack of evidence for dentine tubules, and thus the probable support for the odonte interpretation. The authors provide strong support for the arthropod (aglapid) interpretation. The authors provide hereby a remarkable review of this question, and this may suggest that the rise of the duly recognized vertebrate odontodes may well have started like neurogenic placodes or taste buds. In addition, this review provides invaluable data on the structure and sensitivity potential of arthropod cuticle.
We thank referee #2 for their thoughtful summation of our findings and for highlighting the strong support for the arthropod interpretation of *'Anatolepis'*

Referee #3 (Remarks to the Author):

This is an interesting paper. The title promises insights on the origin of teeth and evolution of sensory systems in vertebrates but almost the entire paper is devoted to demonstrating why Anatolepis is NOT a vertebrate. Eliminating Anatolepis from the vertebrate record is important in terms of determining the origin and nature of tissues, particularly dentine, in the earliest odontodes and the authors provide a wealth of invaluable information on skeletal tissues from a wide range of taxa. However, only in the third paragraph from the end of the paper does the focus change to the sensory capability of early vertebrates such as Astraspis and Eriptychius and the authors might have taken more advantage of their new impressive illustrations of same.
We thank the referee for this note. The sensory hypothesis is introduced in the Abstract, introduction (line 57), and mentioned throughout the results (i.e., Lines 114, 124-128). Finally, three out of the five paragraphs of the discussion are dedicated to the sensory hypothesis.

There's obviously no argument about an extensive Cambrian record of arthropods, including aglaspidids and the like. The authors selected material of Aglaspis from the Cambrian of Wisconsin, a source of well preserved examples. In contrast, the interpretation of mineralized dermal fragments of Cambrian vertebrates is problematic but, given evidence of soft-bodied antecedents in exceptional preservations, the occurrence of

dermal armor in the Cambrian is perhaps not unreasonable. Tomographs of Anatolepis cuticle from the Wilberns Formation of Texas are illustrated for detailed comparison with samples of Aglaspis treated in the same way (Figure 1). The similarities are obvious but the method does not reveal the thin laminae that are a feature of arthropod cuticle in either Anatolepis or Aglaspis.
We thank the referee for pointing this out. The lamellar structure is labeled as "lm" in Figure 1. In addition, Referee 1 raised several comments and suggestions requiring additional description, and discussion of its exact orientation.

Due to these insights, we rescanned and reanalyzed several specimens. This resulted in the same image resolution, but sharper images, boosting the density contrast that we compiled into an additional supplementary figure **(Fig. S5)**, showing the lamellar structure in both *Anatolepis* and *Aglaspidids.*

Laminae in *Anatolepis* material have been shown in Smith et al. 1996. Additionally, Briggs and Fortey, 1982 clearly shows their presence in aglaspidids as well.

In addition, the thickness of the cuticle of Anatolepis is about 4x that of Aglaspis (measured adjacent to the tubercles) and there is the possibility that similarities may be a result of convergence.
We appreciate Referee #3 for alerting us to this issue; we reanalyzed the data and discovered an error relating to the scale bars of *Anatolepis* in Figure 1. The whole dataset with the correct scaling is on Morphosource and Figure 1 has been corrected according to this.

We do not think this convergence is based on the very specific morphology that was found between *Anatolepis* and aglaspidids. This morphology was not found in other samples and taxa, nor does it conform to vertebrate morphology.

The late Cambrian Wilberns Formation material may be arthropodan but I'd be wary of 'definitively aglaspidid' (line 23). Perhaps 'most parsimoniously identified as an aglaspid' is more accurate (line 94). Note, however, that Peel (1979, Ref. 8, p. 114), who ousted Anatolepis from the vertebrates 45 years ago (based on external morphology, not histology), was not prepared to assume that 'all the material in question is congeneric'. Some of the Cow Head specimens (Fortey et al. 1982), which the authors examined but do not figure, are somewhat similar morphologically to Bockelie and Fortey's (1976) original specimens from Spitsbergen where the 'tubercles', at least in some cases, are diamond-shaped blade-like structures unlike those on the Texas specimens. It would be interesting to know whether such specimens show similar internal features. But even if the affinities of Bockelie and Fortey's Anatolepis heintzi could be confirmed as vertebrate, it's early Ordovician in age, not Cambrian, so less of a problem for the odontode story.
Thank you for raising this concern. We added a supplementary figure **(Fig S6)** illustrating the Ordovician material from Cow Head formation. We show that other specimens, including those with "diamond-shaped tubercles" that have been termed *Anatolepis*, share some internal anatomy with the specimens of *Anatolepis* from Texas and with the Aglaspidid specimens from Wisconsin. Importantly, the Anatolepis specimens are more similar to each other and are strikingly different from elements termed "Phosphoannulus" from the same formation. This has been expanded on in the supplemental discussion section.

Additionally, we added a line to explain that the authors agree with Peel 1979 that not all specimens termed *'Anatolepis'* are congeneric. Importantly, the Cambrian specimens of '*Anatolepis'* sampled match those of Smith et al. 1996 and align temporally and anatomically with aglaspidid cuticle and not with vertebrate material.

References:

Anderson, D.J., 1968. Studies on sensory mechanisms in teeth. Ann. R. Coll. Surg. Engl. 43, 331–340.
Briggs, D.E.G., Fortey, R.A., 1982. The cuticle of the aglaspidid arthropods, a red-herring in the early history of the vertebrates. Lethaia 15, 25–29. https://doi.org/10.1111/j.1502-3931.1982.tb01969.x
Hossain, M., Bakri, M., Yahya, F., Ando, H., Unno, S., Kitagawa, J., 2019. The Role of Transient Receptor Potential (TRP) Channels in the Transduction of Dental Pain. Int. J. Mol. Sci. 20, 526. https://doi.org/10.3390/ijms20030526
Johanson, Z., Tanaka, M., Harriman, N., Meredith Smith, M., 2007. Early Palaeozoic dentine and patterned scales in the embryonic catshark tail. Biol. Lett. 4, 87–90. https://doi.org/10.1098/rsbl.2007.0502
Murdock, D., Dong, X., Repetski, J., Marone, F., Stampanoni, M., Donoghue, P., 2013. The origin of conodonts and of vertebrate mineralized skeletons. Nature 502. https://doi.org/10.1038/nature12645
Sansom, I.J., Smith, M.M., Smith, M.P., 1996. Scales of thelodont and shark-like fishes from the Ordovician of Colorado. Nature 379, 628–630. https://doi.org/10.1038/379628a0
Sansom, I.J., Smith, M.P., Armstrong, H.A., Smith, M.M., 1992. Presence of the Earliest Vertebrate Hard Tissues in Conodonts. Science 256, 1308–1311.
Smith, M.P., Sansom, I.J., Repetski, J.E., 1996. Histology of the first fish. Nature 380, 702–704. https://doi.org/10.1038/380702a0