doi:10.1038/nature12645

The origin of conodonts and of vertebrate mineralized skeletons

Duncan J. E. Murdock[1], Xi-Ping Dong[2,3], John E. Repetski[4], Federica Marone[5], Marco Stampanoni[5,6] & Philip C. J. Donoghue[1]

Conodonts are an extinct group of jawless vertebrates whose tooth-like elements are the earliest instance of a mineralized skeleton in the vertebrate lineage[1,2], inspiring the 'inside-out' hypothesis that teeth evolved independently of the vertebrate dermal skeleton and before the origin of jaws[3–6]. However, these propositions have been based on evidence from derived euconodonts. Here we test hypotheses of a paraconodont ancestry of euconodonts[7–11] using synchrotron radiation X-ray tomographic microscopy to characterize and compare the microstructure of morphologically similar euconodont and paraconodont elements. Paraconodonts exhibit a range of grades of structural differentiation, including tissues and a pattern of growth common to euconodont basal bodies. The different grades of structural differentiation exhibited by paraconodonts demonstrate the stepwise acquisition of euconodont characters, resolving debate over the relationship between these two groups. By implication, the putative homology of euconodont crown tissue and vertebrate enamel must be rejected as these tissues have evolved independently and convergently. Thus, the precise ontogenetic, structural and topological similarities between conodont elements and vertebrate odontodes appear to be a remarkable instance of convergence. The last common ancestor of conodonts and jawed vertebrates probably lacked mineralized skeletal tissues. The hypothesis that teeth evolved before jaws and the inside-out hypothesis of dental evolution must be rejected; teeth seem to have evolved through the extension of odontogenic competence from the external dermis to internal epithelium soon after the origin of jaws.

The soft tissue anatomy of euconodonts substantiates their vertebrate affinity[12,13], but homology of euconodont and vertebrate skeletal tissues[1,14,15] remains the subject of controversy[16,17]. The mineralized skeleton of euconodonts consists of an oropharyngeal array of tooth-like elements that are composed of two mineralized structural elements, the crown and basal body which are comprised of tissues that resemble enamel and dentine[8]. Euconodont elements grew through centrifugal appositional growth, with laminae in the crown and basal body added in synchrony, in a manner comparable to enamel and dentine in the teeth of jawed vertebrates. However, knowledge of conodont skeletal tissues is based largely on extremely derived euconodonts and hypotheses of homology to canonical vertebrate skeletal tissues have taken no account of the evolutionary origin of the conodont skeleton. Based principally on similarities in morphology and patterns of growth, an evolutionary series was proposed originally among protoconodonts, paraconodonts and euconodonts[7–11]. Protoconodonts have been recognized subsequently as stem-chaetognaths[18] and excluded from euconodont ancestry, but the hypothesis that euconodonts are derived paraconodonts remains[10,11]. Paraconodont elements are unipart, and have been considered

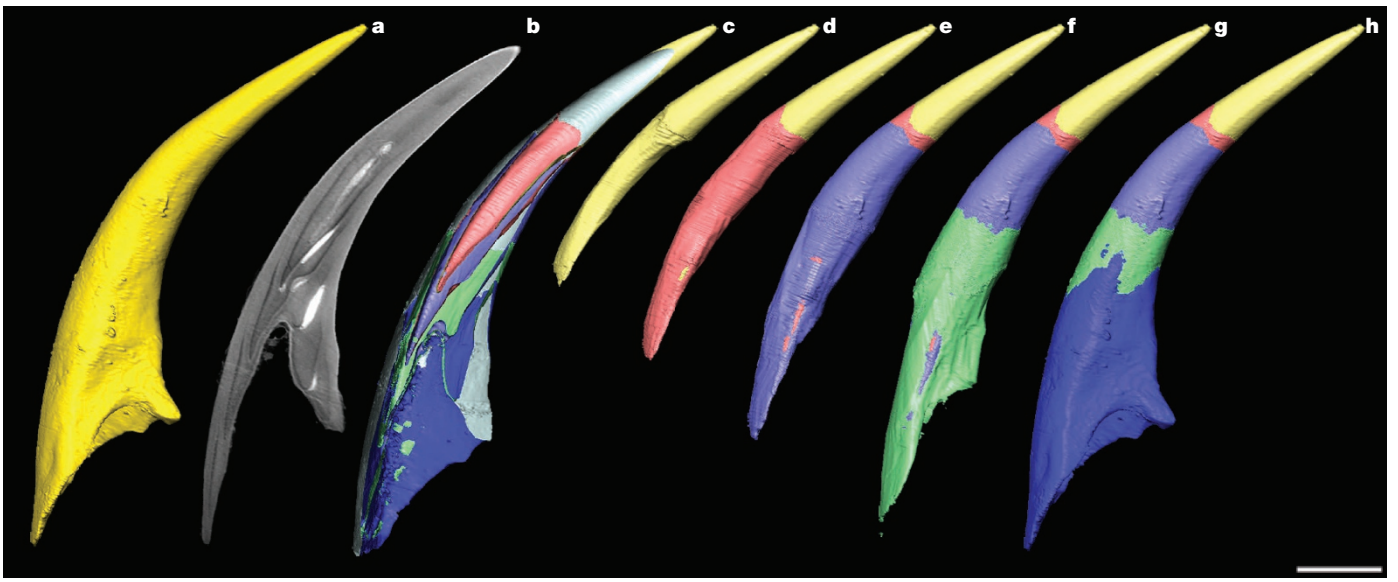


Figure 1 | Element growth and microstructure of the paraconodont *Furnishina*, Threadgill Creek section, Wilberns Formation, central Texas, 1,115 feet above base of Cambrian strata. a–c, The complete element has been subdivided into a number of discrete growth stages delimited by lines showing cessation of growth (**b**, **c**). **d–h**, Initial growth stage, protoelement (**d**), is not enveloped by subsequent growth lamellae, rather lamellae are added to the proximal and lateral margins of the protoelement only (**e–h**). Scale bar, 50 μm.

[1]School of Earth Sciences, University of Bristol, Wills Memorial Building, Queen's Road, Bristol BS8 1RJ, UK. [2]School of Earth and Space Science, Peking University, Beijing 100871, China. [3]State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, Nanjing 210008, China. [4]US Geological Survey, MS 926A, National Center, Reston, Virginia 20192, USA. [5]Swiss Light Source, Paul Scherrer Institut, Villigen 5232, Switzerland. [6]Institute for Biomedical Engineering, University of Zürich and ETH Zürich, Rämistrasse, Zürich 8006, Switzerland.

©2013 Macmillan Publishers Limited. All rights reserved

homologous to the euconodont basal body alone because they grew through apposition of lamella layers to the proximal surface only. However, the histological comparisons of protoconodont and euconodont elements have been vague and aspects of paraconodont element structure and growth remain equivocal. For example, the homology of protoconodont elements and euconodont element basal bodies has been rejected on the basis that a basal body may not be primitive for euconodonts, and therefore could not be homologous to any paraconodont tissues[19,20]. Key to the interpretation of paraconodont morphogenesis is the nature of the earliest stages of growth, or 'protoelement', which forms the distal-most part of the element. If characterized by complete centrifugal growth, this would result in a protoelement stage reminiscent of a euconodont crown plus basal body[8]. By contrast, addition of lamellae to the proximal surface only (that is, basal internal accretion) would result in a morphology reminiscent of the euconodont basal body alone. However, the evidential basis of this characterization has been criticized by some as an analytical artefact[7,10,11]. We used synchrotron radiation X-ray tomographic microscopy (SRXTM) to characterize the element structure of paraconodonts and early euconodonts, non-invasively and at sub-micron resolution. We used the ensuing datasets to characterize the component tissues and uncover the pattern of development recorded in the sclerochronology of the growth arrest lines preserved in the mineralized tissues.

Based on the observed diversity of preserved structure we were able to divide paraconodont elements into three grades, each distinguished by the degree of tissue differentiation. Elements of *Furnishina* sp. exemplify the simplest grade of paraconodont elements. It consists of a single tissue type that exhibits punctuated incremental growth lines which define hollow conical laminae extending around the entire proximal margin and partly around the antapical margins (Fig. 1). Lamellae are oblique to the outer surface of the element and they do not extend over the distal tip, that is, the 'protoelement' is not enveloped by successive laminae (unlike the results in ref. 8). The basal cavity is not evident in the earliest laminae, rather developing in the latter stages; its depth is determined by the ontogenetic stage of development, for example, in elements of *Prooneotodus* sp., in earlier growth lamellae, resulting in a deeper basal cavity (Extended Data Fig. 1). The second grade of paraconodont element organization that we recognize is characterized by elements of *Problematoconites* sp., which is comprised of two tissues that have been identified previously as a distinct 'basal cone' and 'cone-filling'[21]. As in elements of *Furnishina*, the distal part of the element is formed of conical laminae, (basal cone of ref. 21). The proximal part of the element is formed from subsequent laminae extending across the entire proximal surface (cone-filling of ref. 21), forming a series of sub-parallel laminae—extensions of the laminae that comprise the rest of the element (Fig. 2). This is consistent with the model of a single secreting layer, (unlike the results in ref. 21). In our third grade of paracondont element organization, exemplified by elements of *Rotundoconus tricarinatus* (Extended Data Fig. 2a), there are three principal tissue layers. The outermost layer consists of tapering rings that do not extend fully over the outer surface nor are they continuous over the proximal surface. These outer layers are bordered on the inside of the proximal surface by subparallel lamellae; it is unclear whether or not they converge at the apex. Finally, the basal cavity is filled with spheritic mineralization.

All euconodont elements exhibit a clear distinction between basal body tissue and crown tissue (for a guide to terminology see Extended Data Fig. 4). In the earliest euconodont elements, the basal body is indistinguishable from the most derived paraconodont elements. Following initial mineralization of the 'primordial element' subsequent laminae are added to the proximal margins. The basal body is differentiated into two tissue layers, distal hollow conical laminae and subparallel laminae across the proximal surface. These are formed from a single secreting layer (unlike the results in ref. 21). The crown tissue forms a cap over the entire surface of the basal body, thickening towards an enlarged cusp (Fig. 3). The relative size of the crown

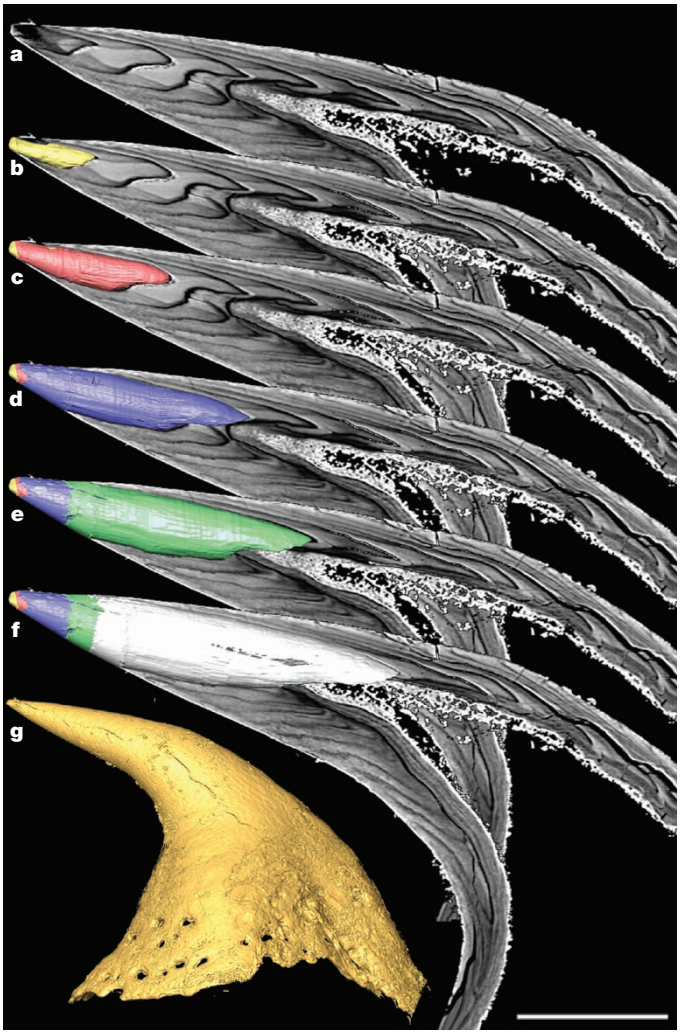


Figure 2 | Element growth and microstructure of the paraconodont *Problematoconites*, Windfall Formation, Tremadocian, Ordovician, Eureka County, Nevada, USA. a–g, Close-up of distal part of the cusp which has been subdivided into a number of discrete growth stages delimited by lines showing cessation of growth (**b**–**f**), with SRXTM rendering of complete element in the same orientation (**g**). Initial growth stage, protoelement, is not enveloped by subsequent growth lamellae, rather lamellae are added to the proximal and lateral margins of the protoelement only. Note the growth lamellae are continuous across the entire basal and margins of the element, not separated into basal cone and cone-filling (unlike the results in ref. 21). Scale bar represents 100 μm (**a**–**f**); 266 μm (**g**).

compared to the basal body is dictated simply by the degree to which the laminae of the crown extend beyond the distal tip of the basal body (compare elements of *Proconodontus serratus*; Extended Data Fig. 3, and *Proconodontus posterocostatus*; Fig. 3). White matter may be present in the crown (for example, in the posterior keel of the cusp of *P. serratus*; Extended Data Fig. 3). Other euconodont taxa retain the distinct three-layer structure of derived paraconodonts, for example, elements of *Granatodontus* sp. The entire element wall is thin and the basal cavity is deep (Extended Data Fig. 2b). A thin crown layer extends over the outer surface of the element, however, the basal body consists of two different tissues; a lamellar layer with sub-parallel lamellae surrounding a poorly defined porous tissue layer (Extended Data Fig. 2b).

Homology of the paraconodont element and the euconodont basal body was first proposed on the basis of simple observations of similarity in morphology and growth[7,8,10,11]. However, these similarities have been insufficient to discriminate convergence from common descent. Our evidence reveals much greater complexity and differentiation in

©2013 Macmillan Publishers Limited. All rights reserved

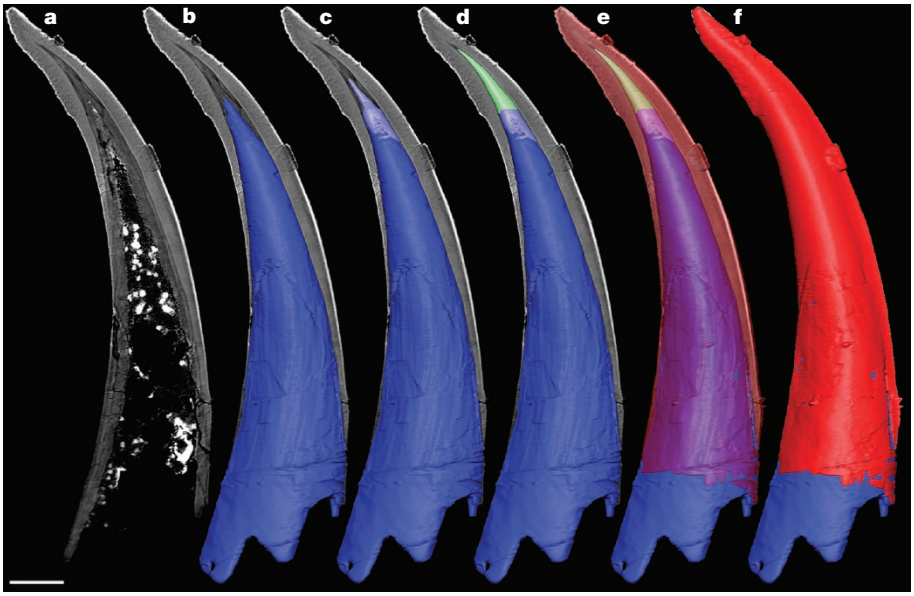


Figure 3 | Element growth of the euconodont *Proconodontus posterocostatus*, Gros Ventre Formation, Late Cambrian, Bighorn Mountains, Wyoming, USA. a, Longitudinal section showing delimitation of element into crown and basal body. **b–f**, SRXTM renderings of the initial two growth layers of basal body and the relationship between the crown (red) and basal body (blue, purple, green). The growth of the basal body continues as in elements of the paraconodont *Furnishina*, but with addition of crown tissue. Scale bar, 50 μm.

the structure and growth of paraconodont elements than has been described previously, corroborating this hypothesis of homology. First, the protoelement of both the paraconodont element and euconodont element basal body is not overgrown at the distal tip. Rather, it is permanently exposed at the tip of protoconodont elements and remains in direct contact with the crown at the core of euconodont elements. Subsequent to the initial mineralization of the protoelement, the ontogeny of both paraconodont elements and the basal bodies of euconodont elements follow the same pattern of growth. The development of structural diversity exhibited by conodont elements is dictated simply by the relative timing of changes in the mode of secretion and, ultimately, through the differentiation of two principal structural elements, the basal body and crown, the latter characterizing the first euconodont elements. The basal cone and cone-filling structure described previously in euconodont basal bodies[21] is manifest also in protoconodont elements, though we show that these are not separate structures and the growth lamellae are continuous between them. Crucially, the range of structures exhibited by the elements of different paraconodont species lie within nested sets of structural complexity, the most complex of which exhibit greater similarity to euconodont elements than other paraconodonts. Indeed, in terms of structure and arrangement of the component tissues, the basal bodies of elements of the early euconodont *Proconodontus* are effectively indistinguishable from the most complex paraconodont elements, such as those of *Problematoconites*. The same comparison can be made of the paraconodont *Rotundoconus* and the euconodont *Granatodontus*.

Direct comparison of ontogeny and tissue organization, coupled with a clear spectrum of complexity through early conodont elements, demonstrates that the similarities between paraconodont and euconodont elements go beyond analogy. Our results corroborate the hypothesis that the structural organization of the euconodont element was not only derived through the evolution of the enamel-like crown tissue from a paraconodont-grade ancestor, but also that characteristics of the euconodont basal body were assembled stepwise among different evolutionary grades of paraconodonts (Fig. 4). Evidently, the proposition of homology between euconodont crown tissue and vertebrate enamel[1,15,22,23] fails a test of phylogenetic congruence[24] and must therefore be rejected. In this light, it is pertinent to question the proposed homology of euconodont basal tissue and vertebrate dentine since this is based largely on the topological and developmental relationship of euconodont basal tissue with crown tissue[14]. Among other early skeletonizing vertebrates, dentine is encountered only in the dermal skeleton, and it appears secondarily and convergently in the pharyngeal and oral cavities of the jawless thelodonts[25] and early jawed vertebrates[26]. Therefore there is no potential homologue of paraconodont elements in other total group gnathostomes. Thus, while it appears that conodonts afford the earliest manifestation of a mineralized skeleton in vertebrates, this skeleton evolved independently of other skeletonizing vertebrates. Although there is a remarkable similarity between euconodont elements and the odontodes of vertebrate scales and teeth, which extends from details of tissue microstructure through to the topological and developmental relationship among these tissues[14,27], it now appears to be a remarkable instance of evolutionary convergence. Euconodonts were influential in the hypothesis that teeth evolved before jaws and the 'inside-out' hypothesis in which dental evolution is independent of the tooth-like 'odontode' structures associated with external dermal scales[3,4,6]. This view now lacks any evidential basis and must be rejected; teeth appear to have evolved through the

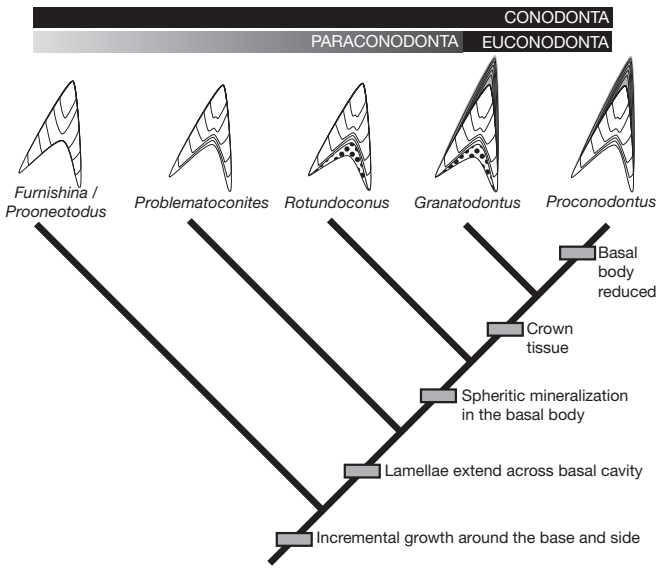


Figure 4 | Proposed phylogenetic hypothesis for the relationship between paraconodonts and euconodonts, and the evolution of conodont skeletal characters. Euconodonts are derived from a paraphyletic assemblage of paraconodonts that exhibit increasing basal body complexity, but are differentiated by the acquisition of the crown. Thus, the euconodont crown cannot be a homologue of vertebrate enamel.

©2013 Macmillan Publishers Limited. All rights reserved

extension of odontogenic competence from the external dermis to internal epithelium soon after the origin of jaws[26].

METHODS SUMMARY

We compared well-preserved, morphologically similar, paraconodont and euconodont elements from Middle Cambrian to Lower Ordovician age deposits; TC 1115, *Furnishina* sp. from Threadgill Creek section, Wilberns Formation, central Texas, 1,115 feet above base of Cambrian strata; USNM 593438, 593439 and 593440, *Prooneotodus* sp., *Problematoconites* sp., and *Proconodontus serratus* from the *Cambrooistodus* subzone of the *Eoconodontus* zone of the Windfall Formation, Tremadocian, Ordovician, Eureka County, Nevada, USA; Lapworth Museum of Geology BU4421 *Proconodontus posterocostatus* from Gros Ventre Formation, Late Cambrian, Bighorn mountains, Wyoming, USA; GMPKU3068, *Rotundoconus tricarinatus* from *Cordylodus intermedius* Zone, Furongian (Upper Cambrian), Panjiazui Formation, Wa'ergang section, Wa'ergangvillage, Taoyuan County, Hunan Province, China; USNM 521006, *Granatodontus* sp. from Steptoe South section, Whipple Cave Formation, uppermost Cambrian, northern Egan Range, White Pine County, Nevada, USA. Specimens were mounted on 3-mm brass stubs using clear nail varnish and volumetrically characterized using SRXTM[28]. Measurements were taken using ×10 and ×20 objective lenses at 10–15 keV. For each data set, 1,501 projections over 180 degrees were acquired, resulting in volumetric data with voxel sizes of 0.74 and 0.36 μm, respectively. These experiments were performed on the TOMCAT beamline[29] at the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland. Figures were prepared using the VSG software Avizo (v6.4–7.1). Discrete growth stages or tissues, delimited by lines showing cessation of growth, were identified in the SRXTM slice data and individually labelled. These labels were then used to generate a three-dimensional surface representing the extent of an individual growth stage or tissue. Successive growth stages are distinguished by (arbitrary) colours.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 24 July; accepted 10 September 2013.
Published online 16 October 2013.

1. Sansom, I. J., Smith, M. P., Armstrong, H. A. & Smith, M. M. Presence of the earliest vertebrate hard tissues in conodonts. *Science* **256,** 1308–1311 (1992).
2. Donoghue, P. C. J. & Sansom, I. J. Origin and early evolution of vertebrate skeletonization. *Microsc. Res. Tech.* **59,** 352–372 (2002).
3. Smith, M. M. & Coates, M. I. Evolutionary origins of the vertebrate dentition: phylogenetic patterns and developmental evolution. *Eur. J. Oral Sci.* **106** (suppl. 1), 482–500 (1998).
4. Smith, M. M. & Coates, M. I. in *Development, function and evolution of teeth* (eds Teaford M. F., Ferguson M. W. J., & Smith M. M.) 133–151 (Cambridge Univ. Press, 2000).
5. Smith, M. M. & Coates, M. I. in *Major events of early vertebrate evolution* (ed. Ahlberg P. E.) 223–240 (Taylor & Francis, 2001).
6. Fraser, G. J., Cerny, R., Soukup, V., Bronner-Fraser, M. & Streelman, J. T. The odontode explosion: the origin of tooth-like structures in vertebrates. *Bioessays* **32,** 808–817 (2010).
7. Bengtson, S. Structure of some Middle Cambrian conodonts, and early evolution of conodont structure and function. *Lethaia* **9,** 185–206 (1976).
8. Müller, K. J. & Nogami, Y. Über den Feinbau der Conodonten. *Memoirs of the Faculty of Science, Kyoto University, Series of Geology and Mineralogy* **38,** 1–87 (1971).
9. Müller, K. J. & Nogami, Y. Growth and function of conodonts in *Proceedings of the 24th International Geological Congress* 20–27 (Montreal, 1972).
10. Szaniawski, H. in *Palaeobiology of Conodonts* (ed. Aldridge R. J.) 35–47 (Ellis Horwood, 1987).
11. Szaniawski, H. & Bengtson, S. Origin of euconodont elements. *J. Paleontol.* **67,** 640–654 (1993).
12. Aldridge, R. J., Briggs, D. E. G., Smith, M. P., Clarkson, E. N. K. & Clark, N. D. L. The anatomy of conodonts. *Phil. Trans. R. Soc. Lond. B* **340,** 405–421 (1993).
13. Pridmore, P. A., Barwick, R. E. & Nicoll, R. S. Soft anatomy and the affinities of conodonts. *Lethaia* **29,** 317–328 (1997).
14. Donoghue, P. C. J. Growth and patterning in the conodont skeleton. *Phil. Trans. R. Soc. Lond. B* **353,** 633–666 (1998).
15. Sansom, I. J., Smith, M. P. & Smith, M. M. Dentine in conodonts. *Nature* **368,** 591 (1994).
16. Blieck, A. *et al.* Fossils, histology, and phylogeny: why conodonts are not vertebrates. *Episodes* **33,** 234–241 (2010).
17. Turner, S. *et al.* False teeth: conodont-vertebrate phylogenetic relationships revisited. *Geodiversitas* **32,** 545–594 (2010).
18. Szaniawski, H. Chaetognath grasping spines recognized among Cambrian protoconodonts. *J. Paleontol.* **56,** 806–810 (1982).
19. Dzik, J. Remarks on the evolution of Ordovician conodonts. *Acta Palaeontol. Pol.* **21,** 395–453 (1976).
20. Dzik, J. in *Problematic Fossil Taxa* (eds Hoffman A. & Nitecki M. H.) 240–254 (Oxford Univ. Press, 1986).
21. Gross, W. Uber die basis der Conodonten. *Palaeont. Zeits.* **31,** 78–91 (1957).
22. Donoghue, P. C. J. Microstructural variation in conodont enamel is a functional adaptation. *Proc. R. Soc. Lond. B* **268,** 1691–1698 (2001).
23. Donoghue, P. C. J., Purnell, M. A. & Aldridge, R. J. Conodont anatomy, chordate phylogeny and vertebrate classification. *Lethaia* **31,** 211–219 (1998).
24. Patterson, C. in *Problems of Phylogenetic Reconstruction. Systematics Association Special Volume* Vol. 29 (eds Joysey K. A. & Friday A. E.) 21–74 (Academic Press, 1982).
25. Rücklin, M., Giles, S., Janvier, P. & Donoghue, P. C. J. Teeth before jaws? Comparative analysis of the structure and development of the external and internal scales in the extinct jawless vertebrate *Loganellia scotica. Evol. Dev.* **13,** 523–532 (2011).
26. Rücklin, M. *et al.* Development of teeth and jaws in the earliest jawed vertebrates. *Nature* **491,** 748–751 (2012).
27. Donoghue, P. C. J. & Aldridge, R. J. in *Major events in early vertebrate evolution: palaeontology, phylogeny, genetics and development* (ed. Ahlberg P. E.) 85–105 (Taylor & Francis, 2001).
28. Donoghue, P. C. J. *et al.* Synchrotron X-ray tomographic microscopy of fossil embryos. *Nature* **442,** 680–683 (2006).
29. Stampanoni, M. *et al.* Trends in synchrotron-based tomographic imaging: the SLS experience. *Proc. SPIE* **6318,** 63180M (2006).

Acknowledgements The SRXTM experiments were performed on the TOMCAT beamline at the Swiss Light Source, Paul Scherrer Institut (Villigen, Switzerland), funded through a project awarded to P.C.J.D. and S. Bengtson (Stockholm). NERC grant NE/G016623/1 to P.C.J.D., a studentship to DJEM funded by NERC and the Paul Scherrer Institut, and NSFC Project 41372015 to X.-P.D. Thanks to R. Stamm (USGS) for reviewing a draft of this manuscript; and thanks to J. E. Cunningham, D. O. Jones and M. Rücklin for assistance at the beamline. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

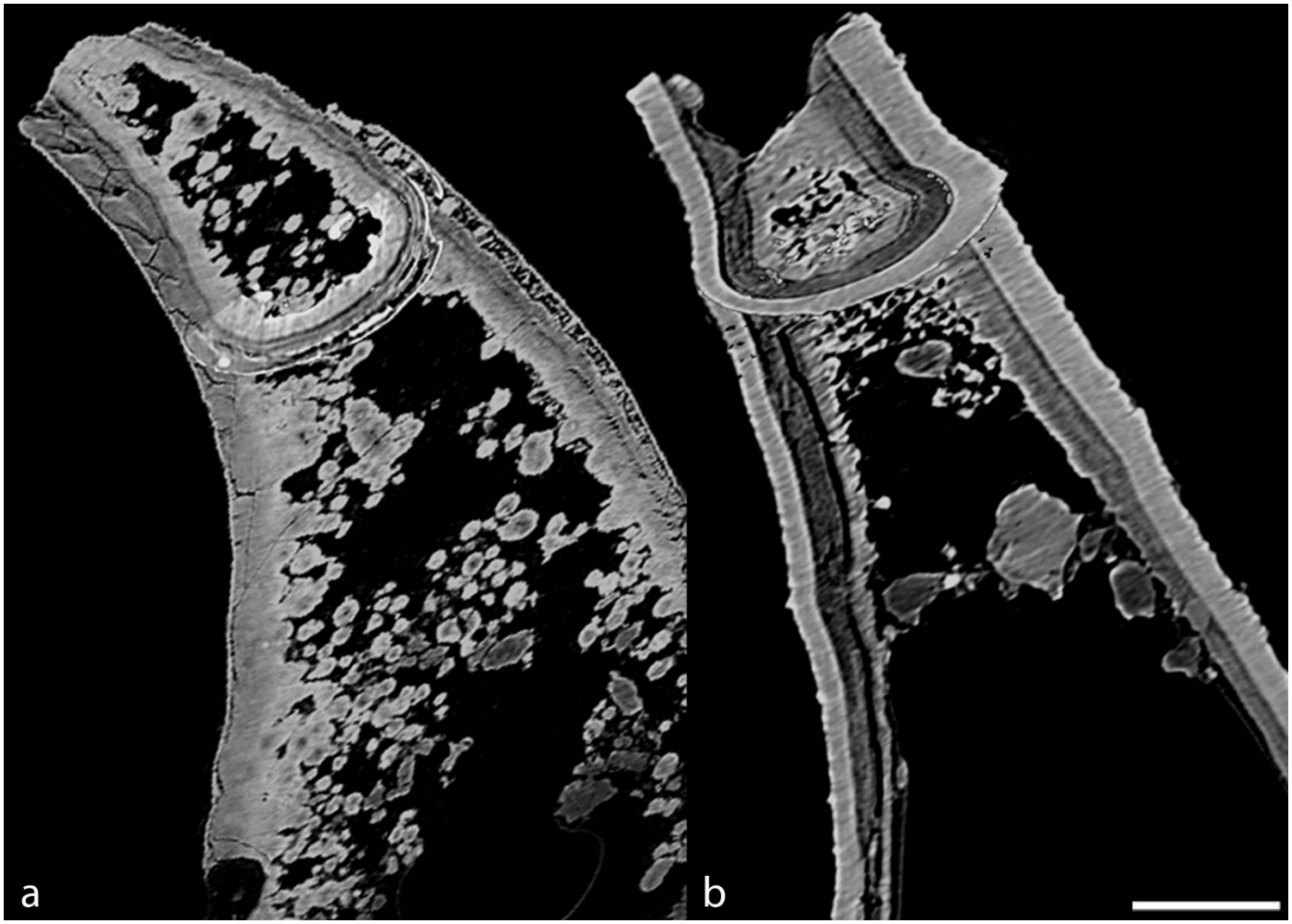
Author Contributions D.J.E.M. and P.C.J.D. conceived and designed the research; D.J.E.M., F.M. and M.S. collected the SRXTM data; J.E.R. and X.-P.D. provided material and taxonomic information; D.J.E.M. analysed the data, prepared the figures and wrote the paper with substantive edits from P.C.J.D. and minor edits from the remaining authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to P.C.J.D. (phil.donoghue@bristol.ac.uk).

©2013 Macmillan Publishers Limited. All rights reserved

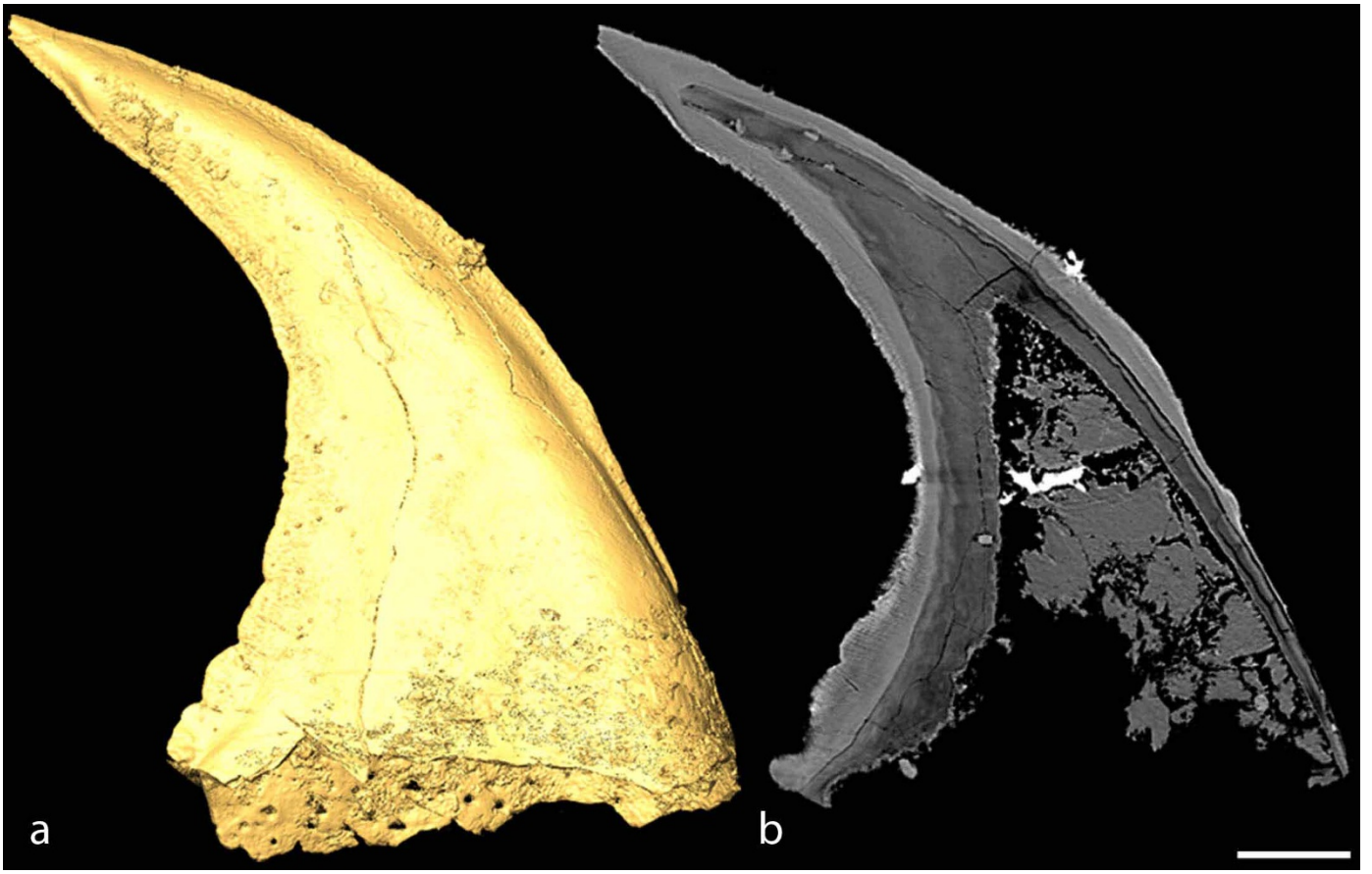
Extended Data Figure 1 | Growth of the paraconodont elements *Prooneotodus*, Windfall Formation, Tremadocian, Ordovician, Eureka County, Nevada, USA. **a**, **d**, **e**, Initial two growth stages highlighted using SRXTM rendering. **b**, **c**, Longitudinal sections through the element showing successive lines of cessation of growth. Note the protoelement is not engulfed by subsequent growth lamellae and basal cavity begins to develop in the second set of lamellae. Scale bar represents 75 μm (**a**, **b**); 50 μm (**c**–**e**).

©2013 Macmillan Publishers Limited. All rights reserved



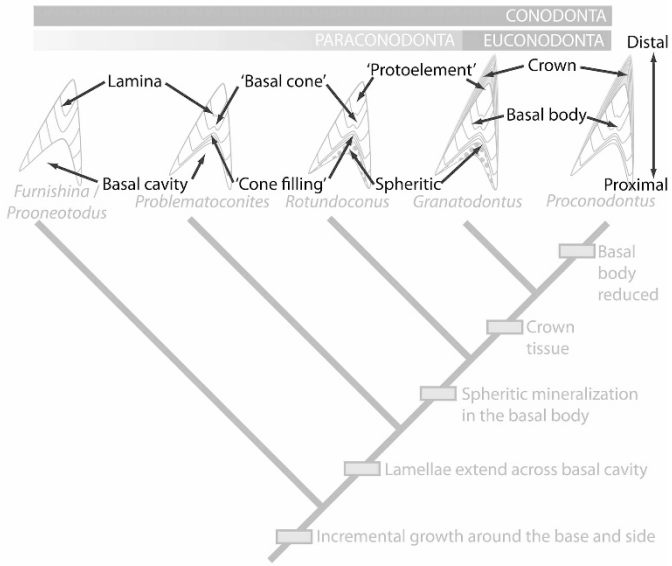
Extended Data Figure 2 | Comparison of the internal structure of the elements of the paraconodont *Rotundoconus tricarinatus* and the euconodont *Granatodontus* sp. **a**, *R. tricarinatus* from the *Cordylodus intermedius* Zone, Furongian (upper Cambrian), Panjiazui Formation, Wa'ergang section, Wa'ergangvillage, Taoyuan County, Hunan Province, China Steptoe South section. **b**, *Granatodontus* sp. from the Whipple Cave Formation, uppermost Cambrian, northern Egan Range, White Pine County, Nevada, USA. Longitudinal and orthogonal sections generated from SRXTM data. In elements of *R. tricarinatus,* wall consists of three layers, the outermost tapering rings that do not extend fully over outer surface nor are continuous over basal surface. In elements of *Granatodontus,* a thin crown extends over the outer surface of the element, basal body consists of a lamellar layer with sub-parallel lamellae surrounding a poorly defined porous tissue layer. Scale bar represents 50 μm (**a**); 30 μm (**b**).

©2013 Macmillan Publishers Limited. All rights reserved



Extended Data Figure 3 | *Proconodontus serratus*, Windfall Formation, Tremadocian, Ordovician, Eureka County, Nevada, USA. a, **b**, SRXTM rendering of external morphology (**a**) and lateral aspect of internal structure (**b**) of an element of the euconodont *Proconodontus serratus*. Note distinction of tissues into crown and basal body. Scale bar represents 100 μm.

©2013 Macmillan Publishers Limited. All rights reserved



Extended Data Figure 4 | Descriptive terminology of paraconodont and euconodont elements. Labels are superimposed over the proposed phylogenetic hypothesis for the relationship between paraconodonts and euconodonts, and the evolution of conodont skeletal characters. Euconodonts are derived from a paraphyletic assemblage of paraconodonts that exhibit increasing basal body complexity, but are differentiated by the acquisition of the crown. Thus, the euconodont crown cannot be a homologue of vertebrate enamel.

©2013 Macmillan Publishers Limited. All rights reserved