

Development of teeth and jaws in the earliest jawed vertebrates

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Teeth and jaws constitute a model of the evolutionary developmental biology concept of modularity¹ and they have been considered the key innovations underpinning a classic example of adaptive radiation². However, their evolutionary origins are much debated. Placoderms comprise an extinct sister clade³ or grade^{4,5} to the clade containing chondrichthyans and osteichthyans, and although they clearly possess jaws, previous studies have suggested that they lack teeth^{6–8}, that they possess convergently evolved tooth-like structures^{9–11} or that they possess true teeth¹². Here we use synchrotron radiation X-ray tomographic microscopy (SRXTM)¹³ of a developmental series of *Compagopiscis croucheri* (Arthrodira) to show that placoderm jaws are composed of distinct cartilages and gnathal ossifications in both jaws, and a dermal element in the lower jaw. The gnathal ossification is a composite of distinct teeth that developed in succession, polarized along three distinct vectors, comparable to tooth families. The teeth are composed of dentine and bone, and show a distinct pulp cavity that is infilled centripetally as development proceeds. This pattern is repeated in other placoderms, but differs from the structure and development of tooth-like structures in the postbranchial lamina and dermal skeleton of *Compagopiscis* and other placoderms. We interpret this evidence to indicate that *Compagopiscis* and other arthrodirans possessed teeth, but that tooth and jaw development was not developmentally or structurally integrated in placoderms. Teeth did not evolve convergently among the extant and extinct classes of early jawed vertebrates but, rather, successional teeth evolved within the gnathostome stem-lineage soon after the origin of jaws. The chimaeric developmental origin of this model of modularity reflects the distinct evolutionary origins of teeth and of component elements of the jaws.

Possible scenarios for the evolutionary origin of teeth and jaws have been influenced heavily by chondrichthyans, in which teeth develop within a deep dental lamina, producing files of replacements performed long ahead of their functional deployment⁶. However, chondrichthyans are not primitive jawed vertebrates⁴ but, along with osteichthyans, represent crown-group gnathostomes. Therefore, the pattern of tooth development and replacement in living chondrichthyans does not necessarily reflect the nature of the earliest jawed vertebrates. The extinct placoderms are the most primitive jawed vertebrates known, comprising either a monophyletic sister lineage to crown gnathostomes³ (Fig. 1a) or, more persuasively, a primitive grade of jawed vertebrates that includes a succession of sister lineages to crown gnathostomes^{4,5} (Fig. 1b). As such, placoderms are crucial to resolving the early evolution of teeth and jaws.

The nature of the dentition in placoderms has been the subject of debate that can be reduced to semantic differences, with some authors advocating a structural diagnosis that identifies teeth in placoderms^{11,12} and others adhering to the use of developmental criteria that preclude the presence of teeth in placoderms^{6–8}. Intuitively, developmental definitions cannot be applied to fossil material, but the

pattern of skeletal development is preserved in the sclerochronology of growth-arrest lines and the polarity of cell lacunae and canaliculi in mineralised skeletal tissues. So far, however, understanding of placoderm jaw and dental development has been limited largely to inference from surface morphology⁷, with only one direct study of development¹². This is because traditionally analyses have been destructive. We used SRXTM¹³ to study jaw, dental and dermal skeletal development in ontogenetic stages of the arthrodiran *Compagopiscis croucheri*, selected because it is known from abundant articulated three-dimensional remains; the smallest and largest specimens known were included in our study.

The lower jaw of *Compagopiscis* is comprised of the infragnathal that rests on the Meckel's cartilage which is ossified only at its proximal (articular) and distal (mentomeckelian) extremes (Supplementary Figs 1 and 2). Tomographic data reveal that the infragnathal is composed of two principal ossifications, the bony shaft of the jaw (axial ossification) and a distal compound dental ossification (Fig. 2). We digitally extracted successive stages of growth in the infragnathal, revealing the sequence of development of the dental ossification (Figs 2d, e, g, h and 3). Growth proceeded through the addition of cusps, proximally, distally and lingually relative to a primordial cusp. Initial addition consists of single cusps and subsequent cusps are associated with the growth of an increasingly expansive sheet of tissue that in later developmental stages extends ventrally around the bony shaft of the infragnathal and, in the largest infragnathal, partially around the Meckel's cartilage (Figs 2 and 3). In most cases, these sheets of tissue are continuous from the proximal to distal rows of cusps (Fig. 3), indicating coordinated growth. More cusps are added to the proximal row than to the distal and lingual rows during each growth episode. However, the

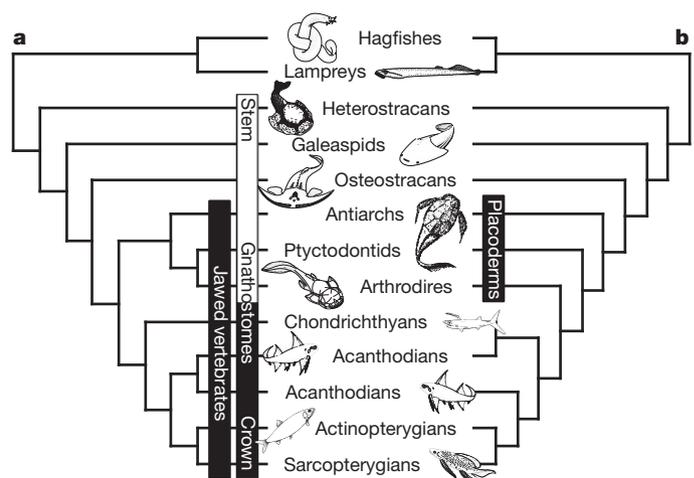


Figure 1 | Evolutionary relationships of principal groups of vertebrates. a, b, Phylogenetic relationships among the principal groups of stem and crown gnathostomes. The traditional view of placoderm monophyly^{27,28} (a) versus the more recent hypothesis of placoderm paraphyly⁴ (b).

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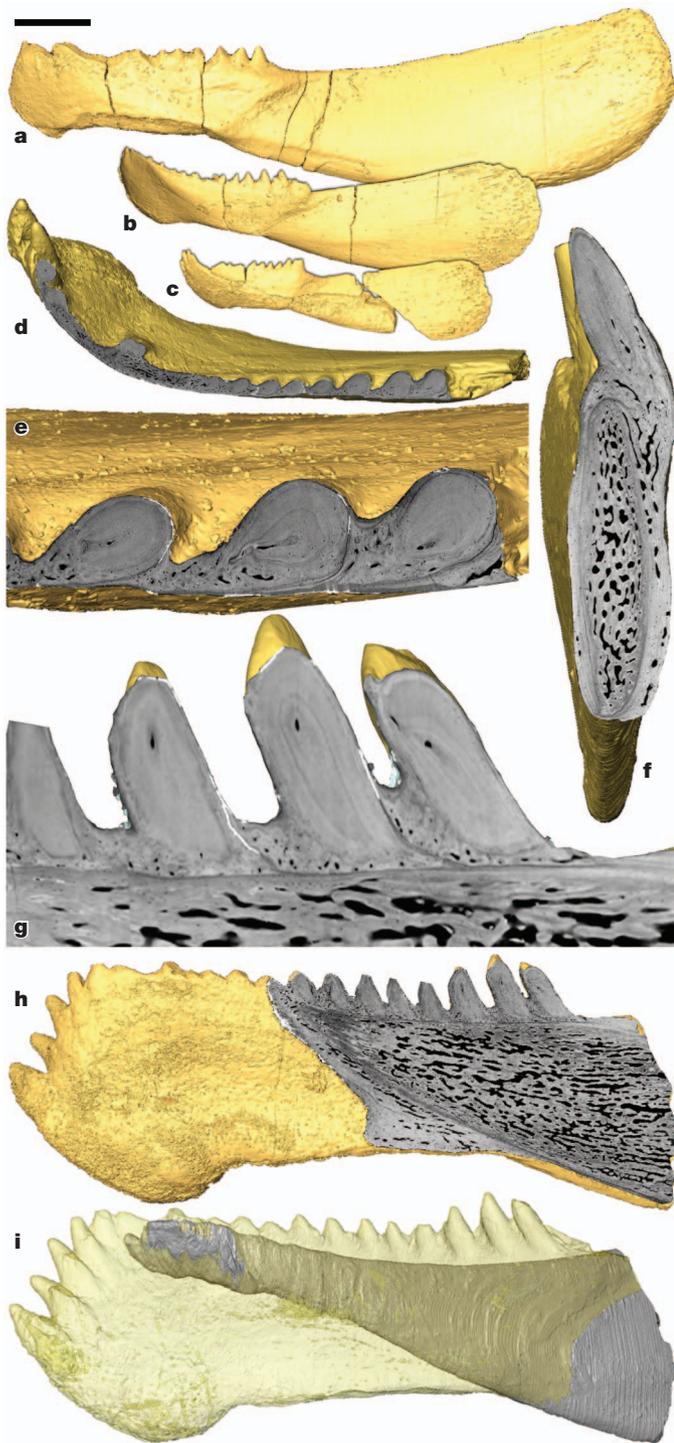


Figure 2 | Lower-jaw element of *Compagopiscis croucheri*, Late Devonian period, Australia. a–c, microCT data; d–i, SRXTM data. Volume rendering of jaws and teeth (a–c, i) and surface cut (d–h). Ontogenetic sequence in lateral view (a) NHMUK PV P.50948. (b) NHMUK PV P.50943 and WAM 91.4.3 (c). Teeth and jaw ossifications WAM 91.4.3: horizontal section (d, e), vertical section (f), longitudinal section (g, h) and labelled sclerochronology as virtual dental ossification (transparent) and bony shaft (shaded, i). Scale bar in a represents 2 mm (a–c), 1 mm (d, h, i), 240 μ m (f), 400 μ m (e, g).

individual cusps are clearly successional (not synchronous) even within each growth episode and tooth row (Figs 2d–g and 3). The cusps are composed of dentine; they have distinct pulp cavities lined with centripetally nested tissue layers permeated by radially arranged and polarized canaliculi, but not cell lacunae (Figs 2e, g and 4a). The pulp cavity of each cusp is infilled progressively through ontogeny

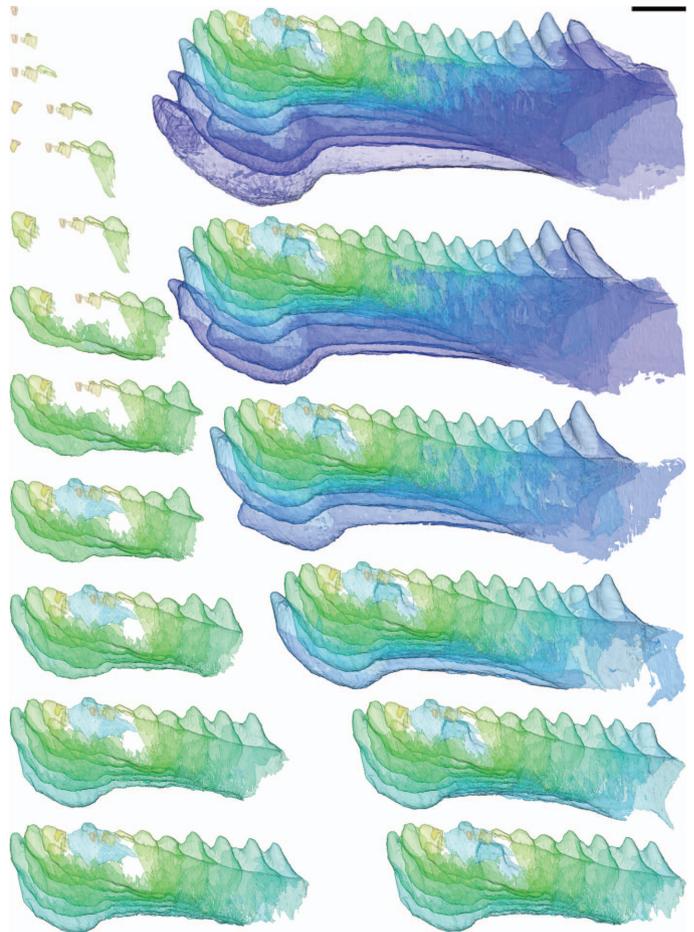


Figure 3 | Virtual development of a *Compagopiscis croucheri* lower jaw, Late Devonian period, Australia. Labelled sclerochronology in the dental element of the lower jaw of WAM 91.4.3. Subsequent ontogenetic stages follow from top left to top right in an anti-clockwise direction. Darker shades represent the addition of developmental stages of teeth and sheets of tissue. Scale bar, 1 mm.

such that the earliest formed cusps are completely infilled. The structure and pattern of development of the supragnathals (Fig. 4a), which attach directly to the roof of the oral cavity, are comparable to the dental ossification of the infragnathal.

The pattern of development of the dental ossification in *Compagopiscis* is compatible with observations made in other arthrodiran placoderms. For example, we can corroborate the identification of distinct dental and axial ossifications comprising the infragnathal of *Plourdosteus*, as well as the coordination of proximal and distal cusp development that was inferred but could not be observed in the same study¹². However, we find no evidence for the widespread resorption and remodelling in *Compagopiscis* that was inferred for *Plourdosteus*¹². An axial and dental ossification, along with sequential cusp development in a proximal row, is present in the infragnathal of buchanoosteids (Fig. 4b), which are distantly related arthrodires¹⁴, indicating that these characteristics are primitive features of arthrodires. Distinct gnathal and dermal jaw ossifications seem to be primitive for placoderms more generally, as a cusp-bearing capping structure occurs associated with a proximal axial ossification in rhenanids¹⁵. An equivalent of the dermal axial ossification in arthrodires occurs also in antiarchs (Fig. 4c), in which the secondary absence of dentine cusps from the infragnathal mirrors their secondary absence from the dermal skeleton¹⁶. However, it may alternatively reflect a primitive absence of teeth deep within the paraphyletic placoderm grade of stem-gnathostomes^{4,5}.

The structure of the individual cusps and their pattern of sequential development within the gnathal elements in *Compagopiscis* and other

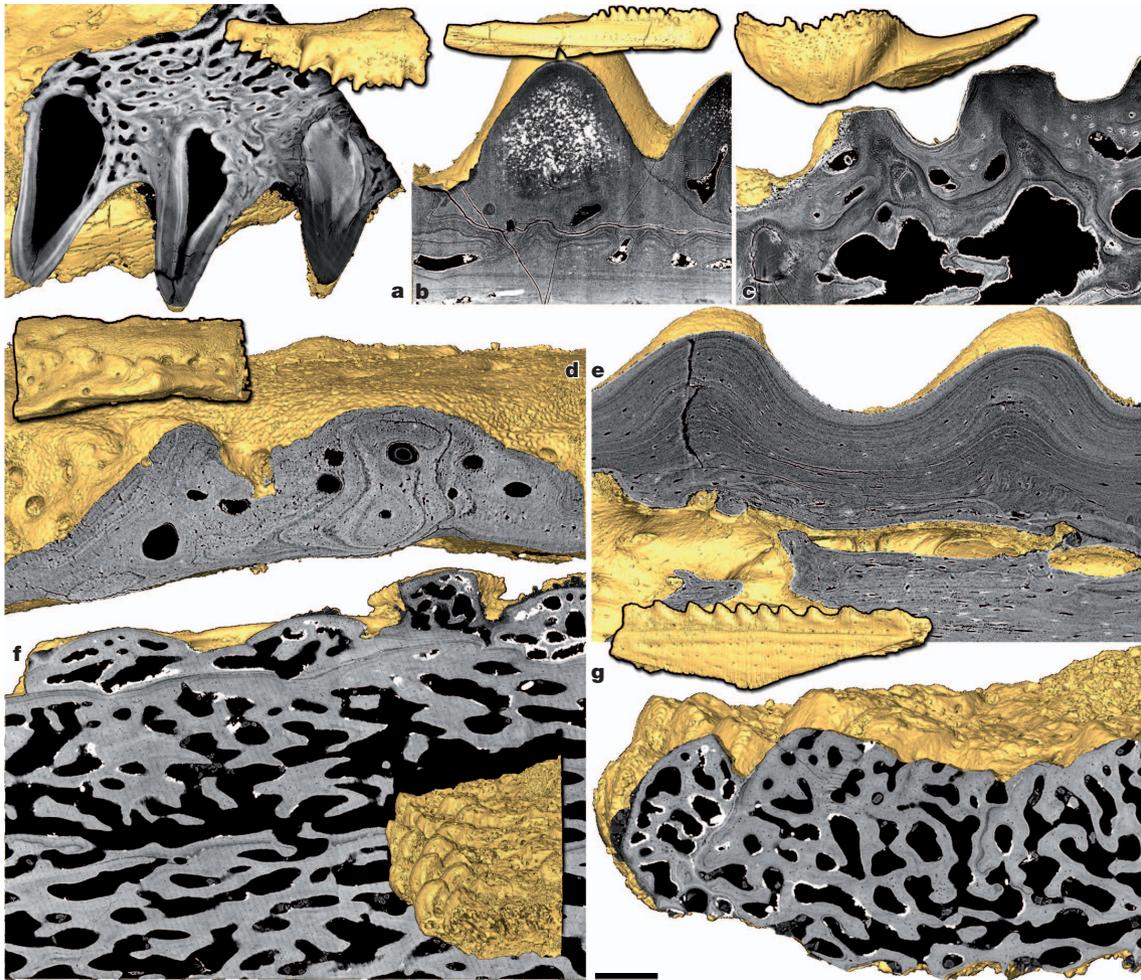


Figure 4 | Histological comparison of the *Compagopiscis croucheri* cusps on jaws, dermal bone and postbranchial lamina, with the jaws and pectoral fins of the antiarch *Bothriolepis* species, both Late Devonian, Australia, and the jaw of a buchanosteid arthrodire, Early Devonian, Saudi Arabia. a–g, Volume rendering of microtomography data (small insets; a, b), volume rendering of SRXTM data (small insets; c–f), surface-cuts showing SRXTM images (large insets; d, e, g) and virtual thin sections of SRXTM images (a–c, f). Cusps on jaws of *Compagopiscis croucheri*, proximal upper jaw (posterior supragnathal) National History Museum London (NHMUK) PV P.57629, small

inset NHMUK PV P.50943 (a), buchanosteid arthrodire, lower jaw, Muséum National d'Histoire Naturelle Paris MNHN.F.ARB 239 (b) and *Bothriolepis* sp. lower jaw NHMUK PV P.50898 (c). Tubercles on dermal plate (marginal) of *Compagopiscis croucheri* NHMUK PV P.50945 (d), marginal cusps on pectoral fin of *Bothriolepis* sp. NHMUK PV P.50898 (e) and tooth-like cusps on postbranchial lamina of *Compagopiscis croucheri* NHMUK PV P.5255.6 (f, g). Scale bar in g represents 500 μm (a, small inset f), 200 μm (g, small inset a), 285 μm (b), 5 mm (small inset b), 167 μm (c–e), 2 mm (small inset c), 400 μm (small inset d), 333 μm (small inset e), 143 μm (f).

placoderms, support comparisons to gnathostome teeth that have been made previously based on surface morphology^{9–11}. This interpretation has been contested on the grounds that tooth-like cusps are not diagnostic of teeth, evidenced by the fact that even in placoderms such as *Compagopiscis*, comparable tooth-like cusps also occur in association with the cranial dermal skeleton^{3,7,8}. To test this comparison we examined the structure and development of the dermal skeleton. The *Compagopiscis* dermal skeleton is similar to that of other placoderms that have been investigated¹⁶, in that they are composed of a basal division of lamellar bone, a middle division of cancellar bone, and in the superficial division of compact bone with surface tubercles. However, unlike the morphogenetically distinct cusps associated with the gnathals, the superficial tubercles of the dermal skeleton are revealed to be focal developments of continuous sheets of bone that are morphogenetically integrated with the underlying dermoskeleton (Fig. 4d). Tooth-like structures associated with the dermal pectoral fin spines of antiarch placoderms could be an exception; when isolated, these structures can be mistaken for complete jaws⁸ (Fig. 4e). However, our analysis reveals that the tooth-like margin of the pectoral spines is again characteristic of the dermoskeleton, comprised collectively of continuous sheets of bone, not from morphogenetically distinct

elements as in the gnathals (Figs 2 and 4a, b). Evidently, structural objections to the identification of teeth in placoderms are unfounded.

It has been suggested that the placoderm dentition fails to meet the definition of a gnathostome tooth because there is no evidence that they develop from a deep and continuous dental lamina^{3,6,8}. However, living jawed vertebrates show great diversity in dental development, with teeth developing in deep or shallow positions, from continuous to discrete epithelial pockets, that persist through life or atrophy and develop anew^{17–19}; the plesiomorphic conditions for jawed vertebrates and crown gnathostomes are unclear. The discrete teeth in *Compagopiscis* and other placoderms developed in a shallow position like those of many living osteichthyans. The key distinction in placoderms is that the dentition is statodont, that is, teeth are not resorbed, shed and subsequently replaced. In this sense, the placoderm dentition is most similar to that of ischnacanthid acanthodians²⁰, holocephalans²¹ and lungfish²², in which teeth develop through marginal apposition to a compound dental plate. The functional limitation of this approach to development is that worn teeth cannot be replaced. The innovation of crown gnathostomes is site-specific tooth replacement.

It has been suggested that successional tooth homologues are present also on the postbranchial wall of placoderms, including

Compagopiscis^{9,10}, coopted convergently to the jaw among different lineages of primitive gnathostomes to serve a tooth function^{9,10}. The cusps are arranged into columns and rows reminiscent of the tooth families of extant chondrichthyans^{9,10}, but the hypothesis of sequential development is an inference based on surface morphology. Our data refute this hypothesis, given that in the dermoskeleton, the rows of tooth-like cusps that occur on the postbranchial wall are simple focal developments of continuous sheets of spongy bone, added episodically to the growing margin of the postbranchial wall (Fig. 4f, g).

Our evidence indicates that teeth are present even in the earliest jawed vertebrates and that within the phylogenetic context of placoderm paraphyly^{4,5} they can be identified as homologous to the teeth of crown gnathostomes. This contrasts with the hypothesis that teeth were absent from the earliest jawed vertebrates, evolved convergently through cooption of oral and pharyngeal denticles^{9–11}. Indeed, our tomographic analyses show that the putative tooth-like pattern of placoderm pharyngeal denticle replacement bears no resemblance to that of their teeth, except in superficial morphology. Thus, the hypothesis of a distinct evolutionary origin of teeth and dermal denticles^{9,10,23} can be rejected, as jawless stem-gnathostomes have been shown to lack homologous dental patterning²⁴ and the assertion of a fundamental embryological distinction between external and oral denticles has been refuted²⁵. Ultimately, teeth and other oral and pharyngeal denticles must be derived through the extension of the odontogenic capacity of the external dermis to the internal dermis and endoderm. However, tooth- and jaw-structure and development is evidently less integrated within the placoderm grade of early jawed-vertebrate evolution than in derived osteichthyans in which teeth, tooth development and jaw structure are intimately interwoven, as part of the process of site-specific tooth replacement. Upper and lower dental ossifications occur in placoderms, but there is no clear homologue of the osteichthyan dentary or coronoid. However, the axial ossification of the infragnathal can be compared to the inner dental arcade of early osteichthyans based on its location relative to the underlying Meckel's cartilage, overlying dental ossification and lateral muscle attachment. *Compagopiscis* and other placoderms evidence an early stage in jawed vertebrate evolution in which the components of the mandible were fewer and more obviously distinct than they are in osteichthyan evolutionary and developmental model organisms. Some processes associated with these more derived taxa, such as tooth resorption (as a necessary precursor to tooth replacement), are absent in placoderms. This stepwise acquisition reflects the fact that character complexes like the gnathostome jaw have been assembled over a protracted episode of evolutionary history and so the modular construction of the mandible¹, for example, reflects the disparate evolutionary origins of its component modules.

METHODS SUMMARY

Museum repositories: National History Museum London (NHMUK); Western Australian Museum (WAM); Muséum National d'Histoire Naturelle Paris (MNHN). The material was scanned using a SkyScan 1172 Micro-CT scanner at the University of Bristol, and using the TOMCAT beamline²⁶ of the Swiss Light Source (SLS), Paul Scherrer Institut, Switzerland. Slice data were analysed and manipulated using Avizo 6.3 (<http://www.vsg3d.com>).

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions M.R., P.C.J.D. and Z.J. conceived the project. K.T. acid-prepared WAM specimens. M.R., P.C.J.D., F.M. and M.S. collected the data. M.R. analysed the data. All authors contributed to the interpretation of the data and the writing of the manuscript.

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 M.R. (m.ruecklin@bristol.ac.uk) and P.C.J.D. (phil.donoghue@bristol.ac.uk).

METHODS

The fossil material used in this study is housed in the National History Museum London, the Western Australian Museum (WAM) and the Muséum National d'Histoire Naturelle Paris (MNHN). It is comprised of specimens of *Compagopiscis croucheri* and *Bothriolepis* species from the Frasnian, Late-Devonian-period Gogo Formation of Western Australia and a bichanosteid arthrodire from the Emsian, Early Devonian, Jawf Formation of Saudi Arabia. The material is acid prepared and was scanned using the SkyScan 1172 microtomography scanner at the University of Bristol (Figs 2a–c, and 4a, b; small insets) and synchrotron radiation X-ray tomographic microscopy (SRXTM)¹³, at the TOMCAT beamline²⁶ of the Swiss Light Source (SLS), Paul Scherrer Institut, Switzerland (Figs 2d–i, 3 and 4). Using a $\times 10$ objective exposure time at 25 keV was 1100 ms, and 3,001 projections were acquired equi-angularly over 360° with the rotation axis positioned at the border of the field of view. In this way it has been possible to almost double the width of the field of view offered by the $\times 10$ objective while preserving spatial resolution (Figs 2d–i and 3). Projections were post-processed and rearranged into flat- and dark-field-corrected sinograms, and reconstruction was performed on a 60-core Linux PC farm, using a highly optimized routine based on the Fourier transform method and a regridding procedure²⁹. The resulting volume, obtained by vertically stacking four tomograms, consisted of $6,645 \times 3,082 \times 3,659$ voxels, with isotropic dimensions of 0.74 μm . The other

SRXTM experiments followed a standard acquisition approach with the rotation axis located in the middle of the field of view and the acquisition of 1,501 projections equi-angularly distributed over 180°. For the larger specimens, a $\times 4$ objective (resulting pixel size = 1.85 μm) and an energy of 20 keV (Fig. 4a, c, e) or 30 keV (Fig. 4f, g) were used. Smaller samples were scanned using a $\times 10$ objective (resulting pixel size = 0.74 μm) at either 20 keV (Fig. 4c), 21.5 keV (Fig. 4d) or 30 keV (Fig. 4b). The microtomography experiments of the largest complete specimens were 360° scans with resulting pixel size of 5 μm at 37 kV (Fig. 2b, c and small inlets of Fig. 4a), 65 kV (Fig. 2a) and 75 kV (small inlet of Fig. 4b). Slice data were analysed and manipulated using Avizo 6.3 (<http://www.vsg3d.com>). Sectional images and 'virtual thin sections' were created using maximum intensity projections (MIP; the voltex module in Avizo), which simulates the casting of light rays from preset sources through a volume of data. Three-dimensional models of the different growth stages were derived by labelling manually the sclerochronology as slightly different grey-scale volumes.

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