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Acanthodian dental development and the origin of gnathostome dentitions

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Chondrichthyan dentitions are conventionally interpreted to reflect the ancestral gnathostome condition but interpretations of osteichthyan dental evolution in this light have proved unsuccessful, perhaps because chondrichthyan dentitions are equally specialized, or else evolved independently. Ischnacanthid acanthodians are stem-Chondrichthyes; as phylogenetic intermediates of osteichthyans and crown-chondrichthyans, the nature of their enigmatic dentition may inform homology and the ancestral gnathostome condition. Here we show that ischnacanthid marginal dentitions were statodont, composed of multicuspidate teeth added in distally diverging rows and through proximal superpositional replacement, while their symphyseal tooth whorls are comparable to chondrichthyan and osteichthyan counterparts. Ancestral state estimation indicates the presence of oral tubercles on the jaws of the gnathostome crown-ancestor; tooth whorls or tooth rows evolved independently in placoderms, osteichthyans, ischnacanthids, other acanthodians and crown-chondrichthyans. Crown-chondrichthyan dentitions are derived relative to the gnathostome crown-ancestor, which possessed a simple dentition and lacked a permanent dental lamina, which evolved independently in Chondrichthyes and Osteichthyes.

he dentitions of most modern chondrichthyans (Elasmobranchii, the sharks and rays) are organized into files of replacement teeth arrayed side-by-side along the jaw. The simplicity of this conveyor-belt system has long been interpreted to reflect the ancestral condition for the dentitions of jawed vertebrates and theories of dental developmental evolution have invariably attempted to rationalize the dentitions of osteichthyans on such a model^{1,2}. Critical to this model is the presence of a permanent dental lamina along the jaw that is responsible for tooth development and replacement, which can be observed in living jawed vertebrates³⁻⁶ and inferred from evidence of comparable tooth replacement patterns in extinct relatives. However, the first fossil evidence of crown-chondrichthyan divergence is from the end-Middle Devonian, later than the first crown-osteichthyans which are late Silurian7. Furthermore, recent fossil discoveries have decisively overturned the view that chondrichthyan morphology is representative of the ancestral gnathostome condition^{8,9}. The extinct acanthodians are recognized as a paraphyletic lineage of stem chondrichthyans⁸ and, as phylogenetic intermediates of the crown-chrondrichthyans and osteichthyans, they have the potential to inform the nature of the dentition in the ancestral crown-gnathostome and, indeed, to address the question of whether it possessed a dentition at all¹⁰. Acanthodians exhibit variation in their dentitions, from acanthodids and diplacanthids lacking teeth entirely, to climatiid dentitions comprised wholly of statodont tooth whorls, to ischnacanthids possessing symphyseal tooth whorls, a marginal dentition^{11,12} and tooth-like scales around the jaw margins^{13,14}. Here we focus on the nature of the dentition in ischnacanthids, which manifest the diversity of dentitions seen in dentate acanthodians.

The development of the marginal dentition of ischnacanthids has been interpreted on the basis of its external morphology^{12,15}, broken surfaces¹⁶ and a few traditional destructive studies, for example^{14,17}. These data have led to divergent interpretations of the development of the marginal dentition (and therefore its homologies). It has been argued that acanthodian marginal dentitions were shed and replaced in toto¹⁸ or that they grew episodically with the teeth developing as continuous projections of the underlying bony plate¹⁹. Confirming either of these hypotheses would reveal tooth development mechanisms without parallel in other gnathostomes, thereby expanding our knowledge of the disparity of early dentitions. A third hypothesis is that each of the cusps (regardless of size) represent distinct teeth that were added sequentially, extending the tooth row distally²⁰. This invites comparisons with arthrodire placoderms (and, to a lesser extent, osteichthyans), raising the possibility that such dentitions are ancestral for gnathostomes as a whole, or that similar dentitions appeared multiple times through convergent evolution. To discriminate among these interpretations we used synchrotron X-ray tomographic microscopy (SRXTM)²¹ to study the structure and infer the development of the marginal and symphyseal dentitions of ischnacanthid acanthodians based on exceptionally well preserved material from the Lower Devonian (Lochkovian) of the Prince of Wales Island in Arctic Canada.

Acanthodian jaws consist of paired upper palatoquadrate cartilages and paired lower Meckel's cartilages that were only rarely ossified (perichondrally) and thus preserved (for example, Fig. 1a). The marginal dentition of ischnacanthids is associated with the oral side of these upper and lower jaw cartilages and comprises a more or less extensively developed ossification, including oral tubercles. The tubercles are organized into two or more rows that diverge distally at about 20° within a horizontal plane, the first approximately parallel to the jaw margin and the second extending lingually in a distal direction (relative to the jaw joint, Fig. 1c,d,f). A ridge occurs between the rows of tubercles, increasing in prominence distally (Fig. 2c,d).

Tomographic data demonstrate that these oral, tooth-like tubercles developed separately from the bony base to which they

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are ankylosed (see ref. ¹⁶). The bony base is comprised of cancellar cellular bone exhibiting frequent spheritic mineralization (Fig. 1d,e), overlying a layer of compact lamellar bone. Each of the overlying tubercles has a prominent conical central cusp and a number of smaller accessory cusplets (Fig. 1c,d; 2c); these increase in size distally. A thin (5–70 μ m) surface layer of highly attenuating hypermineralized tissue that we interpret as enameloid extends across the large central cusp and the smaller marginal cusplets, evidence of their formation as a single morphogenetic unit (Fig. 1i), rather than as separately developing tubercles (see ref. ¹²). The tubercles are otherwise composed of dentine with tubuli extending from a large central pulp-cavity to near the tubercle surface, into the hypermineralized enameloid layer (Fig. 1i). The tubercles in the marginal dentition are therefore compositionally, developmentally and topologically compatible with teeth.

The overlapping relationships of the teeth, delimited by growth arrest lines, allow the development of the dentition to be reconstructed (Fig. 1d,f, 2, 3; Extended Data Fig. 1). The teeth were added sequentially along a proximal to distal vector within each row, as revealed by their overlapping relationship, with each tooth added onto the distal margin of the predecessor. This proximal to distal sequence is also evidenced by the differing degree to which the pulp cavities have been infilled by centripetal layers of dentine. Teeth within the lateral row are overlapped marginally by teeth within the lingual row (Fig. 1g), indicating that the lateral teeth developed earlier and more distally. This arrangement breaks down proximally where teeth exhibit considerable wear and are replaced through superpositional apposition, though they cannot be assigned to any particular row with confidence (Fig. 1f,h, 2, 3; Extended Data Fig. 1). We find no evidence for tooth resorption and our data allow us to reject hypotheses that (1) the dentigerous jawbones of ischnacanthids were episodically shed and replaced in toto¹⁸, (2) the teeth developed episodically as elaborations of the underlying bone¹⁹, and (3) the possibility that each cusp and cusplet constitutes a developmental unit distinct from the principal cusp²⁰.

The structure of the tooth whorls is quite distinct, comprised of monocuspid conical teeth that project from a concave oval base and exhibit an ordered increase in height and width lingually. One row of teeth and paired marginal teeth may occur within any one tooth whorl, reducing in height laterally (Fig. 4). The teeth are distinct from the underlying bony base, which is composed of a layer of cancellar bone on a thin base of compact lamellar bone (Fig. 4c). Each tooth is composed largely of dentine surrounding a central pulp cavity and a thin $(10-50 \,\mu\text{m})$ hypermineralized capping layer, that we interpret as enameloid and that does not extend to encompass adjacent (successional and marginal) teeth (Fig. 4b). Cancellar bone attaches each tooth to the bony base and the lingual margin of

Fig. 1 | Jaw bones and marginal dentition of ischnacanthid acanthodians. a, Mandible of Ischnacanthus kingi lateral view of complete specimen in rock from the National History Museum, London (NHMUK), specimen NHMUK PV P.15362. b,c, Mandible of ischnacanthid acanthodian from the Naturhistoriska Riksmuseet, Stockholm (NRM), specimen NRM-PZ P. 9449 (lateral view in **b** and dorsal view in **c**) of complete ossified bone and teeth. d,e, Detailed lateral view showing the tooth addition in the lateral row, indicated by arrows (d), and detail of the spheritic mineralizations (e). f, Detailed dorsal view with teeth separated by growth arrest line, indicated by arrows. g, The distal-most tooth of the lingual row overlaps a tooth within the lateral row. **h**, Overgrowth of teeth at the centre of ossification and initial sequential addition, indicated by arrow. i, The largest and last-added medial tooth, showing a hypermineralized layer, which we interpret as enameloid, forming the proximal ridge and the smaller marginal cusplets and dentine infilling the pulp cavity. Scale bar in a represents 4.3 mm in **a**, 270 μ m in **b**, **c**, 107 μ m in **d**, 61 μ m in **e**, 156 μ m in **f**, 200 μ m in g, 21 µm in h and 50 µm in i.



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Fig. 2 | Surface and reconstructed growth of marginal tooth rows on an ischnacanthid acanthodian jawbone. Jawbone NRM-PZ P. 9449 Early Devonian, Canada. **a,b**, Lateral view of the surface (**a**) and reconstructed addition of teeth (**b**). **c,d**, Occlusal view of the surface (**c**) and reconstructed addition of teeth (**d**). The colours of the nested boxes reflect the successive stages of tooth development. Scale bar represents 220 µm; prox, proximal; dist, distal; ling, lingual; lab, labial.

the preceding tooth. Successive teeth are distinguished by a growth arrest line, indicating that the largest teeth were added last (Fig. 4b). There is no evidence of apposition of the tooth and its underlying bony base, indicating that the two developed synchronously. A network of vascular canals connects the teeth and the dental pulp cavities exhibit a polarized pattern of infilling, with the earliest (oldest) being completely infilled (Fig. 4b).

Ischnacanthid tooth whorls are comparable to the tooth families of living chondrichthyans, to which they have long been compared, but they are even more similar to the statodont tooth whorls of other acanthodian stem-chondrichthyans (for example, *Climatius* and *Ptomacanthus*) and the symphyseal tooth whorls of stem- and early crown-osteichthyans (for example, *Onychodus*)^{22,23}, which also possess a unifying bony base²⁴ and multiple rows of cusps. However, osteichthyan tooth whorls exhibit distinct growth of the teeth and bony base²³.

The marginal dentitions of ischnacanthids find no counterpart in living chondrichthyans in terms of their association with an ossified mandibular plate, their pattern of addition along the jaw rather than across it, or their pattern of dental replacement. They may be compared to the marginal dentitions of arthrodiran placoderms²⁵ and osteichthyans (for example, *Onychodus* and *Moythomasia*) in being arranged in marginal rows. In contrast to arthrodiran placoderms and osteichthyans in which tooth addition occurs in both a proximal and distal direction^{26,27}, the ischnacanthid marginal dentition shows only distal extension of the tooth rows. Our data show evidence of tooth replacement at the proximal end of the row but, unlike in osteichthyans, this occurs superpositionally and without resorption in ischnacanthids. Thus, although the tooth whorls of dentate acanthodians support inference of a permanent dental lamina, the pattern of superpositional replacement in the dentigerous jaw bones is incompatible with tooth development within a permanent dental lamina, similarly inferred for stem-osteichthyans^{25,28}.

Inferring the nature of the ancestral crown-gnathostome dentition requires resolution of homology among diverse gnathostome dentitions, including those of ischnacanthids. This is a question not merely of structural and developmental similarity, but of phylogenetic congruence²⁹, which is complicated by the uncertainty of phylogenetic relationships among early gnathostomes and acanthodians, in particular. Accounting for this uncertainty, we estimated ancestral states for dental characteristics on the posterior distribution trees from a tip-dated Bayesian analysis of early

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Fig. 3 | Virtual development of teeth on an ischnacanthid acanthodian jawbone. Marginal tooth rows of NRM-PZ P. 9449 Early Devonian, Canada. **a,b**, Labelled sclerochronology of the teeth in possible sequence of addition in oral (**a**) and labial view (**b**). Colours of the nested boxes reflect the successive stages of tooth development. Scale bar represents 150 µm; prox, proximal; dist, distal; ling, lingual; lab, labial. Arrow indicates the sequence of addition.

gnathostome relationships (Figs. 5 and 6). This recovered strong support for the presence of oral tubercles on jaw cartilages in the ancestral crown-gnathostome (Fig. 5, posterior probability P=0.99), and homology of osteichthyan and (conventionally defined) chondrichthyan⁸ teeth as oral tubercles (P=0.95). Loss of oral tubercles is inferred several times in acanthodians (Fig. 5). Testing homology of arthrodiran, osteichthyan and ischnacanthid dentitions, there is evidence for the convergent evolution of marginal tooth rows (Fig. 6a) and tooth whorls among gnathostomes (Fig. 6b). The highest posterior density interval for the number of independent tooth whorl origins was 6–15, and 3–7 for marginal tooth rows. These results are robust to the phylogenetic position of 'psarolepid' osteichthyans (Extended Data Fig. 2), to the status of placoderms as paraphyletic or monophyletic and to different divergence dating methodologies (Extended Data Fig. 3).

Our results suggest that the ancestral crown-gnathostome possessed teeth. However, complex dentitions, a permanent dental lamina and coordinated tooth replacement all evolved multiple times; teeth were also lost multiple times among acanthodians (Figs. 5 and 6). The similarities reported here between tooth rows in ischnacanthid dentitions and those of arthrodiran placoderms and osteichthyans are inferred to reflect convergence rather than homology (in contrast to ref.²⁵). The diversification of crown-gnathostomes is associated with an extremely rapid burst of phenotypic evolution³⁰ manifest in the diversity of early crown-gnathostome dentitions. This may go some way to explain why models of tooth replacement based on crown-chondrichthyans perform so poorly in attempting to rationalize the dentitions of crown-osteichthyans, as well as differences which at least in part inspired the hypothesis that teeth evolved independently within these and other lineages of jawed vertebrates^{10,31}.

Methods

Materials. Fossil material comprises specimens of an ischnacanthid acanthodian from the Lochkovian, Early Devonian, Prince of Wales Island, Canada. Mandibles with tooth rows: NRM-PZ P. 9449: labelled model (Figs. 1b–h, 2). Tooth whorls:

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Fig. 4 | Tooth whorl of an ischnacanthid acanthodian. a–**c**, Tooth whorl NRM-PZ P. 15908, with lateral view of complete bone and teeth (**a**), a virtual section showing tooth and base developing synchronously, separated from successive teeth by a growth arrest line (**b**), and a virtual section through second tooth and side teeth (**c**). Scale bar represents $120 \,\mu\text{m}$ in **a**, $60 \,\mu\text{m}$ in **b** and $42 \,\mu\text{m}$ in **c**.

specimen figured in Rücklin et al.³² from the same locality (NRM-PZ P. 15908, Fig. 3a–c). Ischnacanthid acanthodian jaw from the Downtonian, Upper Silurian, Baggeridge Colliery, South Staffordshire, UK (NHMUK PV P.15362, Fig. 1a)³³.

Tomography. Material from Canada was acid-prepared and scanned using SRXTM²¹ at the TOMCAT (X02DA) beamline³⁴ of the Swiss Light Source, Paul-Scherer Institut, Switzerland. Using a 10× objective, 1,501 projections were acquired equi-angularly over 180°. Projections were post-processed and rearranged into flat- and darkfield-corrected sinograms, and reconstruction was preformed on a Linux PC farm, resulting in isotropic voxel dimensions of 0.74 µm. The complete jaw BMNH P. 15362 was scanned using an x-tex XTH 225ST scanner at Nikonmetronics, Tring. 3,142 projections were acquired and were post-processed resulting in isotropic voxel dimensions of 100 µm. Slice data were analysed and manipulated using Avizo 8.01 (http://www.fei.com). Sectional images were studied and three-dimensional models of the different growth stages were derived segmenting following lines of arrested growth.

Phylogeny and ancestral state reconstruction. The phylogenetic data matrix was based on King et al.³⁰, with a revised taxon and character list incorporating

new information on stem chondrichthyans7,14,35, and improved sampling of sarcopterygian osteichthyans. The analysis was a tip-dated approach performed in BEAST2.5.2³⁶ with BEAGLE likelihood calculation library³⁷. Characters were partitioned according to the number of states. We applied the Mkv model38 gamma distributed among-character rate variation, the sampled ancestor birthdeath model³⁹ and the log-normal relaxed clock⁴⁰. Fossil ages were assigned uniform priors across the range of uncertainty. Analyses were run for 200 million generations with 2,000 trees saved. Convergence was assessed in Tracer41 and RWTY⁴². The analysis strongly supports a sarcopterygian position for the 'psarolepid' osteichthyans, but as previously discussed, this may be an artefact of the relatively sparse coding for the characters supporting a stem osteichthyan position for these taxa⁴³. Therefore, a second analysis was performed in which they were constrained to be stem osteichthyans. We used a backbone constraint, so that Ligulalepis, Dialipina and Janusiscus were free to move into or out of the crown. To additionally assess the robustness of results to different phylogenetic and timescaling methods, an additional undated Bayesian analysis was performed in MrBayes3.2.6 (ref. 44), and the post-burn-in sample of trees was time-scaled using the 'equal' method in the R function timePaleoPhy, package paleotree⁴⁵. All three sets of trees (BEAST2, BEAST2 constrained and MrBayes timescaled) were used for ancestral state reconstruction.

Four characters were used for ancestral state reconstructions, three of which were essentially the same as those found in the data matrix. These were 'oral dermal tubercles borne on jaw cartilages', 'oral dermal tubercles in patterned rows (teeth)' and 'tooth whorls'. The latter two characters were changed from the form in the phylogenetic data matrix by recoding inapplicable (-) taxa as absent (0). This prevents illogical results (in particular, the reconstruction of tooth whorls as present but oral tubercles as absent, even though tooth whorls are a form of oral tubercle). A fourth character was introduced for ancestral state reconstructions to assess the homology of osteichthyan, arthrodiran and ischnacanthid tooth rows. This character was formulated as 'teeth, made of dentine, in organized rows and ankylosed to dermal jaw bones'. Owing to its compound formulation it was not included in the original phylogenetic data matrix, which includes each of these aspects as a separate character. Brazeau and Friedman⁴⁶ demonstrated the importance of phylogenetically constrained comparative analysis, suggesting that oral tubercles and tooth whorls are ancestral for crown-gnathostomes. Our phylogenetic analysis corroborates the ancestral condition of oral tubercles, but disagrees with the conclusion that tooth whorls are ancestral.

Ancestral state reconstructions were performed in BEAST1.10.2 (ref. ⁴⁷) with the BEAGLE likelihood calculation library³⁷, using the post-burn-in sample of trees from the three analyses detailed above. Characters were analysed with a strict clock, and a separate evolutionary rate was calculated for each of the four characters. An exponential prior with mean 0.1 was placed on the evolutionary rate. The analysis produced ancestral state reconstructions mapped onto the sample of trees¹⁸ and a count of the number of state changes⁴⁹. The analysis was run for 10 million generations, with 1,000 trees saved. We tested symmetrical and asymmetrical models of trait evolution using Bayes factors. Marginal likelihoods were calculated using the stepping-stone method⁴⁰ with 100 steps, a chain length of 100,000 per step and alpha 0.3. The Bayes factor⁵¹ support for asymmetrical models was 0.53 ('not worth more than a bare mention'), and we therefore chose the symmetrical model for interpretation. Results using the asymmetrical models are included in Extended Data Fig. 3 for comparison.

Post-analysis processing was performed in R using the packages OutbreakTools²², ape⁵³ and phangorn⁵⁴. The state for each character at the crown gnathostome node in each tree of the post-burn-in sample was assessed, producing posterior probabilities. We also assessed the homology of characters between osteichthyans and chondrichthyans (characters were said to be homologous if they were present at every node linking the two clades).

Transition counts are output by the BEAST analysis⁴⁹, but detailed inspection of the results reveals that some transitions are reconstructed incorrectly (for example, a transition to a state on a branch leading to a taxon that lacks that state, and no reversal reconstructed on the same branch). Therefore, the transition counts were also analysed in R using the ancestral state reconstruction at each node. Transitions



Fig. 5 | The 50% majority rule consensus tree from a tip-dated Bayesian analysis, annotated with ancestral state reconstructions for oral tubercles. Branch widths are proportional to the posterior probability of the reconstructed state. Arrows indicate taxon ages that extend beyond the range displayed on the figure.

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Fig. 6 | The 50% majority rule consensus tree from a tip-dated Bayesian analysis, annotated with ancestral state reconstructions for ankylosed tooth rows and tooth whorls. a,b, Ankylosed tooth rows (**a**) and tooth whorls (**b**). Branch widths are proportional to the posterior probability of the reconstructed state. Arrows indicate taxon ages that extend beyond the range displayed on the figure.

were counted when a node had a state different to the immediately ancestral node. This provides a good estimate of the number of transitions, although it will be a slight underestimate because occasional double hits (that is, two transitions in a single branch) will be missed.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data matrix is available at https://doi.org/10.6084/m9.figshare.14447139. Sources for taxa and age ranges and the phylogenetic character list are available as supplementary information. Tomograms and surface files are archived in the University of Bristol data repository, data.bris, at https://doi.org/10.5523/bris.1557r zkyzst5b2jagjuz9li5er.

Code availability

XML BEAST2 files, MrBayes Nexus files, BEAST1 XML files and R scripts are available at https://doi.org/10.6084/m9.figshare.14447139.

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References

- Smith, M. M. & Coates, M. I. Evolutionary origins of the vertebrate dentition: phylogenetic patterns and developmental evolution. *Eur. J. Oral. Sci.* 106, 482–500 (1998).
- Botella, H., Blom, H., Dorka, M., Ahlberg, P. E. & Janvier, P. Jaws and teeth of the earliest bony fishes. *Nature* 448, 583–586 (2007).
- Debiais-Thibaud, M. et al. Tooth and scale morphogenesis in shark: an alternative process to the mammalian enamel knot system. *BMC Evol. Biol.* 15, 292 (2015).
- Rasch, L. J. et al. An ancient dental gene set governs development and continuous regeneration of teeth in sharks. *Dev. Biol.* 415, 347–370 (2016).
- Smith, M. M., Fraser, G. J. & Mitsiadis, T. A. Dental lamina as source of odontogenic stem cells: evolutionary origins and developmental control of tooth generation in gnathostomes. *J. Exp. Zool. B* 312, 260–280 (2009).
- Tucker, A. S. & Fraser, G. J. Evolution and developmental diversity of tooth regeneration. *Semin. Cell Dev. Biol.* 25, 71–80 (2014).
- Coates, M. I. et al. An early chondrichthyan and the evolutionary assembly of a shark body plan. *Proc. R. Soc. B* 285, https://doi.org/10.1098/rspb.2017.2418 (2018).
- Zhu, M. et al. A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature* 502, 188–193 (2013).
- Giles, S., Friedman, M. & Brazeau, M. D. Osteichthyan-like cranial conditions in an Early Devonian stem gnathostome. *Nature* 520, 82–U175 (2015).
- Smith, M. M. & Johanson, Z. Separate evolutionary origins of teeth from evidence in fossil jawed vertebrates. *Science* 299, 1235–1236 (2003).
- 11. Denison, R. H. Acanthodii (Gustav Fischer, 1979).
- 12. Smith, M. M. Vertebrate dentitions at the origin of jaws: when and how pattern evolved. *Evol. Dev.* 5, 394–413 (2003).
- Blais, S. A., MacKenzie, L. A. & Wilson, M. V. H. Tooth-like scales in Early Devonian eugnathostomes and the 'outside-in' hypothesis for the origins of teeth in vertebrates. *J. Vertebr. Paleontol.* **31**, 1189–1199 (2011).
- Burrow, C. J., Newman, M., den Blaauwen, J., Jones, R. & Davidson, R. The Early Devonian ischnacanthiform acanthodian *Ischnacanthus gracilis* (Egerton, 1861) from the Midland Valley of Scotland. *Acta Geol. Polon.* 68, 335–362 (2018).
- 15. Burrow, C. J. Acanthodian fishes with dentigerous jaw bones: the Ischnacanthiformes and *Acanthodopsis*. *Foss. Strat.* **50**, 8–22 (2004).
- Lindley, I. D. Acanthodian fish remains from the lower devonian cavan bluff limestone (Murrumbidgee group), Taemas district, New South Wales. *Alcheringa* 24, 11–35 (2000).
- Newman, M. J., Burrow, C. J. & den Blaaauwen, J. L. A new species of ischnacanthiform acanthodian from the Givetian of Mimerdalen, Svalbard. *Norw. J. Geol.* **99**, 1–13 (2019).
- Gross, W. Über das Gebiss der Acanthodier und Placodermen. Zool. J. Linn. Soc. 47, 121–130 (1967).
- Ørvig, T. Acanthodian dentition and its bearing on the relationships of the group. *Palaeontographica A* 143, 119–150 (1973).
- Smith, M. M. & Coates, M. I. in *Major Events Of Early Vertebrate Evolution* (ed. Ahlberg. P. E.) 223–240 (Taylor & Francis, 2001).
- Donoghue, P. C. J. et al. Synchrotron X-ray tomographic microscopy of fossil embryos. *Nature* 442, 680–683 (2006).
- Friedman, M. & Brazeau, M. D. A reappraisal of the origin and basal radiation of the osteichthyes. J. Vertebr. Paleontol. 30, 36–56 (2010).

- Doeland, M., Couzens, A. M. C., Donoghue, P. C. J. & Rücklin, M. Tooth replacement in early sarcopterygians. *R. Soc. Open Sci.* 6, https://doi. org/10.1098/rsos.191173 (2019).
- Jarvik, E. Middle and Upper Devonian porolepiformes from East Greenland with special reference to *Glyptolepis groenlandica* n. sp. and a discussion on the structure of the head of porolepiformes. *Medd. Groenl.* 187, 1–295 (1972).
- Chen, D., Blom, H., Sanchez, S., Tafforeau, P. & Ahlberg, P. E. The stem osteichthyan *Andreolepis* and the origin of tooth replacement. *Nature* 539, 237–241 (2016).
- Rücklin, M. et al. Development of teeth and jaws in the earliest jawed vertebrates. *Nature* 491, 748–751 (2012).
- Clemen, G., Bartsch, P. & Wacker, K. Dentition and dentigerous bones in juveniles and adults of *Polypterus senegalus* (Cladistia, Actinopterygii). *Ann. Anat.* 180, 211–221 (1998).
- 28. Chen, D. et al. Development of cyclic shedding teeth from semi-shedding teeth: the inner dental arcade of the stem osteichthyan *Lophosteus. R. Soc. Open Sci.* **4**, 161084 (2017).
- Patterson, C. in *Problems Of Phylogenetic Reconstruction* (eds Joysey, K. A. & Friday, A. E.) Systematics Association Special Volume 21, 21–74 (Academic Press, 1982).
- King, B., Qiao, T., Lee, M. S. Y., Zhu, M. & Long, J. A. Bayesian morphological clock methods resurrect placoderm monophyly and reveal rapid early evolution in jawed vertebrates. *Syst. Biol.* 66, 499–516 (2017).
- Andreev, P. et al. The systematics of the Mongolepidida (Chondrichthyes) and the Ordovician origins of the clade. *PeerJ* 4, e1850 (2016).
- 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).
- White, E. I. The Old Red Sandstone of Brown Lee Hill and the adjacent area. II. Palaeontology. Bull. Br. Mus. (Nat. Hist.) Geol. 5, 245–310 (1961).
- 34. Stampanoni, M. et al. TOMCAT: a beamline for tomographic microscopy and coherent radiology experiments. *AIP Conf. Proc.* **879**, 848 (2007).
- Maisey, J. G. et al. in *Evolution and Development of FIshes* (eds Johanson, Z., Underwood, C. J. & Richter, M.) 87–109 (Cambridge Univ. Press, 2018).
- 36. Bouckaert, R. et al. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **15**, e1006650 (2019).
- Ayres, D. L. et al. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. *Syst. Biol.* 61, 170–173 (2012).
- Lewis, P. O. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50, 913–925 (2001).
- Gavryushkina, A., Welch, D., Stadler, T. & Drummond, A. J. Bayesian inference of sampled ancestor trees for epidemiology and fossil calibration. *PLoS Comput. Biol.* **10**, e1003919 (2014).
- Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, 699–710 (2006).
- Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. Tracer v1.6 http://beast.bio.ed.ac.uk/Tracer (2014).
- Warren, D. L., Geneva, A. J. & Lanfear, R. RWTY (R We There Yet): an R package for examining convergence of Bayesian phylogenetic analyses. *Mol. Biol. Evol.* 34, 1016–1020 (2017).
- King, B. Which morphological characters are influential in a Bayesian phylogenetic analysis? Examples from the earliest osteichthyans. *Biol. Lett.* 15, https://doi.org/10.1098/rsbl.2019.0288 (2019).
- Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542 (2012).
- Bapst, D. W. paleotree: an R package for paleontological and phylogenetic analyses of evolution. *Methods Ecol. Evol.* 3, 803–807 (2012).
- Brazeau, M. D. & Friedman, M. The characters of Palaeozoic jawed vertebrates. Zool. J. Linn. Soc. 170, 779–821 (2014).
- Suchard, M. A. et al. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* 4, https://doi.org/10.1093/ve/ vey016 (2018).
- Lemey, P., Rambaut, A., Drummond, A. J. & Suchard, M. A. Bayesian phylogeography finds its roots. *PLoS Comput. Biol.* 5, e1000520 (2009).
- Minin, V. N. & Suchard, M. A. Counting labeled transitions in continuous-time Markov models of evolution. *J. Math. Biol.* 56, 391–412 (2008).
- Xie, W., Lewis, P. O., Fan, Y., Kuo, L. & Chen, M. H. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Syst. Biol.* 60, 150–160 (2011).
- 51. Kass, E. R. R. & Bayes, A. E. Factors. J. Am. Stat. Assoc. 90, 773-795 (1995).
- Jombart, T. et al. OutbreakTools: a new platform for disease outbreak analysis using the R software. *Epidemics* 7, 28-34 (2014).
- Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290 (2004).
- Schliep, K. P. phangorn: phylogenetic analysis in R. *Bioinformatics* 27, 592–593 (2011).

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Author contributions

M.R. and P.C.J.D. designed the initial research. M.R., J.A.C., P.C.J.D. and F.M. performed scans. M.R. and J.A.C. segmented tomograms. B.K. produced the phylogenetic data matrix, and performed the phylogenetic analysis and ancestral state reconstruction. M.R. and P.C.J.D. drafted the manuscript, to which all authors contributed.

Competing interests

The authors declare no competing interests.

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ARTICLES



Extended Data Fig. 1 | Virtual development of teeth on an ischnacanthid acanthodian jawbone. Tooth rows of NRM-PZ P. 9449 Early Devonian, Canada. Labelled sclerochronology of the lateral row (**a**), lingual row (**b**) and overgrowth of the initial teeth at the centre of ossification (**c**). Colours of the nested boxes reflect the successive stages of tooth development. Scale bar represents 169 µm in **a**, **b**, and 72 µm in (**c**).

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Extended Data Fig. 2 | 50% majority-rule consensus tree from tip-dated analysis of early gnathostome fossils. 'Psarolepids' constrained as stem osteichthyans, annotated with ancestral state reconstruction of tooth whorls.

| A | RTI | ICL | ES |
|---|-----|------------|----|
| | | | |

| Tree source | Tip-dated trees; symmetrical model | Tip-dated trees; asymmetrical model | Undated trees; symmetrical model |
|---|---------------------------------------|---|-------------------------------------|
| Chondrichthyan and osteichthyan teeth homologous as oral tubercles | 0.94 | 0.90 | 0.96 |
| Oral tubercles at crown node | 0.97 | 0.94 | 1 |
| Patterned teeth at crown node | 0.62 | 0.27 | 0.8 |
| Tooth whorls at crown node | 0.06 | 0.63 | 0.1 |
| Tooth rows at crown node | 0.03 | 0.04 | 0.02 |

Extended Data Fig. 3 | Posterior probabilities from ancestral state reconstructions. In column 1, 'chondrichthyans' refers to conventionally-defined chondrichthyans possessing tooth batteries. This includes *Doliodus* and crown chondrichthyans. Posterior probabilities are similar for tip-dated trees, and for undated Bayesian trees time-scaled a posteriori.

nature research

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Software and code

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|--------------------|--|
| Data collection | Avizo 8.01; Mesquite 3.6 |
| Data analysis | BEAST2.5.2; MrBayes3.2.6; BEAST1.10.2; R 4.0 |

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The data matrix, xml BEAST2 files, MrBayes nexus files, BEAST1 xml files and R scripts are available in the Dryad data supplement. Sources for taxa and age ranges and the phylogenetic character list are available as supplementary information. Tomograms and surface files are archived in the University of Bristol Research Data Storage Facility at publication.

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All studies must disclose on these points even when the disclosure is negative.

| Study description | Fossils of jaws of stem-chondrichthyans are studied using Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM). We describe the growth and addition of teeth on the jaws and compare with Palaeozoic Chondrichthyes and Palaeozoic Osteichthyes. We run an ancestral state reconstruction to identify the condition of the gnathostome crown-ancestor. |
|-----------------------------|---|
| Research sample | Exceptionally preserved fossils of marginal and symphyseal dentitions of ischnacanthid acanthodians from the Lower Devonian (Lochkovian) of Prince of Wales Island, Arctic Canada and the Lower Devonian of Brown Clee Hill, Shropshire, UK are studied using SRXTM. |
| Sampling strategy | Several jaws were scanned, the best preserved specimen was segmented following the sclerochronology. |
| Data collection | Tomographic data was collected using Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM) at the TOMCAT (X02DA) beamline of the Swiss Light Source (SLS), Paul-Scherer Institution, Switzerland. Using a 10x objective 1501 projections were acquired equi-angularly over 180°. Projections were post-processed and rearranged into flat- and darkfield-corrected sinograms, and reconstruction was preformed on a 32-node Linux PC farm resulting in isotropic voxel dimensions of 0.74 µm. Overview scan of the large specimen was done with x-tex XTH 225ST scanner at Nikonmetronics, Tring. 3142 projections were acquired and were post-processed resulting in isotropic voxel dimensions of 100 µm. Data collection was done by Martin Rücklin, John Cunningham, Philip C.J. Donoghue and Federica Marone. |
| Timing and spatial scale | Fossils were scanned once and slerochronology reconstructed using the segmentation tool of AVIZO by Martin Rücklin and John Cunningham to test the results of the segmentation. |
| Data exclusions | No data was excluded. |
| Reproducibility | To verify the interpretation of the tomographic data Martin Rücklin and John Cunningham both segmented the specimen. This test of the result was positive and will increase the reproducibility. |
| Randomization | This is not relevant for the study as fossils were segmented and no larger data set available. |
| Blinding | Blinding was not possible for the study as we describe unique fossils and their interpretation. |
| Did the study involve field | d work? Yes Xo |

Reporting for specific materials, systems and methods

Methods

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| \boxtimes | Human research participants | | |
| \boxtimes | Clinical data | | |
| \boxtimes | Dual use research of concern | | |
| | | | |

Palaeontology and Archaeology

Specimen provenance

Ischnacanthid acanthodian from the Lochkovian, Early Devonian, Prince of Wales Island, Canada. Mandibles with tooth rows: NRM-PZ P. 9449. Tooth whorls: specimen figured in Rücklin et al. (2011) from the same locality (NRM-PZ P. 15908). Ischnacanthid acanthodian jaw from the Downtonian, Upper Silurian, Baggeridge Colliery, South Staffordshire, UK (NHMUK PV P.15362.

Ethics oversight

Dating methods

Specimen deposition

No ethical approval is needed.

London (NHMUK).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

No new dates for the fossils described here were taken.