Tomographic data of 'acanthodian' oral structures and a review of dental diversity in early chondrichthyans

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Abstract

The teeth of sharks famously form a series of parallel, continuously replacing files borne directly on the jaw cartilages. In contrast, bony fishes possess site-specific shedding dentition borne primarily on dermal plates. Understanding how these disparate systems evolved is challenging, not least because of poorly understood relationships amongst early chondrichthyans and the profusion of morphologically and terminologically diverse bones, cartilages, splints and whorls that they possess. Here we use tomographic methods to investigate mandibular structures in several early branching 'acanthodian'-grade stem-chondrichthyans. We characterise the dentigerous jaw bones of disparate genera of ischnacanthids as growing bones with non-shedding dentition. Mandibular splints, which support the ventro-lateral edge of the Meckel's cartilage in some acanthodians, are formed from dermal bone and may be an acanthodid synapomorphy. We strengthen the case for *Acanthodopsis* as an acanthodid deeply nested within an edentulous radiation and show that its teeth are borne directly on the mandibular cartilage, unexpectedly representing an independent origin of teeth. Poor resolution of relationships amongst 'acanthodians' represents a major barrier to understanding the evolution and homology of teeth and associated oral structures.

Keywords (3-6): dentitions, early vertebrates, acanthodians, chondrichthyans, tooth evolution, Palaeozoic

1. Introduction

The structure and position of teeth and jaws are amongst the major anatomical distinctions between crown osteichthyans (bony fishes: ray-finned fishes, lobe-finned fishes, and tetrapods) and crown chondrichthyans (cartilaginous fishes: sharks, rays, and chimaeras)[1]. In osteichthyans, teeth are partially resorbed, shed, and replaced in position on dermal bones lateral to and overlying endoskeletal jaw cartilages as part of inner and outer dental arcades. In crownward chondrichthyans, teeth grow, shed, and are replaced in parallel rows of labially-directed series directly on the endoskeletal jaw cartilages. The origins of these dental structures can be traced back to Palaeozoic taxa, which suggest that the last common ancestor of jawed fishes (gnathostomes), as well as crownward stem gnathostomes (a paraphyletic assemblage referred to as ‘placoderms’), possessed non-shedding teeth fused to the underlying dermal jaw bone [2–7].

The advent of micro-computed tomography has led to a renewed interest in tooth evolution and development in Palaeozoic gnathostomes. These have mostly focussed on stem-gnathostome ‘placoderms’ [2,5,6,8] and osteichthyans [9–14]. Early-branching members of the chondrichthyan total-group (including ‘acanthodians’), have received far less attention, with just three taxa described using CT data [7,15–18]. This is despite a staggering array of dermal oral structures across the assemblage including teeth growing on (or absent from) dermal plates of differing constructions, tooth whorls, gracile and molariform teeth, and extramandibular ‘dentitions’ [15,19–26].

Here we use computed tomography to image the teeth, jaws and associated oral structures of several early-diverging stem-group chondrichthyans with the aim of more broadly sampling the diversity of their oral structures. We contextualise our new data within a wider review of stem-group chondrichthyan oral structures and identify those that represent synapomorphies, while also discussing challenges in reconstructing dental evolutionary histories and homologies.

2. Materials and Methods

2.1 Taxa examined

All specimens studied here are housed at the Natural History Museum, London (NHMUK), and comprise: an isolated jaw of *Taemasacanthus erroli* (NHMUK PV P33706); an isolated jaw of *Atopacanthus* sp. (NHMUK PV P.10978); an isolated jaw of *Acanthodopsis* sp. (NHMUK PV P.10383); a partial head of *Acanthodes* sp. (NHMUK PV P.8065); and an isolated jaw of *Ischnacanthus* sp. (NHMUK PV P.40124).

Taemasacanthus erroli is known from eight isolated jaws from the Emsian (Lower Devonian) Murrumbidgee Group in New South Wales, Australia [27]. Other species, also based on isolated jaw bones, have been assigned to the genus [28,29], but no articulated animals are known. *Taemasacanthus* is understood to be an ischnacanthid on the basis of its dentigerous jaw bone. NHMUK PV P.33706, described here (figure 1), is a right lower jaw, and comprises two main parts: a dermal dentigerous jaw bone and the articular

ossification of Meckel's cartilage. The external morphology of this specimen of *Taemasacanthus* has been fully described [27] but is briefly redescribed here to contextualise our new information.

Atopacanthus is known throughout the Middle-Upper Devonian [30]. The type species, *Atopacanthus dentatus*, is known from several dentigerous jaw bones from near Hamburg, New York and is presumed to be an ischnacanthid [21,31]. An articulated specimen of *Atopacanthus* sp. from the Upper Devonian is also known [32], but its attribution to this genus is questionable [33], and it has subsequently been referred to *Serradentus* [34]. The specimen described here, NHMUK PV P.10978 (figure 2), is a dentigerous jaw bone collected from Elgin, Scotland, and is Middle Devonian in age. It was originally labelled as a possible dipnoan toothplate before later being referred to *Atopacanthus*, and its morphology conforms with that of other specimens described as *Atopacanthus*. It is not associated with any endoskeletal material, and it is impossible to tell whether it is from a right lower jaw or upper left jaw. For ease of comparison with other specimens, we describe its morphology as if it were a lower jaw element.

Ischnacanthus is the best known ischnacanthid 'acanthodian', represented by numerous articulated specimens of Lochkovian (Lower Devonian) age from the Midland Valley in Scotland [23]. The material described here (NHMUK PV P.40124; figure 3) is an isolated left lower jaw from the Lower Devonian (Lochkovian) Midland Valley in Tealing, Forfarshire [35]. It is fairly complete, but parts of the dorsal and anterior margins have been lost to the counterpart (which is preserved, but was not CT scanned), and the whole jaw is laterally flattened. It comprises a dentigerous jaw bone and Meckel's cartilage.

Acanthodopsis is known from the Carboniferous of the UK and Australia. *Acanthodopsis* has been previously considered an ischnacanthid on the basis of its “dentigerous jaw bones”, but in terms of its skeletal anatomy it is more similar to acanthodids [21,27]. The material described here, NHMUK PV P.10382 from the Northumberland Coal Measures (figure 4), comprises a laterally flattened lower right jaw, consisting of a Meckel’s cartilage with teeth and a mandibular splint.

Acanthodes is the latest occurring genus of ‘acanthodian’ found as articulated body fossils from the Mississippian (Carboniferous) into the Lower Permian [36]. It is the only genus of ‘acanthodian’-grade animal known from extensively preserved endoskeleton, seen in specimens of *Acanthodes confusus* from Lebach, Germany [37–45]. The material described here (NHMUK PV P.8065) comprises part of the ventral half of the head of a specimen from the Knowles Ironstone of Staffordshire (figure 5). As the dorsal margins of the jaw bones are obscured within the rock, it was originally referred to ‘*Acanthodopsis* or *Acanthodes’*. As CT scanning shows that dentition is absent, we can confirm it to be *Acanthodes* sp. Most of the left jaw is preserved, and of the right jaw only the mandibular splint is preserved, as are some of the lower branchiostegal ray series and isolated dermal gill rakers. Scattered parts of the rest of the head endoskeleton are also present, including parts of ceratobranchial and a hyomandibular.

2.2 CT scanning

CT scanning of two specimens took place at the Imaging and Analysis Centre, NHMUK, using a Metris X-Tek HMX ST 225 with the following settings: *Taemasacanthus erroli*: 3142 projections, 130 kV, 131 *µ*A, 0.1 mm copper filter, voxel size 17.3 *µ*m; *Atopacanthus*: 3142 projections, 130 kV, 154 *µ*A, 0.1 mm copper filter, voxel size 19.508 *µ*m.

CT scanning of three specimens took place at the Bristol University Department of Life Sciences using a Nikon XT H 225 ST with the following settings: *Acanthodopsis*: 3142 projections, 180 kV, 92 *µ*A, no filter, voxel size 22.6 µm; *Acanthodes*: 3142 projections, 215 kV, 165 *µ*A, 0.1 mm tin filter, voxel size 44.9 *µ*m; *Ischnacanthus*: 3142 projections, 222 kV, 105 *µ*A, 0.5 mm copper filter, voxel size 24.6 *µ*m.

Reconstructed tomographic datasets were segmented in Mimics v.19 (biomedical.materialise.com/mimics; Materialise, Leuven, Belgium) and images were generated using Blender (blender.org).

2.3 Phylogenetic analyses

Our dataset is based on Dearden *et al*. [46], the most recently published phylogenetic dataset specifically tackling stem-group chondrichthyan relationships. We have added one taxon and four characters and made minor modifications to some codes (full details given in the supplementary text). We performed a parsimony analysis in TNT [47] with the following settings: a parsimony ratchet with 10,000 iterations, holding 100 trees per iteration, with TBR branch swapping. Galeaspida was set as the outgroup and the following constraint applied: (Galeaspida(Osteostraci(Mandibulate Gnathostomes))).

We also performed a Bayesian analysis, with Galeaspida set as the outgroup, mandibulate gnathostomes constrained as monophyletic, and the following settings. We used a uniform prior with the Mkv model and gamma-distributed rates; searched for 10,000,000 generations, sampling every 1,000 generations; and calculated the majority rule consensus tree with a relative burn-in of 25%.

3. Results

3.1 *Taemasacanthus*

The dentigerous jaw bone of *Taemasacanthus* is approximately half the full depth of the jaw and is sinusoidal in dorsal view (figure 1). A circular ridge, suggested to be for a labial cartilage attachment [27], is present on the lateral surface approximately ¼ of the way along its length (figure 1b,c). Posteriorly, the bone curves laterally and broadens to wrap around the articular. The lateral expansion is larger than the medial expansion, and both are rounded posteriorly. Ventrally, a groove formed by the posterior confluence of these two processes runs underneath the entire length of the dentigerous jaw bone, and would have overlain Meckel's cartilage (figure 1c). The dentigerous jaw bone is approximately trapezoid in cross section, and bears three rows of teeth (figure 1a,b,d,f,g). Histologically the underlying dermal plate comprises heavily vascularised dermal bone, similar to that observed in thin sections of other ischnacanthid dentigerous jaw bones [7,23,48], with a relatively thin layer of less vascularised bone around the plate margins (figure 1f). The vascularisation comprises an interlinked network of tubules, which are

strongly polarised along the anteroposterior length of the element. In transverse section these are near-horizontal, running the width of the element, and are interlinked. The circular ridge is formed of avascular bone, but is otherwise a similar tissue to that forming the outer margin of the dermal plate.

Three rows of teeth are borne on the biting edge of the dermal plate, all starting approximately at the level of the mesial ridge: a lateral, medial, and lingual row (figure 1a,b,d). Teeth within the lateral and lingual rows are fused to the jaw, but the base of the tooth is marked by an increase in the density of randomly-oriented vascular canals (figure 1f). The medial row lies on the mesial ridge and comprises a single row of small disorganised cusps that are continuous with the underlying dermal plate. Only the more posterior few are vascularised. The lateral and lingual rows of teeth are much larger, are ridged, and comprise a vascular base topped with an avascular crown seemingly lacking a continuous enameloid covering (figure 1f). Both grow by the addition of new teeth anteriorly, as evidenced by anterior teeth partially overlying posterior ones, and cusps becoming progressively larger in the direction of growth. The sole exception to this is the penultimate lingual tooth in *Taemasacanthus*. In what is probably a pathology, the eighth cusp of the lingual row is incomplete, and is either damaged or its growth appears to have been aborted (figure 1a). The ninth cusp in the row has instead grown over the incomplete eighth as well as the tenth cusp. This is in fact the youngest cusp in the entire row and is oriented notably more medially. The lateral tooth row comprises around twelve cusps, and its teeth are laterally unornamented and continuous with the lateral surface of the dermal bone, connected to one another via antero-posterior lateral ridges (figure 1b). The lingual side of each

cusp is rounded, and ornamented with a number of ridges, which become progressively more tuberculated on anterior cusps. The lingual tooth row comprises ten cusps, which curve away from the occlusal surface anteriorly.

Only the posteriormost portion of the Meckel's cartilage, the articular ossification, is preserved (figure 1). It is formed from a sheath of perichondral tissue and would have been filled with cartilage in life. Some spongy texture is apparent on the interior surface of the perichondral bone. A shallow groove on the posterior surface does not appear to continue ventrally, making it unlikely to have accommodated a mandibular splint as previously suggested [27]. Articulation with the palatoquadrate appear to be via an open, oval, fossa [27] (figure 1d). The tissue forming this is notably ill-formed, and appears to lack a solid perichondral covering, meaning that it could well, as Burrow [21] suggests, be a typical ischnacanthid process that has lost its tip. An additional ventral fragment of the articular (previously figured [27]) has become detached from the rest of the ossification.

3.2 *Atopacanthus*

The dentigerous jaw bone is robust, trapezoid in cross-section, but flatter and taller proportionate to its length than in *Taemasacanthus* (figure 2). It is slightly medially convex. The anterior fifth of the preserved element (it is broken both anteriorly and posteriorly) is toothless and tapers slightly. A narrow, shallow groove to accommodate the mandibular cartilage runs along the ventral surface of the element (figure 2c). The histology is similar to *Taemasacanthus*, with heavily vascularised dermal bone surrounded by a

less vascular layer, but the vascular tubules are even more strongly polarised in an antero-dorsal direction (figure 2e). Towards the surface of the element vascularisation is less dense, and not polarised. The lingual face of the dentigerous jaw bone supports a distinct thin, lingual, tooth-bearing plate (figure 2a,d,e). This is still heavily vascularised, but tubes are polarised dorso-lingually.

As in *Taemasacanthus*, lateral, mesial and lingual tooth rows are borne on the dorsal surface of the underlying dermal plate (figure 2a,b,d). The medial ridge bears two disorganised rows of cusps along its anterior half, with the posterior half being smooth. All cusps are vascular, and are histologically continuous with the medial ridge. The lateral tooth row comprises eight cusps, which become progressively larger anteriorly, and their lateral surfaces are continuous with the outside of the dermal plate (figure 2b). Their lingual surfaces are rounded and ornamented with untuberculated ridges. The inner tooth row comprises ten main cusps, which curve medially across the element. Two additional small (but histologically similar) cusps are present near the posterior margin of the dermal plate, ventral to the main lingual tooth row. This tooth row lies on top of a lingual plate, which is apposited onto the lingual surface of the main dermal plate (figure 2a,d,e) . As in the lateral row and in *Taemasacanthus*, cusps become larger anteriorly, and are ornamented with ridges. The histology of the teeth of the lateral and lingual rows comprises a vascular base topped with an avascular cap lacking enameloid, with the vascular canals oriented distinctly from the underlying dermal and lingual plates (figure 2e). Teeth in both the lateral and lingual rows were added anteriorly, with anterior cusps partly overlying their posterior fellows.

3.3 *Ischnacanthus*

Only the anterior part of the dentigerous jaw bone is preserved in the part (figure 3), although the mould of the posterior region is visible in outline. The underlying dermal plate is much shallower than in *Taemasacanthus* and *Atopacanthus*. A lateral tooth row and a medial ridge are present. The lateral tooth row preserves four cusps, which are linked by a cuspidate ridge along their lateral faces. Relative size and age are difficult to determine due to the mode of preservation, but the anteriormost cusp is the largest, and cusp overlap indicates that teeth were added anteriorly. Although the ventralmost parts of the dermal plate are missing, the ventral margin of the teeth is marked by a noticeable shift in density and orientation of the vascular canals (figure 3d). The tissue forming the teeth is similar to *Taemasacanthus* and *Atopacanthus*, with a vascularised base and an avascular crown apparently lacking enameloid (contra ref. 7).

The large Meckel's cartilage is near-complete and preserved as a single element (figure 3). It is curved posteriorly and tapers anteriorly. The dentigerous jaw bone is borne on its dorso-lingual surface. A laterally-directed articular condyle is present at the posterior extent, and a shallow groove extends ventral to the condyle. The majority of the Meckel's cartilage is formed of globular calcified cartilage. Parts of its lateral surface, as well as its ventral, anterior, and posterior extents, are covered by a thin, densely mineralised tissue that appears to be perichondral bone [23]. This tissue thickens ventrally and posteriorly and is fractured. A thickened ridge along the posteroventral and posterior margin is continuous with the perichondral rind

that extends onto the lateral surface, but externally gives the appearance of a separate ossification (figure 3bc,). In section this is closely comparable to the so called “mandibular bone” that Ørvig [49] described in *Xylacanthus and* is probably responsible for accounts of mandibular splints in ischnacanthids.

3.4 *Acanthodopsis*

The lower jaw in *Acanthodopsis* comprises a tooth-bearing Meckel’s cartilage and a mandibular splint (figure 4). The Meckel’s cartilage is long and thin and similar in form to that of *Acanthodes* (figure 5; 30,44], with an identical articular cotylus and marked preglenoid process. It tapers anteriorly, terminating in a small, cup-shaped anterior symphyseal fossa (figure 4c,e). The Meckel’s cartilage is formed from a shell of perichondral bone [30] and is internally unmineralized (figure 4g,h), although some mineralisation appears to be present in the jaw articulation. Unlike *Acanthodes*, it is perichondrally mineralised along its entire length (figure 4), rather than in separate articular and mentomandibular sections.

Ten monocuspid, triangular teeth form a row along the dorsal surface of the Meckel’s cartilage (figure 4). The largest tooth is in the middle of the jaw, with teeth becoming smaller and more closely set anteriorly and posteriorly; they are slightly lingually convex, each with a smooth (but possibly weathered) lateral face and a longitudinally striated lingual face. Previous description of the teeth [21] were unclear as to their tissue makeup. Based on our data we infer them to be dermal as they are ornamented and histologically distinct from the Meckelian element. Internally they are formed from a thick outer

layer and a vascular inner tissue with distinct canals, with no obvious pulp cavity (figure 4g,h); more detailed study is needed to establish these tissues' identity. The direction of growth is difficult to infer, but the largest tooth appears to be overlapped by the anterior and posterior teeth, possibly making it the oldest. This contrasts to the order of growth in *Taemasacanthus*, *Atopacanthus* and *Ischnacanthus*, where teeth are added anteriorly. However, as with the identity of the tissue, we express caution at this interpretation.

The mandibular splint in *Acanthodopsis* is an unornamented, slightly sinusoidal bone that fits into a groove on the ventro-lateral part of Meckel's cartilage, extending almost its entire length (figure 4a,b). This groove was likely originally much shallower, and its depth has been exaggerated by lateral flattening of the specimen. The tissue forming the splint is solid, and is organised into multiple concentric lamellae likely representing lines of arrested growth. It is pierced by a series of thin, longitudinally oriented canals (figure 4g,h). This tissue is distinct from that forming Meckel's cartilage, in particular being denser and better organised, and can be interpreted as dermal bone, especially given the ornamentation of the mandibular splints of some acanthodids [50]. Burrow [21] reported that the mandibular splint in *Acanthodopsis* was formed from cartilage and bone based on thin sections, but the cartilage identified in these reports is likely the Meckel's cartilage above the bone (CJB pers. comm. July 2020).

3.5 *Acanthodes*

The left lower jaw comprises ossified articular and mentomandibular parts of Meckel's cartilage, as well as a mandibular splint (figure 5). Mineralised parts of the Meckelian element are formed from thick perichondral bone, and are slightly laterally crushed (figure. 5a-d). The articular is as previously described [44]. The mentomandibular has a distinct cup-like symphyseal fossa at its anterior tip, forming part of the mandibular symphysis (figure 5c). The mandibular splint is unornamented, slightly sinusoidal in shape, and ellipsoid in cross-section. It sits in a groove in the lateral faces of the mentomandibular and articular. Internally it is solid and vascularised by sparse long, thin canals running its length (figure 5e,f), as in *Acanthodopsis*. A single tooth-like cusp sitting in (although separate from) a cushion-shaped base is probably a gill raker (figure 5b,e).

3.6 *Phylogenetic results*

Our parsimony analysis recovered 26101 most parsimonious trees with a length of 704 steps. The strict consensus of these results (figures 6a, S1) is consistent with other recent analyses in finding all 'acanthodians' to be stem-group chondrichthyans. We recover "Acanthodii" *sensu* Coates *et al.* [51] as a clade subtending the remainder of the chondrichthyan total group, with *Euthacanthus* as the sister group to the "Acanthodii". However, acanthodiforms (i.e. cheiracanthids, mesacanthids, and acanthodids) are paraphyletic. Ischnacanthids plus diplacanthids form a clade, but ischnacanthids themselves are paraphyletic, and diplacanthids are a clade to the exclusion of *Tetanopsyrus*). Remaining stem chondrichthyan taxa, including climatiids, *Gladbachus* and Doliodus, are recovered in a polytomy

along with a monophyletic chondrichthyan crown group. Support values aside from the chondrichthyan total group and crown group nodes are typically low. The Bayesian majority rule consensus tree (figure 6b, S2) is broadly consistent with the parsimony strict consensus tree, although “Acanthodii” decay into a polytomy subtending all more crownwards chondrichthyans. *Acanthodopsis* is recovered as the sister-taxon to *Acanthodes* in both analyses.

4. Discussion

4.1 Stem-chondrichthyan relationships

The majority of known chondrichthyan stem-group members are ‘acanthodians’: a collection of poorly understood fishes with even more poorly understood relationships (figure 7). ‘Acanthodian’ phylogeny has undergone major upheavals in the last decade: long considered a monophyletic group of stem osteichthyans (for a brief review see [53]), a radical revision of early gnathostome relationships suggested they might be a paraphyletic group smeared across the gnathostome, chondrichthyan and osteichthyan stem-groups [54]. Subsequent discoveries indicated that all acanthodians are in fact stem chondrichthyans [55,56], which has been upheld by the vast majority of subsequent studies (e.g. refs 7,46,51,52,57,58). However, hypotheses of relationships on the chondrichthyan stem itself are far from settled. There is some evidence that a diplacanthiform, ischnacanthiform, and acanthodiform taxa may form a clade or grade subtending the remainder of the chondrichthyan total-group [51]. ‘Climitiid’ ‘acanthodians’, which have

overlapping character complements with more shark-like chondrichthyans, tend to be recovered in a more crownward position (although see [52], figure 7d). Beyond this, however, different phylogenetic analyses present very different schemes of relationships (figure 6,7), often with low support. These conflicting patterns of relationships present major obstacles to understanding patterns evolution for many of the dental structures seen in early chondrichthyans, although likelihood-based methods provide a possible approach [7].

4.2 Distribution of oral structures in the chondrichthyan stem-group

Teeth and other oral structures in stem-chondrichthyans take on a broad variety of forms, raising questions about their homology and the primitive or derived condition for different features. Here we briefly review this diversity and map key characters onto our phylogeny. However, we do not attempt ancestral state reconstructions or character optimisations given the above caveats concerning early chondrichthyan phylogeny.

A diverse array of **teeth** (light blue icons, figures 6 and 7) are present in the majority of Palaeozoic chondrichthyans, including as tooth whorls, but are also remarkable for the breadth of taxa in which they are absent. Acanthodids (e.g. *Acanthodes*: figure 5, ref. 44) are completely toothless (except for *Acanthodopsis*), as are the likely related mesacanthids (e.g. *Promesacanthus* [59]) and cheiracanthids (e.g. *Cheiracanthus* [60]). In this latter group, tooth-like hyoid rakers have sometimes been mistaken for teeth [61]. Teeth are also absent in diplacanthids [22], with the possible exception of *Tetanopsyrus* [24].

A number of toothless taxa with otherwise diverse anatomies, including *Obtusacanthus*, *Lupopsyrus*, *Euthacanthus*, *Brachyacanthus*, and *Kathemacanthus* [45,62–64], are often resolved as more closely related to the crown-group (figures 6,7). Given the small size and two-dimensional preservation of some of these taxa, it is possible that teeth are present but reduced and so far undetected, as in *Gladbachus adentatus* [51]. Teeth are inferred to be homologous across gnathostomes [7], demanding numerous independent losses of teeth in stem chondrichthyans (figures 6 and 7).

Tooth whorls (dark green icons, figures 6 and 7) are tooth files fused to a bony base, which grow by the lingual addition of new cusps along a single axis [18]. Tooth whorls are understood to be the evolutionary precursor to modern chondrichthyan tooth families, which have a similar morphology but lack a common bony base [53]. Tooth whorls are present in a range of stem-group chondrichthyans with otherwise dissimilar anatomies, including those with dentigerous jaw bones (e.g. *Ischnacanthus* [23]), densely tesserate head skeletons (e.g. *Climatius* [65]), and more shark-like taxa (e.g. *Doliodus* [15]). They have also been described in the acanthodiform-like *Latviacanthus* [66], although as this is based on x-rays these may be mischaracterised hyoid rakers as in *Homalacanthus* [61]. Tooth whorls are also (presumably secondarily) present in some stem-group holocephalans [67], as well as some osteichthyans (e.g. *Onychodus* [14,68]). The main variation between forms is in distribution: in ischnacanthids and osteichthyans, tooth whorls are few in number and limited to the symphysis [23], whereas in more crownward chondrichthyans they are arrayed along the length of the jaw and may even comprise the entire dentition (gold and yellow-green icons, figures 6 and 7).

Osteichthyan tooth whorls shed via resorption, a mechanism not present in statodont chondrichthyan tooth whorls [14,18]. The distribution of tooth whorls across osteichthyans and within chondrichthyans is complex, with whorl-bearing taxa often nested within whorl-less radiations. Probabilistic ancestral state reconstruction indicates that tooth whorls evolved independently multiple times both within chondrichthyans and across gnathostomes [7]. Within chondrichthyans, different phylogenetic topologies have quite different implications for the gain and loss of tooth whorls and their distribution across the group (figure 7).

Some chondrichthyans have **teeth that are not organised into files** but which lie directly on the jaw cartilage (coral icons [in part; this icon also captured teeth arranged in files that are borne on the jaw cartilages], figures 6 and 7). This condition is present in *Acanthodopsis* (figure 4), *Pucapampella*, and *Gladbachus* [16,51], although presents in different ways. In *Acanthodopsis*, teeth are triangular and diminish in size anteriorly and posteriorly (figure 4). In *Pucapampella*, teeth form a single row along the jaw but show a variety of sizes, shapes, and spacings [16]. In *Gladbachus*, teeth are much reduced and individually separate, although possibly aligned linguo-labially [51]. The broad phylogenetic distribution of these forms, as well as their anatomical variation, suggests that they are homoplasious. Although *Gladbachus* and *Pucapampella* are recovered as a clade in our analyses, this is by no means a typical phylogenetic result (figure 7c,d) and we view this result with extreme caution.

Dentition cones — tooth-like cones with smaller denticles attached — are present in the mouths of some stem-group chondrichthyans. They are only

known in partially-articulated ischnacanthids with dentigerous jaw bones: *Zemylacanthus* (*Poracanthodes*), *Acritolepis*, and *Serradentus* [34,69,70]. However, they are absent in the better characterised *Ischnacanthus* and relatives [19,23]. The lack of fully articulated fossils bearing dentition cones leaves open the possibility that they represent a displaced part of the branchial apparatus rather than oral structures [34]. Their presence may unite a subset of acanthodians with dentigerous jaw bones.

Tooth-like scales are present along the oral margin of some stem-group chondrichthyans; and include part of the cheek squamation, the 'lip' and the ventral rostral area. They are best characterised in *Ischnacanthus*-like acanthodians [19] in which they show a variety of morphologies, and in life may have helped with grasping prey. Some of these scales are strikingly similar in organisation to tooth whorls (which are also present within the gape of the same animals), comprising a file of denticles oriented towards the mouth [19,23]. Specialised tooth-like scales have also been identified along the margin of the mouth in *Obtusacanthus* [62]. More generally, tooth-like denticles are common along the oral margin of the tooth row in early osteichthyans [14,71]. Although potentially interesting from a developmental perspective, they seem unlikely to carry any phylogenetic signal.

Dentigerous jaw bones are tooth-bearing dermal jaw bones present in the upper and lower jaws of a number of stem-chondrichthyan taxa [21]. Although isolated dentigerous jaw bones are comparatively common in the rock record, articulated fossils are relatively few and far between: they include *Zemlyacanthus, Nerepisacanthus*, and *Serradentus* [30,34,72]. By far the best anatomically characterised taxa with dentigerous jaw bones are

Ischnacanthus and similar taxa [23,73]. There are few anatomical characters to group taxa possessing dentigerous jaw bones, but all have a complement of oral structures including some combination of symphyseal tooth whorls, dentition cones, and tooth-like cheek scales. Dentigerous jaw bones themselves display anatomical diversity, for example relating to the structure of the bone, the number and shape of tooth rows [21], and variance in dentition shapes likely linked to diet [73]. In our phylogeny, taxa with dentigerous jaw bones (i.e. ischnacanthids) are recovered in a polytomy, in a broader grouping of acanthodians with dermal mouth plates (dark purple icons, figures 6 and 7). Dermal jaw bones, both edentulous and tooth-bearing, are also present in 'placoderms' and osteichthyans, but their homology is unclear.

Occlusal plates are a pair of smooth dermal plates in the gapes of some stem-chondrichthyans (light purple icons, figures 6 and 7). Their detailed anatomy is poorly characterised and in the past they have become terminologically and anatomically confused with the mandibular splint [25]. Occlusal plates are present in *Diplacanthus*, *Rhadinacanthus*, *Milesacanthus*, *Uraniacanthus*, *Culmacanthus*, and *Tetanopsyrus* [22,24,25,74–76]. At least some of these animals have other common morphologies (i.e. similar body shapes, scapular processes with posterior lamina, large postorbital scales, deep, striated dorsal fin spine insertions), and on this basis they are grouped into the diplacanthids [22]. There is some variation in the morphology of occlusal plates. In all taxa but *Tetanopsyrus* [24,77], they are only present in the lower jaws; *Tetanopsyrus* may also have tooth-like denticles along the inner surface of the plates, although this is only known from an isolated

Meckel's cartilage associated with an already complete *Tetanopsyrus* specimen [24]. In *Uraniacanthus* and *Culmacanthus*, a dorsal process is present [74,75]. We recover diplacanthids as monophyletic in our Bayesian analysis (figure 6, S2), but paraphyletic with respect to *Tetanopsyrus* in our parsimony analysis, and occlusal plates appear to be a character uniting diplacanthids (figures 6, 7). *Tetanopsyrus*, with its upper and lower plates, may represent a link between the occlusal plates of diplacanthids and dermal jaw bones of ischnacanthids.

A **mandibular splint** (variously termed dentohyoid, extramandibular spine, splenial, or mandibular bone) is a slightly sinusoidal dermal bone that underlies the Meckel's cartilage ventrolaterally (grey icons, figures 6 and 7). Unlike the other structures discussed here, it did not lie within the gape, and likely reinforced the lower jaw. Mandibular splints are present in *Acanthodes*, *Acanthodopsis*, *Halimacanthodes*, *Howittacanthus*, and *Protogonacanthus* [44,50,78,79]. They have also been incorrectly identified in a variety of other taxa. Mandibular splints in mesacanthids [45,59] are similar in size to gular plates, and may represent displaced elements of this series. In diplacanthids [75,76] they are better interpreted as occlusal plates [25]. Although a mandibular splint has been identified in the putative cheiracanthid *Protogonacanthus* [50], the taxon in question is likely not a cheiracanthid but an acanthodid [60]. Finally, as we show, descriptions of mandibular splints in ischnacanthids [32,49] instead represent a reinforced ventral margin of the endoskeletal mandible. Mandibular splints in acanthodids are very conservative in form, although may be ornamented as in *Acanthodes sulcatus* [50]. Its similarity to the ventral branchiostegal rays in *Acanthodes* (figure

5a,b), which are also dermal, tubular, and slighty sinusoidal, suggests that it may be part of this series that has been co-opted to support the jaw. Our phylogeny suggests that a mandibular splint unites *Acanthodes* and *Acanthodopsis* but either evolved convergently in *Halimacanthodes* (figure. 6) or was lost in *Homalacanthus*; we consider it most likely that it unites acanthodids to the exclusion of other stem-chondrichthyans, and that this distribution is a result of undersampling acanthodids and their characters in our phylogeny.

4.3 Tooth and jawbone evolution in early gnathostomes

Our new data show that the dentigerous jaw bones of all ischnacanthids, including articulated taxa such as *Ischnacanthus* and those only known from isolated jaws (e.g. atopacanthids and taemasacanthids), were united by a common construction. These follow the model of tooth growth supposed by Ørvig [80] and demonstrated by Rucklin *et al.* [7] on the basis of directional wear and overlapping cusps. These teeth were fused to, but distinct from, the underlying bone, which grew with the endoskeletal component of the jaw. This condition and the positions of tooth rows relative to the underlying dermal plate is common to the three different morphologies of dentigerous jaw bone that we describe and we infer it to have been a common feature of ischnacanthid dentigerous jaw bones The presence of a (presumably pathological) out of sequence tooth in *Taemasacanthus*, where the youngest tooth has partially overgrown a cusp anteriorly (figure 1), suggests that non-sequential growth was possible in the otherwise ordered tooth rows. Dentigerous jaw bones are broadly comparable with the condition in stem-

gnathostomes in the sense that non-shedding teeth are growing on a basal bone. However, phylogenetic topologies supporting homology between these conditions are in limited supply (figure. 7), and is not supported by our topology or by more detailed analysis [7].

Although dentigerous jaw bones are typically contrasted with tooth whorls [7], we suggest that dentigerous jaw bones can be usefully interpreted by comparison to “stretched out” tooth whorls. The tooth rows on dentigerous jaw bones are comparable to whorls in that they grow in a single direction in isolated files on an underlying dermal plate. Notably, symphyseal tooth whorls and whorl-like cheek scales in some ischnacanthids could suggest common patterning mechanisms affecting dermal structures in and around the mouth of early chondrichthyans [19]. Teeth and tooth-like structures are added directionally across the gnathostome crown-group (e.g. [6]). However, tooth rows growing in isolated files, on whorls or dentigerous jaw bones, appears to be apomorphic for chondrichthyans (with osteichthyan tooth whorls optimised as homoplasious [7]), and may be a character uniting chondrichthyans more broadly than the presence of tooth whorls.

The row of monocuspid ‘teeth’ borne directly on the Meckelian element of *Acanthodopsis* is unlike that of any other known chondrichthyan or gnathostome. Furthermore, the presence of teeth in a Carboniferous taxon deeply nested within an edentulous radiation (figure 6), the oldest of which are Early Devonian in age, strongly suggests that this represents an independent acquisition of dentition. Previous studies of the jaw of *Acanthodopsis* have interpreted it either as a dentigerous jaw bone [80] or as a perichondrally ossified Meckelian element with ‘teeth’ [21]. Our data confirm the latter view

and suggest that the teeth in *Acanthodopsis* are histologically distinct from the underlying perichondral bone and likely dermal in origin. Our phylogenetic analysis supports the view of Long [27] and Burrow [21] that *Acanthodopsis* is closely related to *Acanthodes*, with the presence of teeth representing the only difference between the genera. A possible morphological comparison to these teeth lies in the branchial and hyoid rakers found in acanthodiform fishes like *Acanthodes*, *Cheiracanthus*, and *Homalacanthus* [36,60]. As with the teeth of *Acanthodopsis*, these rakers are triangular in shape, sometimes striated ([81] fig. 3), and decrease in size from the centre outwards (Dearden *pers obvs*). An alternative to a cryptic homology between teeth in *Acanthodopsis* and other gnathostomes may be the co-option of hyoid and branchial rakers to form a novel “dentition” on the mandibular arch.

This revised picture of ‘acanthodian’ jaw elements adds a critical perspective to hypotheses of dental evolution. The ‘Acanthodii’ grade or clade, members of which are typically (but not always) recovered towards the base of the chondrichthyan total group (figures 6,7) display a plethora of oral structures, including dermal jaw plates, symphysial tooth whorls with joined bases, as well as edentulousness. Mandibular splints and occlusal plates may characterise subclades within this radiation. Dermal jaw bones are absent in taxa more proximate to the chondrichthyan crown, and a dentition comprised entirely of tooth whorls along the whole length of the jaw is also restricted to this node. This may represent a ‘two-step’ development of the stereotypical chondrichthyan dentition, with a shift towards tooth whorls bone exclusively on the jaw cartilages, and the eventual loss of fused bases and concomitant development of tooth shedding at the chondrichthyan crown node. Not all

topologies support this hypothesis, however: Frey *et al.* [52], for example, recover climatiids as remote from the chondrichthyan crown node, with an extensively whorl-based dentition borne on the jaw cartilages rather than dermal bones apparently developing independently or being lost in "Acanthodii". The interposition of many non-shedding stem-chondrichthyan taxa between shedding chondrichthyans and shedding osteichthyans confirms that a shedding dentition evolved twice, in two different ways, in crown-gnathostomes [6,7,11,14]. The teeth of extant chondrichthyans, borne directly on endoskeletal mandibular cartilages, are positionally distinct from both the inner and outer dental arcades of osteichthyans, where teeth are borne on dermal bones.

A comparison of recent phylogenetic hypotheses for the chondrichthyan stem-group (figure 7) as well as the generally low support value within the chondrichthyan stem-group in our own phylogenetic analyses (figures S1,2) illustrate the instability in the deepest parts of the chondrichthyan tree. Similar conflicting topologies in the gnathostome stem group, for example over whether 'placoderms' are monophyletic and the relationships of antiarchs and arthrodires to the gnathostome crown node [6,58,82–84], in conjunction with an array of difficult-to-interpret dental conditions [6], compound this problem. Likelihood-based methods provide one way of overcoming this uncertainty [7], but the proliferation of different tree shapes in conjunction with generally low support values means that results should be considered across multiple potential topologies. This illustrates the challenges of drawing broad scale conclusions for gnathostome tooth evolution on the basis of unstable relationships or tentatively-placed taxa.

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Ethics. This research is based exclusively on specimens from natural history collections.

Data accessibility. Raw data (.vol or .tiff stacks), Mimics files, and 3D PLY files for each specimen are deposited in Dropbox for the purposes of review((Acanthodes_NHMUK_PV_P.8065 PLY files: https://www.dropbox.com/sh/7mmrrdo0shhhkc3/AABG5jhLrbghHeMghpYgizJAa?dl=0; Acanthodes_NHMUK_PV_P.8065 TIFF stack:

https://www.dropbox.com/sh/ao3hua7467kat40/AADUb_etPMoD_CK-c7m-E5kba?dl=0; Acanthodes_NHMUK_PV_P.8065 Mimics file: https://www.dropbox.com/s/x8hfru687hs8lqj/Acanthodes_NHMUK_PV_P.8065_mimics.mcs?dl=0; Acanthodopsis_NHMUK_PV_P.10328 PLY files: https://www.dropbox.com/sh/2ip4lzsd56yjpyz/AAAqqq2BU6EbJ8x8Xm0TJWuTa?dl=0; Acanthodopsis_NHMUK_PV_P.10328 TIFF stack https://www.dropbox.com/sh/n24djy5n4de82bd/AABDEmru9PVyOqSHg-f_GCqNa?dl=0; Acanthodopsis_NHMUK_PV_P.10328 Mimics file: https://www.dropbox.com/s/9y9r7yac7p4gc0n/Acanthodopsis_NHMUK_PV_P.10328_mimics.mcs?dl=0 Atopacanthus_NHMUK_PV_P.10978 PLY files: https://www.dropbox.com/sh/0kgc31bw6b1sb78/AAB0kvTiCeA__Nw4JTrKd5f3a?dl=0; Atopacanthus_NHMUK_PV_P.10978 TIFF stack: https://www.dropbox.com/sh/6ceer4qkelkv8be/AABZGZqJ-tneqFvX8Ff_K7UZa?dl=0 Atopacanthus_NHMUK_PV_P.10978 Mimics file: https://www.dropbox.com/s/gxiyur8qo54mcxw/Atopacanthus_NHMUK_PV_P.10978_mimics.mcs?dl=0; Ischnacanthus_NHMUK_PV_P.40124 PLY files: https://www.dropbox.com/sh/mrskv03iztott7s/AAChCylP9LMOYOXbYDymN_pCa?dl=0; Ischnacanthus_NHMUK_PV_P.40124 TIFF stack: https://www.dropbox.com/sh/x82rgb6dx5zjv1g/AAB8f6psZiuwY-m-R-_SiK76a?dl=0; Ischnacanthus_NHMUK_PV_P.40124 Mimics file: https://www.dropbox.com/s/4d2bboluoes51l4/Ischnacanthus_NHMUK_PV_P.40124_mimics.mcs?dl=0; Taemasacanthus_erroli_NHMUK_PV_P33706 PLY files: https://www.dropbox.com/sh/521y1zjasuff2c7/AABeQ16MiFbNKbSyMuvCG4Rva?dl=0; Taemasacanthus_erroli_NHMUK_PV_P33706 VOL file:

https://www.dropbox.com/sh/tm798rx0ilmutqo/AADob3ja5rCoZV5Hd_D5qlt_a?dl=0; Taemasacanthus_erolli_NHMUK_PV_P33706 Mimics file: https://www.dropbox.com/s/qm90cxvlivd4zf8/Taemasacanthus_erolli_NHMUK_PV_P33706_mimics.mcs?dl=0.) and will be permanently deposited in Dryad upon manuscript acceptance.

Authors' contributions. S.G. conceived the project and selected specimens. S.G. and R.P.D. carried out CT scanning. R.P.D. segmented the specimens, made Blender renders and constructed figures with input from S.G. S.G. and R.P.D. drafted the manuscript. Both authors revised and edited the manuscript, approved the final version and agree to be accountable for all aspects of the work.

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Figure captions

Figure 1. Tomographic model of the left lower jaw of *Taemasacanthus erroli* NHMUK PV P.33706 in (*a*) medial view, (*b*) lateral view, (*c*) ventral view, (*d*) dorsal view, (*e*) posterior view, (*f*) a reconstructed tomogram showing a transverse section through the lower jaw and (*g*) a reconstructed tomogram showing a sagittal section through the lingual tooth row. Teeth in panels (*a*) and (*b*) are coloured separately from the dentigerous jaw bone. Abbreviations: art, articular (Meckel's cartilage); art.con, articular 'condyle'; circ.ri, circular ridge; djb, dentigerous jaw bone; lat.t.r., lateral tooth row; ling.t.r, lingual tooth row; mes.ri, mesial ridge; mes.t.r, mesial tooth row; post.gr, posterior groove; young.t, out-of-order youngest tooth; vent.gr, ventral groove; vas.can, vascular canals. Scale bar = 5 mm.

Figure 2. Tomographic model of a dentigerous jaw bone of *Atopacanthus* sp. NHMUK PV P.10978 in (*a*) medial view, (*b*) lateral view, (*c*) ventral view, (*d*) dorsal view and (*e*) a reconstructed tomogram showing a sagittal section through the lingual tooth row. Teeth in panels (*a*) and (*b*) are coloured separately from the dentigerous jaw bone. Abbreviations: djb, dentigerous jaw bone; lat.t.r., lateral tooth row; ling.pl, lingual plate; ling.t.r, lingual tooth row; mes.ri, mesial ridge; mes.t.r, mesial tooth row; vent.gr, ventral groove; vas.can, vascular canals. Scale bar = 5 mm.

Figure 3. Tomographic model of the left lower jaw of *Ischnacanthus* sp. NHMUK PV P.40124 in (*a*) lateral view, (*b*) medial view, and (*c*), (*d*), (*e*) reconstructed tomograms showing successively deeper sagittal sections. Abbreviations: art.proc, articular process; djb dentigerous jaw bone; lat.t.r., lateral tooth row; Meck.c, Meckel's cartilage; per, perichondral bone; rid, ridge; vas.can, vascular canal. Scale bar = 5 mm.

Figure 4. Tomographic model of the right lower jaw of *Acanthodopsis* sp. NHMUK PV P.10383 in lateral view with (*a*) and without (*b*) mandibular splint, (*c*) medial view, (*d*) dorsal view, (*e*) anteromedial view, (*f*) posterior view, and reconstructed tomograms showing (*g*) a sagittal section through the entire jaw and (*h*) a transverse section through the jaw. Abbreviations: art.cot, articular cotylus; gr.mand.spl, groove for mandibular splint; inn.lay, inner layer; mand.add.fo, mandibular adductor fossa; mand.spl, mandibular splint; Meck.c, Meckel's cartilage; out.lay, outer layer; pgl.pr, preglenoid process; sym.fos, symphyseal fossa; 't', 'teeth'. Scale bar = 5 mm in (*a*)-(*g*), 2 mm in (*h*).

Figure 5. The lower jaws of *Acanthodes* sp. NHMUK PV P.8085 in (*a*) dorsal view against the matrix, (*b*) in ventral view superimposed on a digital mould of the matrix's surface, the left lower jaw isolated in (*c*) medial and (*d*) lateral view, and reconstructed tomograms showing (*e*) a coronal section through the specimen, and (*f*) a transverse section through a lower jaw. Abbreviations: art, articular (Meckel's cartilage); br, branchiostegal rays; mand.spl, mandibular

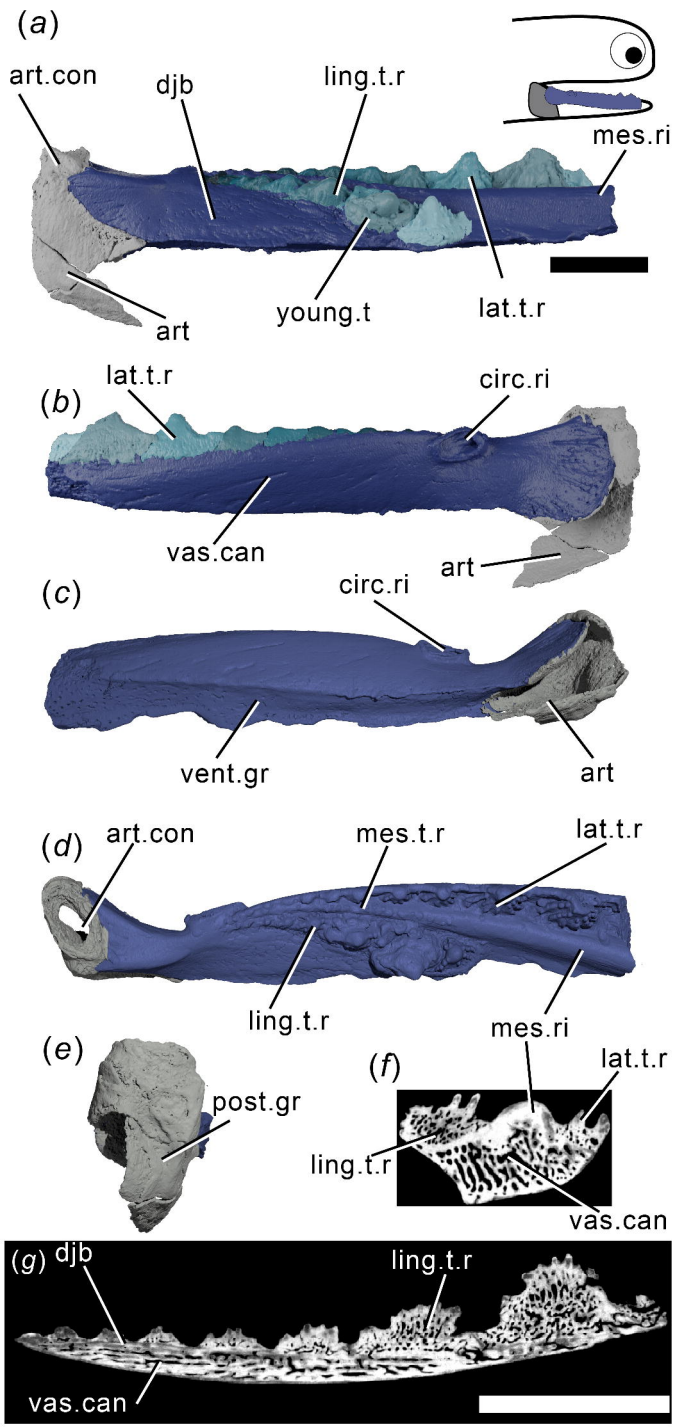
splint; Meck.c, Meckel's cartilage; ment, mentomandibular (Meckel's cartilage); pgl.pr, preglenoid process; rak, gill raker; sym.fos, symphyseal fossa; vis.ar, visceral arch fragments. Scale bar = 5 mm in (*a*)-(*e*), 2mm in (*f*).

Figure 6. Phylogenetic relationships of early chondrichthyans and distribution of oral structures. Strict consensus of 26101 most parsimonious trees, with some non-chondrichthyan taxa excluded (full tree with support values in figure S1). Filled boxes indicate presence of feature; '?' indicates uncertainty; '-'indicates inapplicability.

Figure 7. Summaries of four contrasting recent phylogenetic schemes of early chondrichthyans, with distribution of oral characters. (a) King *et al.* 2016 [58], (b) Chevrinais *et al.* 2017 [57], (c) Coates *et al.* 2018 [51], (d) Frey *et al.* 2020 [52]. Boxes as in Fig. 6 Acanthodiforms includes taxa both with and without mandibular splints.

Supplementary material (separate pdf)

Supplementary information for this manuscript is included as two files. The first includes notes on the phylogenetic analysis (taxon addition, character addition and coding changes) and supplementary figures 1-5. The second is a zipped folder containing all files necessary for replicating our Bayesian and parsimony analyses.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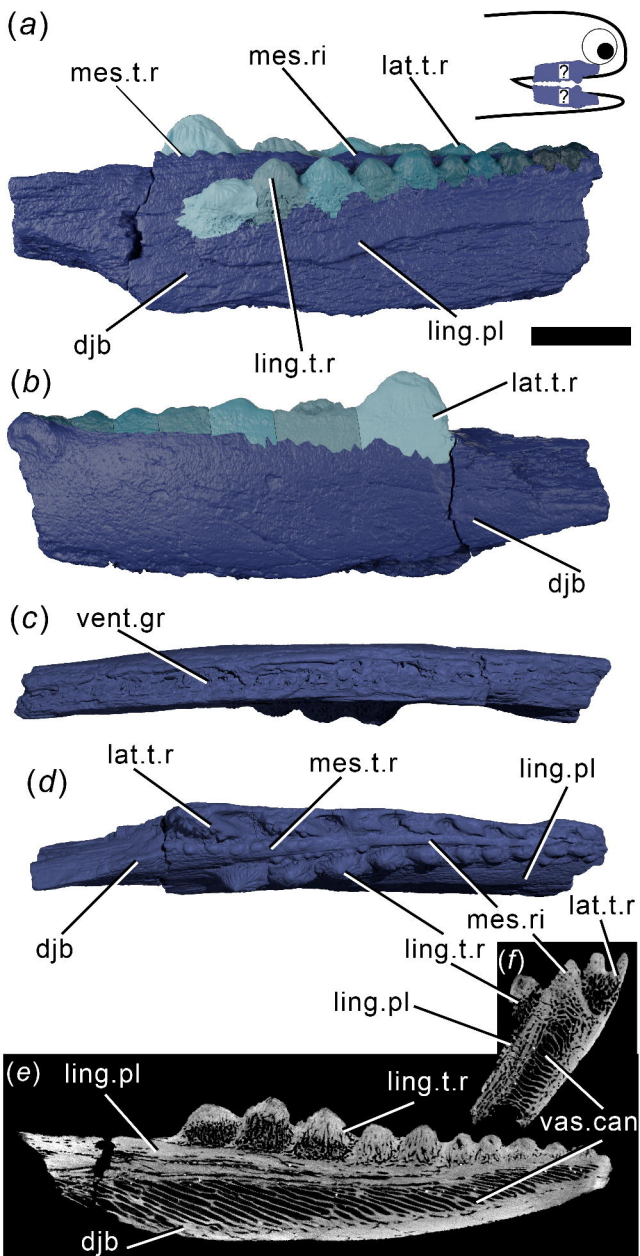
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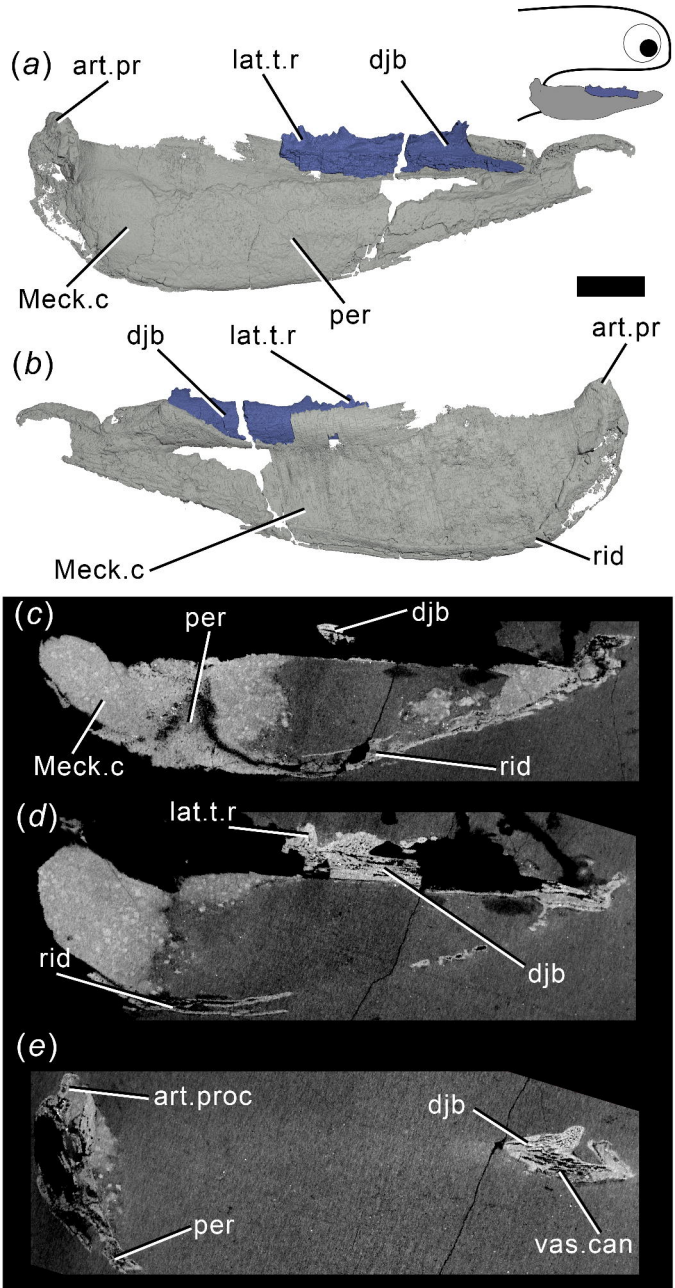
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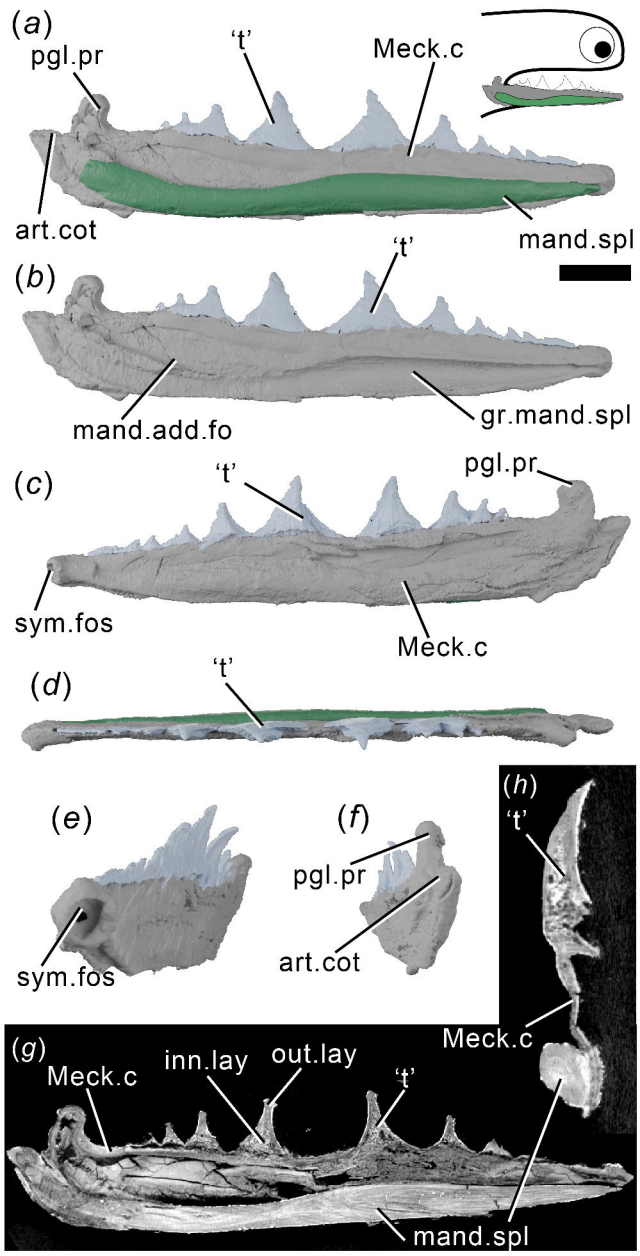
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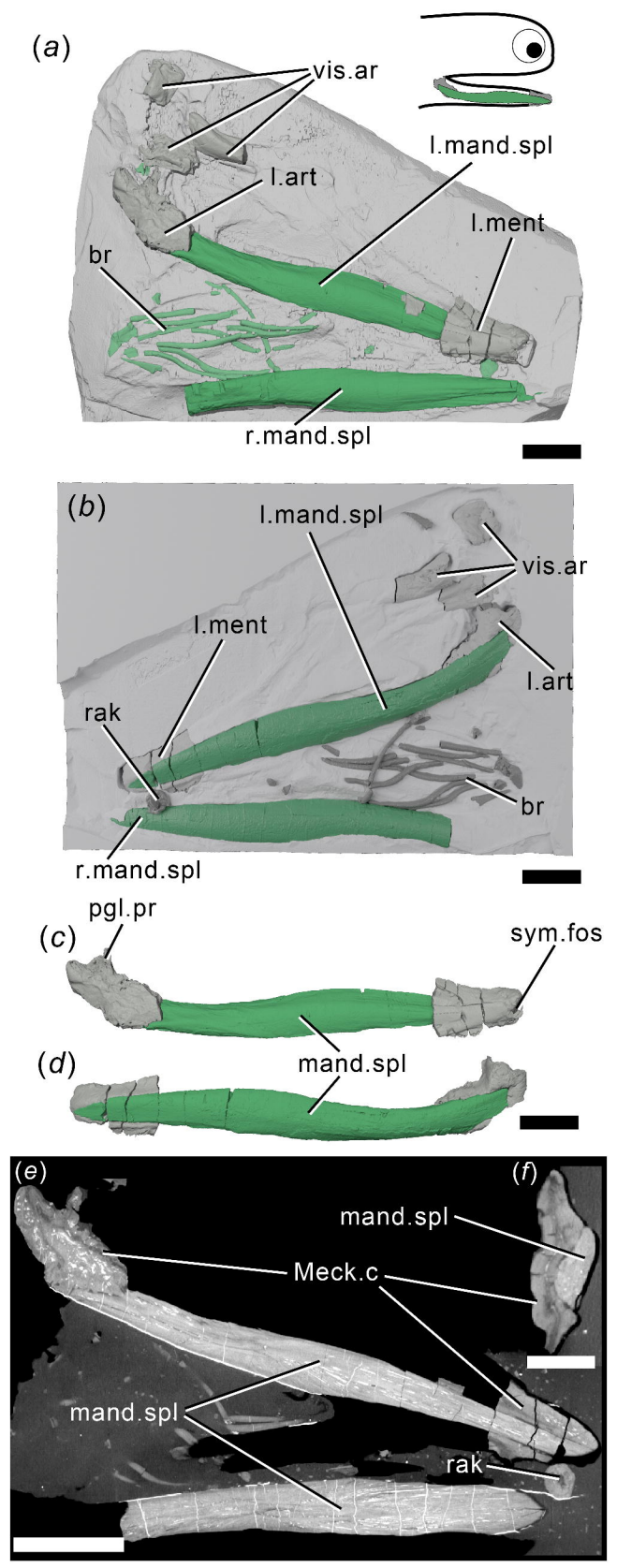
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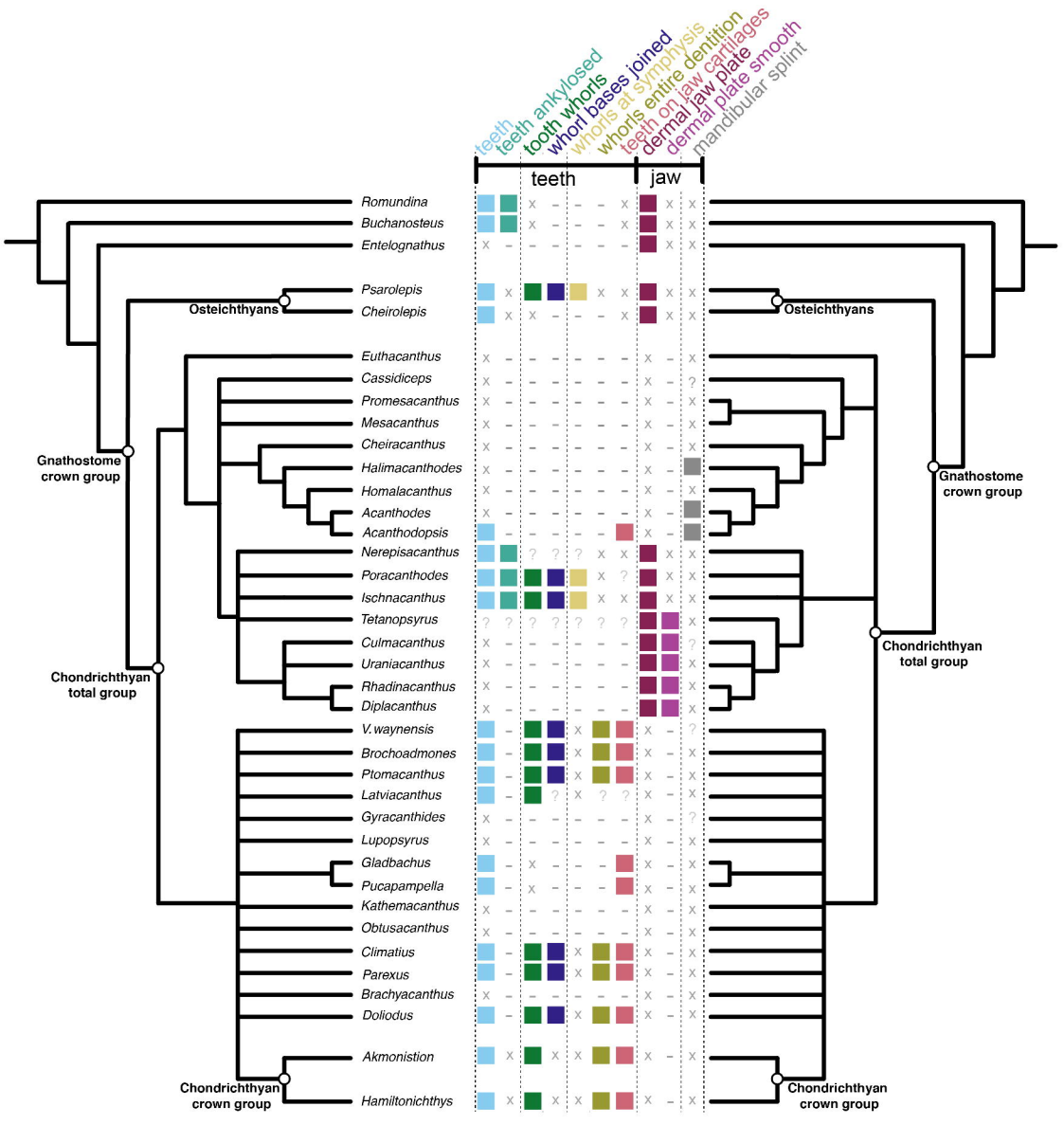
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teeth ankylosed
tooth whorls
whorl bases joined
whorls at symphysis
whorls entire dentition
teeth on jaw cartilages
dermal jaw plate
dermal plate smooth
mandibular splint
teeth
jaw
Romundina
Buchanosteus
Entelognathus
Psarolepis
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Osteichthyans
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Acanthodes
Acanthodopsis
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Tetanopsyrus
Culmacanthus
Uraniacanthus
Rhadinacanthus
Diplacanthus
V. waynensis
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Ptomacanthus
Latviacanthus
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Lupopsyrus
Gladbachus
Pucapampella
Kathemacanthus
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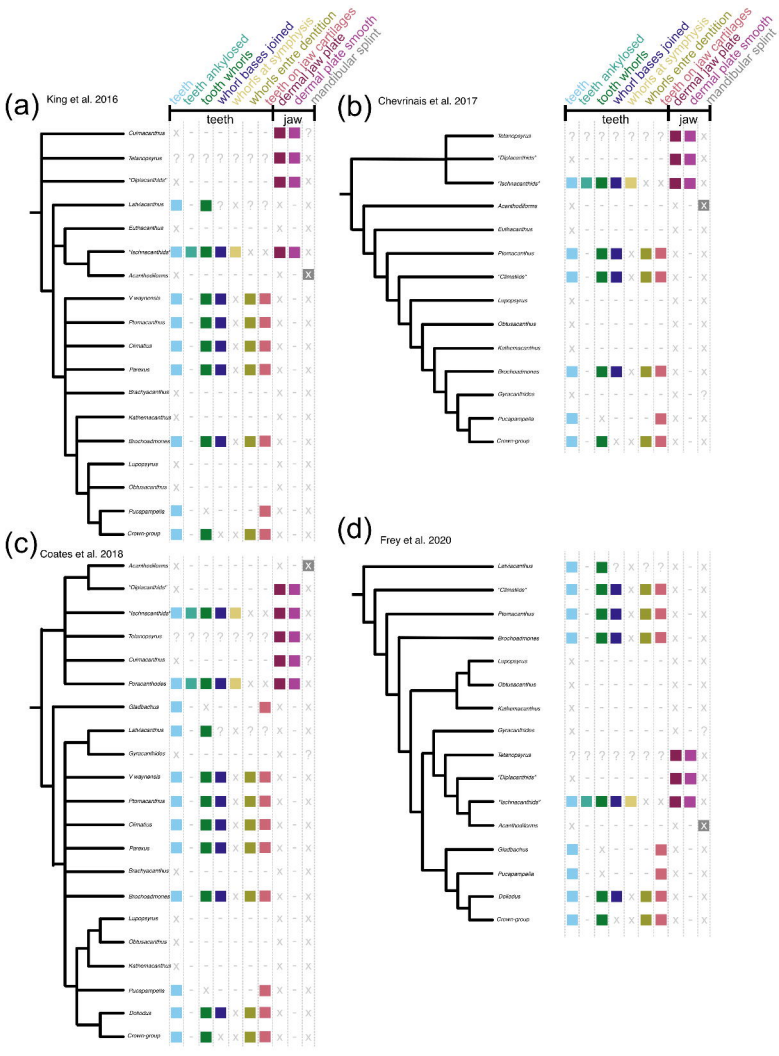

(a)
King et al. 2016
teeth
teeth ankylosed
tooth whorls
whorl bases joined
whorls at symphysis
whorls entire dentition
teeth on jaw cartilages
dermal jaw plate
dermal plate smooth
mandibular splint
teeth
jaw
Curmacanthus
Telanopsyrus
"Diplacanthids"
Labiacanthus
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Kathemacanthus
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Lupopsyrus
Obtusacanthus
Pucapampella
Crown-group
(b)
Chevrinais et al. 2017
Tetanopsyrus
"Diplacanthids"
"Isohnacanthids"
Acanthodiforms
Euthacanthus
Ptomacanthus
"Climatius"
Lupopsyrus
Obtusacanthus
Kathemacanthus
Brochoadmones
Gyracanthides
Pucapampella
Crown-group
(c)
Coates et al. 2018
Acanthodiforms
"Diplacanthids"
"Isohnacanthids"
Telanopsyrus
Curmacanthus
Poracanthodes
Gladbachus
Labiacanthus
Gyracanthides
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Ptomacanthus
Climatius
Parexus
Brachyacanthus
Brochoadmones
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Obtusacanthus
Kathemacanthus
Pucapampella
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Crown-group
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Labiacanthus
"Climatius"
Ptomacanthus
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"Isohnacanthids"
Acanthodiforms
Gladbachus
Pucapampella
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