

Teeth before jaws? Comparative analysis of the structure and development of the external and internal scales in the extinct jawless vertebrate *Loganellia scotica*

Martin Rücklin,^a Sam Giles,^a Philippe Janvier,^b and Philip C. J. Donoghue^{a,*}

^aSchool of Earth Sciences, University of Bristol, Wills Memorial Building, Queen's Road, Bristol, BS8 1RJ, UK

^bMuséum National d'Histoire Naturelle, CNRS, UMR 7207, Paris, 75231, France

* Author for correspondence (email: phil.donoghue@bristol.ac.uk)

SUMMARY Traditional hypotheses posit that teeth evolved from dermal scales, through the expansion of odontogenetically competent ectoderm into the mouth of jawless vertebrates. The discovery of tooth-like scales inside thelodonts, an extinct group of jawless vertebrates, led to the alternative hypothesis that teeth evolved from endodermal derivatives and that there exists a fundamental developmental and phylogenetic distinction between oral/pharyngeal and external odontodes. We set out a test of this latter hypothesis, examining the development of scales of the thelodont *Loganellia scotica* using synchrotron radiation X-ray tomographic microscopy (SRXTM). We reveal that the internal scales are organized into fused patches and rows, a key distinction from the discrete der-

mal scales. The pattern of growth of oral scale patches is polarized, but not along a particular vector, whereas pharyngeal scale rows grew along a vector. Our test of the phylogenetic distribution of oral and pharyngeal scales and teeth in vertebrates indicates that odontodes are first expressed in an external position. Internal scales, where present, are always located near to external orifices; the sequential development of pharyngeal scales in *Loganellia* is peculiar among thelodonts and other stem gnathostomes. It represents a convergence on, rather than the establishment of, the developmental pattern underpinning tooth replacement in jawed vertebrates. The available evidence suggests that internal odontodes evolved through the expansion of odontogenic competence from external to internal epithelia.

INTRODUCTION

Almost all living vertebrates are jawed vertebrates and their rise to dominance over the jawless vertebrates has long been considered contingent on the origin of jaws and teeth. The traditional hypothesis posits that teeth evolved from dermal scales, through the expansion of odontogenetically competent ectoderm into the mouth of jawless vertebrates (Reif 1982). This is based primarily on pre-evolutionary observations that tooth and scale development are very similar (e.g., Williamson 1849), so much so that they have been grouped as a class of structures known as odontodes (or more correctly, odontode derivatives; Donoghue 2002).

This dogma has been challenged by the discovery of arrays of tooth-like scales in thelodonts, an extinct group of jawless vertebrates. Specifically, the thelodont *Loganellia scotica* has been shown to possess a field of buccal and pharyngeal scales that differ in their size, arrangement, and inferred pattern of development, from the scales that comprise the dermal skeleton (Van der Bruggen and Janvier 1993; Smith and Coates 1998). In comparison to the individual scales that comprise the dermal skeleton of *Loganellia*, the buccal and pharyngeal scales are fused into patches and polarized rows, respectively,

described as pharyngeal denticles or tooth whorls associated with gill bars or an extrabranchial duct (Van der Bruggen and Janvier 1993). Based on their location within the mouth and the pharynx, the internal scales have been inferred to have developed from endoderm rather than ectoderm (Smith and Coates 1998). Together with evidence from developmental genetics, this has led to the hypothesis that teeth evolved from endoderm, rather than from ectoderm and, thus, that there is a fundamental developmental and phylogenetic distinction between oral/pharyngeal and external odontodes (Smith and Coates 1998, 2000, 2001; Smith 2003). Teeth and scales are considered to have distinct evolutionary histories that predate mineralizing vertebrates (Fraser et al. 2010).

There remain advocates of the traditional hypothesis who rationalize *Loganellia* as evidencing the inward expansion of ectoderm through the mouth, nasal, and pharyngeal openings of early vertebrates, implying a much more recent common ancestry of these two classes of structures (Huyssene et al. 2009, 2010). The discovery that tooth development is not contingent on endoderm and, in fact, ectoderm, endoderm, and mixed ecto/endodermally derived epithelia are odontogenetically competent (Soukup et al. 2008) has done nothing to dampen the debate. Although these data

remove the developmental distinction between dermal scales and teeth or oral/pharyngeal scales, whether oral odontodes evolved through heterotopy from dermal odontodes (Donoghue and Sansom 2002), or have distinct evolutionary origins (Smith and Coates 1998; Fraser et al. 2010), remains an open question. The presence of internal scales in the thelodont *L. scotica* continues to be influential in supporting the contention that internal and external odontodes have been maintained as distinct developmental modules since before the earliest skeletonizing vertebrates (Fraser et al. 2010). After all, the pharyngeal scales in *Loganellia* have been described as organized into developmentally polarized rows, such as tooth whorls, and quite unlike anything encountered in the external dermal skeleton. If internal and external odontodes are to be considered homologous, under this scenario, they have been united only in a “deep homology” rooted in a gene regulatory network, itself inherited, perhaps, from a cutaneous sensory receptor (Fraser et al. 2010; Fraser and Meredith Smith 2011).

It is astonishing that so much of this debate, including the evolutionary transformation of germ layer boundaries and the interpretation of molecular developmental studies, has been inspired and influenced by a dead fish. Even more astonishing is the fact that the material basis of this debate has yet to be established: (i) the hypothesis of a fundamental distinction of size, shape, and development, between the internal and external scales of *Loganellia* and, (ii) the hypothesis of a fundamental similarity between the development of the pharyngeal scales of *Loganellia* and the tooth whorls of chondrichthyans and osteichthyans. Thus, we set out to effect two tests of the hypothesis that the internal scales of the extinct thelodont *L. scotica* evidence the evolution of teeth before jaws.

First we set out to establish the material basis of the hypothesis of homology by determining whether or not the different classes of internal and external scales in *Loganellia* differ in composition or in terms of their inferred development and whether the internal scales show any clear resemblance to the teeth of jawed vertebrates. Corroboration of these inferences would substantiate the conjecture of homology between the internal compound scales of the extinct jawless thelodont *Loganellia* and the replacement teeth of living jawed vertebrates. However, any such conjecture of homology needs also to be corroborated by phylogenetic congruence to determine whether similarity is a consequence of convergence or common descent (Patterson 1982; de Pinna 1991). Therefore, our second test of homology was to evaluate the evidence for phylogenetic continuity between the internal scales of *Loganellia* and the teeth of jawed vertebrates. Such evidence would be expected to demonstrate the derivation of scales and teeth from a structure present in the most recent common ancestor of *Loganellia* and jawed vertebrates. We reconsider the significance of *Loganellia* for

understanding the evolutionary origin of teeth in light of the outcome of these tests.

MATERIAL AND METHODS

In addition to their dermoskeleton of external scales, three types of internal scales were described by Van der Bruggen and Janvier (1993): (1) minute, forward pointing scales in the snout; (2) backward-pointing scales in the buccal cavity, similar to the minute ones; (3) slender, often fused thin, curved series of posteriorly pointing scales located near the branchial openings. Scales representative of each of the internal and external sites were extracted from the original material described by Van der Bruggen and Janvier (1993; GBP384); scale classes 1 and 2 cannot be distinguished in this material because they differ only in terms of their orientation. Material of *Loganellia scotica* is deposited at the Muséum national d'Histoire naturelle, Paris (MNHN); an ischnacanthid acanthodian tooth whorl used for comparison is deposited at the Department of Palaeozoology, Naturhistoriska Riksmuseet Stockholm (NRM).

Volumetric characterization of 36 fossil scales of one animal (GBP384) was achieved using synchrotron radiation X-ray tomographic microscopy (SRXTM; Donoghue et al. 2006a) at the TOMCAT beamline (Stampanoni et al. 2007) of the Swiss Light Source, Paul-Scherrer Institut, Switzerland. Using a 20× objective, exposure time at 12 keV was 55 ms, and 1501 projections were acquired equi-angularly over 180°. Projections were post-processed and rearranged into flat- and darkfield-corrected sinograms, and reconstruction was performed on a 32-node Linux PC farm. Isotropic voxel dimensions are 0.36 μm. Slice data were analyzed and manipulated using Avizo 6.2 (www.vsg3d.com). Sectional images in different directions were studied and, using the labeling option, three-dimensional models of the different growth stages were derived.

RESULTS

Comparative structure and development

Dermal scales

Scales cover the whole external body surface of thelodonts and vary significantly in their morphology depending on their position and growth stage (Gross 1967). Crown and base are separated by a concave neck (Fig. 1A–C, F). The shape of the crown varies from “oak-leaf” shaped body scales to oval or round head scales, and flat to convex. Examined specimens have six lateral ridges, three on each side. The base is oval and slightly shorter than the crown along an antero-posterior direction, and nearly as wide as the crown. Small openings on the surface of the base open to the spaces for odontoblast cell processes that dominate the structure of the crown (ocp, Fig. 1A). The opening in the base of the scale to the central pulp cavity (pc, Fig. 1A) decreases in size with the age of the scale due to the centripetal infilling of the

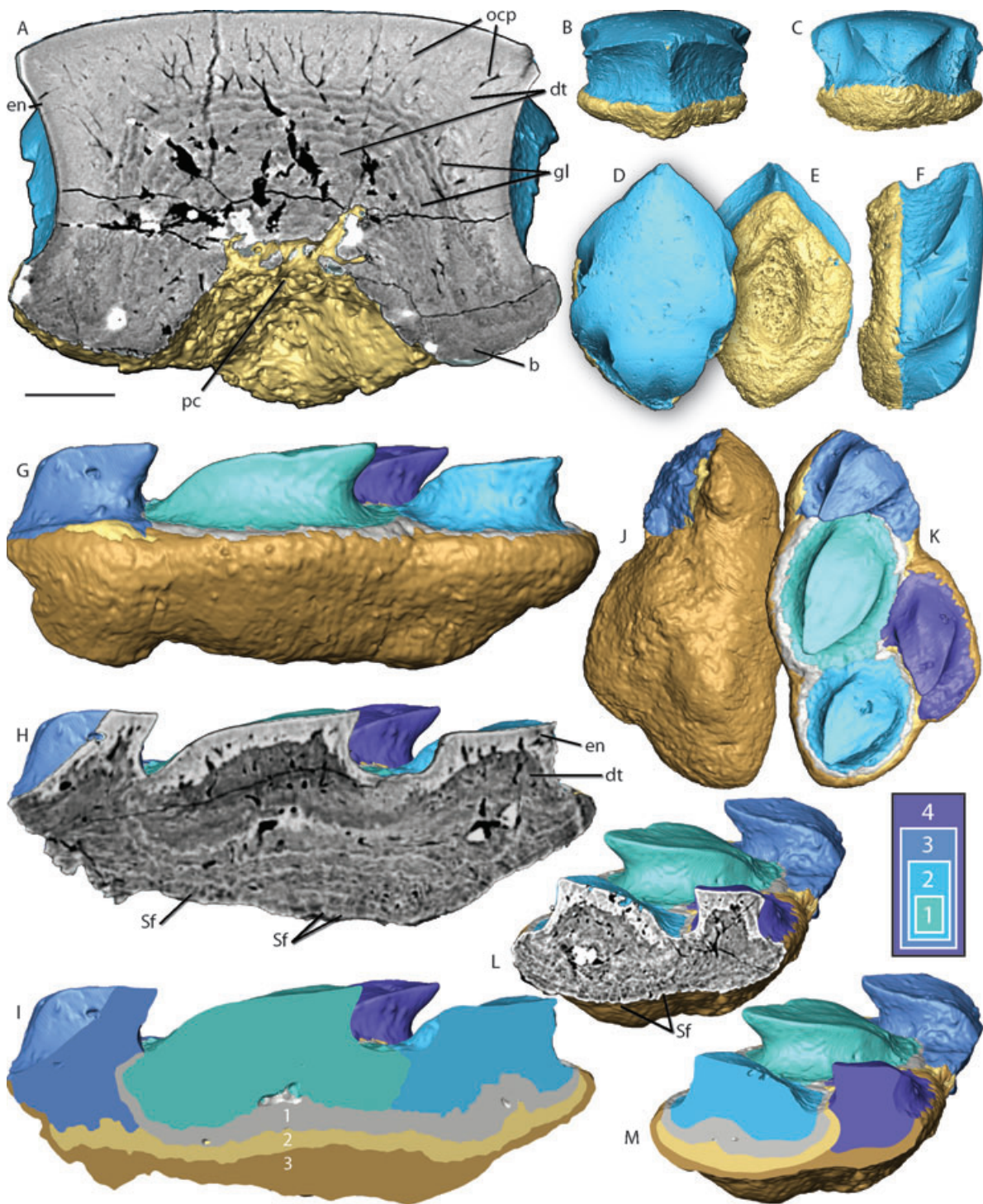


Fig. 1. External and oral scale of *Loganellia scotica* from the Silurian of Scotland (MNHN GBP384). SRXTM images: volume rendering of cusps and bases (B–G, J, K, M), surface-cut showing SRXTM image (A, H, L), and surface-cut showing rendered surfaces (I, M). External scale (A–F): (A) transverse section through volume rendering, (B) posterior, (C) anterior, (D) dorsal, (E) ventral, and (F) lateral view. Oral scale (G–M): (G) lateral view of volume rendering, (H) longitudinal section, (I) longitudinal surface cut, (J) ventral, (K) dorsal view, (L) transverse section, and (M) transverse surface cut through volume rendering. Abbreviations used in this figure 1 and Figure 2: b, base; dt, dentine; en, enameloid; gl, growth line; mr, median ridge; ocp, odontoblast cell process; pc, pulp cavity; and Sf, Sharpey’s fibers. Colors of the nested boxes reflect the successive stages of development of the scales in ascending order. Relative scale bar represents 40 μm (A), 153 μm (B–F), 38 μm (G–I), 60 μm (J, K), 45 μm (L–M).

pulp cavity, as has been described in other thelodonts (Gross 1967).

The crown consists of a very thin surficial layer that appears as a high attenuation phase in the SRXTM data and compares to a thin surface layer of enameloid observed in other thelodont taxa (Donoghue et al. 2006b; Sire et al. 2009). This enameloid layer caps and encapsulates a core of dentine that comprises the bulk of the scale (en, Fig. 1A). Over eight clear growth lines are visible within the dentine, with each growth line approximately 5 μm in thickness (gl, Fig. 1A). The dentine contains an extensive network of spaces for odontoblast cell process and their vasculature (dt, Fig. 1A), opening to the pulp cavity. The base of the scale, traditionally interpreted as acellular bone (e.g., Gross 1967), is composed of a low attenuation phase in SRXTM data, with up to three or four faint growth lines (b, Fig. 1A). Growth layers in the base are typically 20 μm thick, but vary highly.

Oral scales

Component scales are four to six times smaller than external scales. The shape of the scales is also distinct, with a single cusp that expands to define a triangular flat surface with concave sides as it tapers in height to a rounded base (Fig. 1G–M). Some scales possess cusps with one or two low median ridges. The bulk of the tissue comprising the crown is permeated with distinct spaces for odontoblast cell processes and represents orthodentine (dt, Fig. 1H), capped with a 10–15 μm thick layer of enameloid that appears as a distinct, highly attenuating phase in SRXTM data (en, Fig. 1H and L). The base is 40–60 μm thick and composed of several layers, each 5–15 μm . Sharpey's fibers are visible as globular structures in cross-sections and parallel fibers in longitudinal sections (Sf, Fig. 1H and L). As for the external scales, the crown of dentine and enameloid grew first with a pulp cavity in the middle. An acellular base layer formed subsequently, filling in the pulp cavity in larger, thus, older specimens. The key difference in comparison to the external dermal scales is the fusion of adjacent scales.

In our material, scales are fused into patches of up to five in number, although much larger patches of scales have been reported from the same specimen (Smith and Coates 2000). The scales are bound together by a continuous layer of tissue. Cusps on the same patch have approximately the same orientation, but different sizes. The anterior margins of the component scales are obscured by its neighbors. Small openings and pulp openings are rarely visible from the base. SRXTM data reveal the successive growth stages within the tissue that binds the scales, as well as the growth sequence of the component scales. The first formed scales display convex margins, but those that are added to their margin are concavo-convex, their concave margin fitting into the convex margin of the preceding scale (Fig. 1), with the convex edge

forming the free margin onto which the next stage is added. The bases of newly added scales extend beneath the existing scales, uniting them into a plate. There is no polarity to the addition of the scales, other than to the periphery of the pioneer scale. Larger patches are probably the result of smaller, coalescing patches of two or three scales.

Pharyngeal scales

Component scales are four to five times smaller than the external scales and approximately the same size as the oral scales. Pharyngeal scales have a distinct shape, with a rounded bottom and slender cusp that ranges from 50 μm to 80 μm in height, sloping gradually to the base of the crown over the long axis of the scale, sometimes with a median ridge that extends along the back of the cusp (mr, Fig. 2A). The crowns are composed of enameloid and dentine (dt, Fig. 2B) with odontoblast cell processes (ocp, Fig. 2B). The enameloid layer appears as a highly attenuating phase in SRXTM data, ranging from 4–13 μm in thickness (en, Fig. 2B). Scale bases are composed of several layers. Scale growth is reflected in the centripetal infilling of the pulp cavity and the constriction of the neck of the pulp cavity by the growth of the acellular bone of attachment (pc, Fig. 2B). Hence, the growth exhibited by individual pharyngeal scales is similar to that of oral and external scales, but the sequence of addition of adjacent scales is different.

Adjacent scales are united by a thin (<10 μm) basal tissue layer in which growth lines preserve evidence of the sequence of scale addition. Scales are added in sequence along the tooth row with their cusps oriented oblique to the direction of scale addition (Fig. 2F). Each scale is fused to, and accommodates the preformed margin of, its predecessor (Fig. 2B), indicating that, within the developmental sequence, scale development is initiated only after the preceding scale is fully developed. As in the oral scales, basal plate tissue is added in association with each new scale and this underpins all previously developed basal plate tissue. The principal distinguishing characteristic of pharyngeal scales in comparison to oral scales is their strictly polarized sequence of addition.

Interpretation

The original descriptions of the internal scales in *L. scotica* observed clear differences that set the internal scales apart from external scales. The internal scales are much smaller and frequently organized into fused patches or tooth-like rows in which component scales are polarized in their orientation. Though much was inferred from morphology, it has not been known to what degree the fusion and polarity of the internal scales reflected their development. Our observations amplify the distinction between internal and external scales. The internal scales exhibit a distinct morphology, particularly

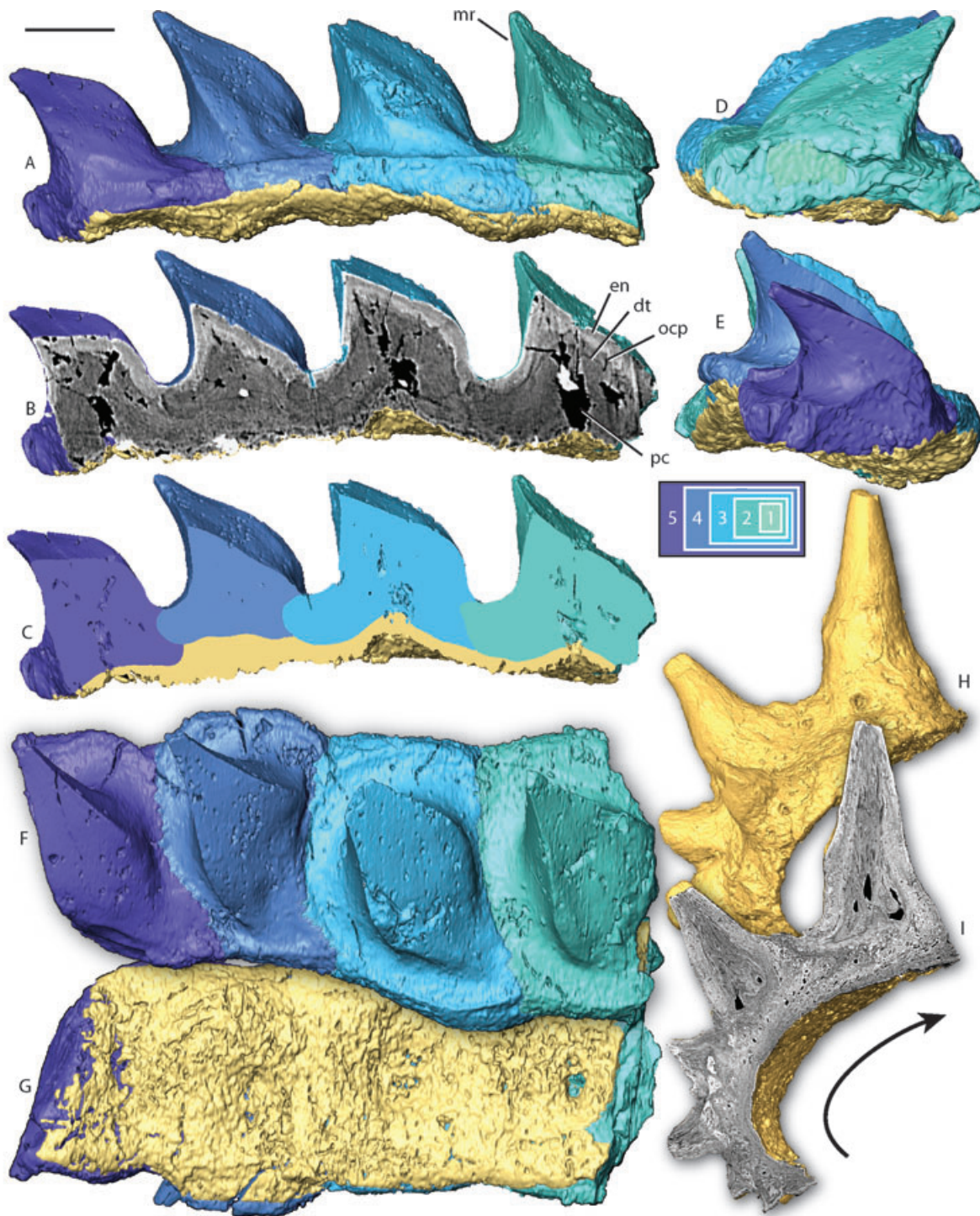


Fig. 2. (A–G) Pharyngeal scale of *Loganellia scotica* from the Silurian of Scotland (MNHN GBP384) and (H–I) an ischnacanthid acanthodian tooth whorl from the Emsian of the Canadian Arctic (NRM-PZ P. 15908) for comparison. SRXTM images: volume rendering of cusps and bases (A, D–H), surface-cut showing SRXTM image (B, I), and surface-cut showing rendered surfaces (C). Pharyngeal scale (A–G): (A) anterior view, (B) longitudinal section, (C) longitudinal surface cut through volume rendering, (D) median, (E) lateral (F) dorsal, and (G) ventral view. (H) Entire volume rendering of the acanthodian tooth whorl in lateral view and (I) longitudinal section through volume rendering; arrow indicates polarity of tooth addition within the tooth whorl. Colors of the nested boxes reflect the successive stages of development of the scales in ascending order. Relative scale bar represents 44 μm (A–G) and 148 μm (H, I).

in terms of the degree to which the morphology of the crown is differentiated. External scales typically exhibit bilaterally symmetrical ridges, whereas the surface morphology of the crown of internal scales is typically undifferentiated, save that it is developed into a cusp. Tissue composition is common to internal and external scales, although the surface layer of enameloid is of the same thickness and, therefore, it is proportionally much thicker in the smaller internal scales.

The organization of the internal scales into fused patches and rows is a key distinction from the external scales. Our data indicate that growth of the component scales and their subtending basal plate is distinct but coordinated, such that each layer of tissue comprising the basal plate is added in coordination with the addition of a new scale. Hence, the basal plate is thinnest beneath the youngest scale and thickest beneath the oldest scale within a patch or row (Figs 1H, I, L, M and 2B, C). Our data suggest that scale patches in the oral region coalesce through marginal addition of scales around separate pioneer scales. In this sense, growth is polarized with respect to the pioneer scales but new scales are not added along any particular vector. In contrast, pharyngeal scale rows grew through the addition of scales along a vector.

Thus, there is good evidence for a distinction, in terms of form and development, between internal and external scales in *L. scotica*. This, along with the original thesis that internal teeth and external scales differ fundamentally due to their distinct epithelial sources, has inspired the idea of a phylogenetic and developmental distinction between teeth and external scales that predated the origin of jawed vertebrates (Smith and Coates 1998). It is now known that no such difference occurs (Soukup et al. 2008). However, similarities have been drawn between the arrangement and inferred development of the pharyngeal scale rows in *Loganellia* and the tooth whorls of crown gnathostomes (Van der Bruggen and Janvier 1993; Smith and Coates 1998, 2000, 2001; Smith 2003). The pharyngeal scale rows in *Loganellia* have been considered as foreshadowing dental patterning before the origin of teeth and, indeed, jawed vertebrates, thereby evidencing a phylogenetic distinction between internal scales and teeth versus external scales (Smith and Coates 1998, 2001; Johanson and Smith 2003; Smith 2003; Johanson and Smith 2005; Fraser et al. 2006; Fraser et al. 2010; Fraser and Meredith Smith 2011).

Our analyses have borne out the inference of polarized sequential addition of scales within the pharyngeal scale rows of the thelodont *L. scotica* (Van der Bruggen and Janvier 1993; Smith and Coates 1998, 2001; Smith 2003). The fusion of adjacent scales within the succession shows superficially greatest similarity to groups in which the teeth are bound together by a common basal ossification, as in the tooth whorls of some Palaeozoic chondrichthyans, as initially suggested by Van der Bruggen and Janvier (1993). The same condi-

tion is seen in ischnacanthid acanthodians (e.g., Ørving 1973) and onychodontid sarcopterygians (Campbell and Barwick 2006). However, the similarity to tooth whorls does not bear closer comparison. The pharyngeal scale rows exhibit only very slight curvature along their principal axis of growth, whereas tooth whorls are strongly re-curved (Fig. 2H and I). In tooth whorls of gnathostomes, such as acanthodians, each tooth is added beneath its predecessor within the sequence, and the cusps of the teeth are oriented in the direction of addition (Fig. 2I). However, in the pharyngeal scale rows of *Loganellia*, each scale is level with its predecessor within the sequence, and the scale cusps are oriented oblique to the direction of addition. Neither of these observations necessarily precludes the hypothesis that the pharyngeal scale rows in *Loganellia* presage the origin of polarized sequential tooth families in crown gnathostomes. All character complexes show evidence of their evolutionary assembly and these pharyngeal scale rows have been touted as evidence in this sense—as an exaptation that was later co-opted to perform a tooth function at the origin of jaws (Smith and Coates 1998, 2001; Johanson and Smith 2003; Smith 2003; Johanson and Smith 2005; Fraser et al. 2006; Fraser et al. 2010; Fraser and Meredith Smith 2011).

Phylogenetic test of homology

To evaluate the homology of the internal scales in *Loganellia* and the teeth of jawed vertebrates through phylogenetic congruence, we must first establish the evolutionary relationships of these taxa. Despite suggestions that thelodonts are close relatives of the jawed vertebrates (Turner 1991), little support for this hypothesis has emerged from formal cladistic analyses (though see Gagnier 1993, 1995; Wilson and Caldwell 1998). The majority of cladistic studies indicate that they are only remotely related, comprising a grade (Janvier 1981, 1996a, 1996b) or a clade (Donoghue and Smith 2001; Gess et al. 2006; Sansom et al. 2010) that constitutes a sister group or a series of sister groups to jawed vertebrates plus osteostracans plus galeaspids (Forey 1984, 1995; Donoghue et al. 2000; Donoghue and Smith 2001; Gess et al. 2006) plus heterostracans (Sansom et al. 2010). It remains possible that the phylogenetic position of thelodonts, remote from crown gnathostomes, is an artifact of their incomplete preservation—anatomical evidence of a more derived affinity may merely not have been preserved (Donoghue and Purnell 2009). After all, even exceptionally preserved articulated thelodonts are little more than flattened integumentary sacks studded with scales. However, the synapomorphies that preclude a closer affinity to crown gnathostomes, such as a mineralized braincase, heterocercal tail, cellular dermal bone, cellular mesodermal bone, endoskeletal sclera, and/or a shoulder girdle, would have been preserved were they present in life. Thus, although there may be some uncertainty

concerning the phylogenetic position of thelodonts, on the available evidence, groups such as the galeaspid, osteostracans, and placoderms are more closely related to crown gnathostomes. Some of the lineages that branch from the gnathostome stem intermediate of thelodonts and jawed vertebrates possess internal scales, but the presence of these structures is not general for these component lineages and no polarized pharyngeal scales are present in any of them (Donoghue and Smith 2001).

Heterostracans possess oral plates that exhibit scales that extend a short distance internal to the orifice from the external (dermal) surface (e.g., Purnell 2002). There is no evidence of scales associated with the oral cavity proper, nor have any been recorded in the pharynx despite knowledge of articulated specimens (e.g., White 1935) and specimens preserving impressions of the gill lamellae (e.g., Janvier and Blicek 1979). Scales were described associated with the median “nostril” of the galeaspid *Polybranchiaspis liaojaoshanensis* (Thanh et al. 1995) and generalized to the group (Janvier 1996b), however, they have not been encountered in other galeaspid and appear to be unique to this species which is relatively derived within Galeaspidia (Zhu and Gai 2006). Tremataspid osteostracans possess a median dorsal patch of scales immediately inside the oral cavity (e.g., Janvier 1985), but this is an extremely derived, not a general or plesiomorphic, character within Osteostraci (Sansom 2008). Placoderms, the most plesiomorphic clade (Young 2010) or grade (Brazeau 2009) of extinct jawed vertebrates include taxa that possess scales (or denticles) associated with their jaws, “palate,” and the posterior wall of the branchial chamber (Johanson and Smith 2003, 2005). It is not clear whether this is a plesiomorphic character for placoderms because of phylogenetic uncertainty (Brazeau 2009; Young 2010) and the absence of pharyngeal scales or denticles in antiarchs (Johanson and Smith 2003, 2005).

Finally, the condition in *L. scotica* is not general to the Thelodonti. Other species of thelodonts have scales associated with their oral cavity and/or their efferent branchial ducts (Turner and Van der Bruggen 1993; Märss and Ritchie 1998; Novitskaya and Turner 1998; Wilson and Caldwell 1998; Donoghue and Smith 2001; Märss et al. 2006; Märss and Wilson 2008), indicating that this is a general character for the group (Märss and Wilson 2008). Both *Pezopallichthyes ritchiei* (Wilson and Caldwell 1998) and *Phlebolepis elegans* (Märss and Wilson 2008) have compound scales associated with the efferent branchial openings. However, no thelodont species other than *L. scotica* has been described to possess internal scales fused into developmentally polarized complexes. Neither is there evidence that *Loganellia* is representative of the primitive condition for Thelodonti, since cladistic analyses place it in a derived position when thelodonts are considered a clade (Donoghue and Smith 2001; Wilson and Märss 2004).

Thus, our test of phylogenetic congruence reveals that, although internal scales are widespread among stem gnathostomes, *Loganellia* is the only jawless vertebrate to exhibit internal scales organized into developmentally polarized complexes (Fig. 3). Given the apparently primitive absence of scales from the mouth or pharynx of galeaspid and osteostracans, which are successively more closely related to jawed vertebrates than are thelodonts, similarities between successional scale and tooth development in *Loganellia* and primitive jawed vertebrates are a consequence of convergence rather than common descent.

SCENARIOS FOR THE EVOLUTIONARY ORIGIN OF TEETH

Our conclusion, that the developmental similarities between the pharyngeal scales of *Loganellia* and the tooth whorls of jawed vertebrates are a consequence of convergence, does not allow us to discriminate between competing hypotheses on the origin of teeth. Teeth evolved through co-option of odontogenically competent oral and/or pharyngeal epithelium. It remains unclear whether this odontogenic competence is inherited from external epithelia (Reif 1982; Huysseune et al. 2009, 2010), or the odontogenic capacity of internal and external epithelia has been acquired independently, through co-option of the capacity of an epithelial source to produce a distinct structure (i.e., neither a scale nor a tooth) (Fraser et al. 2010) within a broader class of epithelial appendages (focal outgrowth of an epithelium; Krejsa 1979; Chuong 1998). However, the evidence on which the original distinction was drawn between internal and external odontodes (Smith and Coates 1998, 2000, 2001) is no longer tenable. *Loganellia* can no longer be used in justification for an historical difference between internal and external odontode derivatives because, aside from their location and the tooth-like mode of polarized successional development in the pharyngeal scales, the internal and external scales are otherwise alike in terms of their structure and morphogenesis. Moreover, Soukup et al (2008) have demonstrated that no fundamental distinction can be drawn between internal teeth/scales and external scales based on the source of the epithelia from which they develop. Therefore, Fraser et al. (2010) “infer homology among all odontodes . . . although we favor a deep molecular homology.” Fraser et al. (2010) come to this view explicitly to “focus attention away from an argument about primacy of location and/or cell type.” However, it is only through resolution of the location within which odontogenic competence first emerged that the homology (deep or shallow) and, therefore, the evolutionary origins of internal and external odontodes, can be determined.

Our phylogenetic survey reveals that internal scales are widespread among stem gnathostomes but that they are

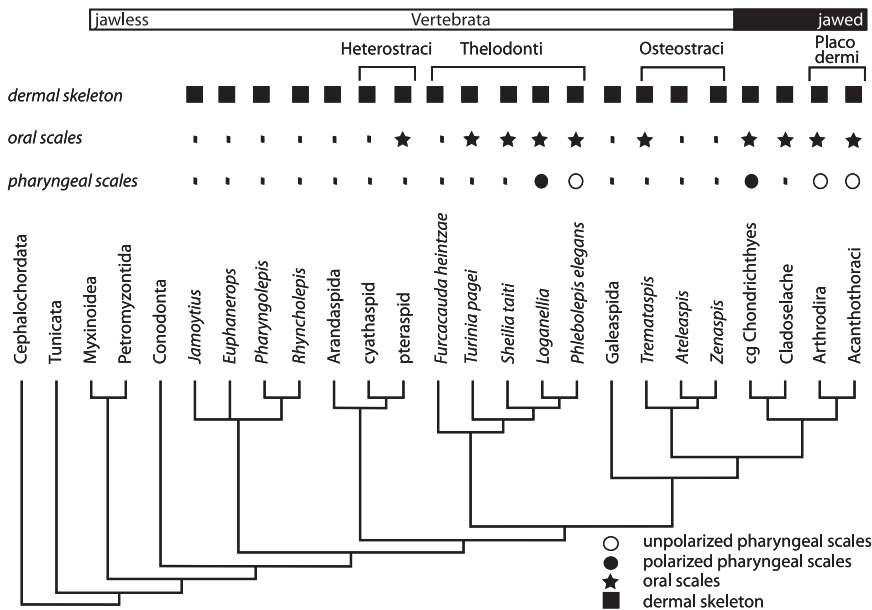


Fig. 3. Distribution of the external and oral scales, plotted on a phylogenetic tree of the vertebrates. Phylogeny after Donoghue et al. (2000) and Donoghue and Smith (2001).

secondary to external scales, and that they are invariably located near to external openings such as the mouth, nostril, or efferent gill openings. Based on the available evidence, it is beyond reason that the odontogenic capacity of internal and external epithelia has been acquired independently, through co-option of an ancestral developmental module that was not linked to scale/tooth development. Rather, the available phylogenetic evidence suggests that odontogenic competence emerged first in the external dermis, and that the odontogenic capacity of internal epithelia is a derived characteristic of skeletonized vertebrates. How odontogenic competence spread from external to internal epithelia is more open to question. However, the location of internal scales in the body of stem gnathostomes, proximal to external openings, is particularly compatible with the modified outside-in hypothesis of Huysseune et al. (2009, 2010).

Fraser et al. (2010) drew homology between internal and external odontodes explicitly so that they could “clarify that an appreciation of deep homology among odontodes may broaden our evolutionary understanding of these structures.” We agree that debate needs to move on from analyzing fossils to testing the predictions of an historical evolutionary distinction between oral/pharyngeal and dermal odontodes using molecular developmental genetics. However, for this to be successful, it will be necessary to refine competing hypotheses and their predictions so that they are more readily falsifiable. On face value, the tests appear simple: we should anticipate that temporal and spatial patterns of gene expression and interaction should be more similar between internal and external odontodes if external odontodes are primitive and internal odontodes arose latterly through expansion of odontogenic competence from external to internal epithelia.

However, the very basis of the hypothesis that internal and external odontodes share only a “deep homology” is that this homology is based in a gene regulatory network (Fraser et al. 2010). Thus, the hypothesis of a “deep homology” cannot be tested simplistically in this way. Fraser et al. (2010) argue that the gene regulatory networks underpinning internal and external odontodes were recruited independently from a common ancestral gene regulatory network underpinning development of a class of unmineralized sensory papillae, such as a taste receptor. An obvious test of this hypothesis would entail a comparative analysis of the gene regulatory networks of internal odontodes, external odontodes, sensory papillae, among potential outgroups from elsewhere within what have been perceived as an evolutionarily coherent family of organs united by a common program of epithelial appendage morphogenesis (Krejsa 1979; Chuong 1998; Donoghue 2002). We are some way from being able to perform such tests, let alone interpret their results. Very few data are available on the developmental genetics of external odontode development and the existing data on comparative tooth development show significant variation even within single dentitions (Borday-Birraux et al. 2006; Debais-Thibaud et al. 2008; Gibert et al. 2010). However, a more fundamental problem is that both scenarios for the origin of internal odontodes would predict the same outcome—that the networks of interacting genes would be more similar in internal and external odontodes than either would be to any other class of structures, including sensory papillae such as taste buds.

Perhaps the only test available at present would consider whether the gene regulatory networks underpinning internal and external odontodes diverged before or after the 2R

genome duplication event, which is widely thought to have occurred in the gnathostome stem lineage after it diverged from the cyclostome lineage. In particular, if the specific transcription factors and cell-signaling molecules implicated in odontode development in both locations include members of paralogy groups that are the product of 2R, it would suggest that the gene regulatory networks diverged after the 2R event—a shallow divergence. This test is imperfect for a number of reasons: (i) the relative timing of 2R is not known conclusively and it is argued that 2R occurred shortly after 1R, prior to the divergence of crown vertebrates (Kuraku et al. 2009); (ii) the two gene regulatory networks could have diverged earlier than 2R but both retained the same diversity of paralogous genes that are the product of 2R; none of the proponents of deep homology have been specific about the timing of divergence of the gene regulatory networks underpinning internal and external odontodes except Fraser et al. (2010) who argue implicitly that this occurred prior to the origin of odontodes. Nevertheless, preliminary descriptions of *Dlx* expression in shark tooth and scale development reveal that they are not distinguishable on this basis (Debiais-Thibaud et al. 2009). Combined with phylogenetic evidence on the distribution of external and internal odontodes among the earliest skeletonized vertebrates, the available data suggest that internal odontodes evolved after external odontodes and the topological distribution is compatible with the origin of internal odontodes through expansion of odontogenic capacity from external to internal epithelia.

CONCLUSIONS

In *Loganellia*, the internal scales exhibit a distinct morphology in comparison to the external scales. The internal scales are organized into fused patches and rows, a key distinction from the scales comprising the external dermal skeleton, which are always discrete. Growth of the component internal scales and their basal plate is distinct but coordinated in such a way that each layer of tissue comprising the basal plate is added in coordination with the addition of a new scale. Scale patches in the oral region coalesce through marginal addition of scales around pioneer scales. Growth is polarized with respect to the pioneer scales but new scales are not added along a particular vector. In contrast, pharyngeal scale rows grew through the addition of scales along a vector. Thus, there is good evidence for a distinction, in terms of form and development, between internal and external scales in *L. scotica*. The sequence of addition of the scales, while polarized, is distinct from the pattern seen in the tooth whorls of early jawed vertebrates, with each scale added level with its predecessor within the sequence. The phylogenetic distribution of internal and external scales in stem gnathostomes indicates

that odontodes are first expressed in an external position. Internal scales, where present, are always located near to external orifices, such as the mouth, nasal, and efferent pharyngeal ducts. The tooth-like patterning of pharyngeal scales encountered in *Loganellia* is unique among thelodonts and other stem gnathostomes. It is likely to represent an instance of convergence rather than reflecting the establishment of the developmental pattern underpinning tooth replacement in jawed vertebrates. The available evidence suggests that internal odontodes evolved through the expansion of odontogenic competence from external to internal epithelia.

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