Testing models of dental development in the earliest bony vertebrates, *Andreolepis* and *Lophosteus*

John A. Cunningham¹, Martin Rücklin¹, Henning Blom², Hector Botella³ and Philip C. J. Donoghue¹,∗

¹School of Earth Sciences, University of Bristol, Wills Memorial Building, Queen’s Road, Bristol BS8 1RJ, UK
²Department of Organismal Biology, Uppsala University, Norbyvägen 18A, 752 36, Uppsala, Sweden
³Department of Geology, University of Valencia, Dr Moliner 50, 46100 Burjassot, Valencia, Spain
∗Author for correspondence (phil.donoghue@bristol.ac.uk).

Theories on the development and evolution of teeth have long been biased by the fallacy that chondrichthyan reflect the ancestral condition for jawed vertebrates. However, correctly resolving the nature of the primitive vertebrate dentition is challenged by a dearth of evidence on dental development in primitive osteichthyans. Jaw elements from the Silurian–Devonian stem-osteichthyans *Lophosteus* and *Andreolepis* have been described to bear a dentition arranged in longitudinal rows and vertical files, reminiscent of a pattern of successional development. We tested this inference, using synchrotron radiation X-ray tomographic microscopy (SRXTM) to reveal the pattern of skeletal development preserved in the sclerochronology of the mineralized tissues. The tooth-like tubercles represent focal elaborations of dentine within otherwise continuous sheets of the dermal skeleton, present in at least three stacked generations. Thus, the tubercles are not discrete modular teeth and their arrangement into rows and files is a feature of the dermal ornamentation that does not reflect a polarity of development or linear succession. These fossil remains have no bearing on the nature of the dentition in osteichthyans and, indeed, our results raise questions concerning the homologies of these bones and the phylogenetic classification of *Andreolepis* and *Lophosteus*.

**Keywords:** Osteichthyes; *Andreolepis*; *Lophosteus*; tooth; development; evolution

1. INTRODUCTION

Chondrichthyan (cartilaginous fishes) have long been considered a model for rationalizing the evolutionary origin of the vertebrate dentition and, consequently, patterns of development and developmental evolution among osteichthyans (bony fishes including tetrapods) [1]. However, the nature of the ancestral gnathostome dentition and its development can only be inferred through comparative analysis of chondrichthyan, osteichthyans and their extinct sister lineages. This aim is challenged most especially by a dearth of evidence on dental development in stem-osteichthyans. The description of a dentary bone of *Andreolepis* and a maxillary bone of *Lophosteus*, both interpreted as stem-osteichthyans [2], holds the promise of a fundamental insight into the mode of dental development primitive to osteichthyans and gnathostomes more generally.

These dermal jaw elements of both *Lophosteus* and *Andreolepis* are covered with tubercles that have been interpreted as organized into longitudinal rows and transverse files, reminiscent of a pattern of successional development, such as that seen in chondrichthyan teeth [2]. Indeed, this inferred pattern of dental development, without shedding or replacement, has been suggested to illustrate an early stage of osteichthyan tooth patterning [2]. Inferences of skeletal development from surface morphology are problematic but the fossil remains are too rare to apply the conventional destructive histological methods needed to test the inferred pattern of development. Therefore, we employed synchrotron radiation X-ray tomographic microscopy (SRXTM), a non-invasive means of obtaining a high-resolution volumetric virtual characterization of the fossil remains in which to investigate skeletal development.

2. MATERIAL AND METHODS

Two specimens were used in the analyses. An incomplete putative right dentary of *Andreolepis helosai* from the Upper Silurian (Ladlow) of Gogs, Gotland, Sweden, housed in the Swedish Museum of Natural History, Stockholm (NRM-PZ P. 15910). A putative right maxillary of *Lophosteus superbus* from an Upper Silurian (Middle–Upper Pridoli) erratic boulder from Germany (also figured by Botella et al. [2, fig. 2a–d]) is repositioned at the Museum für Naturkunde, Berlin (MB.f.17035). Volumetric characterization of the specimens was achieved using SRXTM [3]. The analyses were carried out at the X02DA (TOMCAT) beamline at the Swiss Light Source, Paul Scherrer Institut, Villigen. The virtual slice data were visualized using Avizo 6.3 (www.vsg3d.com). ‘Virtual thin sections’ were created using the voltex module in Avizo, which simulates the casting of light rays from preset sources through a volume of data.

3. RESULTS

The dermal plates of both *Andreolepis* (figures 1a–d and 2a–d) and *Lophosteus* (figures 1e–h and 2e–h) exhibit a similar gross histological architecture comprising a basal division of compact lamellar bone that intergrades with a middle division of vascular or cancellar bone rich in osteocyte lacunae. The superficial division includes tubercles composed of dentine surrounding spurs of the vascular network. In both taxa, the tubercles reflect different developmental generations, evidenced by their vertical or lateral overlap.

In the putative dentary of *Andreolepis*, the vascularization of the bone is thinnest in radial extent in the region of the longitudinal concavity (figure 2a,b) where the compact lamellar basal layers are proportionally more extensively developed. Vascularization increases away from the longitudinal concavity, dorsally and ventrally (following the orientations inferred [2]; figure 2a,b). Growth arrest lines within the middle layer evidence the appositional growth of the plate dorsally and, especially, ventrally, with concomitant growth of the basal and superficial layers (figure 2d).

However, growth of the superficial layer was not limited to the dorsal and ventral margins of the plate, with successive...
generations of dermal tubercles intercalating, overlapping and enveloping those already present (figure 2c). Nevertheless, the dermal tubercles that comprise the entire superficial layer occur as a sequence of superimposed laterally continuous sheets (figure 2a–d). The tubercles exhibit simple undivided pulp cavities and a distinct highly attenuating and therefore hypermineralized capping tissue (figure 2c–d). The height of tubercles increases towards the external face of the dorsal margin of the plate, and those on the extreme margin of the plate (figure 1a–d) are clearly associated morphogenetically with the appositional growth of the whole plate (figure 2d).

In Lophosteus, the putative maxillary plate shows some variation in the proportional development of the spongy vascular bone and the compact lamellar bone at the base, from inferred proximal to distal. Distally, the spongy bone comprises the plate almost wholly (figure 2g), while more proximally the compact basal bone layer comprises as much as half the radial thickness of the plate (figure 2h), with vascular canals extending from the inner surface to the overlying vascular network (figure 2g, h, k). The tubercles are generally rounded in outline and have low relief (figure 1e–h), exhibiting divided pulp cavities with numerous vascular loops (figure 2g–j). The tubercles show evidence of appositional growth in the direction of the presumed dorsal and ventral margins of the plate, and these appositional events are continuous with growth arrest lines in the vascular and compact bone, indicating growth only at the lateral and inner surfaces (figure 2i, j). At the presumed ventral margin, the tubercles have a distinct conical morphology with comparatively high relief (figure 1a, c, e–g); the pulp cavities are undivided and, generally, unfilled (figure 2i, j). There is no evidence for a differentiated capping tissue.

4. DISCUSSION

Evidently, the tubercles comprising the superficial layer of the presumed dentary of Androolepis and maxillary plate of Lophosteus developed in association with marginal accretion of the underlying dermal plate. There is some evidence of tubercles augmenting the central face of the plate, isolated from marginal appositional growth, but these are local morphogenetically continuous associations of tubercles. This mode of development is not compatible with expectations of tooth development. Teeth invariably develop as morphogenetically distinct elements, fused, ankylosed and/or socketed with the associated dermal plate. The tubercles comprising the superficial layer of these dermal plates are more reminiscent of the tubercles that develop in association with the dermal skeleton more generally. The polarization of the tubercles on the surface of the dermal plates does not reflect a pattern of tooth succession but, rather, reflects marginal growth associated with the expansion

Figure 1. Surface renderings (gold) of (a–d) Androolepis and (e–h) Lophosteus based on SRXTM data. (a) Lateral, (b) dorsal, (c) medial and (d) posterior views of the putative right dentary of Androolepis (NRM-PZ P15910). (e) Lateral, (f) ventral, (g) medial and (h) posterior views of the putative right plate of Lophosteus superbus MB.f.17035. Scale bars: (a–c) 1.71 mm; (d) 0.54 mm; (e–g) 2 mm; (h) 0.9 mm.
Figure 2. Surface renderings (gold) of (a–f) *Androlepis* and (g–l) *Lophosteus* cut by ‘virtual thin sections’ based on SRXTM data. (a–d) Transverse, (e) longitudinal, and (f) horizontal surface cuts of the putative right dentary of *Androlepis* (NRM-PZ P. 15910); (c) and (d) are enlargements of the sections shown in (a) and (b), respectively. (g–j) Transverse, (k) longitudinal and (l) horizontal surface cuts of the putative right maxillary of *Lophosteus superbus* MB.f.17035; (i) and (j) are enlargements of the sections shown in (g) and (h), respectively. Scale bars: (a,b) 0.8 mm; (c) 0.3 mm; (d) 0.35 mm; (e,f) 2 mm; (g,h) 1 mm, (i) 0.4 mm; (j) 0.3 mm; (k,l) 2.4 mm.
in size of the dermal plate. In the specimen of *Andreolepis*,
the apparent successive rows of tubercles comprising the
tooth-like margin of the plate are all products of the
same morphogenetic event and so the arrangement
reflects a spatial organization rather than a pattern of
linear succession.

Although the tubercles associated with the dorsal
margin of the presumed dentary and ventral margin
of the presumed maxilla exhibit a tooth-like mor-
phology, in the *Andreolepis* plate, they intergrade
continuously with the morphology of the tubercles
away from this margin (figures 1a–d and 2a–d). The
case for a tooth-like morphology appears clearer in
*Lophosteus*, since these conical marginal tubercles
differ from the rounded tubercles that occur deep
towards the plate margin. However, this distinction flatters to
deceive since the intervening tubercles have been
removed. From what little remains, in terms of the
dimensions of the pulp cavities, it appears that
the tubercles are similar in size gradually, from the tuber-
cles in the plate centre, towards the plate margin
(figure 1e–h).

Therefore, our tomographic data reveal that neither
*Andreolepis* nor *Lophosteus* grew by sequential addition
of tubercles at the medial margin, as previously inferred from external morphology [2]. The structure and pattern of development of the dermal plates is generally representative of dermal bones of these taxa
[4,5]. These findings are incompatible with *Lophosteus*
or *Andreolepis* tubercles growing in files similar to the
tooth ‘families’ of chondrichthians and some
acanthodians, or to the shedding tooth rows of
crown-osteichthians. There is therefore no support
for the presence of tooth ‘capsules’ acting as separate
functional modules. This is not to say that *Andreolepis*
and *Lophosteus* lacked teeth, not least since Gross [6]
has described isolated toothwhorl-like aggregates of
tubercles associated with *Lophosteus*, rather, that there
is no evidence for teeth among the specimens that
we describe. As such, these dermal plates do not evidence an incipient osteichthyan dentition, such as might be expected by hypotheses that seek to explain the evolutionary origin of teeth through the hetero-
topic extension of odontogenic competence from the
external dermis to the oral cavity.

As the bones do not have incipient teeth it is
appropriate to reconsider their homology with
osteichthyan dentary and maxillary bones. This is not
the only line of evidence used to identify the bones
as jaw elements. Botella et al. [2] justify homology on
the presence of a narrow medial horizontal lamina,
the presence of upper and lower overlapping plate mar-
gins, and the general similarity to the maxilla of
osteichthyan maxillary plates. However, the dermal
histology of *Lophosteus* is uncharacteristic of osteich-
thyan and shows greater similarity to the placoderm
dermoskeleton. Typical osteichthyan characters are
absent also from the putative dentary of *Andreolepis*,
such as the absence of a closed sensory-line canal
that is characteristic of osteichthyan lower jaws, though its histological structure is more typically
osteichthyan. These observations have implications for
resolving the phylogenetic affinities of *Andreolepis*
and *Lophosteus*, which have proven to be contentious.

*Andreolepis* has generally been interpreted as a primitive actinopterygian [4,7,8] or a stem osteichthyan
[2,9], but similarities to sarcopterygians and acantho-
dians have also been noted [4,10]. Apart from the
interpreted dentary bone, the only remaining charac-
ters used to place *Andreolepis* in the osteichthyan
total group are rhombic scales with ganoine/enamel,
possible fulcral scales, and a possible cleithrum [9].
*Lophosteus* has been compared with crown or stem-
osteichthians [2,5,6,11–13], placoderms [14], and
acanthodians [6,13,15]. Rhombic scales are the only
osteichthyan character currently known in *Lophosteus*
other than the maxillary bone [9]. If the jaw characters
are removed, the remaining osteichthyan characters of
both taxa are few in number and their identification is
often tentative. The chimaeric assemblage of charac-
teristics exhibited by *Lophosteus* and *Andreolepis* may
reflect the paucity of our understanding of the phyloge-
netic distribution of these characters, some of which
may be crown-gnathostome, or primitive to jawed
vertebrates more generally.

5. CONCLUSIONS

The tomographic data presented here show that the
tubercles of *Andreolepis* and *Lophosteus* were neither added sequentially at the medial margin, nor arranged
in single tooth files. Instead there is evidence that
tubercles represent focal elaborations of dentine
within continuous sheets of dermal bone. This demonstr-
ates that the tubercles of these taxa did not grow in a
similar way to the teeth of either chondrichthians or
crown group osteichthians. Instead their ontogeny is
comparable to that observed in dermal scales. As a
result, these structures are uninformative regarding the
origin of osteichthyan dentition.

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