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The Origin of Tooth Replacement

Three-dimensional Synchrotron Histology Visualizes the Dental Development of Silurian Stem Osteichthyans

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UPPSALA
2017

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, Evolutionsbiologiskt centrum, Norbyvägen 14, Uppsala, Friday, 31 March 2017 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Dr Zerina Johanson (Natural History Museum, London).

Abstract
Chen, D. 2017. The Origin of Tooth Replacement. Three-dimensional Synchrotron Histology Visualizes the Dental Development of Silurian Stem Osteichthyans. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1481. 53 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9829-0.

Mechanisms of tooth replacement distribute incongruently among extant gnathostomes (jawed vertebrates): a permanent tooth-generating dental lamina exists in chondrichthyans (cartilaginous fish) and tetrapods but not teleosts, whereas tooth shedding by basal hard tissue resorption occurs in tetrapods and teleosts but not chondrichthyans. Theories about the evolution of tooth development have been biased towards the chondrichthyan conveyor-belt replacement, since there has been no fossil evidence for the origin of osteichthyan (bony fish and tetrapods) tooth replacement until now. 3D virtual dissections with submicron-scale resolution, based on propagation phase contrast synchrotron microtomography (PPC-SRμCT), reveal the growth history of the dentitions of *Andreolepis* and *Lophosteus*, 423-Myr-old Silurian stem osteichthyans close to the common ancestor of tetrapods and teleosts. Their marginal jawbones and "tooth cushions" (possible homologues of coronoids) shed teeth by *in situ* cyclic basal resorption, the earliest examples of osteichthyan-style tooth replacement. The replacement cycles were site-autonomic, and occurred in broad irregular multi-row tooth fields, including at sites separated from the margin of the bone by intervening teeth, showing that the production of replacement teeth did not occur in a single deep dental lamina, but in pockets associated with each tooth, as in many teleosts. It suggests that the functionally and anatomically similar laminae of chondrichthyans and tetrapods are convergent. The marginal jaw bones of both genera carry an initial non-shedding dentition arranged in alternate transverse files, labial to the shedding tooth field, overgrown by later dermal ornament and probably not belonging to the oral domain, but bearing *in vivo* biting damage showing that they functioned as teeth. The most lingual of these odontodes have been resorbed apically and are overlain by shedding teeth. The first-generation teeth on the tooth cushions display basal resorption in *Andreolepis*, but semi-basal resorption in *Lophosteus*. The latter leaves a basal dentine ring from each tooth, implying only odontoclasts are involved in the semi-basal resorption, which is probably the first step towards evolving a site-specific resorption. The polarized displacement of each generation of resorption surfaces reflects the fact that the cyclic replacement, as well as the sequential addition of tooth sites, is closely related to bone growth. Resorption surfaces and growth arrest surfaces also record the life history and the replacement rate. These data provide unique insights into the origin of osteichthyan tooth replacement.

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*To all the creatures that ever contributed to
the beauty of this planet*

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I   **Chen, D.**, Blom, H., Sanchez, S., Tafforeau, P. & Ahlberg, P. E. (2016) The stem osteichthyan *Andreolepis* and the origin of tooth replacement. *Nature*, 539(7628):237–241

II   **Chen, D.**, Blom, H., Sanchez, S., Tafforeau, P., Märss, T. & Ahlberg, P. E. *in review*. Development of cyclic shedding teeth from semi-shedding teeth: the inner dental arcade of the stem osteichthyan *Lophosteus*. *Royal Society Open Science*.

III   **Chen, D.**, Blom, H., Sanchez, S., Tafforeau, P. & Ahlberg, P. E., *manuscript*. Microstructures of the most primitive form of inner dental arcade and life history of the Silurian stem osteichthyan *Andreolepis*.

IV   **Chen, D.** & Ahlberg, P. E., *manuscript*. The marginal dentition of *Lophosteus superbus* Pander 1856, a Late Silurian stem osteichthyan from Estonia.

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Contents

Introduction

The evolution and development of teeth has long been a source of interest for both paleontologists and neontologists. However, when, where and how teeth emerged, and the definition of "true teeth", remains controversial. There have been several theories on the origin of teeth. The classic "outside-in" theory regards teeth as modified scales in the oral cavity, in the context of jaws. The opposing "inside-out" theory hypothesizes that teeth appeared as organized denticles in the pharyngeal cavity, prior to the origin of jaws. These two consider teeth to be ectodermal and endodermal in origin, respectively. More modified theories, like "both inside and outside" (Fraser et al., 2010), have been proposed based on whether cell population identity really is important for tooth formation, and whether teeth originated only once or several times.

The recent consensus is that teeth occur both in the gnathostome crown groups, chondrichthyans and osteichthyans, and in the jawed stem gnathostome placoderms. However, the evolutionary relationship of these dentitions is unclear. Placoderm teeth are surprisingly only recognized in a single derived clade, arthrodires, giving rise to the hypothesis that the teeth of stem-group and crown-group gnathostomes had independent origins (Smith and Johanson, 2003b). Chondrichthyan teeth and their dermal denticles are both highly specialized and decoupled from dermal bone, which has biased our understanding of the primitive condition of vertebrates. Osteichthyan teeth, which like those of placoderms are intimately associated with macromeric dermal bones, are characterized by a shedding-replacing cycle where shedding is effected through basal resorption of hard tissues. Unfortunately, the derived models of the boneless chondrichthyans, and the extant osteichthyan lab models, such as the chicken without teeth, the mouse without replacement teeth, and the zebra fish with neither jaw-teeth nor dermal odontodes, are unable to illuminate the poorly understood primitive state of the osteichthyan dentition. This severely limits our ability to investigate vertebrate dental evolution.

Fortunately, the development of new 3D imaging technology is allowing us to address this challenge by investigating the dentitions of two of the oldest and phylogenetically most basal osteichthyans from the late Silurian of the Baltic region. *Andreolepis hedei* Gross 1968 from Gotland, Sweden (Gross, 1968), and *Lophosteus superbus* Pander 1856 from Saaremaa, Estonia (Pander, 1856), both represented exclusively by well-preserved but com-

pletely disarticulated microremains, used to be enigmatic vertebrates that were generally identified as primitive actinopterygians because of their the rhombic scales, but were also argued by different authors at different times to have some sort of connections to all other gnathostome major groups except the conventional (crown) chondrichthyans (Gross, 1969, 1971, Janvier, 1971, 1978, Schultze, 2015, 1977; Otto, 1991; Burrow, 1995; Märss, 2001; Schultze and Märss, 2004; Chen et al., 2012; Qu et al., 2013). A new phylogenetic perspective on early and problematic vertebrates began to develop with the recognition, about 20 years ago, that many such forms might belong to the stem groups of extant clades and could potentially be identified by their possession of parts of the crown group character complement. This idea was first tested in the tetrapod stem group, where osteolepiform fishes and elpistostegids were shown to be paraphyletic arrays of stem tetrapods (Ahlberg and Jonanson, 1998). The discovery of new jaw material of *Andreolepis* and *Lophosteus* prompted a similar analysis (Botella et al., 2007), where these genera were assigned to the stem group of osteichthyans on the basis of their tooth-bearing marginal jaw bones, which were interpreted as differing from those of crown osteichthyans in lacking tooth replacement.

When the actinopterygian-sarcopterygian divergence had been pushed earlier back to the Silurian by the discoveries of osteichthyans with mosaic characters (Zhu et al., 2009), and both placoderms and acanthodians had been reinterpreted as paraphyletic (Brazeau, 2009), the stem group of osteichthyans again came into focus. Several late Silurian–early Devonian "actinopterygians" were tentatively reinterpreted as stem osteichthyans (Friedman and Brazeau, 2010). Soon afterwards, the long-established hypothesis that the macromeric dermal skeletons of osteichthyans and placoderms are non-homologous was challenged head-on by the discovery of the so-called the maxillate placoderms, *Entelognathus* and *Qilinyu*, the Silurian of Yunnan, China (Zhu et al., 2013, 2016). These genera combine osteichthyan-like marginal jaw bones, homologous with the osteichthyan outer dental arcades but without other bones internal to them, with an otherwise placoderm-like dermal skeleton and (at least in *Entelognathus*) braincase morphology. This suggests a close affinity between maxillate placoderms and osteichthyans, and further indicates that Osteichthyans have retained the ancestral nature of jawed vertebrates more than chondrichthyans, the other crown gnathostomes. The "tooth cushions" of *Andreolepis* and *Lophosteus*, small domed tooth-bearing elements, appear to represent an incipient form of osteichthyan inner dental arcade. Therefore, *Andreolepis* and *Lophosteus* are potentially not only close to the common ancestor of actinopterygians (e.g. teleosts) and sarcopterygians (e.g. tetrapods), but may also represent the milestones in the placoderm-osteichthyan transition.

In order to evaluate the significance of the various early vertebrate dentitions, we need to understand not only their morphology but also their ontogenetic histories. The marginal dentitions of *Andreolepis* and *Lophosteus*

have been interpreted as non-shedding (Botella et al., 2007) or dermal tubercles (Cunningham et al., 2012), on the basis of external morphology and 2D virtual thin sections, respectively. However, only 3D paleohistological reconstruction allows the microanatomy to be fully understood and the ontogenetic history of the dentition to be inferred. For this PhD project, propagation phase contrast synchrotron microtomograpy (PPC-SRμCT) synchrotron scans with sub-micron resolution were carried out on their marginal jaw bones and tooth cushions of both genera, in order to provide a full 3D palaeohistological data set.

Albeit only known from isolated dermal elements, *Andreolepis* and *Lophosteus* exhibit exceptional 3D histological preservation. The developmental processes, such as tooth addition, replacement cycles, tissue resorption, odontode overgrowth and appositional bone growth, can be inferred with confidence from these 425-million-year-old animals. The results overturn the previous findings: on one hand, *Andreolepis* and *Lophosteus* represent the earliest examples of tooth replacement by in situ basal resorption; on the other hand, they display tooth-like structures that are at present unknown elsewhere but may be primitive for osteichthyans or even gnathostomes, challenging the definition of teeth. *Andreolepis* and *Lophosteus* are the first taxa whose dentitions have been investigated by manually modeling various subtle embedded histological structures, such as resorption surfaces. The modeling process has revealed exquisite 3D information that has neither been obtained from the previous exhaustive studies on *Andreolepis* and *Lophosteus*, nor from other well-documented fossil or even extant species examined by traditional techniques. The overall aim of this groundbreaking project, together with the related studies on the dentitions and dermal skeletons of other early vertebrates, is to revolutionize the field of early vertebrate paleontology and dental evolution by broadening the horizons both technically and theoretically.
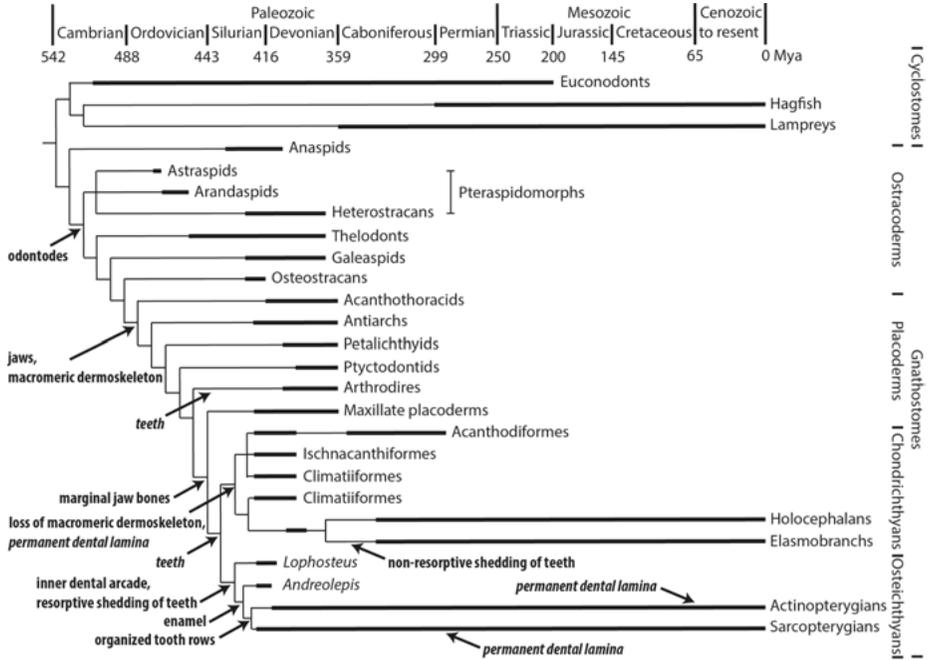
Figure 1. Time-calibrated phylogeny of jawless and jawed vertebrates, based on Brazeau and Friedman (2015) and Janvier (2015).

Evolution and development of odontodes

Mineralized skeleton is one of the major evolutionary novelties of vertebrates. The skeletons of mammals are mostly endoskeletons, like our long bones, which form initially as cartilage that is then broken down and replaced by endochondral ossification. But our skull roof, facial skeletons and dentary are formed in the dermis of the skin by intramembranous ossification without the presence of a cartilage template, and thus called **dermoskeleton**. Mineralized dermoskeleton appears to be both stratigraphically and phylogenetically older than mineralized endoskeleton, and exhibits a great variability, especially in fossils where it is often well preserved (Francillon-Vieillot et al., 1990). In non-mammalian vertebrates (fishes, amphibians and reptiles), dermal bones display pattered tubercles or ridges, known as **ornamentation**, which are believed to have protective and sensory functions. Although in our own bodies only the teeth are composed of dentine and enamel, the dermoskeletal ornamentation of non-tetrapod vertebrates (jawed and jawless fishes) are often covered with dental tissues. The basic unit of these dental components is an **odontode** that consists of a cone of dentine, a pedicle of vascularized bone-like tissue, and sometimes a cap of hypermineralized tissue such as enamel. Ornamentation is often built up of **odontocomplexes**, each of which formed by the areal and superpositional growth of consecutive generations of odontodes.

Odontodes have an evolutionary history of 500 million years, and odontode-derived structures from different parts of the body in different vertebrate groups have been dramatically modified in morphology, function, tissue composition and growth pattern. Although odontodes and dermal bones develop as two modules (Smith and Hall, 1993), and may originally have evolved as as separate entities (Janvier, 1996), odontodes are already the constituents of the dermal skeleton in the earliest known unambiguous vertebrates, the Ordovician (c. 488-443 Mya) jawless fish, such as the pteraspidomorph *Eriptychius*, in which separated odontodes have accreted into odontocomplexes (Ørvig, 1958, 1989), substantially thickening the dermal elements. Various Paleozoic ostracoderms (osteostracans, galeaspids, heterostracans, thelodonts, and anaspids), and placoderms, the jawless and jawed vertebrates that together constitute the gnathostome stem group, are heavily armored by dermal bones, scutes, scales, spines, tesserae and other types of dermoskeleton, which are combinations and rearrangements of a wide range of superficial **odontogenic** (tooth-forming) and deep **osteogenic** (bone-

forming) components (Sire et al., 2009). In early vertebrates, dental tissues divide into orthodentine, mesodentine and semidentine; capping tissues are mainly enameloid, enamel and acrodin; bony tissues include cellular and acellular bones, aspidin, galeaspidin, isopedine, elasmodine and cementum. There are many other less common and poorly understood forms, with false concepts of transition series, of different grades, or specific to a certain taxa (Smith and Sansom, 2000). More terms have been created for tissue complexes, for instance, ganoine and cosmine refer to enamel homologue that possesses a specified structural relationship with dentine or a canal system. Terminologies can be confined by adjectives, like prismatic enamel, which in some cases are even turn to be an ambiguous, like "true enamel". The homology, classification and definition of vertebrate hard tissues have been much debated (Ørvig, 1967). Many basic tissues have emerged in the very early history of vertebrate evolution, but quite a few are innovations of jawed vertebrates, such as, semidentine (unique to placoderms), enamel and acrodin (synapomorphies of osteichthyans), and elasmodine (characterizing the elasmoid scales of actinopterygian and sarcopterygian fish). In limbed tetrapods, however, the diversity of hard tissues has generally decreased, and only two types of dermoskeleon, dermal bones and teeth, are present.

In fact, reduction is the evolutionary trend of odontodes (Huysseune and Sire, 1998). Taking osteichthyan scales as an example (Schultze, 1977), the ancestral rhombic scales of stem osteichthyans (e.g. *Andreolepis*) carry successive generations of odontodes. The ganoid scales of basal actinopterygians (e.g. *Polypterus*) have the odontocomplex flattened into multiple layers of enamel overlying a single layer of dentine; lepisosteoid scales (e.g. *Lepisosteus*) even lose the layer of dentine; elasmoid scales (e.g. *Amia*) further lose the bony base. Among sarcopterygians, the elasmoid scales of coelacanths (e.g. *Latimeria*) lose the bony base but keep the overgrowing odontodes, whereas lungfish scales lose both (Sire et al., 2009). With regard to dermal bones, thelodonts and crown chondrichthyans have largely reduced or entirely lost the osteogenic tissues; galeaspids have lost the odontogenic contribution, and dentinal ornamentations was replaced by bony sculptures through the rise of tetrapods (Witzman, 2009).

It seems an aquatic environment is necessary for a diversity of vertebrate dermal hard tissues, especially dental tissues to flourish. In marked contrast to the extensive differentiation of hard tissues in fish, oral odontodes, or teeth, is the sole form for dental tissues to persist in tetrapods. This is possibly because their location in the oro-pharynx allows the dental tissues to be kept hydrated in the terrestrial life. But it is certain that odontodes, bearing dental tissues that are harder than bone, would have been selected for by gaining a feeding function at the entrance of the digestive tract. Dentine is retained in oral or pharyngeal teeth of teleosts, even though it has been lost in the scales. Therefore, the oral cavity is the refuge of dentine, enamel,

enameloid and acrodinin the two dominant modern taxa, tetrapods and teleosts.

The oral cavity and dermal integument have been considered as two independent developmental fields (Reif, 1982), with dentition and integumentary skeletons patterned as two regulation systems (Fraser and Smith, 2011). Additional to the **oral-dermal boundary**, there is a **head-body boundary** between dermal bones and scales that relate to the development and evolution of odontodes. Although the latter might have been lost in acanthodians and crown chondrichthyans, along with the loss of large dermal bones, the two boundaries demarcate the three developmental domains in placoderms and osteichthyans, as can be seen in the acquisition of enamel: the primitive stem osteichthyan *Lophosteus* has no enamel at all; the stem osteichthyan *Andreolepis* only possesses enamel on the body scales; the derived stem osteichthyan *Psarolepis* bears enamel on both scales and dermal bones, but naked teeth; crown osteichthyans (except tetrapods and teleosts) carry enamel all over the scales, skull and teeth (Qu et al., 2015).

All types of odontodes share a deep molecular homology by a core odontogenic gene regulatory network (oGRN) (Martin et al., 2016). They are all initiated from a well-delimited condensation of mesenchymal cell population, the **odontogenic (dental) papilla**, which is absent in the development of bony tissue. The osteogenic primordium locates deep within the mesenchyme, the connective tissue below epithelium, without relation with epithelial cells. Conversely, morphogenesis and differentiation of the odontogenic papillae, which always situate next to the epithelial-mesenchymal boundary, requires inductive **epithelial-mesenchymal interactions**. How dental tissues are deposited in a polarized manner provides a good example: growth factors spread through the superficial vascular network mediate the differentiation of the dental papilla cells into **odontoblasts**, which will first synthesize a woven-fibered tissue, enameloid, against the epithelial-mesenchymal boundary; then a parallel-fibered tissue, dentine, centripetally against the enameloid surface; the formation of dentine induces the differentiation of epithelial basal layer cells into **ameloblasts**, which will contribute proteins to the loose enameloid matrix, or if there is no enameloid, produce a homogeneous matrix, enamel, against the dentine surface. The difference among the elasmoid scales of teleosts, the elasmoid scales of coelacanths, and the elasmoid-like young polypterid scales is the lack of avascular connection between epithelium and mesenchyme in teleost scales, which explains why enamel and dentine are absent in teleost scales but present in the others (Sire and Huysseune, 2003). Blood vessels surrounding the developing odontodes will be integrated into the supporting bone and attachment bone. Fiber bundles in the mesenchyme will also be incorporated. When mesenchymal cells from the base of the odontogenic papilla differentiate into **osteoblasts** and **fibroblasts**, bone of attachment will be formed with ligaments linked to the dentine base. A fully differentiated tooth cannot erupt until the precipitation of

the bone of attachment, which prolongs the tooth base, but does not depend on whether the tooth has been attached to other dermal bones or not (Huysseune, 1983).

The disparity of tissue origin not only exists between odontogenic and osteogenic components, but also among the odontodes from the three developmental domains. Scales develop in a well-structured mesenchyme, where they can be anchored firmly with Sharpey's fibers. Tubercles, denticles and teeth that are based on a bony support only need an unstructured mesenchyme. The first indication of their formation is the thickening of the epithelium, and the odontogenic papilla will form once the differentiated basal epithelial layer invaginates. An invagination into the mesenchyme indicates the morphogenesis of a tooth. But for dermal odontodes, there is just a shallow invagination within the differentiated basal region of epithelium. The deep epidermal invagination, called **dental lamina**, is the organ to generate tooth buds prior to the shedding of the functional teeth, guaranteeing the supply of sharp processers for feeding. By contrast, dermal odontodes cannot be replaced until the predecessor has been shed, according to the "odontode regulation theory" (Reif, 1982). Therefore, the presence of a dental lamina has conventionally been taken to defines "true teeth". However, this mode of tooth development is largely based on the studies on modern chondrichthyans. Chondrichthyan-like scales have a much longer fossil record than chondrichthyan teeth (Janvier, 1996; Miller et al., 2003), suggesting that teeth could have evolved within the chondrichthyan stem group, independently of osteichthyan teeth.

Origin and diversity of teeth

The origin of teeth remains unresolved, even after decades of study. New discoveries have provided evidences for various theories, but did not close the argument. It is not a time either to cling to the old theoretical frameworks or to erect new ones. Instead of attempting to discriminate the competing hypotheses as has been done by many excellent review articles (Huysseune et al., 2009, 2010; Fraser et al., 2010; Donoghue and Rücklin, 2014; Witten et al., 2014; Smith and Johanson, 2015), the increasing knowledge on the diversity of vertebrate teeth, together with the new data from *Andreolepis* and *Lophosteus*, is herein integrated and analyzed with a minimum of prior expectations, in order to build the understanding from the ground up.

When attempting to investigate the evolutionary history, we immediately encounter a problem that seems paradoxical given the familiarity of the anatomical term: what exactly do we mean by "a tooth"? On one hand, it is necessary to constrain the deeply entrenched meaning in everyday language to a precise scientific definition of "true teeth". "Tooth" is such an ancient and general term that its use has come to encompass tooth-like stuff beyond biology. Among metazoans we find numerous examples of tooth-shaped structures that are used for food processing, composed of a wide range of materials and borne by non-homologous structures: an extreme example of convergent evolution. At the same time, the teeth of jawed vertebrates are so diverse that they are not necessarily tooth-like in appearance. We thus need to define a set of criteria by which to identify a true tooth, irrespective of its morphology. On the other hand, if "true teeth" are put into a rigid typological box, how to recognize the transitional forms? Teeth are more likely to be modified from some sort of "non-tooth odontodes", probably the common ancestral odontodes of modern teeth and dermal odontodes with more generalized characters, rather than to have evolved *de novo* for multiple times. When teeth are defined as it were in opposition to other odontodes, entirely on the basis of characters that differ between them, the possible homologies will be obscured.

The term "teeth" was originally used to describe human teeth, and more recently shark teeth have been used as developmental study models, but primitive teeth would presumably not show all the characteristics of these teeth. The problem is whether a subset of characteristics is sufficient to recognize earliest teeth, and if so, which subset. Criteria of true teeth have been proposed and questioned, but only two points are accepted here: 1) teeth are

odontodes; 2) teeth never overgrow each other. These are merely the prerequisites for true teeth, and the contrary is not true: not all odontodes are teeth, and monoodontode scales don't overgrow either, but teeth can be overgrown by dermal odontodes.

Below I consider a range of criteria that can potentially be used to identify teeth, each discussed and illustrated with examples of different teeth and None of these criteria alone can indicate true teeth.

Function

"Tooth" in the common language includes different kinds of functional analogues related to food uptake and processing. That is to say, a tooth-like feeding organ is not necessarily a true tooth. The chelicerae fangs of arachnids are chitinous cuticle, while the rasping teeth of hagfish and lamprey, and the serrations along the beak of modern turtles and birds are keratinized structures. **Chitin** and **keratin** are non-mineralized skeletal collagen proteins that are differentiated outside the epidermis (**exoskeleton**), rather than within the dermis (**dermoskeleton**) and thus are not even regarded as true skeleton (Francillon-Vieillot et al., 1990).

"Teeth" have other functions that are crucial to survival, for example, the egg tooth for hatchlings to pierce the eggshell, is also found in spiders, frogs, retiles, birds and monotremes. The egg tooth of turtles is a modified keratinized scale, but those of platypuses and echidnas are true shedding teeth with enamel, as in lizards and snakes, even though the teeth for feeding have been lost for millions of years in adults (Davit-Béal et al., 2009).

Not all the feeding styles require teeth or tooth-like structures. Tooth loss in mammals is typically associated with the ant- and termite-feeding with long sticky tongue, exemplified by s echidnas, anteaters and pangolins, and the filter-feeding of baleen whales (Davit-Béal et al., 2009). Some actinopterygians, such as sturgeons, paddlefish and armored catfish possess functional teeth for catching preys in the juvenile stage, but lose them when they switch to filter-feeding in the adult stage (Sire and Davit-Béal, 2002; Smith et al., 2015a).

The functionality of fossil teeth or tooth-like structures can be indicated by the evidence of wear (Reisz, 2006). In some placoderms, the biting areas of the supragnathal and infragnathal plates may carry denticles, large tusks and blade-like cutting edges (Johanson and Smith, 2005). These structures display abrasion, and evidently function as mandibular or pharyngeal teeth. The blades of the gnathal plates may be formed through remodeling of worn teeth, however, they are made of sheets of bone or pleromic dentine, which grows continuously throughout life to accommodate the wear (Ørvig, 1980b), and hence are not odontodes and not true teeth (Rücklin et al., 2012).

Antiarchi placoderms have headshield ornamentations that grades into denticles at the biting edge, suggesting that to recruit dermal odontodes to serve as a functional dentition is a primitive alternative solution to having teeth (Johanson and Smith, 2005). The first-generation odontodes of the marginal jawbone of *Andreolepis* bear chipped tips, which are preserved by the overgrowth of successive generations of odontodes. This provides an intriguing example of the function of a dentition being initially carried out by tooth-like dermal odontodes, prior to the developing of true teeth (Paper I).

Morphology

Teeth of an animal can be of the same morphology (**homodont**). Sexual dimorphism (Kajiura and Tricas, 1996; Underwood et al., 2015b) and onto-genetic variation (Clemen and Greven, 1994) can be reflected in tooth shape. Excluded from these factors, more elaborate tooth shapes with varied functions (**heterodont**) are reported in all major non-mammalian taxa (Huysseune and Sire, 1998), and became a shared character in synapsids. In mammals, tooth shape is determined by tooth position, but the degree of variation varies between taxa. Carnivore teeth are highly differentiated with sharp crests for piercing and slicing, but omnivore have multi-purpose teeth of more generalized forms or continuous gradient, and herbivore teeth are more or less uniform with complicate occlusal surfaces for crushing and grinding (Scott and Turner, 2000). Whilst fish display not only the numerous fine pointed teeth of cardiform and villiform, but also the caniniform and molariform teeth (Thomson et al., 2000; Bemis and Bemis, 2015).

The complexity of the **multi-cuspid** mammalian molars results from the folding of inner epithelium by the secondary enamel knots, namely, the modifying of the epithelial-mesenchymal boundary, against where dentine and enamel will be deposited (Catón and Tucker, 2009). Whereas in non-mammalian vertebrates multi-cuspid teeth are not led by enamel knots, such as, the bicuspid teeth of squamate are produced by the asymmetrical deposition of enamel (Zahradnicek et al., 2012), and the tricuspid teeth of shark are created by the independent expression of proliferative and anti-proliferative signals (Debiais-Thibaud et al., 2015). In urodeles, monocuspid teeth are replaced by bicuspid teeth after metamorphosis (Clemen and Greven, 1994); in cichlids, the first generation teeth are monocuspid, but the replacement teeth are tricuspid (Fraser et al., 2013; Tucker and Fraser, 2014). Actually, multi-cuspid (with side cusps) odontodes are not uncommon in placoderm, acanthodian, chondrichthyan and osteichthyan scales and ornamentations, apart from their dentitions.

The general trend of early vertebrate teeth are uniformly **monocuspid** (conical), distinct from the ornamentation. Placoderms and *Lophosteus* with

multi-cuspid stellate tubercles are the typical examples. The conical first-generation odontodes on the marginal jaw bone in *Lophosteus*, become flattened, elongated and labially (Paper IV). Whereas some placoderms have organized rows of tooth-like odontodes, which are also multi-cuspid but modified to be blade-like (personal observation). Although teeth can have various shapes, it is significant to look specifically at morphological differences between teeth and dermal odontodes in taxa that have both.

Composition

The oldest mineralized skeleton is the tooth-like grasping elements of **conodonts**, which are presumably the stem vertebrates or stem cyclostomes (Janvier, 2015). The vertebrate affinities of euconodonts have been confirmed by soft-tissue characters, and paraconodonts have been demonstrated as ancestor of euconodonts, but prtoconodonts have been reinterpreted as the stem-chaetognaths (arrow worms) (Szaniawski, 1982). The earliest form, prtoconodont elements, is already in conical shape, but it is poorly mineralized. The paraconodont elements have atubular dentine-like tissue, but its punctuated distal-proximal growth by the enveloping of successive laminae is different from the centripetal dentine deposition of odontodes, and may have evolved independently. The most derived euconodont elements are capped by a thick enamel-like crown, but their phylogenetic position rejects the homology with tooth enamel, instead suggesting that skeletal tissues were absent in the common ancestor of conodonts and gnathostomes (Janvier, 2013; Murdock et al., 2013). Therefore, conodonts are considered irrelevant to the origin of gnathostome teeth by its tissue composition (Donoghue and Rücklin, 2014).

Compared to the tooth shape, which is closely linked with diet and subjected to strong selective pressures, the tissue diversity and internal microstructure of teeth has been much more conserved. Nevertheless, interspecific variation occurs and might be of phylogenetic significance. As an odontode, a tooth is constituted by a dentine cone, the attachment structures and sometimes a capping layer.

Dentine is divided into the outer **mantle (pallial) dentine** and the inner **bulk (circumpulpal) dentine**, or into **primary dentine** and **secondary dentine** that are deposited before and after the tooth becomes functional, and **tertiary dentine** that responds to stimuli. Mantle dentine, the layer with interglobular spaces (globular zone of dentine), is less calcified and softer, and thus functions as a shock absorption system. These structures, first known from human teeth can be distinguished in early vertebrates as well (LeBlanc and Reisz, 2013). For example, a survey of various dinosaur teeth show that the thickness of mantle dentine relative to bulk dentine and enamel

varies among genera of saurischians, but that ornithischian teeth have little mantle dentine (Wang et al., 2015).

Mantle and bulk dentine together, with no vascular or cells enclosed, are called **orthodentine**. It is the dominant dentine type of vertebrate teeth, the sole dentine type of tetrapod teeth (Huysseune and Sire, 1998), and is sometimes referred to as true dentine, regular dentine or gnathostome-type dentine. Although it was considered as the most advanced form of dentine in the classical classification (Ørvig, 1967), it is actually already present in the dermal odontodes of the Ordovician jawless fish pteraspidomorphs, believed to be part of the sensory system (Sansom et al., 1997; Smith and Sansom, 2000). However, its absence in osteostracans, which are closer to jawed vertebrates, suggests convergent evolution (Donoghue and Sansom, 2002). **Plicidentine**, the dentine infolded at the base of sarcopterygian fish and amphibian teeth, and **vasodentine**, the dentine permeated by capillaries in teleost teeth, are just modifications of orthodentine (Ørvig, 1967). **Mesodentine**, with odontoblasts being trapped within the matrix (**odontocytes**), is another ancient dentine tissue that have already existed in the Ordovician (Smith and Sansom, 1997, 2000). It is common in osteostracans, but not retained in jawed vertebrates and thus not a component of any true teeth known so far. **Semidentine**, which appears to be an intermediate form between the cellular mesodentine and the acellular orthodentine, is usually regarded as a a synapomorphy of placoderms (Brazeau and Friedman, 2014). But orthodentine also occurs in different lineages of placoderms, notably in the arthrodire teeth, even though it is rarely occurs in dermal tubercles and may have evolved independently within the subgroup. Orthodentine has been central to the controversy about distinguishing true teeth from tubercles in placoderms (Deninson, 1978; Burrow, 2003; Smith and Johanson, 2003a, 2003b; Young, 2003; Johanson and Smith, 2005; Burrow et al., 2016). In consideration of the great plasticity of the dentinal tissues and the uncertainty of their homologies, it is legitimate to question whether the type of dentine should be a criterion of true teeth. On one hand, the arguably separate origin of placoderm teeth from those of the crown gnathostomes raises doubts about the necessity to exclude the possibility of a placoderm tooth composed of semidentine. On the other hand, semidentine has not been observed associated with an **undivided pulp cavity**, only with **pulp canals**. An undivided pulp cavity is often seen in true teeth, though it also occurs in certain dermal odontodes like thelodont scales. It is probably because a tooth needs a wide cavity to house numerous vessels and nerves from the large canals of the basal plate, which is why a tooth can never overgrow on other teeth or dermal odontodes, otherwise it can connect to the basal canal system only through small canal branches. In *Lophosteus*, the pulp cavities of both the replacement teeth and the first-generation biting odontodes at the marginal jawbones are always open, while those of the non-shedding dermal odontodes on both the facial lamina, as well as on the scales (Jerve et al., 2016),

become infilled by denteones of osteodentine in the younger generations (Paper IV).

When the tissue interior to the mantle dentine consists of **denteones** (dentinal osteons), which are formed by filling the pulpal spaces around the blood vessels, it is called **osteodentine (trabecular dentine)**, for example the solid infilling in the pulp cavity of the saw-teeth from the batoid ray *Schizorhiza* (Smith et al., 2015b). In flat non-shedding crushing teeth, such as, the tooth plates of ptyctodontid placoderms (Ørvig, 1980a), holocephalans (Didier et al., 1994) and lungfish, it is specialized into a group of hypermineralized wear-resistant tissues, the **pleromic dentine (pleromin)**, which is as hard as enamel (Ørvig, 1967). It is deposited secondarily in response to abrasion by infilling the pulp cavity and grows continuously in a basal direction, resembling the reparative tertiary dentine of human teeth. In lungfish tooth plates, it is called **petrodentine**, secreted by petroblasts, which are differentiated from the odontoblast layer, with very fine cell processes. It grows beneath the primary dentine cones that would be worn away, and progressively into soft tissue spaces of the bone below, linking the older worn teeth from the same radial tooth row into a biting ridge, while only the youngest tooth of the row is still covered by enamel. In this way, some lungfish produce "pseudoteeth" that are not generated by dental papillae (Smith, 1984, 1985; Smith and Sansom, 2000; Ahlberg et al., 2006). In arthrodires, when tooth addition came to an end, worn teeth would be incorporated into a shearing edge, which is maintained by the ingrowing of pleromic dentine (Ørvig, 1980b). The existing rows of teeth offer the growth sites for pleromic dentine, while the tubercles never shows invasive growth of pleromic dentine (Johanson and Smith, 2005). All this seems to point to a developmental and evolutionary association with true teeth, even though pleromic dentine does not make up true teeth itself, and develops within mesenchyme without contact with epithelium. On the other hand, skeletal repair by the infilling of reactionary dentine, such as the secondary and the tertiary orthodentine observed in the jawless fish heterostrancans, has been a widespread and conserved process not restricted to dentitions (Ørvig, 1976; Johanson et al., 2013).

The typical hypermineralized capping tissues of teeth are **enameloid** and **enamel**. Their mechanical properties are similar, and they are speculated to be homologous to the extent of deriving from the same developmental system (Smith, 1995). Notice that the former is an epithelial-mesenchymal product like dentine (and even shares a common set of extracellular matrix in teleosts), also secreted by odontoblasts, but before the deposition of dentine; while the latter is of purely epithelial origin, laid down by ameloblasts after the first formation of dentine. By contrast, the pleromic tissues are hypermineralized without the contribution of epithelium, and the **acrodin**, uniquely found on the tip of some actinopterygian teeth (Ørvig, 1973b), is mineralized by a different programme as well (Qu et al., 2015). Enameloid is

prevalent in chondrichthyan and basal actinopterygian teeth, but has primitively occurred in the integument of many jawless fish (Sire et al., 2009). Enamel is a synapomorphy of osteichthyans (Friedman and Brazeau, 2010), and sarcopterygians have enamel only, except for the coexistence of enameloid and enamel in the teeth of urodele larvae (Davit-Béal et al., 2007). Teleosts lack enamel, but occasionally bear collar enamel on the teeth. Crown-group actinopterygian teeth are capped by another tissue, acrodin. There are finer-scale variations among different taxa, such as enameloid in actinopterygians differs from that in chondrichthyans, chondrichthyan enameloid differs between sharks and rays, mammalian enamel differs from that of the other osteichthyans. Placoderms, as well as some jawless fish, have neither enameloid nor enamel (Ørvig, 1980a, 1980b; Giles et al., 2013; Burrow et al., 2016). The lack of pattern to the phylogenetic distribution suggests these capping tissues, just as the dentinal tissues, have evolved independently in various lineages (Donoghue and Sansom, 2002, 2006).

The shape of the pulp cavity of the odontodes of *Andreolepis* and *Lophosteus* is related to the location of the odontodes. The pulp cavities of teeth and first-generation odontodes on the lingual margin of the marginal jaw bones are conical in shape, but those of the first-generation odontodes on the facial lamina become flatened and elongated like the horizontal canals in scales, and those of scale odontodes have fine ascending canals entrapped in the infilling dentine matrix like arcades (Qu et al., 2016). Cell spaces of odontoblasts are partially entrapped at the border of the pulp cavity of the functional replacement teeth of *Andreolepis* and *Lophosteus*, which may indicates the deposition of dentine was progressing in life, but not at that of the first-generation odontodes of the marginal jaw bones. Regional and interspecific variations of the interglobular spaces are also observed in the dentine of *Andreolepis* and *Lophosteus* teeth (Paper III). But no enamel is found in their teeth, only on the scales of *Andreolepis*, which supports their stem-group position in osteichthyans. Their teeth often show pulp cavities open at the surface that imply serious abrasion, and this may not only be the result of postmortem transportation, since intact teeth also exist. However, there is no further filling by tertiary dentine or wear-resistant tissue. It demonstrates the significance of tooth replacement as a novel strategy for dental repair that it has even evolved prior to the covering of enamel (Paper I-IV).

Location

Osteichthyan teeth are typically known as a linear row parallel to the jaw margin on the dermal bones premaxilla, maxilla and dentary, arranged as the **outer dental arcades**. Multiple marginal tooth rows can be found in all major taxa of osteichthyans (Fraser et al., 2004; Reisz, 2006). There are additional tooth rows on the vomer, dermopalatine, ectopterygoid and coronoid

series in non-mamalian and non-crocodilian osteichthyans (Schwenk, 2000), which form the **inner dental arcades**. In mammals, the job of holding and mashing food performed by the palatal dentition is taken over by the palatal rugae, the transverse, cornified ridges on the secondary palate (Pantalacci et al., 2008). Turtles are primitively dentate by the presence of palatine teeth, in spite of the absence of the marginal teeth (Davit-Béal et al., 2009). In addition to the large conical teeth, basal aquatic tetrapods (Ahlberg and Clack, 1998) and osteichthyan fishes (Clemen et al., 1998) have **denticle fields**, clustered patches of toothlets, over the surfaces of dermal bones in the oropharyngeal cavity, probably to prevent prey escaping in the water. Tetrapods have lost the **pharyngeal denticles** on the branchial arches along with the loss of gill. In contrast, many teleosts, like zebrafish, only possess pharyngeal teeth, with the reduction of mandibular and palatine teeth as the general trend.

Chondrichthyans are famous for the multiple rows of marginal teeth, and in fact each row parallel to the jaw margin can belong to different generations of multiple transverse tooth files. In primitive crown chondrichthyans and stem chondrichthyans (acanthodians), teeth from the same tooth files are fused at the base into a **tooth whorl**, which are lined up along the cartilaginous jaw in some taxa, and small tooth whorls are interpreted as coming from the brahchial-hyoid arches of acanthodians (Denison, 1979). In contrast, osteichthyans only have tooth whorls at the symphysial or parasymphysial position, and placoderms have no tooth whorls (Smith and Coates, 2001). In spite of the lack of the dermal bones, some holocephalans have palatal tooth plates (Didier et al., 1994; Finarelli and Coates, 2012), as well as the mandibular tooth plates. No pharyngeal dentition is present in holocephalans, but pharyngeal denticles are joined as whorl-like sets or separated in many modern or primitive sharks (Smith and Coates, 2001). Some acanthodians carry dentigerous jaw bones (Burrow, 2004), more similar to the macromeric dermoskeleton of placoderms and osteichthyans.

The only accepted true teeth of placoderms are those on the gnathal plates of arthrodires (Ørvig, 1980b; Smith and Johanson, 2003b). Because the infragnathal and the posterior supragnathal are carried by the Meckelian cartilage and the palatoquadrate in conventional placoderms, they have traditionally been homologized with the osteichthyan inner dental arcades. The recently discovered maxillate placoderms *Qilinyu* and *Entelognathus* exhibit facial laminae on their gnathal plates, as the facial lamina on the premaxilla, maxilla and dentary of osteichthyans, demonstrating that the gnathal plates of placoderms are in fact homologous to the osteichthyan outer dental arcades. There is no inner dental arcade in placoderms, but a **palatal lamina** on the gnathal plates that may or may not be dentigerous, which has been lost in the dentary of the most derived member, *Entelognathus* (Zhu et al., 2013, 2016). Placoderms have no pharyngeal denticles either, but almost all placoderms have organized bony denticles on the **postbranchial lamina** of

the anterior-facing pectoral girdle, and bony denticle fields are observed on the surface of the gnathal plates of some taxa (Johanson and Smith, 2003, 2005).

The isolated specimens of both the stem osteichthyans *Andreolepis* and *Lophosteus* contain a considerable amount of cushion-shaped tooth-bearing elements, which were usually regarded as the pharyngeal tooth plates. But their specific anatomy argues that they probably represent the most primitive form of inner dental arcades, bridging the evolutionary gap between placoderms and crown osteichthyans (Paper II and III).

The replacement teeth, as well as the first-generation odontodes, on the marginal jaw bones of *Andreolepis* and *Lophosteus*, were overgrown by invasive dermal ornaments at a certain stage of development. As a result, the shedding cycles was terminated, and these older sites became post-functional positions. This indicates that the oral/dermal boundary, which undulates along the jaw margin, is not static, but fluctuates along with the growth of the jaw bones. As for whether the first-generation odontodes that are located at the labial margin of the shedding tooth fired should be considered as teeth or biting odontodes, it fundamentally questions the validation of the oral-dermal boundary at the early stage of tooth development and evolution (Paper I and IV).

The facts that the conveyance of teeth out of the jaw margin to the facial lamina will cause the overgrowth by ornaments in stem osteichthyans, the integration with tesserae in acanthodians (Miles, 1973), and the tooth shedding through the deterioration of Sharpey's fibers in modern chondrichthyans (Reif, 1982; Williams, 2001), all imply that there must be specified signals within the oro-pharyngeal epithelium to keep odontodes being replaced and patterned as the so-called true teeth, for example, the developmental link with taste buds (Martin et al., 2016). In contrast, jaw seems not a prerequisite for dental patterning, since there are extra-mandibular dentitions within the oro-pharynx. The origin of teeth and jaw are likely independent evolutionary events, contradicting the canonical **"outside-in"** theory, which hypothesizes tooth originated from modified scales that invaded the oral cavity once the jaw evolved. However, all the candidate evidences of teeth in jawless fish, conodonts and thelodonts, have been rejected. Consequently, the **"inside-out"** theory, which advocates that teeth have evolved before jaws, loses its support (Donoghue and Rücklin, 2014). Along with the question why teeth could arise from the oro-pharyngeal cavity of gnathostomes, rather than from that of agnathas, comes the question of the dual epithelial origin of teeth (Soukup et al., 2008). The jaw (oral) is lined by ectoderm, and the gill slit (pharyngeal) by endoderm. Tooth competence requires the contact between the ectodermal and endodermal epithelium (Huysseune et al., 2010), but it is uncertain to what extent the ectoderm migrates inward and endoderm outward even in living animals (Koentges, 2008; Smith and Johanson, 2015).

Needless to say, the position of the **ectoderm-endoderm boundary,** where the regulatory genes are initiated, is irremediably unknown in fossils.

Nevertheless, not all the "teeth" are located within the oro-pharyngeal cavity either, and there are **extra-oral teeth** occurring in all the major groups. Cheek scales with whorl-like arrangements are reported in an ischnacanthid acanthodian. They display a transitional morphology such that the nearer to the jaw margin, the more tooth-like they are (Blais et al., 2011). In this case, it is plausible that the oral signals have been spread beyond the mouth, but decrease externally, suggesting the oral/dermal boundary is not demarcated.

Several teleost lineages, like the armored catfish, swordfish, billfish, bearded silverside and denticle herring, have aligned small teeth covering cranial or postcranial dermal bones. Just like the oral teeth, they are also developing in epithelial invagination, shed and replaced by basal resorption of the bone of attachment and supporting bone (Sire and Huysseune, 1996; Sire et al., 1998; Sire, 2001; Sire and Allizard, 2001). They are postulated to function for improving hydrodynamics, instead of facilitating ingestion (Sire, 2001). The chondrichthyan rostrum saw-teeth have evolved multiple times in sawsharks, sawfishes, rays and chimaeras (Underwood et al., 2015a; Welten et al., 2015). They are all regularly spaced, and especially those of the extinct batoid ray *Schizorhiza* reflect precise timing and spatial control, with the replacement teeth developed into a cone-in-cone stack directly belong the functional tooth (Smith et al., 2015b). But these extra-oral teeth, irrespective of whether they occur in teleosts or chondrichthyans, are examples of secondary specialization and convergent evolution, rather than intermediate forms between skin odontodes and oral teeth. Instead, they reveal the potential plasticity of odontodes, and suggest the competence of patterning is not restricted within the oro-pharynx. The question is what kind of odontodes are they modified from, oral teeth or dermal odontodes, or a more generalized form.

Organization

The developmental patterning of teeth reflected by their ordered organization, from which the spatio-temporal regulation of the dental lamina can be inferred even in the absence of experimental data, has been regarded as one of the most important criteria of true teeth (Burrow, 2003; Smith and Johanson, 2003b).

The dental lamina is a sub-epithelial tissue that has the function of prefabricating tooth buds lingually before the functional teeth are shed. In different taxa, it can be a **continuous dental lamina** that stretches all along the whole jaw, or a **discontinuous dental lamina** that forms by each tooth family, both are forms of **permanent dental lamina** that produces a battery of replace-

ment teeth, whereas a **nonpermanent (successional, transient) dental lamina** generates only a single tooth (Reif, 1982). This concept was proposed based on chondrichthyans, but phylogenetic evidence indicates that it does not represent the primitive condition of gnathostomes, and is not an ideal model for the early evolution of teeth. However, extant actinopterygians, including the primitive representatives bichir *Polypterus* (Vandenplas et al., 2014), paddlefish *Polyodon* (Smith et al., 2015a) and some teleosts (Fraser et al., 2004; Huysseune and Witten, 2008; Abduweli et al., 2014), lack any kinds of dental lamina at all. Although some other teleosts have the successional dental lamina that is regressive after each round of tooth replacement (Huysseune, 2006; Fraser et al., 2013), and tetrapods have a permanent dental lamina that is formed by linked successional dental laminae sequentially branching from the previous tooth buds (Handrigan et al., 2010; Gaete and Tucker, 2013), the permanent dental lamina was probably not shared by the common ancestor of osteichthyans and chondrichthyans, and is unlikely to have occurred in the earliest dentitions. The molecular regulatory network and the mechanisms for tooth patterning may have arisen independently to dental lamina, in different taxa and even in various dentate bone. Dermal denticles have been distinguished from teeth by their superficial formation without a deep invagination, but this is only applicable to chondrichthyans. In fact, tooth buds can also be generated superficially by the stem cells, the progenitors of odontogenesis, within the **odontogenic band** in osteichthyans, and probably in extinct taxa like acanthodians and placoderms (Smith et al., 2009). While the dental lamina is a distinct compartment that provides a niche for the odontogenic stem cells, as its equivalence, this restricted band can be a shallow invagination or indiscernible histologically, but recognized by the specific dental gene expression within the dental epithelia of the previous tooth buds (Fraser et al., 2004).

The presence or absence of a dental lamina cannot be verified in fossils, but what the tooth organization can actually indicate is the sequential addition of teeth. Osteichthyan teeth on the marginal jaw bones are arranged as longitudinal tooth rows. But each tooth has its replacement teeth underneath, initiated lingually, and the whole set together form a **tooth family**, as a tooth file in chondrichthyans. A single-row tooth whorl of sarcopterygians, crown chondrichthyans and acanthodians represents a single tooth family. The holocephalan tooth plates are in fact a compound tooth stacked up by dentine bands of successors from the same tooth family (Didier et al., 1994; Finarelli and Coates, 2012). In both osteichthyan and chondrichthyan jaw-teeth, a tooth family is transverse to the jaw margin. Longitudinally along the jaw margin, sequential initiation of each tooth family starts with an initial primordium (pioneer tooth) for each bone, which is the symphyseal or parasymphyseal tooth germ in chondrichthyans (Underwood et al., 2015b, 2016), and progress to the sides disto-proximally (Smith, 2003). This left/right spaced pattern is retained in the marginal dentition of larval lungfish, where

the resorption of teeth follows the same sequence, first with the symphyseal tooth, even if no replacement teeth will be generated (Reisz and Smith, 2001). Since the first-generation teeth of each tooth family (tooth position) are not initiated simultaneously, a functional tooth row parallel to the jaw margin may contain teeth from different generations, while a synchronous developmental row may be arranged obliquely (Underwood et al., 2016). When teeth at the odd- and even-numbered positions are initiated alternatively (Berkovitz, 1977), two adjacent tooth families make up a clonal generative unit, forming an **alternate tooth pattern**, opposed to the **single file tooth pattern**. Single file addition seems more common in basal chondrichthyans, suggesting it is more primitive (Smith et al., 2012), but it may actually have evolved on several occasions. Ontogenetic observations show that single file arrangement can develop from the alternate pattern formula, and both patterns can occur in the upper and lower jaws, in the embryonic or adult stages of an animal, respectively (Underwood et al., 2016).

In the cases of non-shedding dentitions, as in scales, the adding of new odontodes is associated with the expanding of the bony base (Rücklin et al., 2011; Qu et al., 2013). New teeth are added in the direction of bone ossification. Teeth increase in size with every addition, as the bone grows wider at the new zone. In the acanthodian dentigerous jaw bones, teeth are added anteriorly (Ørvig, 1973a); In arthrodire gnathal plates, teeth are added in different directions to the three tooth rows that radiate from an initiator tooth, for example, posteriorly, anteriorly and internally in the marginal, symphyseal and medial tooth row of the infragnathal, respectively (Johanson and Smith, 2005); in lungfish tooth plates, teeth are added at the end of each radial tooth row (Smith, 1985). Different from the acanthodian jaw teeth, both arthrodire (Ørvig, 1980b) and lungfish (Smith and Krupina, 2001) teeth are also added near-vertically such that the larger younger teeth are tilted obliquely to the horizontal plane of the basal plate, which is due to the bone growing more in depth than in length.

Regular spacing of teeth or cusps in rows is a common biological phenomenon, generally associated with the cutting-in function for feeding or defending. Euconodonts have the tooth-like units arranged neatly in different patterns, lampreys have many consecutive circular rows of horny teeth, and hagfish have two rows of horny teeth that look like two rami of a jaw. Placoderms may have small pointed units overspread their armor (Burrow, 2003; Young, 2003), notably on the fin spines and the postbranchial lamina (Johanson and Smith, 2003, 2005), but these ordered units are not even odontodes (Rücklin et al., 2012; Murdock et al., 2013). The pharyngeal denticles of the jawless fish thelodonts have odontodes composed of dentine and enameloid that are added in a strict polarized sequence like tooth whorls, but this is just a peculiar feature that only occurs in the derived taxa of this group, and these pharyngeal denticles cannot be considered as true teeth, even if they are within the oro-pharyngeal cavity (Brugghen and Janvier,

1993; Smith and Coates, 2001; Rücklin et al., 2011). The cheek scales of ischnacanthid acanthodian outside the oral cavity have a similar arrangement as the tooth whorls inside the oral cavity (Blais et al., 2011). Other extra-oral teeth of teleosts (Sire and Huysseune, 1996; Sire et al., 1998; Sire, 2001; Sire and Allizard, 2001) and saw-teeth of chondrichthyans (Smith et al., 2015b; Underwood et al., 2015a; Welten et al., 2015) are also organized in rows.

   The first-generation odontodes of the scales of *Andreolepis* and *Lophosteus*, in a uniform morphology with increasing size, are also added in sequence along a row, in the direction of the growth of bony base, beginning with primordial odontodes at the growth center of the bone (Qu et al., 2013; Jerve et al., 2016). Even the successive generations of odontodes on the scale of *Lophosteus*, which is more primitive (judging by its lack of enamel), are in a quite regular pattern and shape, albeit restricted within each generation. In the marginal jawbones of both taxa, the elongated or multi-cuspid first-generation odontodes on the facial lamina gradually transform into the conical ones at the labial margin of the oral lamina (the assumed jaw margin at the early stage), and are arranged in transverse files with an alternate pattern. They are probably the positional template of the replacement teeth that develop later at a more lingual field, even though they do not set up a pattern for the overgrowing odontodes to follow (Paper I, IV). The dermal tubercles on an ovoid ornamented plate attributed to the acanthothoracid placoderm *Romundina*, which were interpreted as teeth on a gnathal plate originally, are neatly organized. They constitute a single generation of odontodes, contrary to the multiple generations of odontodes irregularly arranged on its scales (Rücklin and Donoghue, 2015; Burrow et al., 2016). Notably, the sequential addition is not necessarily synonymous with unidirectional addition, but depends on the direction of bone growth. In the ornamented plate of *Romundina*, the multi-cuspid tubercles are added radially, in other words, perpendicularly to the plate margin. This is reminiscent of the addition of arthrodire teeth to the three radial rows, along with growth of the gnathal plate. By comparing the distance of the dorsal/ventral margin to the primordial odontode that are associated with the vertical basal canals, *Andreolepis* scales grow more ventrally than dorsally, so most of the first-generation odontodes are added ventrally, except one of medium size dorsally (Qu et al., 2013). While *Lophosteus* scales grow almost equally in the ventral and dorsal directions, getting taller but also wider at a constantly high speed, so increasingly larger overgrowing odontodes are added alternately in both directions (Jerve et al., 2016). Tooth addition in osteichthyans and chondrichthyans is always unidirectional, probably just because the bones invariably grow lingually (Paper III). Chondrichthyan odontodes do not receive directional growth cues from underlying bone, but there are also organized initiator scale rows in the earliest developmental stage, where odontodes (monoodontode scales) are added unidirectionally along the notochord, myomere, lateral line canal and multidirectionally along the leading edges of fins (Miyake et al., 1999;

Johanson et al., 2007; Martin et al., 2016), or in the direction of cartilage mineralization, like denticles along the rostrum (Welten et al., 2015). Therefore, a regular pattern is likely a general feature of the first-generation odontodes, no matter where they are located. The timing of sequential addition is intrinsic to the first-generation odontodes too, and the direction is associated with bone growth or body axis.

In both scales and jaw bones of *Andreolepis*, this pattern is broken by the superimposition of younger-generations odontodes that overgrow in a gap-filling manner. Teeth can be covered with dermal odontodes when they are moved externally to a facial post-functional position by the growth of the jaw bone, but they never overgrow on each other. Through heterochrony (paedomophosis), teeth might have been modified from the first-generation odontodes by the subsequent programmes of generating overgrowing generations being deleted. Therefore the sequential addition of teeth is likely homologous with that of the ancestral first-generation odontodes, and this ordered organization would be selected for the feeding function of teeth, including for the prey capture of saw-teeth and saw-denticles (Smith et al., 2015b; Underwood et al., 2015a; Welten et al., 2015). In contrast to teeth, the successive generations of dermal odontodes do not retain the spatial polarity of the first-generation odontodes. To keep adding new odontodes to cover the entire external surface might be a primitive character of dermal bone. Since there are newly grown narrow edges around the plate, successive generations of dermal odontodes are generated to fill it up, as well as to fill the gaps between the existing odontodes. Correspondingly, they would turn into an irregular shape, probably also due to the lack of an even depositing floor and a full contact with bone. In derived taxa, when the first-generation odontodes have been lost, ontogenetically or phylogenetically, teeth and dermal odontodes, like those in modern adult chondrichthyans, would appear as two distinctive developmental systems (Paper I, IV).

Replacement

Mammals are characterized for their **diphyodont** dentitions that teeth are usually replaced once, involving two generations of teeth. The incisors or molars of rodents grow continuously, and they are called **monophyodont** teeth. But kangaroos, elephants and manatees are **polyphyodont** animals that have multiple tooth replacement cycles. In fact, most non-mammalian vertebrates are polyphyodonts, with their teeth replaced throughout life, and diphyodonty is derived from polyphyodonty. The phenotypic plasticity of teeth has been reduced as the reduction of the replacement cycles. Because teeth may adjust their size and shape with every cycle (Clemen and Greven, 1994; Fraser et al., 2013; Tucker and Fraser, 2014), polyphyodonty is re-

garded as a preadaptation to produce generational variations for the change of environment (Kajiura and Tricas, 1996).

However, some early vertebrates, like placoderms, acanthodians and primitive crown chondrichthyans, never shed their teeth, and this is the ancestral **statodont** status. Lungfish have evolved statodont teeth secondarily on the tooth plates, from their osteichthyan ancestors who have shedding dentitions. Actually the marginal dentitions of larval lungfish can be shed, but never be replaced (Reisz and Smith, 2001). In the non-shedding non-replacing dentitions, old teeth are simply worn away, and the biting area has to shift towards the new tooth position at a newly grown zone of bone, as in the acanthodian dentigerous jaw bone (Ørvig, 1973a). Or multiple worn teeth are resorbed and "replaced" by a wear-resistant edge or ridge of pleromic tissues, as in arthrodire placoderms and some lungfish. Notably, resorption in these cases is either extensive or randomly, but never targeted at individual teeth (Johanson and Smith, 2005; Ahlberg et al., 2006; Johanson and Trinajstic, 2014).

Both the gnathostome clades with living representatives, crown chondrichthyans and osteichthyans can shed their teeth, but they do so in different ways, depending on the mode of attachment. Crown chondrichthyans probably have lost the programme to produce cellular bone, but since "bone of attachment" is a constituent of odontodes, their odontodes still possess a mineralized base (Underwood et al., 2015a; Welten et al., 2015), probably made of osteodentine (as a bone substitute) (Smith et al., 2015b), or kind of basal tissue secreted by odontoblasts (Miyake et al., 1999), attaching to the cartilaginous jaws by Sharpey's fibers. Replacement teeth are transported to the functional position by the moving of the mat of connective tissue, in a conveyor-belt fashion, and functional teeth are shed simply by the deterioration of the Sharpey's fibers (Reif, 1982; Williams, 2001). Teeth of a functional row are usually shed as a connected suite (Underwood et al., 2016). In primitive taxa, post-functional teeth are not shed, but slid beneath the skin, pushed to the facial lamina, and tightly packed with the preceding post-functional teeth (Williams, 2001). In other early forms (Maisey et al., 2013) and the stem group, acanthodians, teeth of a tooth family are fused into a tooth whorl; and the functional teeth are moved out of, and the replacement teeth in to, the jaw margin by rotating the tooth whorl. Tooth replacement by rotation characterizes the chondrichthyan total group, by which teeth can be replaced without shedding. Tooth whorls with a similar rotation mechanism also occur at the parasymphysial position of sarcopterygians, but the post-functional teeth and their underlying part of whorl are shed by basal resorption (Gross, 1957; Jarvik, 1972; Andrews et al., 2006).

Tooth shedding of osteichthyans is characterized by the basal resorption of the attachment bone that anchors the tooth root to the supporting bone. There is considerable diversity in the mode of attachment in various taxa and various bones for different feeding styles, with regard to the arrangement of

the components - the bone of attachment, the supporting bone, the periodontal ligament, the Sharpey's fibers and the tooth itself - such as fibrous attachment, pedicellate attachment, hinged attachment and so on (Shellis, 1982; Huysseune and Sire, 1998). The **bone of attachment**, a pedicel-like structure made of vascularized bone-like tissue, can be spongy or lamellar (Bemis and Bemis, 2015). Superficially it is a ring with a rough surface, which may reflect the insertion of ligaments (Sire and Huysseune, 1996) or depression left by osteoblasts (Jerve et al., 2016). It forms at the final stages of tooth development, after dentine and enamel deposition and before eruption. It is specified into the **alveolar bone** in amniotes, which builds up the tooth sockets for the cementum coated tooth root to be deeply implanted in. This is the **thecodont** attachment, the most histologically complex mode. The shedding of a tooth can result in resorption of alveolar bone, mineralized periodontal ligament, cementum and root dentine (LeBlanc and Reisz, 2013). But resorption may be incomplete, and remnants of basal dentine buried by new bone tissue has been reported (Bemis et al., 2005). New alveolar bone or bone of attachment of the replacement tooth will be redeposited on the resorption pit. A surface between the remaining tissue of the previous generation and the redeposited tissue can be recognized histologically, and the floor of the alveolar bone or bone of attachment is accumulated by successive generations of tissue (Sire and Huysseune, 1996; LeBlanc and Reisz, 2013), demarcated by such surfaces, which evidence repeated resorption and redeposition.

In mammals, the formation of alveolar bone leaves an interbony canal (gubernaculum dentis) for the gubernacular cord to extend into the bony crypt, bringing nerves, blood and lymphatic vessels, as well as the dental lamina to generate the permanent tooth bud (Marks and Schroeder, 1996; Philipsen and Reichart, 2004). This mode with replacement teeth developing within the bony sockets, cavities or trench is called **intraosseous (intramedullary) replacement**, versus the plesiomorphic **extraosseous replacement**, in which replacement teeth develop in the soft tissue outside the bone. The intraosseous positioning may better protect each individual developing tooth, allowing it to form a precise and delicate shape, and also makes room for more functional teeth. It has also evolved several times in teleosts, in marginal, palatal or pharyngeal dentitions (Trapani, 2001; Bemis et al., 2005; Witten and Huysseune, 2009; Tucker and Fraser, 2014; Bemis and Bemis, 2015). First-generation teeth never develop intraosseously (Sire and Davit-Béal, 2002), including in croakers and cichlids, which bear intraosseous replacement teeth (Govoni, 1987; Huysseune and Sire, 1997), and this advanced mode of replacement starts with the migration of the dental lamina. Ontogenetic studies of bluefish and wolfish show that bone resorption allows the replacement tooth bud from the successional dental lamina to invade into the bone of attachment through the replacement pore, further bone formation closes the pore to encase the tooth bud, and more bone remodeling is re-

quired for the tooth bud to arrive at the functional position (Bemis et al., 2005; Bemis and Bemis, 2015). Therefore, precise control of resorption by the dental follicle plays an important role in osteichthyan tooth replacement, especially if the growth of replacement teeth is combined with complicated displacement.

Probably because of the spatial limitation, in some extraosseous species, as well as in many intraosseous taxa, the replacement teeth are formed at 90 degrees to the functional teeth, lying against the bony ridge and pointing caudally (Trapani, 2001), and have to rotate to the functional orientation during development. Strikingly, a similar initial position of replacement teeth is observed in the convergent saw-teeth (or rostral denticles) of modern sawsharks (Welten et al., 2015) and a Cretaceous Batoid ray *Schizorhiza*, and a highly controlled multiple rotation of replacement teeth outside the oro-pharyngeal cavity is performed by the latter (Smith et al., 2015b). This suggests that the dental lamina and "denticle lamina" (Miyake et al., 1999) are homologous structures that invaginate in a caudo-rostral direction, no matter where they develop. *Schizorhiza* exhibits cone-in-cone stacks of saw-teeth (Smith et al., 2015b), which implies the saw-teeth are able to be shed and replaced rapidly and continuously like the oral teeth. Other chondrichthyan skin denticles can also be shed, and may or may not be replaced (Reif, 1982). The extra-oral teeth on the head or body of some teleosts can be shed by basal resorption, acting like the oral teeth too, albeit replaced less frequently (Sire and Huysseune, 1996; Sire et al., 1998; Sire, 2001; Sire and Allizard, 2001).

In the chondrichthyans with alternate replacement, the same generation of teeth from the odd-numbered families may form a functional row, with those from the even-numbered ones forming another, and a functional row can be shed as a whole and replaced immediately. Due to the complexity of the replacement process, osteichthyans may have a lower replacement rate than chondrichthyans. Thanks to the alternate replacement, osteichthyans can guarantee at least half of the teeth to carry out the job of feeding through the replacement cycles of the others, namely, when the odd-numbered teeth are in various stages of shedding-replacing, the even-numbered teeth have fully formed, and vice versa (Edmund, 1960). But in some taxa, teeth are replaced simultaneously and feeding is thus temporarily limited, usually corresponding to the breeding or cold season (Bemis and Bemis, 2015). Whatever the strategy, the tooth replacement of crown chondrichthyans and osteichthyans is highly regulated. While the modern chondrichthyan skin denticles will not be replaced until they are sporadically lost from the fiber attachment and space is available (Reif, 1982), and the integument is thus scattered with replacement denticles of different generations with varied size (Johanson et al., 2007). The osteichthyan dermal odontodes will not be shed but overgrown by successive generations. To trigger tooth shedding is the key step,

which is likely acquired independently in these two extant gnathostome groups.

In the marginal jaw bones of *Andreolepis*, a shedding tooth field is formed lingual to the first-generation odontodes. Multiple shedding cycles of each individual tooth are illustrated by stacks of resorption surfaces. However, the shedding tooth sites are not arranged in longitudinal rows, even less regularly than the first-generation odontodes. The number of the shedding cycles varies among tooth sites. In the tooth cushions of *Andreolepis* and *Lophosteus*, older tooth sides are generally replaced more times than the younger ones, but the overlapping of resorption surfaces between neighboring tooth sites indicates the asynchronous timing of replacement. That is to say, the first-generation odontodes and the first-generation shedding teeth (tooth sites) are of alternate addition, but the shedding teeth are not of alternate replacement. Tooth shedding is site-autonomous, but since empty sockets of shed teeth are rare, feeding would not be influenced by tooth loss. A semi-regular multi-row arrangement with jaw-teeth in various sizes, which may indicate the coexistence of different generations, can still be found in Triassic actinopterygians (Ørvig, 1978). In *Andreolepis* and *Lophosteus*, the dentitional division of these tooth-bearing bones is thickened by the accumulation of successive generations of bone of attachment between tooth sites, there is no bony crypt to house the tooth buds, and not even a lingual lamina to accommodate a permanent dental lamina. Replacement tooth buds can only be generated by a successional dental lamina or a superficial odontogenic band associated with individual teeth (Paper I-IV).

Temporally, tooth replacement is subjected to local control, while spatially, it is related to the growth of bone, just like the tooth addition. Stacks of resorption surfaces, which represent the position of shed teeth from each tooth site, display a polarized shift that reflect the direction and extent of bone growth. Especially in the marginal jaw bone of *Lophosteus*, which has a rather high bone growth rate and tooth replacement rate, a tooth site can show a large number of replacement cycles at the fastest growing area of bone; tooth replacement is truncated at the area, where the bone stops being thickened, by the overgrowing of dermal ornaments, which smooths the bone surface (Paper I-IV).

Tooth replacement is probably triggered by the slowing down of bone growth rate, for instance, after sexual maturation, whereas the tooth addition rate intrinsic to the species has been selected not to be reduced. When the bone growth rate cannot keep up with the tooth addition rate, new sharp teeth can only be added to the existing tooth sites. The overgrowing of dermal odontodes on scales and dermal bones might be stimulated for the same reason that there was not enough space to accommodate another first-generation odontode, so they could only be added on top. To change the overgrowing of odontodes into replacing the odontodes is the innovation of osteichthyans and the origin of replacement teeth (Paper III). The replace-

34

ment teeth can be kept from being randomly distributed like the overgrowing odontodes, by the directly depositing on the supporting bone surface like the first-generation teeth. The addition of the first-generation teeth in the tooth cushions of *Lophosteus* might exemplify an intermediate step. In this case, tooth addition of first-generation teeth is already faster than bone growth from the beginning, and the new teeth have to overlap on the previous one(s) to a considerable extent. Instead of letting the new tooth to be added like an overgrowing odontodes, which is prevented in the oral cavity, the previous tooth was shed, so the new tooth only overlaps on the remnant of the basal periphery of the previous tooth and is able to connect to the basal canal system directly. Meanwhile, replacement teeth would also be inserted into the ring of the dentine remnant to join the pulp cavities of the first-generation teeth (Paper II).

All kinds of resorption observed in the tooth-bearing bones of *Andreolepis* and *Lophosteus* is distinctive from the extensive resorption in the gnathal plates of placoderms. The first-generation odontodes on the marginal jaw bones of *Andreolepis*, as the successive generations of dermal odontodes, show **apical resorption**, which occurs in a random pattern that conforms to the development of the odontodes overgrowing on top, rather than that of the first-generation odontodes themselves. **Basal resorption** of teeth might have originated by a similar mechanism, but with the resorption induced by the migration and eruption of the replacement tooth bud from the epithelium at the base of the functional tooth. Studies of various animals by thin sections have claimed that the basal resorption begins at the side of the function tooth, where the replacement tooth bud develops. This is how we understand our milk teeth being pushed out by the permanent teeth. However, the phenomenon of double tooth rows resulting from permanent teeth coming in behind milk teeth, commonly known as "shark teeth", is not unusual, and is caused by the roots of milk teeth not being resorbed. This implies that the shedding of functional teeth is not the inevitable result of the physical pressure of the replacement teeth. In fact, the presence of a replacement tooth bud is not a prerequisite for tooth resorption either. Human milk teeth can be resorbed without a permanent successor (Harokopakis-Hajishengallis, 2007). In the osteichthyans that will become edentate in the adult stage, such as the loss of the jaw-teeth in lungfish (Smith and Krupina, 2001) and the loss of all the jaw and pharyngeal teeth in American paddlefish (Smith et al., 2015a), the juvenile teeth are invariably shed by the resorption of bone of attachment, albeit never replaced. Accordingly, tooth shedding is an active process of the functional teeth themselves and a programme activated well in advance by molecular signals. All the resorption surfaces visualized in *Andreolepis* and *Lophosteus* are generally symmetrical in shape, and more or less deviate from the replacement (currently functional) teeth situated above. This suggests that the resorption was initiated before the approaching of the replacement teeth and from center of the tooth, not

from the side; the resorption surfaces are the "footprints" of the shed teeth, representing their size and position. In the tooth cushions of *Lophosteus*, the last added first-generation teeth are also shed in the semi-basal style like the previous ones, but without more teeth added marginally to the files. **Semi-basal resorption**, exclusively discovered in *Lophosteus* so far, does not attack the osteocyte-containing dentinal tissue at the base of the tooth, or the surrounding bony tissue. It is likely to be launched internally from the pulp cavity, and merely involves **odontoclasts**, which differentiate from the circulating progenitor cells that reside within the pulp cavity (Sahara et al., 1996). This makes the resorption process target an individual tooth, activated by a signal from within this tooth. The same process is also adopted in the basal resorption in the replacement teeth, around which more radial vessels develop. **Osteoclasts,** which differentiate from hematopoietic precursor cells in the bone marrow (Fujikawa et al., 1996; Wang and Mccauley, 2011), can be brought in through the radial vessels, and the bone of attachment can be resorbed too. Therefore, basal resorption, is more likely to evolve from semi-basal resorption by introducing radial vessels and osteoclasts. In *Andreolepis*, the first-generation teeth, which are already surrounded by well-developed radial vessels, are thus able to undergo basal resorption as in crown-group taxa (Paper I-IV).

Conclusions, assumptions & future directions

In summary, the data from *Andreolepis* and *Lophosteus* cast new light on the early evolution of the osteichthyan dentition and potentially on the origin of teeth:

1. *Andreolepis* and *Lophosteus* carried true shedding teeth on both the marginal jaw bones and tooth cushions.

2. Tooth shedding and replacement was acquired before capping by enamel, organization of the dentition into linear tooth rows and the regulation of alternate replacement, no later than the emergence of the oldest stem osteichthyans.

3. The incipient tooth replacement is not under strict global control, but site-autonomous.

4. A permanent dental lamina is absent in stem osteichthyans and most actinopterygians, and has evolved independently in chondrichthyans, tetrapods and some teleosts.

5. Osteichthyans replace their teeth through tooth shedding, while acanthodians through tooth whorl rotation, and modern chondrichthyans though both. Osteichthyans shed their teeth via hard tissue basal resorption that targets at individual teeth, which probably originates from the semi-basal resorption that initiates internally from a tooth and affects only on this tooth. Semi-basal resorption is at present only known in *Lophosteus*.

6. The radial vessels, a primitively shared feature, which penetrate the bone of attachment in all kinds of odontodes of various vertebrates, might have made a significant contribution to the origin of osteichthyan-type basal resorption.

7. The resorption surfaces are the skeletal "footprint" that represent the size and position of each shed tooth, and a stack of resorption surfaces at a tooth site is a "trackway" that reflects the displacement of each generation of functional teeth.

8. The tooth-like first-generation odontodes, which are organized in an alternate pattern of sequential addition, gradual become flattened and elongated as they are located progressively further away from the oropharyngeal cavity, and are probably comparable with the ridged fusiform first-generation odontodes on the scales. This demonstrates odontodes have the plasticity to adjust the morphology according to the location and function. If the developmental programmes are also adjusted, this might be how teeth and dermal odontodes start to diverge.

9. Teeth never overgrow, but can be overgrown by the gap-filling dermal odontodes, which are more or less tooth-like in shape when they are close to the biting area.

10. Odontode/tooth addition without shedding, as seen for example in arthrodires, relies on the fast appositional growth of the supporting dermal bone. This mode of development may be a plesiomorphy of vertebrates. Tooth replacement occurs once the bone growth slows down, but the displacement of replacement teeth through each replacement cycles is also associated with the direction and extent of bone growth.

11. Distinctive from the dentinal/lamellar-spongy-lamellar three-layered structure of most dermal bones, osteichthyans tooth bearing bones are comprised of a superficial dentinal layer and supporting bone layer(s). The dentinal layer can occupy a large proportion of the bone, and grow externally rather fast by accumulating successive generations of bone of attachment.

12. Life history can be inferred from tooth-bearing dermal bones, even if resorption has taken place in part of the bone. The replacement cycles indicated by the resorption surfaces are an indirect age indicator, in addition to the surfaces of growth arrest.

13. Although *Andreolepis* and *Lophosteus* are often discussed together, the interspecific differences between them can also be recognized in their dental microstructures, such as the shape of pulp cavities and resorption surfaces, and the inferred process characters, such as tooth replacement rate and the growth of jaw margin, as well as other histological characters, like the size of osteocyte lacunae. Some of these characters are subjected to regional differences within a taxon, for example, the configuration of dentine and enamel, but the others are characteristic for the taxon. Therefore, these dental characters can be used to diagnose species and attribute the isolated specimens.

According to the clues give by the condition of *Andreolepis* and *Lophosteus*, a possible scenario of the evolution of osteichthyan tooth replacement is tentatively proposed here.

1. Dermal bones of some vertebrates are covered with a single layer of first-generation odontodes through alternate sequential addition along with bone growth. When the bone grows wider, more odontode families are added, and when the bone grows longer, more odontodes are added at the end of each family.

2. As the bones grow towards the margin of the oral cavity, odontodes become conical and pointing lingually and obtain a biting function.

3. When bone growth slows down and cannot catch up with the pace of tooth addition, the future newer larger tooth-like odontodes would have to partially overgrow on top of previous odontodes as the second genera-

tion, from the growth center to the margin of the bone again, as they would do in other parts of the body.

4. But multiple rows of odontodes within the oral cavity have odontoclasts differentiate from the pulp cavity and resorb their own crowns from the inside in advance. Then the successive odontodes can directly be deposited on the supporting bone and connect to the large basal canals.

5. When the odontodes are laid down, a complex vessel system that radiates from the pulp cavity to the epithelium is entrapped in the bone of attachment. After a functioning period, when the odontoclasts differentiate inside, osteoclasts are brought in through the radial vessels, and resorb the bone of attachment from the surface of the radial vessels.

6. Then functional odontodes are shed by resorption at their bases. New odontodes can be inserted into the same site to replace the shed ones. This happens cyclically, just as the first-generation odontodes are added at timed intervals. New odontodes are all superficially initiated from the odontogenic stem cells that aggregate in the dental epithelia at the side of the previous odontodes.

7. The band of odontogenic stem cells becomes a deep subepithelial invagination, the successional dental lamina, to produce a single tooth bud for functional tooth (by this step, the odontodes are identical to the teeth of conventional definition).

8. An additional successional dental lamina extends from the tooth bud to generate another tooth bud. In this way, several tooth buds are linked by a permanent dental lamina to a single functional tooth. This structures can better regulate a sequence of tooth replacement.

9. Combined with the bone remodeling, the dental lamina migrates into a bone crypt beneath the functional tooth and lets the tooth bud develop inside the bone. The deep invagination of the dental lamina and the intraosseous replacement can better protect the unmineralized tooth bud, allowing the animal not to change to soft preys during the replacement cycle, and has evolved multiple times because of these functional advantages.

In order to test this hypothesis and develop a more detailed picture, ontogenetic studies on fossil and extant taxa, should be carried out in the future.

3D examination of the jaw bones of maxillate placoderms and other most basal placoderms by high-resolution synchrotron scans and reconstruction of internal structures.

Structures like the first-generation odontodes, which may have been lost in the extant taxa are probably primitively present in stem gnathostomes in the form of tooth-like ornaments or ornament-like teeth, and their developmental process will be possible to infer from 3D histology. 2D synchrotron data have been acquired from bones of placoderms that bear teeth and tooth-like

structures (Rücklin et al., 2012). However, these data derive mainly from *Compagopiscis* and *Bothriolepis*. *Compagopiscis* is a derived taxon from a derived clade of placoderms, the eubrachythoracid arthrodires, which is unlikely to have a close evolutionary relationship with osteichthyans. *Bothriolepis* is from a basal clade of placoderms, but again a most derived taxon within that clade. Neither species is the ideal model for the original form of teeth. That is to say, out understanding of placoderms is very limited and probably suffering from sample bias.

4D observation of tooth resorption in extant osteichthyans by 3D imaging combined with time-lapse or video recording.
Most of dental developmental researches only focus on tooth initiation. The process of hard tissue basal resorption is only illustrated either by thin sections sampled at a random stage and a random position, or by SEM photographs on the dehydrated specimens with soft tissues removed. A tempospatial tracking should be performed by labeling the clast cells, odontogenic cells and gene expression fields, in order to find out the relationship between cell differentiation and migration, molecular signaling, tissue remodeling, vascularization and tooth initiation in the shedding programme. Comparisons can be made between lineages, including those without replacement teeth, like paddlefish, those with intraosseous replacement teeth, like cichlid, those with shedding extra-oral teeth, like armored catfish, and those associated with reparation mechanism, like lungfish.

Ontogenetic investigations on the very early stage of scale development in extant chondrichthyans.
Dermal odontodes of living osteichthyans have either been lost or greatly modified (e.g. ganoid scales). Chondrichthyan dermoskeletons have decoupled from dermal bones, but dermal odontodes are retained as monoodontode scales, which can directly reflect the intrinsic features of odontodes. Organized scale rows have been reported in the earliest embryonic stage in various chondrichthyans (Johanson et al., 2007). They are similar to the initiator scale rows of osteichthyan fish, which is probably led by the formation of a primordial odontode, as observed in *Andreolepis* and *Psarolepis* (Qu et al., 2016). The initiator scales of chondrichthyans sequentially added like a tooth family might be homologous with the first-generation odontodes of *Andreolepis* and *Lophosteus*, a putative plesiomorphy of gnathostomes. Whilst the later developed randomly organized scales might be comparable with the successive generations of dermal odontodes of osteichthyans. The ordered scales are replaced by irregular ones before hatching. Notably, the shedding of monoodontode scale is a derived character after the reduction of odontodes and the loss of the bony base. How the shedding processes work in the two types of scales and the teeth, and why the succeeding scales do not overgrow on the initiators as those in the osteichthyans, but likewise

40

have a disorderly arrangement, may illuminate the evolutionary relationship among the three odontodes and the three major gnathostome groups, placoderms, osteichthyans and chondrichthyans. A study on catshark embryos has tried to answer what are the differences between the patterning signals of the scales and teeth. It shows that a stem cell marker sox2 is specifically expressed in teeth, suggesting a collaboration of gene regulatory network with tooth buds (Martin et al., 2016). The absence of sox2 expression in scales, however, might have been biased by the lack of dental lamina in scales, and should be proved by gene knockout, or repeated in osteichthyan fish that are without dental lamina, like *Polyodon* (Smith et al., 2015a) and *Polypterus* (Vandenplas et al., 2014). Additionally, it doesn't distinguish the patterning signals between the two types of scales, and between the initiator scales and initiator teeth. Conditions in pharyngeal teeth and extra-oral teeth (e.g. sawteeth) have not been explored. To explain how teeth may have possibly evolved from dermal odontodes, we may experiment whether the initiator scales can turn to be periodically shed and replaced by manipulating the developmental environment, even without a deep invagination.

Svensk sammanfattning

Tändernas uppkomst har länge varit en fråga av stort intresse för evolutionsbiologin, men vi har ännu inte några entydiga svar på hur, när och var det skedde. Den under lång tid dominerande teorin, som givits namnet "utifrån och in", hävdar att tandvävnader först utvecklades i huden och att tänder evolverat från modifierade dentinfjäll (också kallade hudtänder) längs munhålans kanter i samband med käkarnas uppkomst. En alternativ teori, "inifrån och ut", argumenterar att tänder utvecklades från dentikler i munhålan redan innan käkarnas upkomst, utan någon närmare relation till hudtänderna på djurets utsida, och att de i själva verket kan ha evolverat innan hudtänderna ens uppkom.

Under flera årtionden dominerades forskningen på tändernas uppkomst och utvecklingsbiologi helt av föreställningen att de nulevande hajarnas kombination av tänder och hudtänder är representativ för primitiva gnathostomer (käkförsedda ryggradsdjur) och därför informativ om tändernas evolution. De senaste årens forskning har dock kraftigt ifrågasatt detta och visar i stället att nulevande osteichthyider (benfiskar och landryggradsdjur) har bevarat primitiva karaktärsdrag som gått förlorade hos hajarna. Detta leder dock till problem för forskningen på tändernas utvecklingsbiologiska evolution, eftersom alla de etablerade osteichthyid-labbmodellerna (t.ex. mus, tamhöns, zebrafisk) saknar hudtänder och flera antingen saknar muntänder eller har mycket modifierade sådana. Två av de viktigaste evolutionära frågorna, nämligen hur osteichthyidernas muntänder relaterar till hudtänderna och hur deras unika sätt att tappa utslitna tänder genom att resorbera själva tandbasen har uppkommit, kan inte besvaras på ett tillfredsställande sätt genom att studera dessa djur.

Ett annat sätt att närma sig problemställningen har blivit möjligt genom utvecklingen av synkrotronmikrotomografi, en typ av skiktröntgen som tillåter mycket höga upplösningar (ned till ungefär en tusendels millimeter). Fossila ben och tänder av tidiga ryggradsdjur bevarar normalt sin inre mikrostruktur: hålrummen efter bencellerna finns kvar, liksom t.ex. dentinrör, inbäddade fibrer och begravda resorptionsytor (där benet resorberats och sedan täckts med ny benvävnad). Genom att visualisera dessa strukturer i tre dimensioner med synkrotronmikrotomografi är det möjligt att inte bara beskriva benens inre arkitektur utan också att rekonstruera deras tillväxtprocess. Vi får alltså tillgång till direkta ontogenetiska data from tidiga ryggradsdjur.

Denna avhandling fokuserar på synkrotronmikrotomografiska studier av käkben från *Andreolepis* och *Lophosteus*, två av de äldsta kända fossila benfiskarna. *Andreolepis* kommer från Gotland och *Lophosteus* från Estland; båda härrör från silurperioden och är ungefär 425 miljoner år gamla. Fossilmaterialet är välbevarat men består uteslutande av isolerade ben som frigjorts från kalkstenen med hjälp av ättikssyra. Käkbenen kan indelas i två kategorier, dels kantben som satt i käkarnas kanter och bär både tänder och hudtänder, dels kuddformade tandbärande ben som satt längre in i munhålan. Hos både *Andreolepis* och *Lophosteus* visar båda kategorierna tydliga spår av osteichthyidernas unika typ av tandersättning, i form av travar av skålformade begravda resorptionsytor under tänderna. Varje resorptionsyta representerar ett tillfälle då tanden tappades genom basal resorption och sedan ersattes av en ny. Resorptionsprocessens exakta form skiljer sig dock något mellan *Andreolepis* och *Lophosteus*. Hos *Andreolepis*, liksom hos nulevande osteichthyider, är den helt basal och tar bort hela tanden. I de kuddformade benen hos *Lophosteus* däremot utgår den första resorptionen från tandens pulpa och lämnar kvar en ring av dentin, medan senare tänder resorberas basalt. Både hos *Andreolepis* och *Lophosteus* föregås utvecklingen av de riktiga tänderna på kantbenen av ett mer externt "tandgarnityr" som förefaller bestå av modifierade hudtänder. Dessa ersätts aldrig utan begravs gradvis av överväxande konventionella hudtänder. Liknande "pseudo-tänder" har aldrig observerats hos andra benfiskar.

Det som gör dessa resultat särskilt intressanta är att *Andreolepis* och *Lophosteus* sannolikt båda hör till osteichthyidernas stamgrupp, alltså de gemensamma förfäderna till strålfeniga fiskar, kvastfeniga fiskar och landryggradsdjur. *Lophosteus* förefaller vara mer primitiv än *Andreolepis* eftersom den helt saknar emalj, en vävnad som är unik för osteichthyider. *Andreolepis* har emalj på fjällen men inte på skallbenen eller tänderna. Det är alltså sannolikt att deras unika karaktärsdrag visar på ursprungstillståndet för osteichthyidernas tanduppsättning. Till dessa drag hör det första "tandgarnityret" av modifierade hudtänder, som kan utgöra stöd för "utifrån och in"-teorin, den semi-basala tandresorptionen på de kuddformade benen hos *Lophosteus*, och det faktum att de riktiga tänderna på kantbenen inte är organiserade i en längsgående tandrad som hos senare osteichthyider (inklusive vi själva) utan bildar ett brett fält. Varje tand i fältet har ersatts upprepade gånger, men tydligen utan någon koordinering med angränsande tänder. Jämförelser mellan dessa resultat och tanduppsättningarna hos andra tidiga gnathostomer, och med tandutvecklingsdata från moderna osteichthyider, kommer förhoppningsvis att tillåta långtgående nya slutsatser om tändernas tidiga evolution.

Acknowledgements

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