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Periderm fate and independence of tooth formation are conserved across osteichthyans

A. Huysseune[1,2*], A. Horackova[1], T. Suchanek[1], D. Larionova[2] and R. Cerny[1]

Abstract

Background Previous studies have reported that periderm (the outer ectodermal layer) in zebrafish partially expands into the mouth and pharyngeal pouches, but does not reach the medial endoderm, where the pharyngeal teeth develop. Instead, periderm-like cells, arising independently from the outer periderm, cover prospective tooth-forming epithelia and are crucial for tooth germ initiation. Here we test the hypothesis that restricted expansion of periderm is a teleost-specific character possibly related to the derived way of early embryonic development. To this end, we performed lineage tracing of the periderm in a non-teleost actinopterygian species possessing pharyngeal teeth, the sterlet sturgeon (*Acipenser ruthenus*), and a sarcopterygian species lacking pharyngeal teeth, the axolotl (*Ambystoma mexicanum*).

Results In sturgeon, a stratified ectoderm is firmly established at the end of gastrulation, with minimally a basal ectodermal layer and a surface layer that can be homologized to a periderm. Periderm expands to a limited extent into the mouth and remains restricted to the distal parts of the pouches. It does not reach the medial pharyngeal endoderm, where pharyngeal teeth are located. Thus, periderm in sturgeon covers prospective odontogenic epithelium in the jaw region (oral teeth) but not in the pharyngeal region. In axolotl, like in sturgeon, periderm expansion in the oropharynx is restricted to the distal parts of the opening pouches. Oral teeth in axolotl develop long before mouth opening and possible expansion of the periderm into the mouth cavity.

Conclusions Restricted periderm expansion into the oropharynx appears to be an ancestral feature for osteichthyans, as it is found in sturgeon, zebrafish and axolotl. Periderm behavior does not correlate with presence or absence of oral or pharyngeal teeth, whose induction may depend on 'ectodermalized' endoderm. It is proposed that periderm assists in lumenization of the pouches to create an open gill slit. Comparison of basal and advanced actinopterygians with sarcopterygians (axolotl) shows that different trajectories of embryonic development converge on similar dynamics of the periderm: a restricted expansion into the mouth and prospective gill slits.

Keywords Periderm, Ectoderm, Oropharynx, Pharyngeal pouches, Gill slits, Mouth, Sturgeon, Axolotl, Teeth

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Background

Vertebrates, like all bilaterian animals, are built on a three-layered plan, the three germ layers of embryonic tissue: ectoderm, endoderm and mesoderm [1, 2]. The concept of germ layers has been an essential, albeit also debated, foundation of our understanding of animal organization in the last 150 years and continues to do so [2–5].

The primary germ layers, as a rule, form either the outer (ectoderm) or inner (endoderm) epithelial lining

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of the vertebrate body, and serve multiple developmental functions. For example, the epithelial lining of the oropharynx gives rise—in conjunction with the underlying mesenchyme—to a complex assemblage of organs vital to feeding (e.g., teeth [6–8]), breathing (e.g., gills, lungs and gas bladders [9, 10]), sensing (e.g., taste buds [11]), homeostasis (e.g., pituitary [12]) or immune responses (e.g., thymus [13]). The germ layer origin of the epithelia involved (whether ectoderm or endoderm) has played a central role in elucidating the development and evolution of these organs, as well as in understanding their disorders. Yet germ layer boundaries have been notoriously difficult to identify, requiring most often transgenic reporter lines [5, 14–17]. This is especially the case for the oropharynx, where ectoderm and endoderm directly appose each other at multiple sites. These include the stomodeum (prospective mouth) and the contact zones between endodermal pharyngeal pouches and skin (prospective gill slits) [18–21].

Data collected on teleosts have indicated that ectoderm and endoderm do not simply abut each other at the different prospective head orifices (mouth and gill slits). Rather, their interface presents a much more complex architecture [22]. In the zebrafish, one of the teleost models most thoroughly studied for early development, the embryo is covered at the end of gastrulation by a two-layered epithelium (prospective epidermis). Only the deep (or basal) layer (resting on the basal lamina) has been regarded as ectoderm, the surface layer (called periderm) has been considered a transient extra-embryonic layer, derived from the enveloping layer, a layer that becomes lineage restricted at the onset of gastrulation [23]. Recently however, the extra-embryonic nature has been questioned and the periderm has been argued to be ectodermal (reviewed in [22]). Moreover, the periderm in zebrafish has been shown to expand, albeit to a limited extent, into the oropharynx via mouth and pouches [17, 24]. This observation has been especially relevant—and discussed—in the context of teeth. The evolutionary origin of teeth from (ectodermal) dermal denticles has indeed elicited the question how external epithelia may have transferred odontogenic competence to internal epithelia to give rise to oral (and pharyngeal) teeth, in conjunction with neural crest mesenchyme [22, 25–30].

Teleosts, including zebrafish, are highly advanced in their own lineage, the actinopterygians. They possess a derived type of early embryonic development including partial (meroblastic) cleavage, absence of a blastocoel, gastrulation without invagination nor blastopore, absence of an archenteron, early lineage restriction of ectoderm, late separation of endoderm from mesoderm, and an alimentary tract developing independently from the massive, extra-embryonic yolk cell [31–33]. One may thus question whether the development of the teleost ectoderm (in which we include the periderm) is representative for ray-finned (actinopterygian) fishes. For example, one may hypothesize that a limited expansion of periderm into the oropharynx, as observed in zebrafish, could be a regressive character linked to the derived type of early embryogenesis in teleosts. Actinopterygians whose lineages originated shortly after the key-divergence of Actinopterygii and Sarcopterygii (for the sake of simplicity further referred to as 'basal actinopterygians'), such as the Senegal bichir (*Polypterus senegalus*) or sturgeon species (e.g., the sterlet, *Acipenser ruthenus*), display total (holoblastic) cleavage, formation of a blastocoel, gastrulation by involution, formation of an archenteron, and a gut wall formed from the yolk-rich endodermal cells [34–37]. Thus, these taxa are very well placed to investigate the fate of periderm in the mouth and gill slit region and to infer the relationship between the periderm and oral and pharyngeal tooth formation. On the other hand, sarcopterygians that undergo holoblastic cleavage yet have lost pharyngeal teeth, e.g., salamanders such as the axolotl (*Ambystoma mexicanum*), present an appropriate case to place these findings in a wider evolutionary context.

The aims of the present paper are threefold. Focusing first on sterlet sturgeon, we assess stratification of the ectoderm prior to mouth and gill slit opening in these regions. We next use lineage tracing to track the fate of the periderm during opening of the mouth and gill slits. Finally, we examine whether periderm covers the oropharyngeal epithelium in prospective tooth-forming areas in sterlet sturgeon, an actinopterygian species that has both oral and pharyngeal teeth, and compare these data to the axolotl, a sarcopterygian species that also forms gill slits but lacks pharyngeal teeth. Together, these data must provide thorough insights into the fate of the periderm in key vertebrate species, and assess its relationship to the development of oral and pharyngeal teeth.

Results

Stratification of the ectoderm in the oropharyngeal region of the sterlet sturgeon

Shortly after closure of the neural tube (occurring at stage 23), the ectoderm in the sterlet sturgeon is already stratified (Fig. 1A). At this stage, the epithelium is not yet clearly separated from the underlying mesenchyme by a basement membrane. At stage 28 (Fig. 1B), the ectoderm in the head region is bilayered and clearly set off against the underlying mesenchyme. Cells in both layers contain numerous yolk platelets, while pigment granules predominate in the surface layer. The apical surface of the latter cells is free of yolk and contains numerous vesicles. This stratification is maintained in later stages (Fig. 1C–I).

In the sterlet sturgeon, like in the Russian sturgeon *Acipenser gueldenstaedtii*, the first embryos of a clutch hatch at stage 35; mass hatching occurs at stage 36, just prior to mouth opening and first gill perforation at stage 37 [35]. The mouth opens 2 days post-hatching in the sterlet sturgeon [38]. At stage 34–35, i.e., immediately before hatching, the stratified ectoderm of the head region possesses a surface layer, composed of cuboidal cells with distinct characters, further referred to as periderm (Fig. 1D–F). On the lateral head surface, in the vicinity of future pouch–ectoderm contact areas, the ectoderm is essentially bilayered, with occasionally intermediate cells present (Fig. 1D, E). The number of tiers increases in areas of contact between endodermal pharyngeal pouches and ectoderm (Fig. 1D, F). The first pouch to develop an extensive contact area with the ectoderm is pouch 2, separating prospective hyoid arch from branchial arch 1 (Fig. 1G and inset). A higher magnification of this area indicates that a basal lamina is present just outside the contact area (Fig. 1G, H) but absent in the contact zone, blurring the boundary between endoderm and ectoderm (Fig. 1G, I). Still, like elsewhere on the head, periderm cells cover these areas. Whatever their location, periderm cells adopt a more or less cuboidal shape and are characterized by numerous mitochondria and prominent vesicles at their apical surface (Fig. 1H, I).

In the absence of transgenic reporter lines for periderm, such as used for zebrafish studies [17, 24], it is nevertheless possible to specifically label the periderm using a fluorescent stain. Soaking decapsulated sterlet sturgeon embryos for 4 h with CDCFDA prior to hatching homogeneously labels the periderm in a long-lasting way, while leaving the basal ectodermal layer unlabeled (Fig. 1J, K). The periderm cells display the typical cuboidal shape already observed in histology (compare Fig. 1E, F with Fig. 1K), with one clear exception: the area of the pre-oral gut [39]. Here the periderm cells adopt a typical 'umbrella' shape (Fig. 1L). In our hands, periderm cells in sterlet sturgeons can maintain a strong fluorescent signal for at least 18 days (Fig. 1M), even in areas where the periderm underwent flattening and/or fragmentation of staining (Fig. 1N).

Fate of the periderm in sterlet sturgeon

To investigate the behavior of the periderm in the oropharynx, we first examined the ectoderm–endoderm contact areas in the region of the prospective mouth and gill slits at the stage just prior to, during, and after their opening to the exterior.

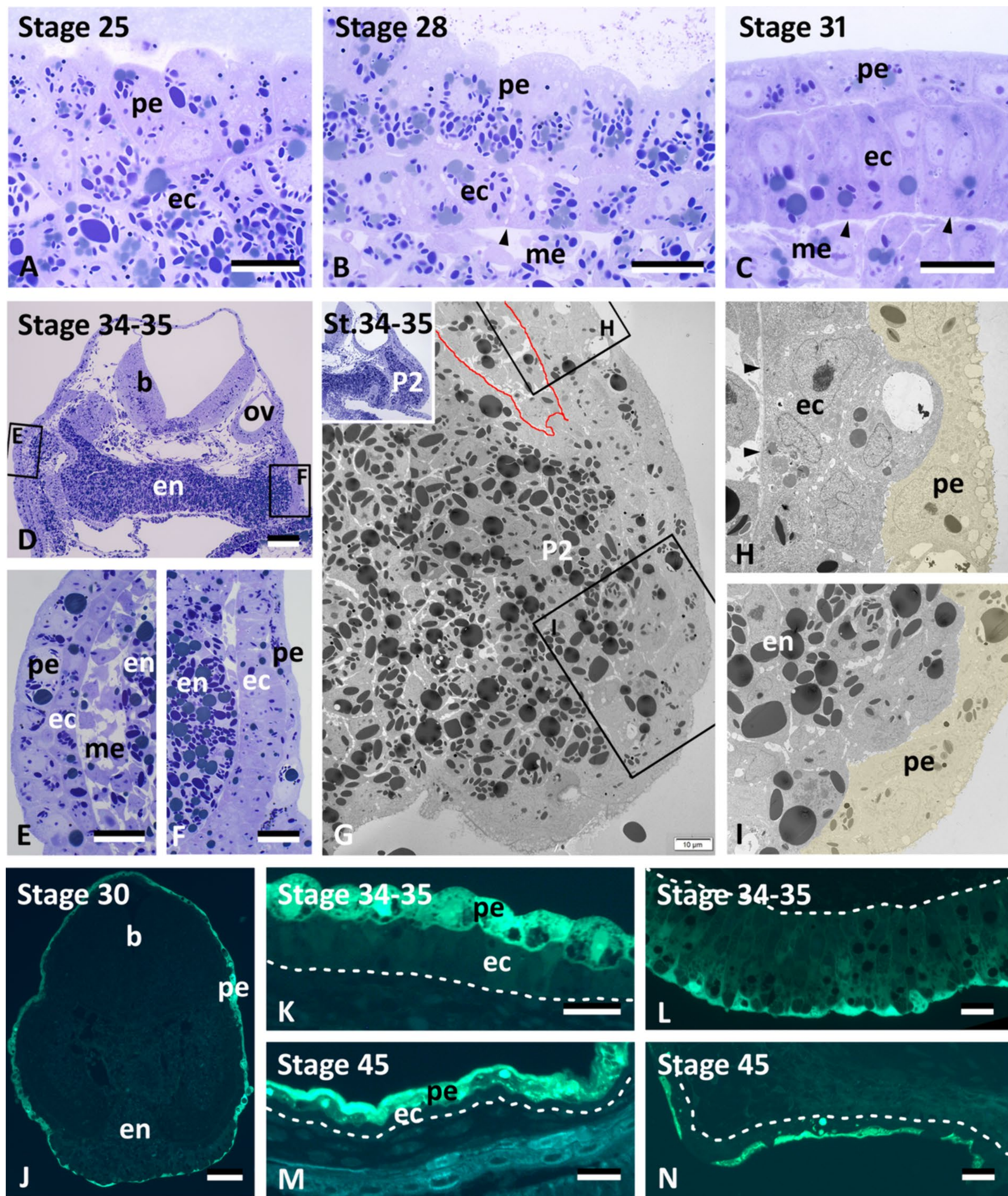
Prior to mouth opening, at an early stage 37, the head surface at the location of the prospective mouth presents as a deep recess but does not communicate internally with the prospective oral cavity (Fig. 2A–B″). At this stage the latter is not more than a thin slit in an apparently homogeneous cluster of endodermal cells. One stage later, the slit has widened into a large cavity, but is still not in open communication with the exterior (Fig. 2C–D″). Indeed, a thin strand of epithelial tissue still closes off the prospective mouth opening (Fig. 2E). Only when this epithelial bridge breaks through (Fig. 2F, G), the oral cavity opens widely to the exterior.

The pouches are constituted of endodermal cells, and extend from the midsagittal plane towards the ectoderm (Fig. 2H–K). Each pouch runs in an oblique anteroposterior direction and terminates posteriorly in a very broad contact zone with the ectoderm (Figs. 1G, 2H, J, K). The pouches are also interconnected in their distalmost portion, so that each prospective gill arch is completely encircled by endodermal epithelium (Fig. 2I).

A study of successive developmental stages revealed that opening of the pharynx to the exterior via opening of the gill slits appears to occur in two phases that are also spatially distinct. First, a deep cleft is formed on the head surface, carving into the broad, posterior region of the endodermal pouch that will open first, i.e., pouch 2 (P2) (Fig. 3A–C, E–G). In this way, the distal, posterior

(See figure on next page.)

Fig. 1 Stratification of the ectoderm in the sterlet sturgeon. **A**–**C** Successive developmental stages show clear stratification of the ectoderm (**A** around yolk sac; **B**, **C** on the head surface) (arrowheads: basal lamina). **D** Cross section (slightly oblique) through the head region of a stage 34–35 sturgeon showing stratified ectoderm with the surface layer, i.e., the periderm, clearly distinct from the basal layer; boxes indicate details shown in **E**, **F**. Endodermal cells are easily recognized by their high yolk content (dark blue); lipid droplets are greenish. **G** Low power TEM image of the contact area of pouch 2 with the skin (cf. Inset); boxes indicate details shown in **H** and **I**. A red line indicates the basal lamina. **H** Detail of ectoderm outside pouch–skin contact; note presence of a basal lamina (arrowheads). **I** Detail of ectoderm where it contacts the pouch and where the basal lamina is absent; periderm highlighted in yellow in **H**, **I**. Note abundance of apical vesicles and of mitochondria in the periderm cells. **J**–**N** CDCFDA labeling in sterlet sturgeon. **J** Uniform labeling of the periderm prior to hatching (soaking at stage 25, fixation at stage 30). The periderm has not entered the body. **K**, **L** Detail of the skin immediately after labeling at stage 34–35; the periderm cells are homogeneously labeled. They are cuboidal-shaped (**K**) except in the area of the pre-oral gut (**L**), where periderm cells adopt a typical 'umbrella' shape. **M** After 13 days of chasing (at stage 45), the periderm has maintained its label uninterruptedly on the dorsal head surface. **N** By contrast, on the ventral head surface, the periderm consists of flattened cells and is partly fragmented. Dotted lines indicate position of the basal lamina. *b* brain, *ec* basal ectodermal layer, *en* endoderm, *me* mesenchyme, *ov* otic vesicle, *P2* pouch 2, *pe* periderm. Scale bars in **A**–**C**, **E**, **F**, **K**–**N** = 20 µm; in **D**, **J** = 100 µm



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part of the hyoid arch becomes separated from the head surface, forming the anlage of the opercular fold. At this moment, the pharyngeal cavity is still represented by a thin slit-like opening in the medial endoderm, without any connection to the exterior environment (Fig. 3G). In a second phase, the slit-like opening expands into the pouch, widens and eventually connects to the cleft that had opened from outside inwards (Fig. 3D, H, Q–S). In this way, an open communication is established between the pharynx and the external environment. These events

are repeated for each prospective gill and proceed in an antero-posterior sequence. Thus, when the cleft within P2 has separated the opercular fold from the head surface, a cleft has started in pouch 3 (P3) to separate the next gill arch, and so on. Likewise, an open communication between the pharynx and the external environment is first established for P2, shortly after a cleft has separated the next gill arch, and so on.

To investigate the behavior of the periderm during these events, we started soaking embryos with CDCFDA at successive developmental stages from stage 25 onwards (i.e., one stage before the first two pouches contact the ectoderm [35]) and examined embryos at regular intervals until stage 45, by the latest. We never observed any labeled cells inside the head prior to stage 36, whatever the time point at which the embryos were labeled. Thus, neither the prospective mouth region, nor the (still solid) endodermal pouches showed any sign of labeled cells inside (Fig. 1J). Labeled cells were observed within the forming gill slits from stage 37 onwards. Thus, a second series of experiments was conducted in which embryos were soaked at stage 35, and a number of embryos fixed at an 8-h interval for a length of time of 5 days.

In the branchial region, the forming clefts were covered with labeled periderm (Fig. 3I–K, M–O). Labeled periderm cells covered the external and internal surface of the distal part of the gill as it separated from the body surface, as well as the head surface from which it had just separated (Fig. 3K, O). After the pouch had opened to the exterior, periderm cells remained restricted to the distal part of the gill slit (Fig. 3L, P). At no stage investigated could labeled periderm cells be observed in more medial parts of the pharynx (i.e., the medial endoderm, Fig. 2J). To assess whether periderm cells actively move inwards or whether bulging out of the prospective gills pushes the periderm inwards, we measured the distance from the medial-most point of periderm expansion to the mediosagittal plane of the body (Fig. S1A). Active invasion would reduce this distance. However, the distance between left and right medial-most labeled cell at the same cross-sectional level did not change appreciably between stages prior to cleft formation and after gill slits had opened (Fig. S1B).

Initially, labeled cells formed a continuous monolayer around the distal part of the outgrowing gill and the head surface below the outgrowth. Yet, these cells were clearly more flattened than the periderm cells forming the outer ectodermal layer elsewhere in the skin (Figs. 3K, O, 4A). At later stages, and deeper into the forming gill slits, staining of this layer became fragmented. More generally, the staining in the branchial region presented a more fragmented aspect than, e.g., on the dorsal head surface (Fig. 4B, C, compare with Fig. 1M). On the developing gill filaments, several cells displayed a typical 'umbrella' shape, with long cell extensions reaching between the endodermal cells towards the basal lamina (Fig. 4B). Such cells were also observed at the level of the pre-oral gut (compare with Fig. 1L), another area of intimate periderm–endoderm contact. Periderm cells deeper inside often adopted an irregular shape with long cell extensions (Fig. 4C, D). Dispersed labeled periderm cells were also observed around the mouth (Fig. 4E–G). The cells formed an interrupted layer of flattened or 'umbrella-shaped' cells at the mouth entrance or inside the upper and lower lip (Fig. 4F, G). As in the forming gill slits, occasionally cells were observed of very irregular shape (Fig. 4H).

Staining for proliferation by PCNA just prior to the start of cleft formation revealed abundant labeled cells in the basal ectodermal layer, but sparse labeling in the periderm. This sparse labeling was maintained in stages corresponding to ongoing and completed cleft formation (Fig. S2).

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Fig. 2 Opening of the mouth in sterlet sturgeon. Midsagittal (**A**, **C**) and serial cross sections (**B**–**B″**, **D**–**D″**) of sturgeon embryos at stage 37 (prior to mouth opening, **A**, **B**–**B″**) and stage 38 (last stage of mouth opening, **C**, **D**–**D″**). At stage 37, a deep recess marks the position of the future mouth opening (**A**, **B**, arrowheads); there is no connection to the thin slit that has appeared within the endodermal foregut (**B**–**B″**). Endoderm is recognizable by a darker blue staining and presence of numerous yolk platelets. At stage 38, the mouth cavity (asterisks) is still separated from the exterior by a thin epithelial bridge (**C**, **D**, arrowhead). Deeper inside, the oropharyngeal cavity has substantially widened (**D′**, **D″,** asterisks). **E**–**G** Sagittal (**E**) and transverse (**F**, **G**) sections of the epithelial bridge that forms the last barrier between external environment (arrowheads) and oral cavity (asterisks). As can be seen on adjacent sections (**F**, **G**) this epithelial bridge is at the verge of breaking through. **H** Transverse, slightly oblique, section of a stage 35 sturgeon embryo, at the level of pouch 2 and anterior portion of pouch 3. The cavity marked by 'X' is a preparation artifact. **I** Sagittal section of a stage 38 sturgeon embryo showing pouches 2 to 4. The oropharyngeal cavity (asterisk) is now already wide. Note interconnection of pouches in their distalmost portion (arrows). **J**, **K** Three-dimensional reconstruction of the pouches of a stage 37 sturgeon in a slightly oblique dorsal (**J**) and a ventral view (**K**). Spiracular pouch (P1) and pouches 2–5 (P2–P5) are clearly distinguishable. Dotted lines demarcate medial pharyngeal endoderm from more lateral pouch endoderm, following the distinction made by Kopinke et al. [82]. Distal interconnections between the pouches are not included. *An* anterior, *b* brain, *eb* epithelial bridge, *en* endoderm, *n* notochord, *ov* otic vesicle, *P1–P5* pouches 1 to 5, *Po* posterior, *y* yolk. In all sagittal sections, anterior is to the left. Scale bars in **A**–**D″**, **H**, **I** = 100 µm; in **E**–**G** = 50 µm

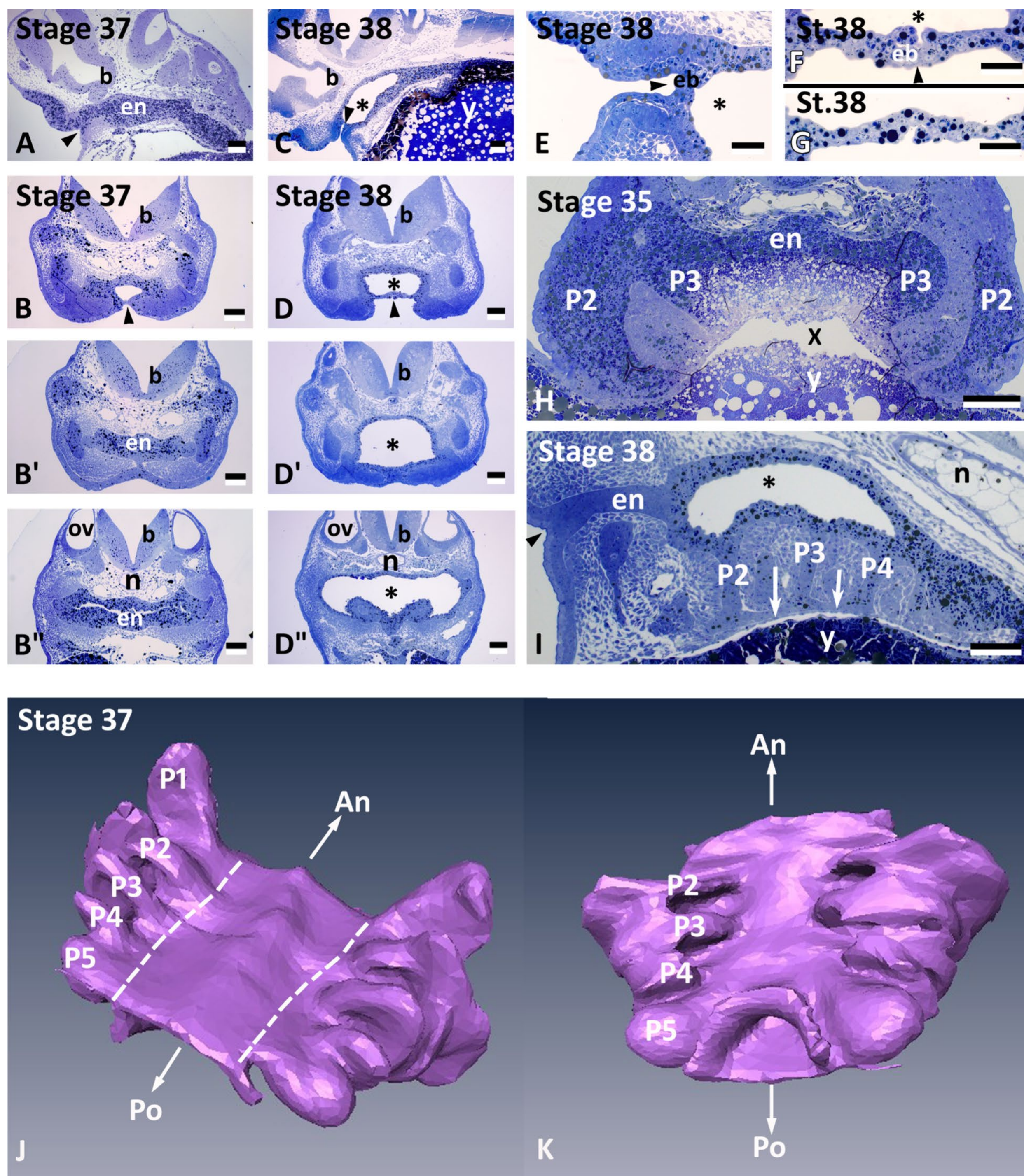

Fig. 2 (See legend on previous page.)

Relationship of the periderm to odontogenic epithelia in sterlet sturgeon and axolotl

Sterlet sturgeons develop six different tooth fields [40]. They encompass oral teeth (on the dermopalatine and dentary bones), palatal teeth (on the palatopterygoid) as well as pharyngeal teeth (infrapharyngobranchials and hypobranchials 1 and 2). Based on marker gene expression, odontogenic bands are first detected for the prospective dermopalatine and dentary tooth fields at stages 35 to 37, with the first individual tooth loci becoming

visible at stage 38. Next appear the palatopterygoid and hypobranchial 1 tooth fields at stage 39, along with the first visible tooth germs on the latter. The odontogenic bands of the infrapharyngobranchial field (and its individual tooth loci) become apparent at stage 40 [40]. Hypobranchial 2 teeth are the very last to appear.

We observed periderm cells in sturgeons scattered over the epithelium covering both the dermopalatine (Fig. 5A–A‴) and dentary tooth fields (Fig. 5B–B‴). However, labeled periderm cells were not observed in the other (i.e., palatal and pharyngeal) tooth fields, not even after chase times of 13 days, when all the dental fields are well underway in their development (Fig. 5C–D‴).

Using lineage tracing with CDCFDA, we also examined the fate of the outer ectodermal layer (periderm equivalent) in axolotl. The oral area at stage 36 consists of a double-layered ectoderm, while the inner region of the prospective mouth is filled with a compact mass of endoderm [14, 19]. The mouth opens at stage 43, when the oral membrane is perforated [14, 19]. The first pouches contact the ectoderm already at stage 24 [41], but gill slits are wide open only at stage 43.

Teeth in larval axolotls are associated with the premaxillary, vomerine and palatine bones in the upper jaw/pharynx roof, and with the dentary and coronoid in the lower jaw [14, 15, 42, 43]. Later in development also the maxillary acquires teeth. The tooth fields are organized into outer (premaxillary and dentary) and inner dental arcades (vomerine, palatine, and coronoid). The first teeth to develop are those from the inner arcade at stage 37. Dentary teeth start to develop at stage 40–41 only, and premaxillary teeth at stage 41 [15]. Thus, oral teeth develop long before the mouth opens. Unlike sturgeons, axolotls do not develop pharyngeal teeth.

We soaked axolotl embryos at stage 29 and examined the embryos at stage 38 and 39 (Fig. 5E–F‴). Under the same conditions of CDCFDA staining as in sturgeon, periderm cells in axolotl showed a more fragmented and granular labeling with CDCFDA, but were nevertheless clearly recognizable (Fig. 5E′, E‴, F′, F‴). Oral tooth germs could be observed prior to opening of the mouth and with periderm restricted to the outer body surface (Fig. 5E–E″). As in sturgeon, the labeled outer ectodermal layer covered inside and outside surfaces of the external gills (Fig. 5F–F‴). Labeled cells were not observed inside the oropharynx.

Discussion

Here we have tested the hypothesis that restricted expansion of periderm into the oropharynx, as observed in zebrafish [17, 24], is specific for teleosts and related to the derived way of teleost early embryonic development. Using lineage tracing, we demonstrate that the sterlet sturgeon, a non-teleost actinopterygian, and the axolotl, a basal sarcopterygian, show a restricted expansion of the periderm into the oropharynx, similar to what is observed in zebrafish. Comparison between sturgeon and axolotl furthermore reveals that the periderm in the branchial region behaves similarly, independent of the presence or absence of pharyngeal teeth. Axolotl data also show that periderm is not required for oral tooth formation, a question that could not be studied in zebrafish given its lack of oral teeth. To arrive at these conclusions, it was necessary, prior to lineage tracing, to assess stratification of the ectoderm in the two species. This will be discussed first.

The outer ectodermal layer in sturgeon and axolotl embryos can be considered a periderm

The data presented here show that, prior to opening of the mouth and of the gill slits, the ectoderm in sterlet sturgeon is at least bilayered. The most precise information regarding the early stratification of ectoderm in basal actinopterygians is available for bichirs. In *Polypterus senegalus*, gastrulation movements (ending at stage 21) involve overgrowth of the yolk (epiboly) by animal cells (i.e., prospective ectoderm) [36]. Already at stage 19, the epidermis is stratified into an upper and a basal (called subepidermis) layer, although the features of these layers differ depending on the region of the body [36].

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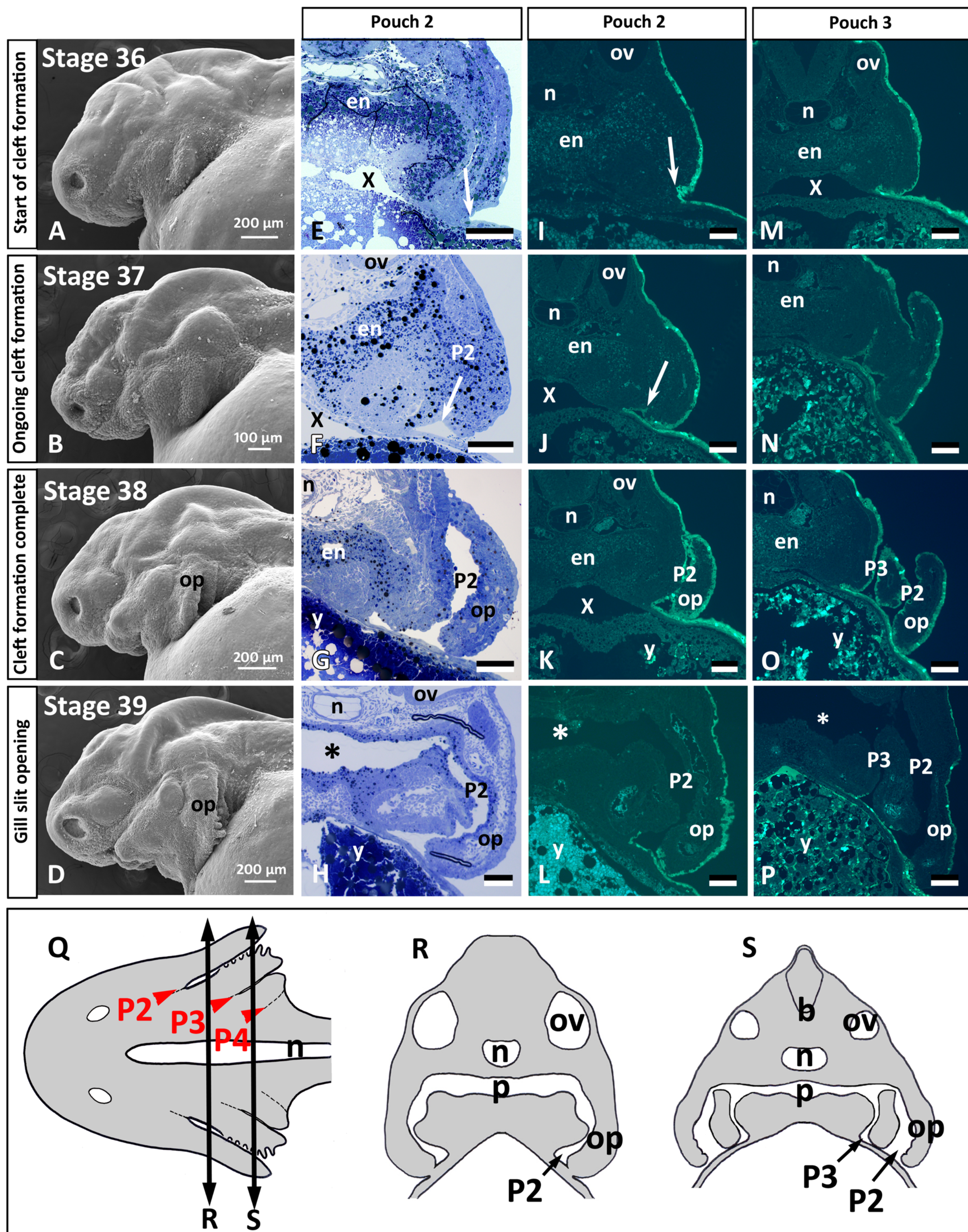
Fig. 3 Gill slit opening in sterlet sturgeon between stage 36 and 39. **A**–**D** SEM pictures showing lateral view of the head surface of sturgeon embryos as shown on corresponding sections in **E**–**P**. **E**–**P** Cross sections of successive stages of gill slit opening as illustrated in histological sections for pouch 2 (**E**–**H**, opercular gill slit, between hyoid and first branchial arch) and lineage tracing with CDCFDA in the corresponding stages (pouch 2, **I**–**L**, and pouch 3, **M**–**P**). First, a deep cleft is formed on the head surface (**E**, **F**, arrow), concomitant with expansion of the periderm (**I**, **J**, **M**, **N**). The distal tip of the arch becomes completely separated from the head surface (**G**), and periderm covers all exposed surfaces (**K**, **O**). The pharyngeal cavity, first not more than a slit-like opening in the endoderm, widens and expands into the already opened pouch (**H**, **L**, **P**), eventually forming an open communication between the pharyngeal cavity (asterisks in **G**, **H**, **L**, **P**) and the external environment. These events are repeated for each prospective gill slit and proceed in an anterior-to-posterior manner. The cavity marked by 'X' in **E**, **F**, **M**, **J** and **K** is a preparation artifact. **Q**–**S** Cartoons representing a horizontal view of the head of a newly hatched sturgeon (**Q**) with cross sections at the level indicated, and comparable to those shown in **H**, **L** and **P** (**R** corresponding to **H**, **L**, and **S** corresponding to **P**). *b* brain, *en* endoderm, *n* notochord, *op* opercular flap, *ov* otic vesicle, *p* pharyngeal cavity, *P2–P4* pouches 2–4, *y* yolk. Scale bars in **E**–**P** = 100 µm



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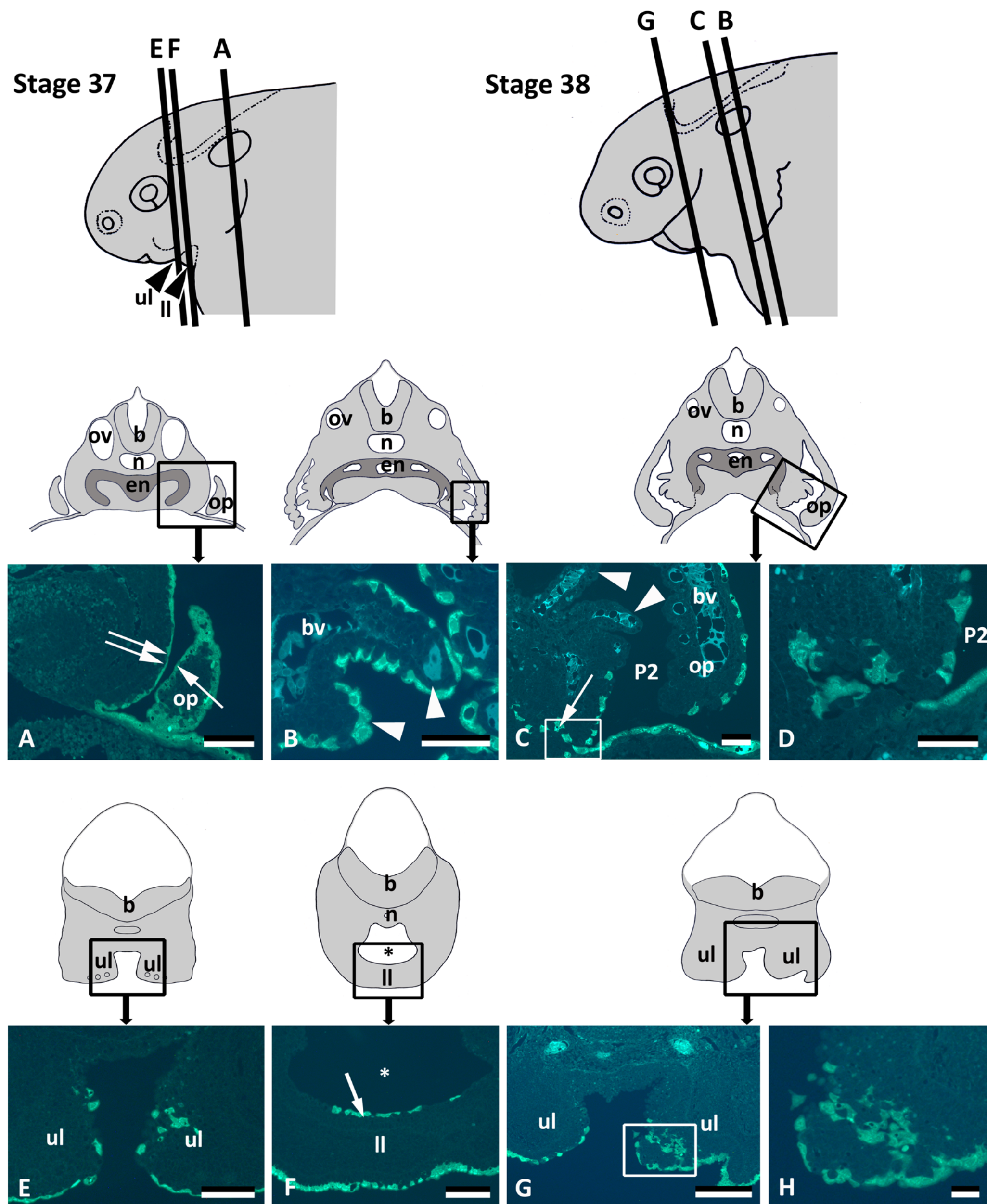

Fig. 4 Details of periderm expansion in sterlet sturgeon. Details of CDCFDA lineage tracing of periderm in the opening gill slits (**A**–**D**) and mouth (**E**–**H**), and cartoons of corresponding sections from which details were taken. Cartoons of a lateral view of the head of stage 37 and 38 sturgeon embryos show the level of sectioning for micrographs **A**–**C** and **E**–**G**. **A** Periderm covers both the internal surface of the outgrowing gill (arrow) as well as the body surface beneath (double arrow). **B** Fragmented periderm covers the forming gill filaments (two individual filaments indicated by arrowheads). **C** Individual periderm cells penetrate into the still closed pouch 3 and 4 (arrow). Arrowheads point to two individual gill filaments. **D** Magnification of the cells that lead the expansion into the pouch, similar to the boxed area in **C**. **E**–**H** CDCFDA lineage tracing of periderm in the opening mouth, and cartoons of corresponding sections from which details were taken. **E** Only a fragmented periderm covers the upper lip surrounding the mouth opening. **F** The inside of the lower lip (arrow) is partially covered by periderm. **G** At the upper lip edge, individual cells take on an irregular shape; boxed area shown in detail in **H**. *asterisks* oral cavity, *b* brain, *bv* blood vessel, *en* endoderm, *ll* lower lip, *n* notochord, *op* opercular flap, *ov* otic vesicle, *P2* pouch 2, *ul* upper lip. Scale bars in **A**, **E**, **F**, **G** = 100 µm; in **B**–**D** = 50 µm; in **H** = 20 µm

Different from bichir, the number of cell layers progressing over the yolk during gastrulation in sturgeon is not clear. However, at the end of gastrulation, the epidermis is stratified into an upper and a basal layer. Both are considered as ectodermal layers in sturgeon [35]. A detailed description of the fine structural details of the sturgeon

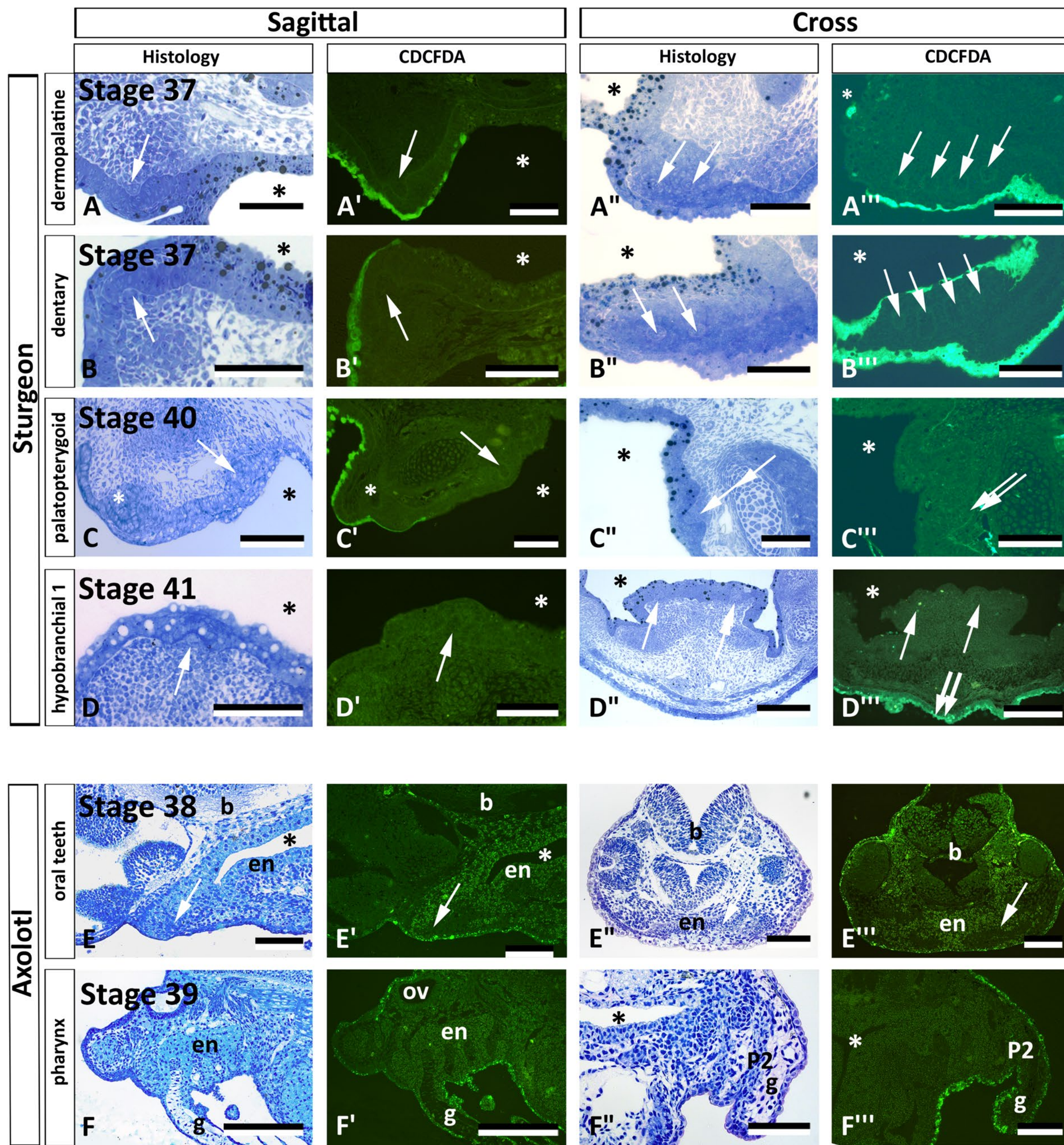

Fig. 5 Periderm partly covers odontogenic epithelia in sterlet sturgeon, but not in axolotl. Sagittal and cross sections (histology and lineage tracing with CDCFDA, resp.) of the four first tooth fields that develop in sturgeon: dermopalatine (**A**–**A‴**), dentary (**B**–**B‴**), palatopterygoid (**C**–**C‴**) and hypobranchial 1 (**D**–**D‴**) tooth fields. Arrows indicate tooth germs in the respective fields. Note that the epithelium of the oral (i.e., dermopalatine and dentary) teeth is covered with labeled periderm, but not the epithelium covering the palatopterygoid or hypobranchial 1 tooth germs, which are positioned deeper within the oropharynx. The low magnification of the hypobranchial tooth germs in cross sections (**D″**, **D‴**) clearly shows the difference between unlabeled odontogenic epithelium and strong labeling of the periderm in the skin (double arrow), 7 days after labeling. In axolotl embryos, teeth are restricted to oral and palatal fields (there are no pharyngeal teeth). Oral teeth (arrows) develop in the absence of periderm, which is still covering the body surface (**E**–**E‴**). Labeled periderm is present around the outgrowing external gills and in the distalmost part of the pouches (**F**–**F‴**). *asterisks* oral cavity, *b* brain, *en* endoderm, *g* external gill, *ov* otic vesicle, *P2* pouch 2. In all sagittal sections, anterior is to the left. Scale bars in **A**–**D′** = 100 µm; in **E**–**F′** = 500 µm; in **D″**, **D‴**, **E″**, **E‴**, **F″**, **F‴** = 200 µm

epidermis is mostly restricted to post-hatching or adult stages (e.g., [44, 45]), or relates to a specific region (e.g., [46]). In larval specimens of the Lake sturgeon, *A. fulvescens*, superficial cells, similar in characters to the periderm cells shown here, are referred to as keratinocytes [47].

The axolotl embryonic epidermis has been thoroughly characterized biochemically (e.g., [48, 49]), but fine structural studies of the epidermis are mostly limited to larvae or adults (e.g., [50, 51]). Pre-hatch axolotl embryos are said to possess a single layer of ectodermally derived epithelial cells that surround the embryo, while after hatching (stage 41, [52]) the epidermis consists of multiple layers of epithelial cells interspersed with Leydig cells [53]. Northcutt et al. [54] report a stratified ectoderm in the 48 h following the end of neurulation (stage 21). With some exceptions (e.g., [55]), generally speaking, only rarely is a distinction made in salamanders between surface and inner layer of the epidermis. Yet, the superficial squamous layer in the skin of direct-developing frogs has been termed periderm [56]. The protective and secretory functions of the skin superficial cell layer in the axolotl, as well as in the anuran amphibian *Xenopus*, have been compared to the mammalian periderm [57, 58]. Other authors refer to the superficial layer of the bilayered epidermis of tadpoles of *Xenopus* merely as outer ectodermal layer, or outer cells (e.g., [59–61]). Differences in histological appearance of the epidermis across species and stages may well be due to the quality of fixation and embedding (cf. [62]).

In zebrafish, at the end of gastrulation the body is covered by a bilayered epithelium. The outer, or enveloping layer (EVL), henceforth called periderm, arises by early lineage restriction and has generally been considered as extra-embryonic [23, 33]. Several recent lines of evidence suggest that the interpretation of the zebrafish periderm as an extra-embryonic layer needs to be revised. First, contrary to what is traditionally assumed, periderm cells persist in the zebrafish epidermis until at least 1 month of age [63–65]. Second, recent findings show that the EVL gives rise to other cell types of the organism, notably to the so-called 'forerunner cells' that in turn give rise to Kupffer's vesicle (whose cells later disperse and assume caudal notochord or muscle fates) [66]. Third, periderm cells partially expand into the mouth and the pharyngeal pouches and assist in clefting of the gill slit [17]. Collazo et al. [31] suggested that the teleost EVL either constitutes a novelty, or could be homologized with the superficial ectodermal layer in other taxa, yet acquired a new fate. Detlaff [67] regarded the stratification of the ectoderm merely as an early (sturgeon) or late (urodele) segregation of the epithelium, together with possible early specialization of the outer layer (in teleosts). In this way, she clearly homologized the superficial ectodermal layer in sturgeon or in salamanders with the teleostean periderm. Likewise, Warga and Kane [66] considered the possibility that the zebrafish periderm is not an extra-embryonic tissue but may be the homologue of the superficial ectodermal layer of the ancient actinopterygian embryo. Together, the analyses of sturgeon, axolotl and teleost embryonic epidermis lead us to interpret the outer layer of the epidermis in embryonic sturgeon and axolotl as a true periderm.

Limited expansion of the sterlet sturgeon periderm into the oropharynx mimics the situation in zebrafish

Lineage tracing revealed that periderm expansion in the sterlet sturgeon is restricted to the mouth entrance and to the distal parts of the pouches, but does not reach the medial pharyngeal endoderm, a situation that is very similar to that seen in zebrafish [17, 24]. A possible explanation for this restricted distribution may be that periderm cells in sturgeon are not proliferating, or do so to a very limited extent, an interpretation supported by our PCNA data. Several other arguments support this idea: the extended period of label retention in the periderm cells and the absence of any variation in signal strength (a weakening signal would be the natural consequence if cells divide). Chan et al. [68] described a process of asynthetic fission in the periderm of the zebrafish skin, although they labeled the layer as 'superficial epithelial cells'. These cells were shown to display a limited proliferation (one cell giving rise to four progeny only) and to continue expanding with asynthetic fission. That the initially uninterrupted layer of labeled periderm in sturgeon becomes locally fragmented after certain chase times, adds to the idea that proliferation of the periderm does not, or cannot, compensate to cover a steadily enlarging surface area. Fragmentation occurs especially in the ventral head region, the cranial vault presenting an uninterrupted labeling (compare, e.g., Fig. 1M, N). A lack of proliferation necessarily results in an arrest of expansion because periderm cells would be 'used up'. Interruptions in the periderm layer can also be caused by apoptosis, or through radial intercalation by cells of the basal layer [69, 70]. Likewise, the extreme cell flattening can be interpreted as a way to compensate for the lack of new cells. Inflicting full-thickness wounds in adult zebrafish skin causes epidermal cells outside the wound to undergo progressive radial intercalation, flattening and elongation [71]. The latter two features are reminiscent of what is observed in the sturgeon periderm.

Is the periderm in sturgeon transported into the oropharynx in a passive way as a result of the outgrowth of the gills, or do the cells actively migrate into the pouches? The quantitative approach that we used did not yield

conclusive answers. The elaborate shape of the periderm cells inside the gill clefts is reminiscent of migrating cells and could be indicative for an active migration. The clear arrest of expansion of the periderm in the distal parts of the prospective gill slits nevertheless speaks in favor of passive transport. Considering that periderm proliferation is likely limited, the two interpretations are not necessarily mutually exclusive: active movement could initially transport cells inside but insufficient proliferation could arrest migration and subject the cells to passive movements. Live imaging will be required to elucidate this question.

Like in zebrafish [17], the gill slit in sturgeon opens concomitantly with periderm expansion, and the function of the periderm cells could well be to separate the epithelia and promote lumen formation, as is inferred for zebrafish. In support of a potential role for periderm in separating epithelial surfaces are other instances where periderm appears to play a non-adhesive role, notably in mammals, even when periderm here originates late and in a distinct way [72]. E.g., periderm disruption is necessary to allow fusion of epithelia and to prevent cleft palate during palatogenesis (reviewed in [73, 74]). Periderm also assists in separating digits [75]. In fact, the expansion of the periderm into the forming gill slits in sturgeon bears a striking resemblance to the process of separating digits in mammals.

Opening of the gill slits in primarily aquatic osteichthyans is very little studied, despite its crucial importance to establish a respiratory water flow. In a recent paper, Rees et al. [76] examined gill slit opening in a cartilaginous fish, the little skate, *Leucoraja erinacea*. The first phases appear to be similar to what is observed here in sterlet sturgeon: as in the skate, pouch endoderm and ectoderm, once in close apposition, fuse into a single disorganized epithelium, enabled by disappearance of the basal lamina. Based on our high-resolution images of sturgeon pouches, we find no evidence for cell death that would allow lumen formation, as is also the case in the little skate. Instead of apoptosis, Rees et al. [76] suggest epithelial remodeling as the last phase in the process of gill slit opening, but do not elaborate on this epithelial remodeling. We propose that, at least in sturgeon, herein lies a role of the periderm, a layer that is perhaps absent in little skate, considering the monolayered aspect of the ectoderm in the images presented by Rees et al. [76]. In how far FGF signaling plays a role in opening of the gill slits in sturgeon, as has been demonstrated in the little skate, is a matter of further investigations.

The particular 'umbrella' shape that some periderm cells adopt (whether at the mouth entrance, the gills, or the pre-oral gut), and especially the cell extensions that reach down towards the basal lamina, call for attention. Similar cell shapes have been observed for the 'periderm-like cells' described in zebrafish [22]. Whether these cell extensions merely serve an anchoring function, or are involved in signaling, is not known at present. The 'umbrella' shape is also reminiscent of that of mammalian superficial urothelial cells. These, however, form an uninterrupted layer securing a high-resistance barrier function [77].

Tooth formation is independent of periderm expansion in the pharynx

Based on our lineage tracing experiments, we find that odontogenic epithelia in the sterlet sturgeon present spatial differences with respect to the presence of a periderm covering. The epithelium of developing oral (i.e., dermopalatine and dentary) teeth is covered by (albeit fragmented) periderm; the epithelium of developing palatopterygoid teeth as well as pharyngeal (hypobranchial and infrapharyngobranchial) teeth is never covered with periderm. By contrast, oral teeth in axolotl develop long before periderm can expand into the mouth. The most parsimonious interpretation is that both oral and pharyngeal tooth initiation/formation is independent from the presence of periderm on the surface of the tooth placode. The presence of periderm covering prospective oral teeth in sturgeon may well be simply the result of timing of their development and of their location. Indeed, oral teeth in sturgeon form very close to the mouth entrance, within reach of the expanding periderm. By contrast, palatal and pharyngeal teeth lie much deeper within the oropharynx and are not reached by periderm. In zebrafish, which lack oral teeth, pharyngeal tooth formation likewise does not depend on periderm expanding into the gill slits, as this happens only after the teeth have been initiated; in addition, periderm never reaches far enough inside to reach the odontogenic areas [17, 24].

Together, the data on sturgeon and axolotl support the conclusion that periderm expansion is not correlated with the presence of either oral or pharyngeal teeth. How can these data be reconciled with the idea that an ectodermal signal is required for tooth formation even when the enamel organ is derived from endodermal epithelium? Minarik et al. [39] showed that the endodermal pre-oral gut in stage 30 sturgeon possesses a constriction in the form of a ring, constituted of ectoderm. Interestingly, the oral teeth form exactly in this ring-shaped ectodermal constriction, suggesting a relationship with—and perhaps dependence on—the position of the ectoderm. Transplant studies using transgenically labeled axolotl have revealed that the first tooth initiated in each tooth field forms either in ectoderm or at the ectodermal–endodermal boundary, even when the enamel organ is endoderm-derived [15]. In zebrafish, the suprabasal

layer of odontogenic epithelia bears a morphological and molecular signature that is similar to the periderm and has been labeled 'periderm-like'. This layer originates endogenously, likely as a result of cooption of endoderm into an ectodermal function [17]. The suprabasal layer of the endoderm in the pharyngeal region of sturgeon and the oral region of axolotl has been described to display ectodermal features [22], and may behave similarly as the 'periderm-like' cells in zebrafish. Whether this layer is truly 'epidermalized' or 'ectodermalized' endoderm [7, 78], is subject of further research.

Conclusions

We have presented arguments that justify in our view the term 'periderm' for the superficial layer of ectoderm, both in sturgeon and in axolotl. Periderm expansion into the oropharynx is restricted in both taxa to the distal parts of the pharyngeal pouches and does not reach the medial pharyngeal endoderm. Restricted expansion of the periderm into the oropharynx has thus been established now for three lineages of primarily aquatic vertebrates with open gill slits: a basal and an advanced actinopterygian (zebrafish and sturgeon, resp.) and a sarcopterygian (axolotl). It therefore appears to be an ancestral feature of osteichthyans, not a regressive feature specific for teleosts. Periderm behavior does not correlate with presence or absence of oral or pharyngeal teeth, whose induction may depend on 'ectodermalized' endoderm. The co-occurrence of periderm expansion with opening of the mouth and/or the gill slits suggests a function in separating opposing epithelia and formation of a lumen.

Methods

Animal collection

Fertilized eggs of sterlet sturgeon (*Acipenser ruthenus*) were kindly provided by the Research Institute of Fish Culture and Hydrobiology (Vodňany, University of South Bohemia, Czech Republic). Embryos were transferred to well-oxygenated tanks and kept at 17 °C in E2 Pen/Strep zebrafish embryo medium [79] until the desired stage was reached. Embryos and early post-embryonic stages of sturgeon were staged according to Dettlaff et al. [35]. Wild-type axolotl (*Ambystoma mexicanum*) embryos were acquired from the axolotl colony maintained at the Department of Zoology of Charles University (Prague, Czech Republic). Eggs were put into sterile Steinberg solution containing antibiotics and staged according to Bordzilovskaya et al. [52].

Embryos of both species were manipulated in accordance with the institutional guidelines for the use of embryonic material and international animal welfare guidelines (Directive 2010/63/EU).

Histology and transmission electron microscopy

Sturgeon and axolotl embryos of the desired stage were killed using an overdose of MS222. They were fixed in a mixture of glutaraldehyde and paraformaldehyde and processed for embedding in epon according to Huysseune et al. [80]. Serial semithin (1 and 2 µm) cross and sagittal sections were made of 15 specimens of sturgeon embryos (ranging between stage 22 and 41) and one axolotl embryo (stage 38) using a diamond knife mounted on a Microm HM360 (Marshall Scientific, Hampton, New Hampshire, USA) automated microtome and stained with toluidine blue (1% toluidine blue in 2% sodium tetraborate). Ultrathin (70 nm) sections of one sturgeon embryo were cut using a diamond knife mounted on a Reichert Ultracut E microtome (Reichert-Jung, Buffalo, New York, USA), contrasted with uranyl acetate and lead citrate and analyzed with a Jeol JEM 1010 transmission electron microscope (Jeol Ltd., Tokyo, Japan) operating at 60 kV. Microphotographs were taken with a Veleta camera (Emsis, Muenster, Germany).

Scanning electron microscopy

Sturgeon embryos of stages 35 to 38 (two specimens per stage) were fixed in PFA, washed in PBS for 10 min and then dehydrated in an ascending ethanol series (35%, 50%, 70%, 80%, 96% and 99%; 15 min for every step). The embryos were next transferred into acetone through an acetone–ethanol mixture series (1:2, 1:1, 2:1; 10 min for every step). The embryos were further dried and gold-coated, mounted on a metal support and imaged on a Jeol-JSM-IT 200 scanning electron microscope, operating at 15 kV.

Lineage tracing

To label the outer epithelial layer, embryos of sterlet sturgeon were decapsulated and soaked in a 1:1000 dilution of [5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate, succinimidyl ester, mixed isomers] (CDCFDA) (cat. No. 22026, AAT Bioquest, Inc.) in embryo medium following [21]. When applied as a live staining, CDCFDA enters the cells that are in contact with the compound, but does not diffuse further. Hence, soaking animals in CDCFDA for an appropriate period of time followed by thorough rinsing of the embryos, can be used for lineage tracing of the periderm. Soaking was started at different time points (stage 25, 28, 32 and 35 for sturgeon; at least a dozen specimens per stage), and performed for 4 h in the dark, followed by rinsing in embryo medium and maintenance (in the dark) in embryo medium for variable chase times (fixation at stages ranging between 30 and 45, depending on the time point of soaking the embryos). Axolotl embryos of stage 29, 30 and 33 (at

least a dozen per stage) were decapsulated and soaked in CDCFDA under the same conditions as for sturgeon (1:1000 CDCFDA, 4 h), rinsed and maintained in embryo medium until fixation at stage 38 and 39.

After the appropriate chase time, specimens were killed with an overdose of MS222, fixed in PFA, and processed for embedding in glycol methacrylate (GMA) according to Oralová et al. [81]. GMA blocks of CDCFDA treated ($n=23$) and control ($n=6$) sturgeon embryos, and of CDCFDA treated ($n=5$) and control ($n=1$) axolotl embryos were serially sectioned (2–5 µm) either on a Microm HM360 automated microtome or manually on a Leica RM2155 rotatory microtome. Throughout the entire procedure (from live staining to sectioning of the blocks), the specimens were sheltered from light.

PCNA staining

For the study of proliferation, developmental stages of sturgeon (between stages 36 and 40) were fixed in PFA, dehydrated and conserved in methanol at −20 °C until further processing. Dehydrated specimens ($n=9$) were processed for paraffin embedding. Sections of 7 µm were blocked in 3% BSA, 1% milk in TBS, or, alternatively, in 10% BSA, 0.03% Triton. An anti-PCNA antibody, clone PC10, from mouse (Sigma CBL407) was applied in the blocking solution (1:200) for 1 h at room temperature. After washing, a goat-anti-mouse secondary antibody (ThermoFisher A11005) was applied for 2 h at room temperature or overnight at 4 °C. Sections were coverslipped using Vectashield Antifade Mounting Medium with DAPI.

Imaging

Semithin epon sections were observed and imaged under transmitted light using an Axio Imager-Z1 compound microscope (Carl Zeiss, Oberkochen, Germany) equipped with an Axiocam 503 color camera (Carl Zeiss, Oberkochen, Germany). GMA sections prepared from the animals labeled with CDCFDA were temporarily mounted with PBS and photographed under epifluorescence on an Axio Imager-Z1 compound microscope equipped with a 5MP CCD camera using a GFP filter (filterset 38, EX BP 470/40, BS FT 495, EM BP 525/50) and ZEN software, or an Olympus BX51 compound microscope with GFP filter, equipped with a DP74 digital camera and using cellSens software. The latter was also used for observing and photographing immunostained sections.

Measurements

To assess how deep labeled cells penetrate into the pouches after specific chase times with CDCFDA, the distance was measured from the medial-most point of periderm expansion to the mediosagittal plane of the body, on serial cross sections ranging from pouch 2 to pouch 6 (4 specimens) (Fig. S1).

Supplementary Information

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Figure S1. (A) Distance between deepest point of periderm expansion (A, A′) to the mediosagittal plane (M), taken on both body sides (A–M and A′–M), as measured on cross sections. (B) Graphical representation of the measurements shown in A for four developmental stages; lower lines were obtained by connecting all points A from anterior to posterior between pouches 2 to 6; upper lines by connecting all points A′; midline = mediosagittal plane.

Figure S2. PCNA staining of sturgeon embryos at the start of cleft formation (A), ongoing (B) and completed cleft formation (C). Note abundant labeling in the basal ectodermal layer (arrows) but only sparsely labeled cells in the periderm (arrowheads). Scale bars = 100 µm.

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Author contributions

AHu, AHo, TS and RC performed the experiments. AHu, AHo, TS and DL prepared the material for analysis. Ahu, AHo and RC analyzed the data. Ahu and RC wrote the manuscript. All authors reviewed the manuscript and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Embryos of sturgeon and axolotl were manipulated in accordance with the institutional guidelines for the use of embryonic material and international animal welfare guidelines (Directive 2010/63/EU).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Hashimshony T, Feder M, Levin M, Hall BK, Yanai I. Spatiotemporal transcriptomics reveals the evolutionary history of the endoderm germ layer. Nature. 2015;519:219–22.
2. Steinmetz PRH, Aman A, Kraus JEM, Technau U. Gut-like ectodermal tissue in a sea anemone challenges germ layer homology. Nat Ecol Evol. 2017;1:1535–42.
3. Hirasawi T, Kuratani S. Evolution of the vertebrate skeleton: morphology, embryology, and development. Zool Lett. 2015;1:2.
4. Hall BK. Germ layers, the neural crest and emergent organization in development and evolution. Genesis. 2018;56: e23103.
5. Teng CS, Cavin L, Maxson Jnr RE, Sanchez-Villagra M, Crump JG. Resolving homology in the face of shifting germ layer origins: lessons from a major skull vault boundary. Elife. 2019;8: e52814.
6. Krivanek J, Soldatov RA, Kastriti ME, Chontorotzea T, Herdina AN, Petersen J, et al. Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. Nat Commun. 2020;11:4816.
7. Huysseune A, Cerny R, Witten PE. The conundrum of pharyngeal teeth origin: the role of germ layers, pouches, and gill slits. Biol Rev. 2022;97:414–47.
8. Chen D, editor. Odontodes: the developmental and evolutionary building blocks of dentitions. 1st ed. Boca Raton: CRC Press; 2023.
9. Gillis JA, Tidswell ORA. The origin of vertebrate gills. Curr Biol. 2017;27:729–32.
10. Sagai T, Amano T, Maeno A, Kimura T, Nakamoto M, Takehana Y, et al. Evolution of Shh endoderm enhancers during morphological transition from ventral lungs to dorsal gas bladder. Nat Commun. 2017;8:14300.
11. Barlow LA. Progress and renewal in gustation: new insights into taste bud development. Development. 2015;142:3620–9.
12. Fabian P, Tseng K-C, Smeeton J, Lancman JJ, Dong PDS, Cerny R, et al. Lineage analysis reveals an endodermal contribution to the vertebrate pituitary. Science. 2020;370:463–7.
13. Boehm T, Bleul CC. The evolutionary history of lymphoid organs. Nat Immunol. 2007;8:131–5.
14. Soukup V, Epperlein HH, Horácek I, Cerny R. Dual epithelial origin of vertebrate oral teeth. Nature. 2008;455:795–8.
15. Soukup V, Tazaki A, Yamazaki Y, Pospisilova A, Epperlein H-H, Tanaka E, et al. Oral and palatal dentition of axolotl arises from a common tooth-competent zone along the ecto-endodermal boundary. Front Cell Dev Biol. 2021;8: 622308.
16. Rothova M, Thompson H, Lickert H, Tucker AS. Lineage tracing of the endoderm during oral development. Dev Dyn. 2012;241:1183–91.
17. Rosa JT, Oralová V, Larionova D, Eisenhoffer GT, Witten PE, Huysseune A. Periderm invasion contributes to epithelial formation in the teleost pharynx. Sci Rep. 2019;9:10082.
18. Grevellec A, Tucker AS. The pharyngeal pouches and clefts: development, evolution, structure and derivatives. Semin Cell Dev Biol. 2010;21:325–32.
19. Soukup V, Horácek I, Cerny R. Development and evolution of the vertebrate primary mouth. J Anat. 2013;222:79–99.
20. Frisdal A, Trainor PA. Development and evolution of the pharyngeal apparatus. WIREs Dev Biol. 2014;3:403–18.
21. Shone V, Graham A. Endodermal/ectodermal interfaces during pharyngeal segmentation in vertebrates. J Anat. 2014;225:479–91.
22. Huysseune A, Cerny R, Witten PE. The conquest of the oropharynx by odontogenic epithelia. In: Chen D, editor. Odontodes: the developmental and evolutionary building blocks of dentitions. Boca Raton: CRC Press; 2023. p. 49–67.
23. Kimmel CB, Warga RM, Schilling TF. Origin and organization of the zebrafish fate map. Development. 1990;108:581–94.
24. Oralová V, Rosa JT, Larionova D, Witten PE, Huysseune A. Multiple epithelia are required to form teeth deep in the pharynx. Proc Natl Acad Sci USA. 2020;117:11503–12.
25. Huysseune A, Sire J-Y, Witten PE. Evolutionary and developmental origins of the vertebrate dentition. J Anat. 2009;214:465–76.
26. Huysseune A, Sire J-Y, Witten PE. A revised hypothesis on the evolutionary origin of the vertebrate dentition. J Appl Ichthyol. 2010;26:152–5.
27. Rücklin M, Giles S, Janvier P, Donoghue PCJ. Teeth before jaws? Comparative analysis of the structure and development of the external and internal scales in the extinct jawless vertebrate *Loganellia scotica*. Evol Dev. 2011;13:523–32.
28. Rücklin M, Donoghue PCJ, Johanson Z, Trinajstic K, Marone F, Stampanoni M. Development of teeth and jaws in the earliest jawed vertebrates. Nature. 2012;491:748–51.
29. Murdock DJE, Dong X-P, Repetski JE, Marone F, Stampanoni M, Donoghue PCJ. The origin of conodonts and of vertebrate mineralized skeletons. Nature. 2013;502:546–9.
30. Donoghue PCJ, Rücklin M. The ins and outs of the evolutionary origin of teeth. Evol Dev. 2016;18:19–30.
31. Collazo A, Bolker JA, Keller R. A phylogenetic perspective on teleost gastrulation. Am Nat. 1994;144:133–52.
32. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995;203:253–310.
33. Cooper MS, Virta VC. Evolution of gastrulation in the ray-finned (actinopterygian) fishes. J Exp Zool (Mol Dev Evol). 2007;308B:591–608.
34. Ballard WW, Ginsburg AS. Morphogenetic movements in acipenserid embryos. J Exp Zool. 1980;213:69–103.
35. Dettlaff TA, Ginsburg AS, Schmalhausen OI. Sturgeon fishes: developmental biology and aquaculture. Berlin: Springer; 1993.
36. Diedhiou S, Bartsch P. Staging of the early development of *Polypterus* (Cladistia: Actinopterygii). In: Kunz YW, Luer CA, Kapoor BG, editors. Development of non-teleost fishes. Plymouth: Science Publishers; 2009.
37. Ostaszewska T, Dabrowski K. Early development of *Acipenseriformes* (Chondrostei: Actinopterygii). In: Kunz YW, Luer CA, Kapoor BG, editors. Development of non-teleost fishes. Plymouth: Science Publishers; 2009.
38. Wegner A, Ostaszewska T, Rozek W. The ontogenetic development of the digestive tract and accessory glands of sterlet (*Acipenser ruthenus* L.) larvae during endogenous feeding. Rev Fish Biol Fish. 2009;19:431–44.
39. Minarik M, Stundl J, Fabian P, Jandzik D, Metscher BD, Psenicka M, et al. Pre-oral gut contributes to facial structures in non-teleost fishes. Nature. 2017;547:209–12.
40. Pospisilova A, Stundl J, Brejcha J, Metscher BD, Psenicka M, Cerny R, et al. The remarkable dynamics in the establishment, rearrangement, and loss of dentition during the ontogeny of the sterlet sturgeon. Dev Dyn. 2022;251:826–45.
41. Cerny R, Meulemans D, Berger J, Wilsch-Bräuninger M, Kurth T, Bronner-Fraser M, et al. Combined intrinsic and extrinsic influences pattern cranial neural crest migration and pharyngeal arch morphogenesis in axolotl. Dev Biol. 2004;266:252–69.
42. Adams AE. An experimental study of the development of the mouth in the amphibian embryo. J Exp Zool. 1924;40:311–79.
43. Berkovitz B, Shellis P. The teeth of non-mammalian vertebrates. Amsterdam: Academic Press, Elsevier; 2017.
44. Saadatfar Z, Shahsavani D, Fatemi FS. Study of epidermis development in sturgeon (*Acipenser persicus*) larvae. Anat Histol Embryol. 2010;39:440–5.
45. Shooraki HF, Saadatfar Z, Shahsavani D. Skin development in *H. huso* Larvae. J Morph Sci. 2018;35:37–43.
46. Zeiske E, Kasumyan A, Bartsch P, Hansen A. Early development of the olfactory organ in sturgeons of the genus *Acipenser*: a comparative and electron microscopic study. Anat Embryol. 2003;206:357–72.
47. Shute L, Huebner E, Anderson WG. Microscopic identification of novel cell types in the integument of larval Lake Sturgeon, *Acipenser fulvescens*. J Morphol. 2016;277:86–95.
48. Slack JMW. Regional biosynthetic markers in the early amphibian embryo. J Embryol Exp Morphol. 1984;80:289–319.
49. Stigson M, Kjellén L. Large disulfide-stabilized proteoglycan complexes synthesized by the epidermis of axolotl embryos. Archs Biochem Biophys. 1991;290:391–6.
50. Jarial MS. Fine structure of the epidermal Leydig cells in the axolotl *Ambystoma mexicanum* in relation to their function. J Anat. 1989;167:95–102.
51. Gerling S, D'haese J, Greven H. Number and distribution of Leydig cells (LC) in the epidermis of the growing axolotl, *Ambystoma mexicanum* (Amphibia: Urodela). Vert Zool. 2012;62:97–111.
52. Bordzilovskaya NP, Dettlaff TA, Duhon S, Malacinski G. Developmental stage series of axolotl embryos. In: Armstrong JB, Malacinski GM, editors. Developmental biology of the axolotl. New York: Oxford University Press; 1989.
53. Seifert AW, Cook AB, Shaw D. Inhibiting fibroblast aggregation in skin wounds unlocks developmental pathway to regeneration. Dev Biol. 2019;455:60–72.
54. Northcutt RG, Catania KC, Criley BB. Development of lateral line organs in the axolotl. J Comp Neurol. 1994;340:480–514.

55. Shi D-L, Riou J-F, Darribère T, Boucaut J-C. A study of cell interactions involved in *Pleurodeles watlii* epidermal differentiation. Roux's Arch Dev Biol. 1988;197:247–57.
56. Richardson MK, Carl TF, Hanken J, Elinson RP, Cope C, Bagley P. Limb development and evolution: a frog embryo with no apical ectodermal ridge (AER). J Anat. 1998;192:379–90.
57. Furlow JD, Berry DL, Wang Z, Brown DD. A set of novel tadpole specific genes expressed only in the epidermis are down-regulated by thyroid hormone during *Xenopus laevis* metamorphosis. Dev Biol. 1997;182:284–98.
58. Page RB, Monaghan JR, Walker JA, Voss SR. A model of transcriptional and morphological changes during thyroid hormone-induced metamorphosis of the axolotl. Gen Comp Endocrinol. 2009;162:219–32.
59. Stubbs JL, Davidson L, Keller R, Kintner C. Radial intercalation of ciliated cells during *Xenopus* skin development. Development. 2006;133:2507–15.
60. Walentek P, Quigley IK. What we can learn from a tadpole about ciliopathies and airway diseases: using systems biology in *Xenopus* to study cilia and mucociliary epithelia. Genesis. 2017;55: e23001.
61. Tomankova S, Abaffy P, Sindelka R. The role of nitric oxide during embryonic epidermis development of *Xenopus laevis*. Biol Open. 2017;6:862–71.
62. Kurth T, Weiche S, Vorkel D, Kretschmar S, Menge A. Histology of plastic embedded amphibian embryos and larvae. Genesis. 2012;50:235–50.
63. Fukazawa C, Santiago C, Park KM, Deery WJ, de la Torre Canny SG, Holterhoff CK, et al. *poky/chuk/ikk1* is required for differentiation of the zebrafish embryonic epidermis. Dev Biol. 2010;346:272–83.
64. Fischer B, Metzger M, Richardson R, Knyphausen P, Ramezani T, Franzen R, et al. p53 and TAp63 promote keratinocyte proliferation and differentiation in breeding tubercles of the zebrafish. PLoS Genet. 2014;10: e1004048.
65. Lee RTH, Asharani PV, Carney TJ. Basal keratinocytes contribute to all strata of the adult zebrafish dermis. PLoS ONE. 2014;9(1): e84858.
66. Warga RM, Kane DA. Wilson cell origin for Kupffer's vesicle in the zebrafish. Dev Dyn. 2018;247:1057–69.
67. Dettlaff TA. Evolution of the histological and functional structure of ectoderm, chordamesoderm and their derivatives in Anamnia. Roux's Arch Dev Biol. 1993;203:3–9.
68. Chan KY, Yan C-CS, Roan H-Y, Hsu S-C, Tseng T-L, Hsiao C-D, et al. Skin cells undergo asynthetic fission to expand body surfaces in zebrafish. Nature. 2022;605:119–25.
69. Walck-Shannon E, Hardin J. Cell intercalation from top to bottom. Nat Rev Mol Cell Biol. 2014;15:34–48.
70. Rauzi M. Cell intercalation in a simple epithelium. Philos Trans R Soc B. 2020;375:20190552.
71. Richardson R, Metzger M, Knyphausen P, Ramezani T, Slanchev K, Kraus C, et al. Re-epithelialization of cutaneous wounds in adult zebrafish combines mechanisms of wound closure in embryonic and adult mammals. Development. 2016;143:2077–88.
72. M'Boneko V, Merker H-J. Development and morphology of the periderm of mouse embryos. Acta Anat. 1988;133:325–36.
73. Lan Y, Xu J, Jiang R. Cellular and molecular mechanisms of palatogenesis. Curr Top Dev Biol. 2015;115:59–84.
74. Hammond NL, Dixon J, Dixon MJ. Periderm: life-cycle and function during orofacial and epidermal development. Semin Cell Dev Biol. 2019;91:75–83.
75. Kashgari G, Meinecke L, Gordon W, Ruiz B, Yang J, Ma AL, et al. Epithelial migration and non-adhesive periderm are required for digit separation during mammalian development. Dev Cell. 2020;52(6):764–78.
76. Rees JM, Palmer MA, Gillis JA. Fgf signalling is required for gill slit formation in the skate, *Leucoraja erinacea*. Dev Biol. 2024;506:85–94.
77. Jafari NV, Rohn JL. The urothelium: a multi-faceted barrier against a harsh environment. Muc Immunol. 2022;15:1127–42.
78. Hayashi H. Opening mechanism of stomodeum cavity and transformation of yolk platelet proteins to various subcellular structures in developing newt embryos, *Triturus pyrrhogaster*. J Electr Microsc. 1972;21:246 (**abstract**).
79. Brand M, Granato M, Nüsslein-Volhard C. Keeping and raising zebrafish. In: Nüsslein-Volhard C, Dahm R, editors. Zebrafish: a practical approach. Oxford: Oxford University Press; 2002.
80. Huysseune A, Soenens M, Sire J-Y, Witten PE. High resolution histology for craniofacial studies on zebrafish and other teleost models. Meth Mol Biol. 2022;2403:249–62.
81. Oralová V, Rosa JT, Soenens M, Bek JW, Willaert A, Witten PE, et al. Beyond the whole-mount phenotype: high-resolution imaging in fluorescence-based applications on zebrafish. Biol Open. 2019;8(5):UNSP bio042374.
82. Kopinke D, Sasine J, Swift J, Stephens WZ, Piotrowski T. Retinoic acid is required for endodermal pouch morphogenesis and not for pharyngeal endoderm specification. Dev Dyn. 2006;235:2695–709.

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