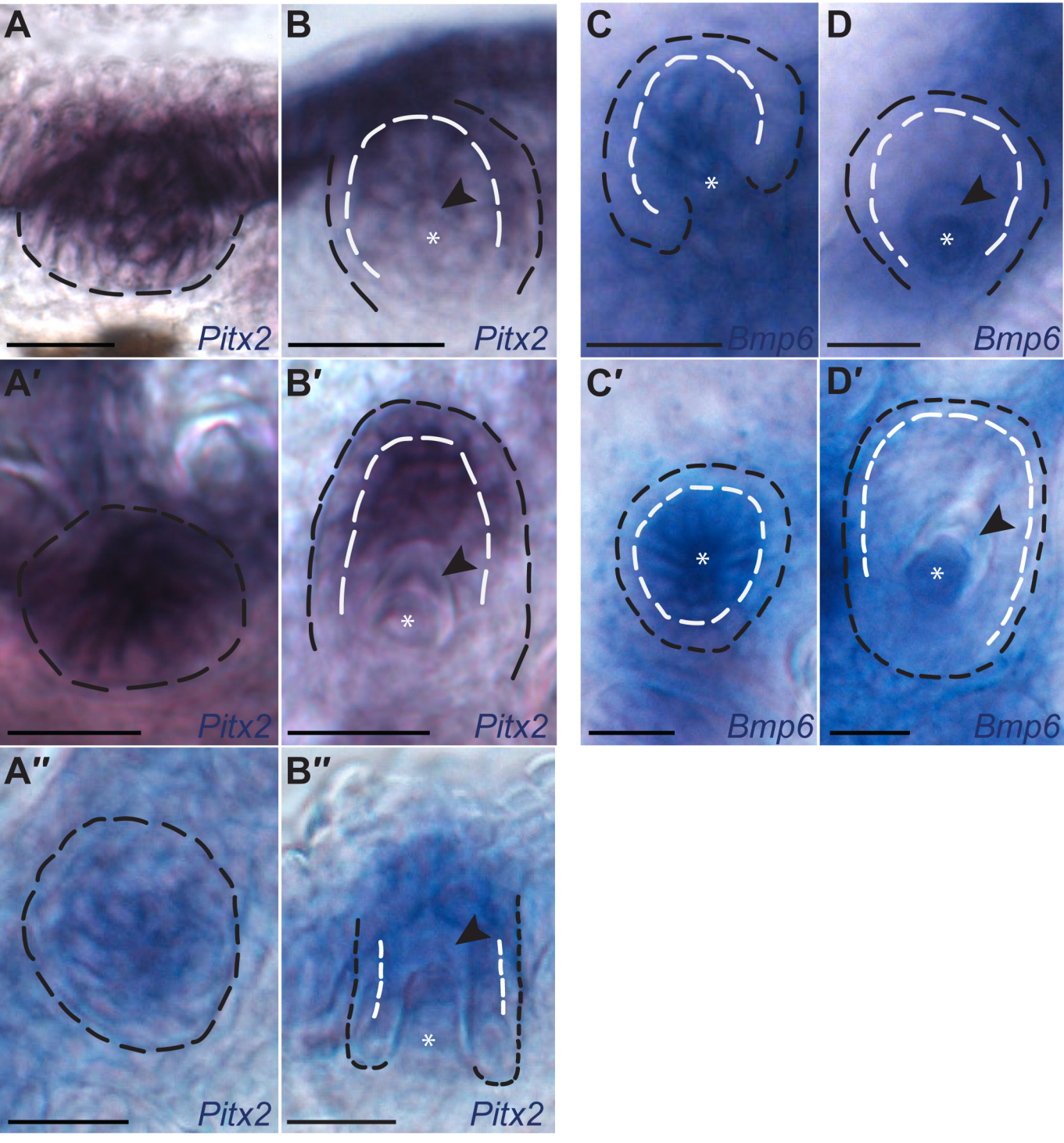
through the first ventral pharyngeal tooth replacement event in a 30 day marine larvae. The eighteenth tooth to form is the replacement of the initial pioneer tooth forming lateral to and at the base of position one, within the fifth ceratobranchial bone. Bone resorption (black arrowheads) has occurred at the base of position one adjacent to the replacement tooth. (C,D) Examples of adult tooth replacement occurring similarly. Bone resorption is present on the side with the replacement tooth (black arrowheads). (E) Primary tooth germs form at the interface of the epithelium and the mesenchyme (left tooth germ, white caret), replacement teeth form deep in the mesenchyme (right germ, white asterisks), but with deeply invaginating epithelium that is continuous with the lumenal pharyngeal epithelium (see Fig. S3). (E') Adjacent serial section showing the adult tooth above the replacement germ (white asterisks). (F, G) Stickleback replacement teeth form intraosseously, though not completely encased in bone (tooth germs marked with black caret). (C-F) Adult freshwater, (G) adult marine. Scale bars = 50 μm.

Fig. S1: *Pitx2* and *Bmp6* tooth expression in section and whole mount.

In situ hybridization for *Pitx2* (A-A", B-B") and *Bmp6* (C, C', D, D') in section (A-D) and whole mount (A'-D', A", B"). Tooth developmental stages are vertically matched for early germs (A, A', C, C') and late germs (B, B', D, D') for pharyngeal (A-D, A'-D') and oral (A", B") teeth. *Pitx2* expression is detected in the dental epithelium (A-B, A'-B', A"-B") while *Bmp6* expression is detected in the inner dental epithelium and mesenchyme early (C, C'), and appears restricted to the mesenchyme only late (D, D'). The black dotted line outlines the developing tooth germ while the white dotted line separates the inner and outer dental epithelium (when discernable). Black arrowheads denote mineralized teeth and white asterisks denote mesenchyme. Scale bar = 15 μm.

Fig. S2: Dorsal pharyngeal tooth plates develop on pharyngobranchial cartilage templates. (A) Alizarin red (bone) and Alcian blue (cartilage) stained 11 days post fertilization (dpf) marine dorsal pharyngeal tooth plates. The pharyngobranchial cartilages chondrify before dorsal pharyngeal teeth form adjacently. (B) Transverse H&E sections of 20 dpf marine dorsal and ventral pharyngeal tooth plates. The dorsal pharyngeal tooth plate contains chondrocytes (white arrowhead) and teeth ossify directly on the thin layer of perichondral bone surrounding them (white arrow). The fifth ceratobranchial contains chondrocytes (black arrowhead), but the ventral pharyngeal tooth plate does not (black arrow), instead forming from subsequent ossification ventral to individual teeth. Note the medial tooth germ not surrounded by bone (black caret). Scale bars = 50 μm.

Fig. S3: Replacement teeth form deep but with invaginated epithelia that is continuous with lumenal pharyngeal epithelium. H&E stained 6 μm serial sections from adult sticklebacks showing pharyngeal teeth forming deep in the mesenchyme with an epithelial connection to the lumenal pharyngeal epithelium. (A-A", B-B", C-C", D-D") Each series indicates an individual tooth germ forming deep in the mesenchyme (A, B, C, D), yet with a clear epithelial connection to the lumenal pharyngeal epithelium in subsequent serial sections (A', A", B', B", C', C", D', D"). (A) Arrowhead indicates the stratum compactum, the boundary between the pharyngeal epithelium and the underlying connective tissue. Scale bar is 50 μm and applies to all panels. (A-C) Adult freshwater, (D) adult marine. Note B and C' appear in Fig. 7C and Fig. 7F respectively.


A
Pitx2
B
Pitx2
C
Bmp6
D
Bmp6
A′
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B′
Pitx2
C′
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A″
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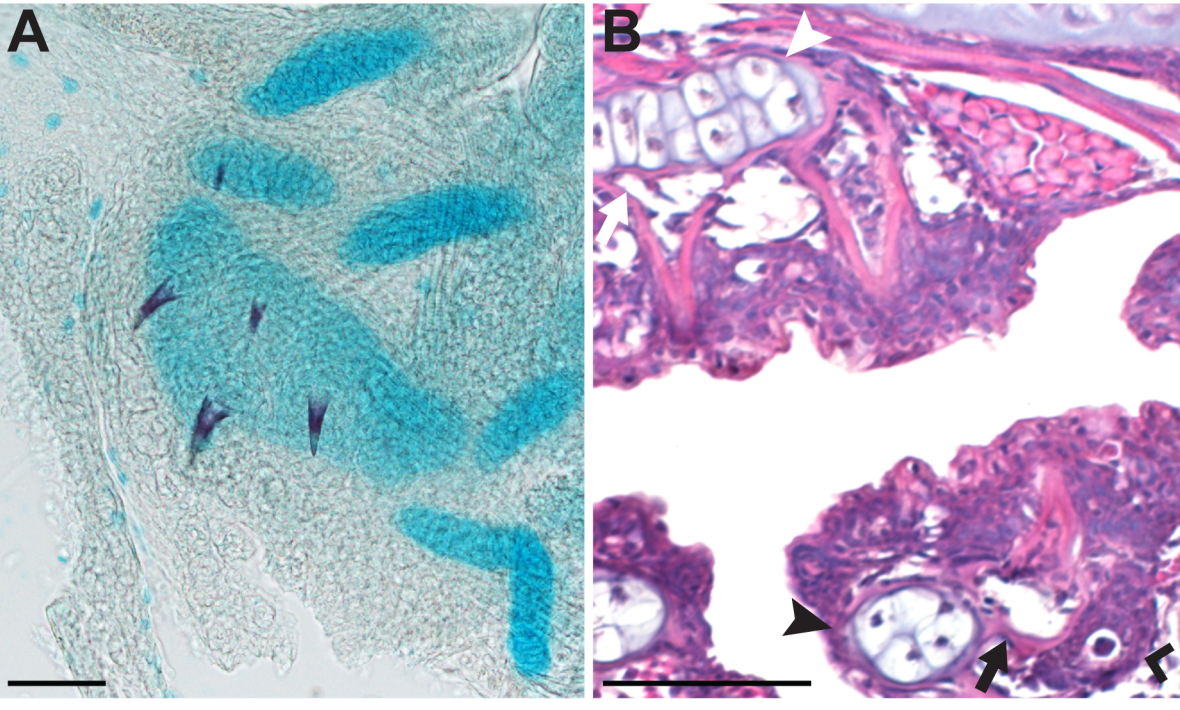
A
B

Fig. S3