The morphology and evolution of tooth replacement in the combtooth blennies (Ovalentaria: Blenniidae)

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Keiffer Logan Williams

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Andrew M. Simons

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DEDICATION

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Abstract

This research investigates the morphology and evolution of tooth replacement in the combtooth blennies. Blennies exhibit complex dentition that is not easily categorized by previously established metrics of teleost tooth replacement (extraosseous and intraosseous replacement). Most blennies are heterodont, possessing a single row of comb-shaped feeding teeth on the anterior portion of their jaws, and enlarged canines on the posterior portion of their lower jaws used for agonistic interactions. However, this bizarre dentition has been intentionally overlooked in classic surveys of teleost dentition due to its complexity. In Chapter 1, I investigate how feeding teeth are replaced in salariin blennies by establishing a descriptive model of tooth replacement for the Pacific Leaping Blenny, *Alticus arnoldorum*. This fish exhibits tooth replacement and tooth attachment that defy the discrete categorizations used for most other teleost dentitions. Using my descriptive model, I then propose hypotheses of how feeding teeth function in this fish. In chapter 2, I investigate how modes of tooth replacement have evolved in blennies. I find that canines in blennies are consistently replaced intraosseously, while feeding teeth across the family vary from intraosseous to a derived form of extraosseous replacement. These results further support the concept of teleost tooth replacement as a continuum rather than discretely classified modes. My results also suggest teleost tooth replacement needs to be carefully examined within a phylogenetic context to better understand how trophic morphological novelties evolve, as modes of tooth replacement likely play a key role in ecological and functional morphological shifts in teleosts.

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Chapter 1. The morphology of tooth replacement and attachment in the Pacific Leaping Blenny, *Alticus arnoldorum* (Blenniiformes: Blenniidae: Salariini) with a discussion on tooth function.

Introduction

Tooth replacement in teleost fishes is diverse and complex, with little known regarding the full diversity of patterns across the group (Huyssuene & Witten, 2006). Historically, modes of teleost tooth replacement are classified as extraosseous, where replacement teeth develop in soft tissue outside the bone of attachment, or intraosseous, wherein replacement teeth develop in sockets within the bone of attachment (Trapani, 2001). Trapani (2001) considered extraosseous replacement to be the plesiomorphic state in teleosts while intraosseous is derived and thought to have evolved at least three separate times in actinopterygians (Trapani, 2001). Intraossseous replacement is also thought to be associated with heterodonty and enlarged tooth size (Bemis & Bemis, 2015; Kolmann et al., 2019). However, recent work has suggested that these modes of tooth replacement are opposite extremes on a continuum rather than discrete classifications (Conway et al., 2015).

Tooth replacement is contingent upon a dental lamina, an infolding of the oral epithelium upon which replacement teeth develop at specific positions (Reif, 1982; Moriyama et al, 2010; Berkovitz & Shellis, 2017). In fishes, the dental lamina was first categorized as either continuous or discontinuous by Reif (1982). A continuous dental lamina stretches all along the dentigerous (tooth bearing) bone or cartilage and is found in most elasmobranchs, while a discontinuous lamina consists of individual epithelial invaginations and are common in actinopterygians (Reif, 1982; Moriyama et al., 2010). Alternatively, Reif (1982) proposed that the dental lamina can be categorized as permanent, in which the dental lamina replaces several teeth, or non-permanent, in which it only produces one tooth. An additional classification dichotomy is the successional dental lamina in which a new tooth germ is formed from the epithelium of a preceding tooth generation (Huysseune, 2006), and the direct dental lamina that derives directly from the oral epithelium and is not associated with a previous tooth generation (Bemis et al. 2019). This diversity of dental lamina classifications underscores the range and complexity of teleost dentitions.

Once developed, replacement teeth must attach to a tooth-bearing bone. Tooth attachment in teleosts was broadly surveyed in Fink (1981). Fink proposed four major modes of tooth attachment ranging from tooth ankylosis to the dentigerous bone, to a hinged-collagen attachment at the posterior base of the functional tooth (Figure 1.1; Fink, 1981). Although useful for broad classification purposes, these discrete patterns leave out more complex modes of tooth attachment. Fink (1981) acknowledges this by making brief reference to the “unattached” dentition found in the lips of some combtooth blennies but does not include this group in the study.

Combtooth blennies are a family of small, cryptobenthic fishes found in shallow, mostly marine communities in both tropical and temperate climates worldwide (Hastings & Springer, 2009). The group is comprised of two major clades, the Springerichthys and

Salariini (Hundt et al., 2014), the latter of which will be the focus of this study. These fishes are aptly named for the arrangement of their feeding teeth, which are positioned along the premaxilla and dentary in a single row, resembling a comb (Figure 1.2). Tooth attachment in blennies ranges from fusion of functional teeth to the bone of attachment to lineages in which functional teeth are not in direct contact with the jawbones, and instead are weakly attached via connective tissues (Figure 1.3, Figure 1.4; Bath 2001, 2002). Most species also possess recurved canines located posteriorly on the dentary, thought to be used for intraspecific combat and interspecific defense (Figure 1.2D; Kotrschal & Goldschmid, 1992). However, canines will not be examined in this study.

Despite numerous studies alluding to the complexity of tooth attachment in combtooth blennies (Springer, 1968; Fink, 1981; Bath, 2001, 2002; Christiansen et al., 2010; Hundt et al., 2018), there is a paucity of research investigating tooth replacement in blennies. To date, only Christiansen et al. (2010) and classic descriptions of Blenniidae osteology have examined tooth replacement in these fishes (Springer, 1968; Smith-Vaniz & Springer, 1971; Bath, 2001, 2002). However, these studies are restricted in their capacity to inform of tooth replacement processes, as they examine only gross morphological characteristics.

The objectives of this study are two-fold: (a) establish a descriptive model of feeding tooth replacement in the Pacific Leaping Blenny, *Alticus arnoldorum,* a salariin blenniid exhibiting extreme tooth detachment from the oral jaws, (b) use the descriptive model of tooth replacement for *A. arnoldorum* to hypothesize how teeth function in salariin blennies. The results of my study further challenge the tooth replacement classifications proposed by Trapani (2001) and the tooth attachment modes described by Fink (1981). I also propose how teeth function in salariin blennies using the descriptive model established in this study.

Materials and Methods

2.1 Terminology

Here, I use the term “replacement tooth” to describe a tooth undergoing the process of development as it moves towards the terminal position in the jaws. The term “functional tooth” is used to describe a tooth that is in the terminal position and used in feeding. I use “tooth locus” to describe the terminal position where a replacement tooth becomes a functional tooth. The term “tooth family” describes all teeth that will develop and become attached at a particular tooth locus throughout life (Reif, 1982; Huysseune, 2006). Unless otherwise stated, all of the above terms only refer to feeding teeth. Canine tooth replacement was not assessed in this study.

Tooth attachment in salariin blenniids was examined previously by Bath (2001, 2002) wherein they described tissue associated with attachment near the posterior bases of functional teeth. They described this tissue using a variety of terms including “rodlet-like bony particles”, “rod-like bony particles”, “rodlets”, “rodlet-shaped bony particles”, “rodlet-like bony inlays”, and “rodlet-like particles” (Bath 2001, 2002), and I find these structures present in *A. arnoldorum*. For simplification, I refer to these structures as attachment rods, reflecting their associated attachment to functional teeth and their rod-like shape.

The osteology and soft tissues associated with the jaws of blenniids was assessed by Springer (1968). They describe the functional teeth and connective tissues associated with the teeth in both the premaxilla and dentary the “premaxillary dental plate” and “dentary dental plate”, respectively. I adopt the terminology used by Springer (1968), although I expand upon this definition to describe the plates as a complex of tissues composed of epithelium, vascular tissue, and connective tissues that contains replacement teeth tissues, functional teeth, and attachment rods.

2.2 Gross Morphology

One specimen of *A. arnoldorum* (SL= 56.78 mm) was used for gross examination of tooth attachment and external lip morphology in distal oral jaw tissues. A left parasagittal incision was made on the distal lip tissue of the upper jaw. Forceps and a probe were used to manipulate the incised tissue in order to determine the position of functional teeth in relation to the position of the maxillary valve.

One cleared and stained specimen (SL= 59.12 mm) was used to determine both replacement and functional tooth positions relative to the oral jaws. The premaxilla and dentary bones along with surrounding tissues were dissected and removed from the specimen and manipulated with forceps and dissection pins to determine the position of incoming replacement teeth, and the position of attachment rods associated with functional teeth.

2.3 Histology

Histological samples in this study were prepared from formalin-fixed specimens from the Bell Museum of Natural History fish collection. A total of 12 specimens of *A. arnoldorum* were examined, ranging in size from 35.2-57.8 mm SL (Table 1.1). The oral jaws were removed via dissection, with incisions made around the premaxilla and dentary bones to remove the jaws. For longitudinal sections, upper and lower jaw tissues were kept intact, while in transverse sections upper and lower jaw tissues were separated to allow for individual examination of the tissues.

Samples were decalcified using a range of decalcifying agents including Decal® Overnight Bone Decalcifier, Formical-2000, and 10% ethylenediaminetetraacetic acid (EDTA). Decalcification time varied, both decalcifying agents and decalcification times are available in Table 1.1. Following decalcification, samples were dehydrated in an ethanol series to 100% ethanol, infiltrated with toluene, and subsequently embedded in paraffin. Paraffin sections were cut at six µm thickness using a Leica Jung 820 Histocut Rotary Microtome and mounted on slides. Sections were stained with Hematoxylin and Eosin, Milligan's Trichrome, and Toluidine Blue stain using protocols modified from Humasson (1972).

2.4 MicroCT

Two specimens of *A. arnoldorum* were scanned with a Bruker Skyscan 1173 at the Karel F. Liem Bioimaging Center at Friday Harbor Laboratories. A non-stained specimen was scanned at 65 kV and 123µA, at a voxel size of 8.9 µm. To visualize soft tissues in the jaws, a second specimen was stained with phosphomolybdic acid (PMA) for approximately 48 hours and scanned at 65 kV and 123µA, at a voxel size of 8.5µm. Scans were reconstructed as .bmp stacks and extracted using Dataviewer software (2012-2017 Bruker microCT, version 1.5.6.2). Specimens were then segmented in the visualization software *Amira* (1999-2019 Thermo Fisher Scientific, version 6.4.0).

2.5 Scanning Electron Microscopy

The premaxillary and dentary tissues of one *A. arnoldorum* specimen (SL= 50.49 mm) were extracted for SEM analysis. Tissues were dehydrated in an acetone series and dried with hexamethyldisilazane (HMDS), sonicated, placed on carbon stubs and then sputtered with gold. Images were acquired on a Jeol JCM-5000 Neoscope at the Karel F. Liem Bioimaging Center at Friday Harbor Laboratories.

Results

3.1 Oral jaw morphology

The oral jaws of *A. arnoldorum* are labeled in Figure 1.3. The upper jaws consist of the premaxilla and maxilla, with the maxilla discontinuous from the premaxilla. The premaxilla consists of two ventrally concave plates that are medially separated via adipose tissue (Figure 1.5B). Each plate of the premaxilla has a medially positioned ascending process that is very loosely articulated with the premaxillary plate and surrounded by adipose tissue. The dorso-anterior edges of the ascending processes extend beyond the anterior margins of the premaxillary plates (Figure 1.3B, F), while the dorsoposterior edge of the ascending processes attach to a sphere of rostral cartilage (Figure 1.4B, Figure 1.5B).

The lower jaws in *A. arnoldorum* include the dentary and anguloarticular bones (Figure 1.3B). The dentary is composed of two anteriorally concave plates separated medially by loose connective tissue. The anterior-medial margin of each plate consists of a bony ridge, upon which connective tissues associated with the functional teeth attach (Figure 1.3F, 1.4B). Each plate of the dentary has a posterior process that extends dorso-posteriorly and which terminates medial to the posterior margins of the maxilla, and dorsal to the posterior margins of the anguloarticular (Figure 1.3B). The anterior margin of the anguloarticular articulates with the dentary, ventral to the base of the posterior dentary process (Figure 1.3B). In males, canine teeth develop and attach at the base of the posterior dentary processes (Figure 1.2D, Figure 1.3F).

Both the upper and lower jaws are enveloped in soft tissue that forms a continuous membrane across both respective jaw bones (the dental plate, highlighted in yellow in Figure 1.4B). These continuous membranes are composed of a complex network of vascular tissue, tooth replacement cells, connective tissue, and oral epithelium (Figure 1.4). In both jaws this tissue is distinct from the lips, which are composed of loose connective tissue and epidermis (Figure 1.4A, Figure 1.5A). In both the premaxilla and dentary the dental plate extends beyond the lateral margins of the jaw bones to the lateral-most edge of the external lip tissue. I define this tissue as “distal lip tissue”, as it extends beyond the distal margins of both the upper and lower jaw bones.

3.2 Tooth development and replacement

Replacement teeth in both the premaxillary and dentary tissue initiate their development at the tooth germ (Figure 1.4, Figure 1.5, Figure 1.6). The tooth germ of *A. arnoldorum* consists of individual epithelial invaginations for every replacement tooth of a tooth family to be generated. The tooth germ in the premaxillary tissue is located in close proximity to the medial-ventral position of the premaxilla and positioned dorsally to developing replacement teeth and functional teeth (Figure 1.3C, D, Figure 1.4). In the dentary tissue the tooth germ is positioned in close proximity to the anterior-inferior margin of the dentary and positioned posterior-ventrally to replacement and functional teeth (Figure 1.4). The tissue surrounding the tooth germ is highly vascularized, allowing blood to flow in close proximity to tooth replacement tissue (Figure 1.4A, Figure 1.5).

As replacement teeth develop in *A. arnoldorum,* they progress beyond the tooth germ toward the tooth locus via a successional dental lamina (Figure 1.6). The dental lamina is permanent, because the jaws exhibit continuous tooth replacement, and discontinuous because each epithelial invagination upon which the dental lamina is formed is independent from adjacent epithelial invaginations. The dental lamina in *A. arnoldorum* can be visualized as individual epithelial tracks upon which each replacement tooth progresses (Figure 1.6). These individual epithelial tracks originate at the tooth germ and are traceable to the position of the tooth locus for every individual functional tooth. In both the premaxillary and dentary tissues of specimens examined, I found an average of three developing replacement teeth present upon each dental lamina, including a replacement tooth initiating development at the tooth germ (Figure 1.5, Figure 1.6). These developing replacement teeth make up a tooth family that is responsible for functional tooth replacement at one individual tooth locus in the jaws.

The replacement teeth in *A. arnoldorum* exhibit a pattern of grouped tooth replacement. As seen in Figure 1.5 and Figure 1.6, developing replacement teeth have a staggered appearance, with incoming replacement teeth arranged in a grouped cascade. In this grouped cascade, the most developed replacement tooth is in closest proximity to its respective functional tooth locus and the least developed tooth is furthest away from its respective functional tooth locus (i.e., closest to the tooth germ) (Figure 1.5, Figure 1.6). However, each individual replacement tooth in the group is associated with its own respective discontinuous dental lamina, and so each replacement tooth in the cascade is independent from the other teeth (Figure 1.6). Therefore, the anterior-most replacement tooth in each group is the next tooth to be replaced. Taking into account all tooth replacement groups in the upper and lower jaws, I found that approximately every fifth functional tooth is undergoing replacement at the same time in *A. arnoldorum*.

3.3 Primary and secondary tooth attachment

In both the premaxilla and dentary, the functional teeth of *A. arnoldorum* exhibit a primary and secondary mode of attachment to the jaws. I define the primary mode of attachment as the tissues which connect functional teeth directly to the jaw bones. In the upper jaws, this attachment occurs at the posterior-inferior margin of the premaxilla, and the posterior-superior margin of the dentary in the lower jaws. Primary tooth attachment is composed of an attachment rod that articulates at the posterior base of each functional tooth (Figure 1.4, Figure 1.7). The attachment rod is a weakly calcified structure extending from the posterior base of the functional tooth. Surrounding the attachment rod is a band of collagen fibers, which connect the attachment rod and its associated functional tooth to the posterior-inferior margin of the premaxilla and the posterior-superior margin of the dentary (Figure 1.4, Figure 1.7). This band of collagen fibers is continuous, connecting all functional teeth in the dental plates to the jaw bones.

I define the secondary mode of tooth attachment as epithelial cells which maintain the functional position of developmentally mature teeth. This secondary mode is composed of three morphologically distinct regions of oral epithelium which I denote using the following terminology: gum epithelium, plate epithelium, and buccal epithelium (Figure 1.8).

The gum epithelium is labially positioned and attaches directly to the lip tissues in both the upper and lower jaw (Figure 1.4, Figure 1.8). This epithelium surrounds the anterior portion of each functional tooth, although the anterior-most edge and the buccal surface of the teeth are exposed (Figure 1.2B, Figure 1.8B). The teeth are only loosely attached to the gums, with observable space between the teeth and gums present at the anterior bases of functional teeth (Figure 1.2B, 1.8C). The plate epithelium surrounds the posterior portion of each functional tooth. It consists of a vascularized mesh that fully encases the posterior portion of the tooth, contrary to the non-vascularized and flexible gum tissue (Figure 1.8). The buccal epithelium is ventral to the plate epithelium in the upper jaw, and dorsal in the lower jaw. This epithelium is composed of pseudostratified epithelial cells directly exposed to the buccal cavity, with the outermost layer of cells exhibiting mucosal glands (Figure 1.8). The buccal epithelium covers the posterior portion of the functional tooth and continually extends lingually throughout the buccal cavity (Figure 1.4, Figure 1.8).

3.4 Distal lip tissue tooth replacement

Functional teeth in *A. arnoldorum* undergo tooth replacement in the distal lip tissue of both the upper and lower jaws (Figure 1.9). Using histology, functional teeth were observed in the distal lip tissue. In both the premaxilla and dentary, teeth in this tissue are smaller in size than more medially positioned functional teeth, and the teeth progressively decrease in size in a proximal-distal orientation (Figure 1.9C, D). Components of tooth replacement in the distal lip tissue, including the tooth germ, developing replacement teeth, dental lamina, and oral epithelium are all present in the distal lip tissue (Figure 1.9D).

Discussion

The ‘unattached’ dentition of salariin blennies has intrigued generations of morphologists (Regan, 1912; Springer, 1968; Fink, 1981; Bath, 2001). However, the specific nature of tooth attachment and the mechanism of tooth replacement in these fishes has been overlooked. Previous authors examined salariin dentition with the primary goal of inferring relationships among taxa (Springer, 1968; Smith-Vaniz & Springer, 1971; Bath, 2001, 2002). While informative for establishing general patterns of dentition across salariin blennies, these studies were restricted to gross morphological observations and therefore limited in their capacity to describe the complexities of tooth replacement and attachment in blennies. In his survey of osteichthyan tooth attachment Fink (1981) makes only a passing mention of blenny teeth and omits them entirely from his study due to their perceived complexity. My study is the first to document the complexity of tooth replacement and tooth attachment in the salariin blenny *A. arnolodorum*. Teeth are replaced extraosseously upon a novel discontinuous, permanent dental lamina. Teeth are continuously replaced within distal lip tissue far removed from dentigerous bone margins, a process previously undocumented in any teleost fish. Functional teeth exhibit both a primary and secondary mode of attachment, with the former involving a direct fibrous attachment to the dentigerous bone and the latter associated with attachment to the dental plate *sensu* Springer (1968). Taken together this descriptive study of tooth replacement for *A. arnoldorum* can be applied across salariin blennies to provide a useful model to generate hypotheses of tooth function for these vital marine detritivores.

4.1 Challenges to historical tooth replacement classifications

Feeding teeth in *A. arnoldorum* are replaced upon a discontinuous, permanent dental lamina. A discontinuous dental lamina is defined by individual epithelial invaginations with no epidermal tissue connecting adjacent tooth families (Reif, 1982; Moriyama et al., 2010). In contrast, a continuous dental lamina consists of a single epithelial folding that stretches across the entire dentigerous bone, with all tooth families connected to each other (Reif, 1982). *Alticus arnoldorum* fits the criteria for a discontinuous dental lamina because each tooth germ and associated dental lamina exhibits clear separation from its adjacent neighbor (Figure 1.6). Although in close proximity to one another, there is no evidence of epidermal tissue connecting adjacent tooth families (Figure 1.6). The dental lamina of *A. arnoldorum* is also permanent. Reif (1982) classifies permanent dental laminae as those that replace at least one but often many teeth, with all continuous and many discontinuous dental laminae fitting this category. In contrast, a nonpermanent dental lamina replaces only one tooth. My evidence shows that *A. arnoldorum* actively generates several replacement teeth at once upon each dental lamina (Figure 1.6).

Tooth replacement in *A. arnoldorum* exhibits similar patterns observed across teleosts. The freshwater gobiid *Sicyopterus japonicus* also possesses a discontinuous, permanent dental lamina in its upper jaw with all functional teeth arranged in a single tooth row (Moriyama et al. 2010). The replacement teeth in in the upper jaw are contained within a capsule of fibrous connective tissue termed a “tooth sac”, a structure that appears analogous to the dental plate in *A. arnoldorum*. However, *S. japonicus* differs from *A. arnoldorum* in the position and shape of the dental lamina, in addition to the number of replacement teeth undergoing active development upon the dental lamina at one time. Tooth replacement in numerous loricariid catfishes also bear a strong resemblance to *A. arnoldorum*. As in *A. arnoldorum*, all functional teeth in loricariids are replaced extraosseously upon a permanent dental lamina and arranged in a single row (Geerinckx et al., 2007). Replacement teeth in both groups can be traced from the tooth germ to tooth loci via epithelial tracks that individual replacement teeth progress upon (Figure 1.6; Geerinckx et al, 2007). However, regulation of tooth replacement in loricariid catfishes is unclear, whereas in *A. arnoldorum* approximately every fifth tooth is undergoing replacement at the same time, due to the observed pattern of grouped tooth replacement. Grouped tooth replacement is only known in two other species of closely related percomorph fishes, *Lobotes surinamensis* and *Datinoides polota* (Hilton & Bemis, 2005). The lateral teeth of both *L. surinamensis* and *D. polota* are replaced in groups positioned beneath the functional tooth loci. However, these species replace their teeth intraosseously. My investigation into *A. arnoldorum* is the first record of grouped extraosseous tooth replacement in teleost fishes.

Feeding teeth in *A. arnoldorum* undergo replacement in distal lip tissue far removed from the margins of the dentigerous bone (Figure 1.9). This demonstrates that the tooth germ and dental lamina have become extremely disassociated with the dentigerous bone, allowing for teeth to develop in extraneous tissue not previously associated with tooth development. This expansion of the tooth germ into novel tissue suggests an advantage over bone remodeling to generate space for additional functional teeth, and the oral epithelium required to generate them (Huysseune & Witten, 2006). The extension of functional teeth into distal lip tissue could also provide an advantage in gape expansion that allows for a greater surface area to acquire food while feeding.

This study underscores the complexity of placing teleost tooth replacement modes into discrete categories. The dental lamina of *A. arnoldorum* fits the definition of discontinuous and permanent, although its position in the jaws and shape differs from that of *S. japonicus* (Moriyama et al, 2010). The presence of teeth that develop in soft lip tissue that do not attach directly to the dentigerous jaw bones also presents a challenge to the current definition of extraosseous tooth replacement, wherein replacement teeth develop in soft tissue outside the bone to which they attach (Trapani, 2001). However, teeth replaced in the distal lip tissue exhibit no direct attachment to the dentigerous bone, instead these teeth develop and attach only to soft epithelial tissue (Figure 1.9). This extreme mode of tooth replacement does not easily fit the discrete extraosseous tooth replacement category. Conway et al. (2015) came to a similar conclusion in their study of gobiesocid tooth replacement, in which they conclude that gobiesocid tooth replacement is an intermediate between the extra- and intraosseous state. They propose expansion of discrete tooth replacement modes into a tooth replacement continuum in which extraosseous and intraosseous tooth replacement represent extremes upon the continuum (Conway et al., 2015). Extraneous tooth replacement in *A. arnoldorum* lends further support to the continuum classification system, further pushing the extremity of the extraosseous tooth replacement definition.

4.2 Two modes of tooth attachment

Alticus arnoldorum exhibits both a primary and secondary mode of functional tooth attachment. The possession of two tooth attachment modes is previously undescribed in teleost fishes. Fink (1981) observed four major modes of tooth attachment in actinopterygians, ranging from complete tooth ankylosis to the dentigerous bone, to a hinged-collagen attachment at the posterior base of the functional tooth (Figure 1.1; Fink, 1981). These modes of tooth attachment do not easily fit with my observations of primary attachment in *A. arnoldorum*, due in part to the extremely elongate shape of *A. arnoldorum* feeding teeth. The majority of actinopterygian teeth are conical in shape and conform to a circular tooth base, unlike the teeth of *A. arnoldorum*. These are anterio-posteriorly elongated and laterally compressed, and do not resemble a typical tooth base. Instead, the base at which collagen attaches on *A. arnoldorum* teeth is ventro-posteriorly located (Figure 1.7). There is also no attachment bone (*sensu* Fink, 1981) present between the dentigerous bone or the collagen band that connects all functional teeth. This lack of an attachment bone for *A. arnoldorum* feeding teeth is an additional novelty among teleost tooth attachment, further complicating the task of classifying *A. arnoldorum* tooth attachment using Fink's (1981) criteria.

The secondary mode of tooth attachment in *A. arnoldorum* also bears little resemblance to the attachment modes described by Fink (1981). Across his survey of teleosts, teeth are attached to the dentigerous bone at the base of the tooth, and any epithelium associated with the tooth is involved either in tooth development or is denoted as gum epithelium (Fink, 1981). The gum epithelium is associated with the anterior areas of type 4 attachment teeth (Figure 11; Kerebel et al. 1979; Fink, 1981), and is hypothesized to be associated with force absorption when the teeth are depressed. The gum epithelium in *A. arnoldorum* is also anteriorly positioned and the teeth are only loosely attached (Figure 1.4, Figure 1.8), suggesting a role in force absorption when teeth are depressed. However, gum epithelium attachment in *A. arnoldorum* is more extensive, surrounding both the anterior tooth base and the lateral sides of the functional tooth in an epithelial sheath (Figure 1.4, Figure 1.8). The embedded posterior portion of the functional tooth in plate and buccal epithelium further demonstrates the extent to which epithelium is involved in tooth attachment in *A. arnoldorum*, more so than type 4 tooth attachment.

The fibrous primary attachment and epithelial secondary attachment of *A. arnoldorum* feeding teeth highlight the complexity of salariin blenny tooth attachment with respect to other teleost fishes. Categorizing the tooth attachment of *A. arnoldorum* into a discrete mode, *sensu* Fink (1981) risks over-simplifying this fish's complex dental morphology. Instead, I propose considering tooth attachment in teleosts in an attachment continuum. As with tooth replacement, a tooth attachment continuum allows for all modes of attachment to be considered, without over-simplifying the morphology. In an attachment continuum ranging from ankylosis to hinged collagen attachment, *A.*

arnoldorum tooth attachment might be considered an extreme of the hinged attachment category, or the polarized opposite of ankylosis attachment (Figure 1.1).

With a few exceptions, the results of my investigation largely support the findings of Bath (2001, 2002) regarding tooth attachment in salariin blennies. Bath (2001, 2002) describes the attachment rod structures at the base of functional teeth in numerous salariin blennies as bone-like structures, though my evidence suggests that the attachment rods in *A. arnoldorum* are composed of mineralized collagen. Using cleared and stained specimens it is evident that the attachment rods are located at the bases of functional teeth (Figure 1.7), however the structures are only lightly stained by alizarin red. I interpret this light staining by alizarin red as indicative of low levels of calcification. In addition, in histological sections, the attachment rod position more closely resemble tightly packed collagen fibers, rather than bone (Figure 1.4A), and attachment rods are not detected at very high resolution microCT scans (8.9 µm, Figure 1.3F). Bath (2001, 2002) also notes the presence of irregular sized bony inlays located between the bases of the functional tooth row and the posterior-inferior margin of the premaxillary and the posterior-superior margin of the dentary. He suggests these structures are artifacts of a lost bony connection between the margins of the premaxilla and dentary and their respective functional tooth rows. These structures are not present in either the premaxilla or dentary tissues in *A. arnoldorum*. However, these structures are observable in several other salariin blenny genera, particularly those with fewer functional teeth (personal observations, Chapter 2 results in this thesis). The loss of bony inlays in *A. arnoldorum* could relate to a variation in habitat occupancy across the salariin blennies, with supratidal species exhibiting little to no inlays and subtidal species exhibiting more complete inlays. The relationship between the distance of the posterior margins of the functional tooth to the margins of the dentigerous bone in comparison to the presence or absence of bony inlays blennies also remains to be studied and could provide further insight into the evolution of tooth attachment in salariin blennies.

4.3 Inference on tooth function

The function of feeding teeth in salariin blennies remains largely unknown. Christiansen et al. (2010) made observations on the abrasions present on functional teeth following feeding and took tooth measurements of bite gape using the salariin blenny *Salarias fasciatus*. They suggest that feeding teeth in blennies function as scrapers rather than combs, although the authors did not examine tissue associated with tooth attachment, and only observed replacement teeth that were externally exposed from soft tissue. Determination of tooth function is beyond the scope of my study; however, morphology and attachment of these teeth provide a guide to function.

I hypothesize that the feeding teeth in *A. arnoldorum* function by both compressing and scraping against the substrate using the anterior portion of their teeth (Figure 1.10). As the mouth opens to feed, the premaxillary tooth row swings anteriorly, and the dentary tooth row swings posteriorly (Figure 1.10A). Upon contact with the substrate, the anterior portions of both the premaxillary and dentary teeth compress into the gum epithelium. Once compressed, the jaws will return to the closed position, scraping the teeth along the substrate and dislodging food items in the process (Figure 1.10B).

Several observations of functional tooth morphology lend support to this hypothesis. The feeding teeth of *A. arnoldorum* exhibit mineralization at the anterior tooth tip, most likely iron (Christiansen et al. 2010; Figure 1.11A). This mineralization suggests an adaptation for resistance to wear (Christiansen et al. 2010). SEM analysis of feeding teeth also revealed what could be artifacts of tooth compression in the anterior portion of the tooth adjacent to the mineralized tip (Figure 1.11B). The observable space between the functional tooth base and gum epithelium suggests teeth are compressed while feeding, with the gum epithelium acting as a cushion for the tooth. Tooth compressibility and dentin hypomineralization (soft dentin) is observed in several loricariid catfish species and allows for a reduction in brittleness and increased flexibility of feeding teeth (Geerinckx et al., 2012). Manipulation of cleared and stained *A. arnoldorum* feeding teeth revealed the teeth as quite flexible and able to bend laterally without breaking, and even maintaining a bent position after manipulation. Soft dentin in *A. arnoldorum* feeding teeth could explain the paradox of tooth flexibility in scraping teeth as suggested in Geerinckx et al. (2012). An increase in tooth slenderness and flexibility also appears to correlate with a decrease in tooth attachment strength in loricariid catfishes (Geerinckx et al., 2012). The slender, elongate tooth morphology and flexible primary and secondary attachment modes in *A. arnoldorum* suggests support for this correlation. A comparative study of elongate tooth morphology and attachment types associated with scrape feeding in teleost fishes would better clarify this relationship (Geerinckx et al., 2012; Bellwood et al. 2014).

Conclusion

The complexity of tooth replacement in salariin blennies has long been overlooked by fish morphologists. In this study I describe the morphology of tooth replacement in the Pacific Leaping Blenny, *Alticus arnoldorum*. This fish exhibits novelties in tooth generation, tooth replacement, and tooth attachment that provide further support for tooth replacement and attachment continuums, rather than discrete classification schemes. This study also establishes a descriptive model of tooth replacement that can be used to propose hypotheses of tooth function in *A. arnoldorum* and other salariin blennies. In the future, comparative tooth function studies on salariin blennies and other long-toothed teleosts will better elucidate the evolution of the long-toothed feeding morphology, and better elaborate the functional role of long-toothed fishes in aquatic ecosystems.

Species	**Museum catalogue no.**	**Standard length (mm)**	**Decalcification solution**	**Time decalcified (hours)**	**Stain**
Alticus arnoldorum	JFBM 46263	49.53	Decal® Overnight Bone Decalcifier	25	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	47.21	Decal® Overnight Bone Decalcifier	24	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	55.51	Formical-2000	66	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	51.86	Decal® Overnight Bone Decalcifier	22	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	44.02	Decal® Overnight Bone Decalcifier	22	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	35.21	Formical-2000	93	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	51.37	Decal® Overnight Bone Decalcifier	24	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	46.38	Decal® Overnight Bone Decalcifier	48	Milligan's trichrome
Alticus arnoldorum	JFBM 46263	44.64	Decal® Overnight Bone Decalcifier	48	Milligan's trichrome
Alticus arnoldorum	JFBM 46263	43.56	10% EDTA	264	Hematoxylin & Eosin

Table 1.1 Specimens used for histology, including sample decalcification time and stain used.

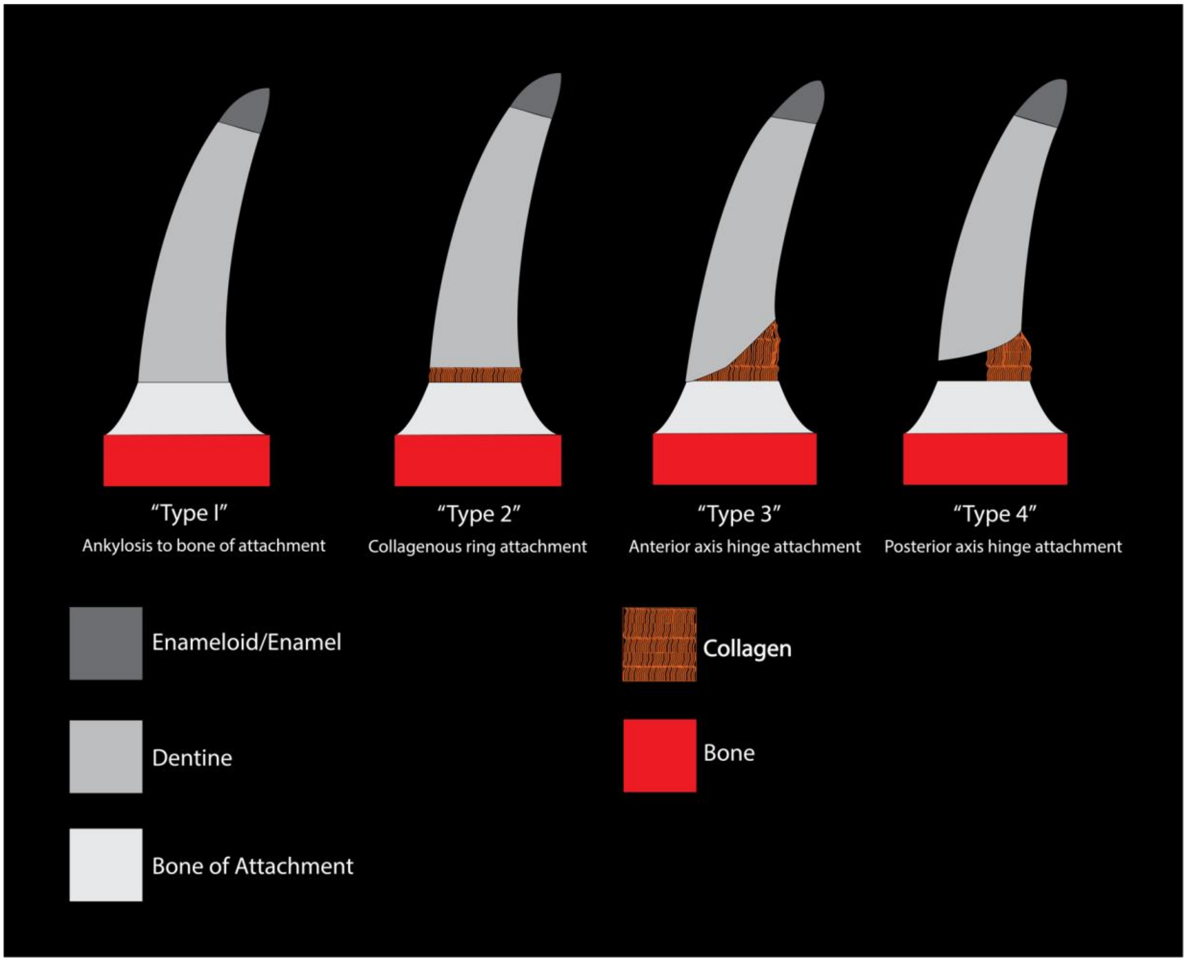

Figure 1.1. Modes of Teleost tooth attachment (modified from Fink, 1981). The modes of attachment described by Fink are: Type 1, ankylosis of functional tooth to the bone of attachment. Type 2, attachment of tooth to the bone of attachment via a collagenous ring at the tooth base. Type 3, anterior axis hinge attachment with collagen extending posteriorly along the tooth base. Type 4, Posterior axis hinge attachment, in which the anterior tooth base is unattached, but the posterior tooth base is attached via collagen.

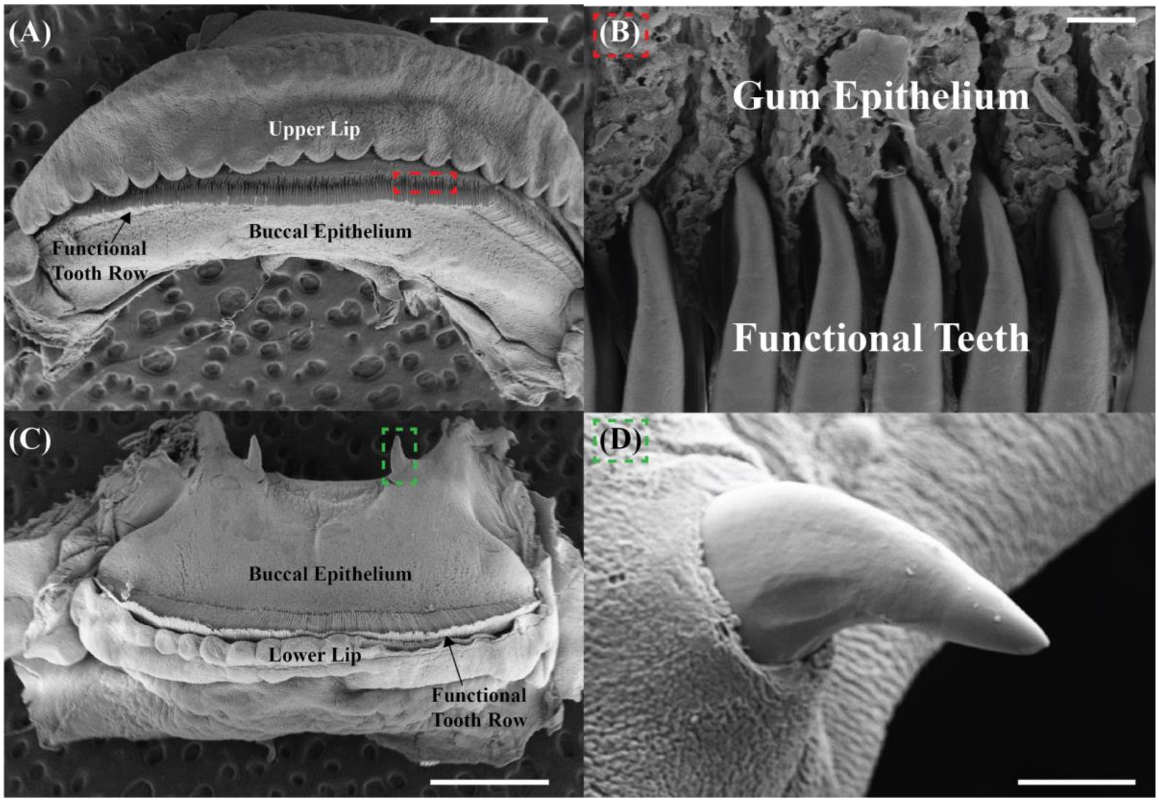

Figure 1.2. SEM imagery of *Alticus arnoldorum* external oral jaw morphology. **(A)** Anterior view of upper jaw with components labeled. Scale bar = 1 mm. **(B)** Close-up of section outlined in red-dashed box in (A), showing gum epithelium and anterior view of functional feeding teeth. Scale bar = 20 µm. **(C)** Anterior-dorsal view of lower jaw with components labeled. Scale bar = 1 mm. **(D)** Close-up of canine tooth outlined in (C). Scale bar = 100 µm.

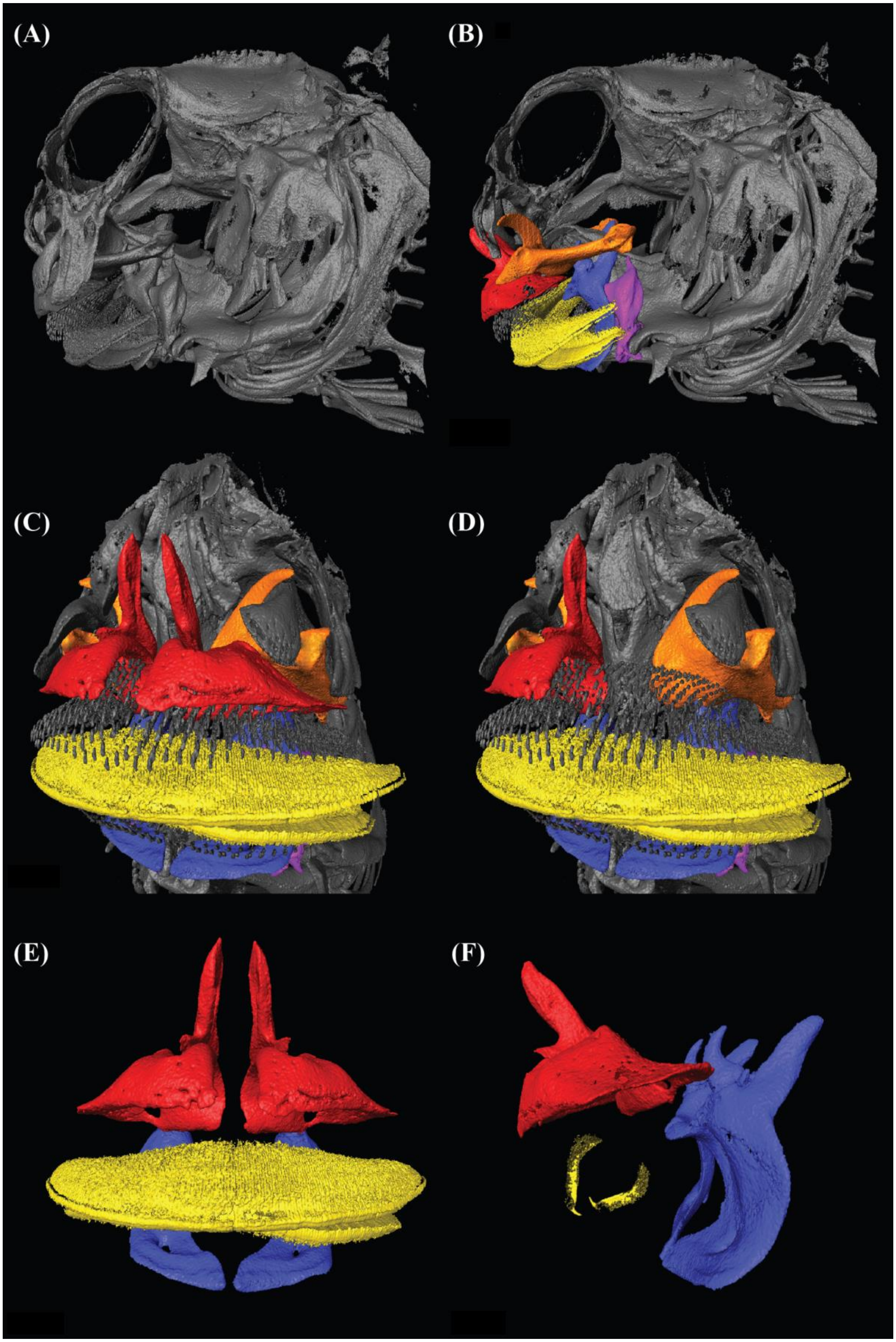
(A)
(B)
(C)
(D)
(E)
(F)

Figure 1.3. MicroCT scans showing the oral jaws of *Alticus arnoldorum*. **(A)** Left lateral view of *A. arnoldorum* skull. **(B)** Left lateral view of *A. arnoldorum* with lachrymal and infraorbital bones removed to show oral jaws. Components of the oral jaws are colored as follows: Red = premaxilla, Orange = maxilla, Blue = dentary, Purple = anguloarticular, Yellow = functional feeding teeth. **(C)** Anterior view of skull, showing rows of replacement teeth positioned between the premaxilla and functional tooth row. Replacement teeth between dentary and dentary tooth row are also visible at the bottom of the image. **(D)** Anterior view of skull with left premaxillary plate removed, showing all stages of replacement tooth development. **(E)** Anterior view of oral jaws, with skull and replacement teeth removed. **(F)** Left lateral view of oral jaws with skull and replacement teeth removed. Image shows one individual functional feeding tooth on both the premaxilla and dentary. Note the lack of bony connection and distance between the functional teeth and their respective dentigerous bones

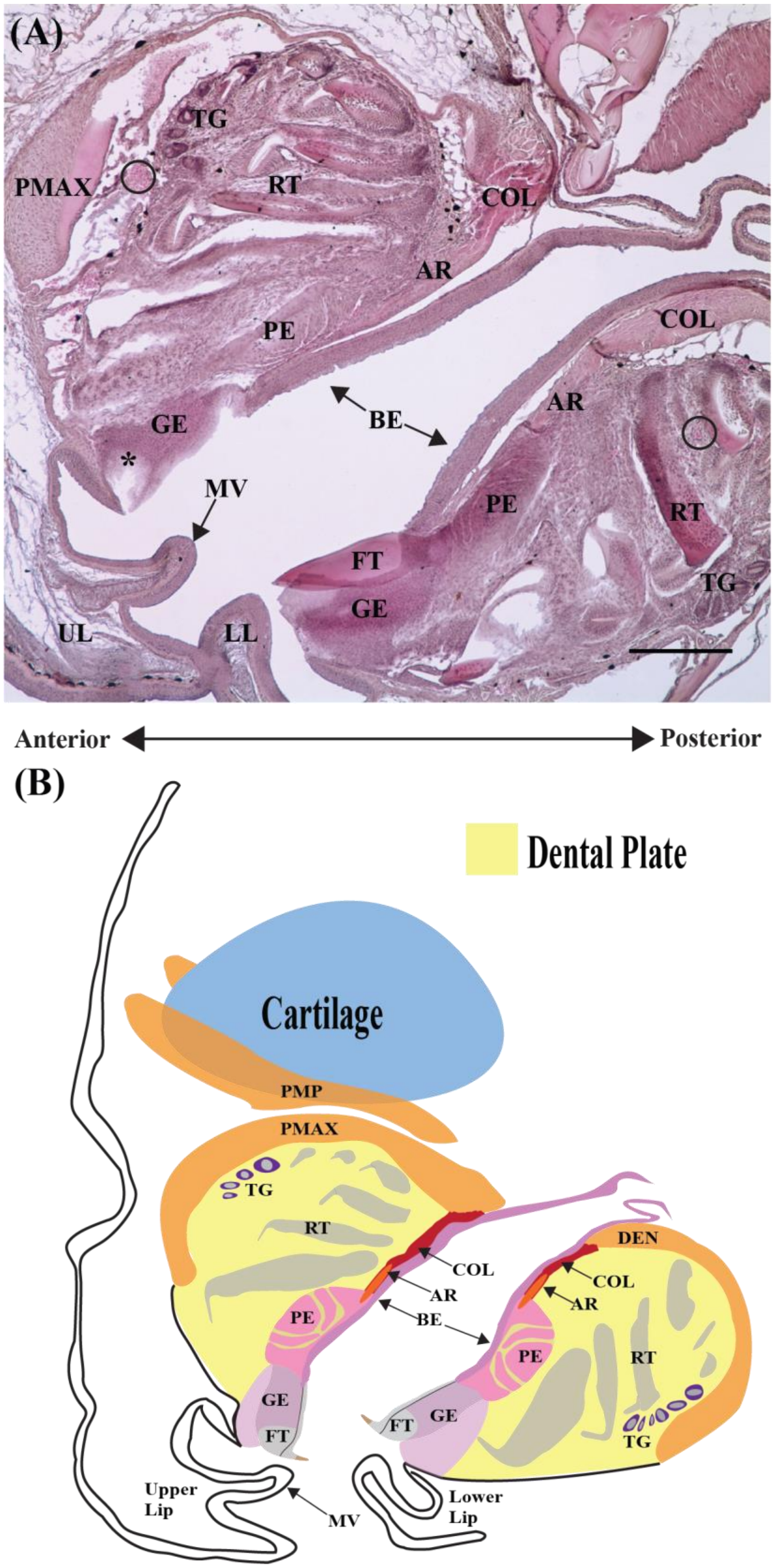
(A)
TG
PMAX
RT
COL
AR
PE
GE
*
BE
MV
COL
AR
PE
RT
FT
GE
TG
UL
LL
Anterior
Posterior
(B)
Dental Plate
Cartilage
PMP
PMAX
TG
RT
COL
AR
BE
PE
GE
FT
DEN
COL
AR
PE
RT
GE
FT
TG
Upper Lip
MV
Lower Lip

Figure 1.4. The morphology of tooth replacement in *Alticus arnoldorum*. **(A)** Left sagittal view of hematoxylin & eosin stained *A. arnoldorum* oral jaw tissues. In the upper jaw tissue of this section, the functional tooth broke free while sectioning. '*' denotes where the functional tooth would be if present in the image. Black circles denote vascularized tissue. Scalebar = 0.2 mm. **(B)** Illustration of *A. arnoldorum* oral jaw tissues. Abbreviations for both (A) and (B) are as follows: UL = upper lip, LL = lower lip, MV = maxillary valve, GE = gum epithelium, PMAX = premaxilla, TG = tooth germ, RT = replacement teeth, COL = collagen, AR = attachment rod, PE = plate epithelium, BE = buccal epithelium, FT = functional tooth, AP = ascending process of premaxilla, DEN = dentary. Yellow box denotes the dental plate, highlighted in yellow in both the upper and lower jaw tissues in the illustration.

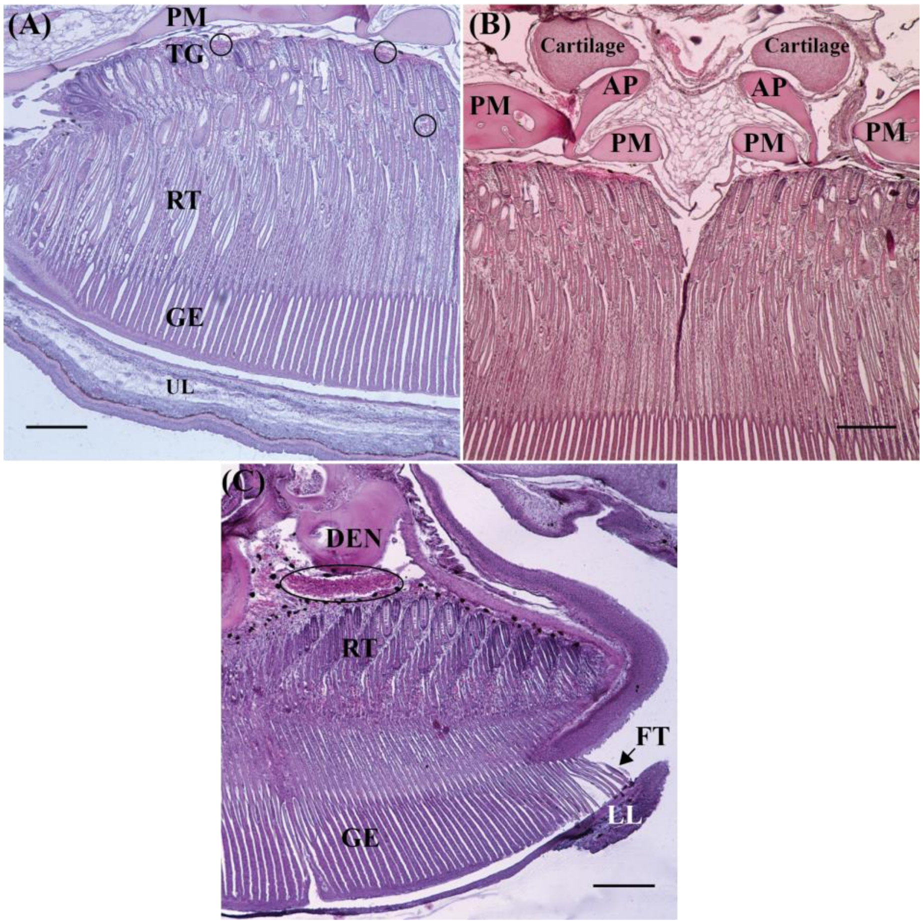

Figure 1.5. The upper and lower jaw morphology of *Alticus arnoldorum*. **(A)** Ventral orientation of a section of the left side upper jaw. Circles indicate vascularized tissue Scale bar = 0.2 mm. **(B)** Ventral orientation of the medial upper jaw, showing loose connective tissues situated between components of the upper jaw. Scale bar = 0.2 mm. **(C)** Dorsal orientation of the left side lower jaw tissues. Circle denotes pocket of vascularized tissue. Scale bar = 0.2 mm. All sections stained with hematoxylin and eosin and sectioned at 6μm thickness. Abbreviations: PM = premaxilla, TG = tooth germ, RT = replacement teeth, GE = gum epithelium, UL = upper lip, AP = ascending process of premaxilla, DEN = dentary, FT = functional teeth, LL = lower lip.

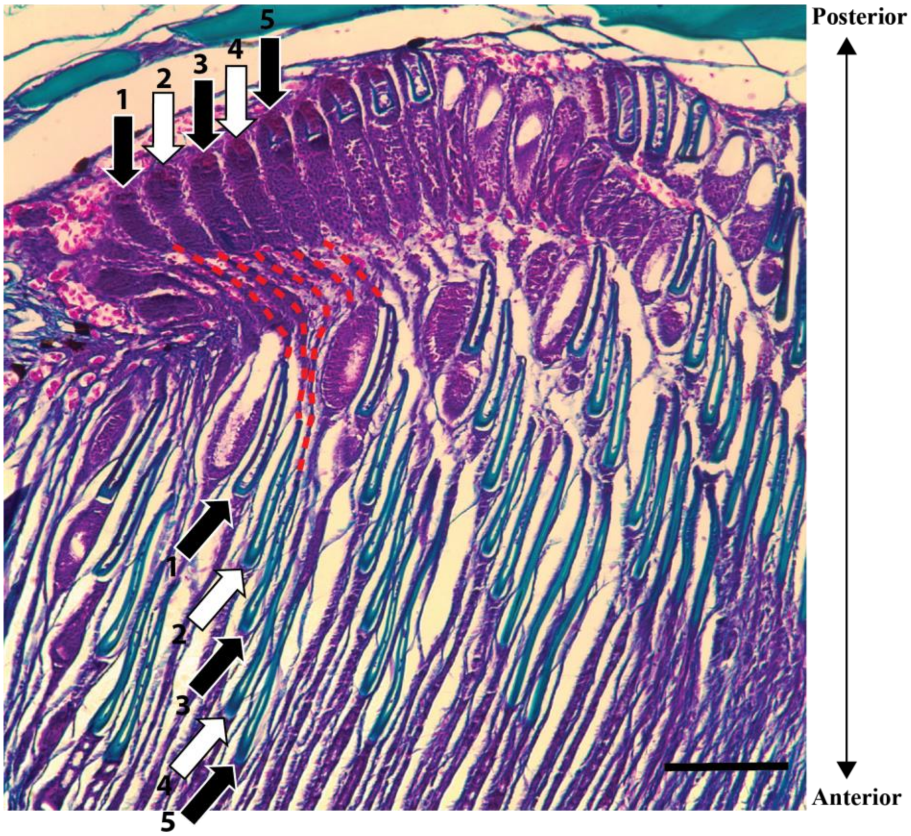

Figure 1.6. Milligan's trichrome stain of tooth replacement in *Alticus arnoldorum*. The alternating black and white arrows near the top of the image denote the individual epithelial invaginations of the dental lamina. Each arrow numbered 1-5 can be traced along the red-dotted line to the alternating black and white arrows near the bottom of the image, which denote the incoming replacement tooth associated with each respective dental lamina. The epithelial tracks associated with each respective epithelial invagination and replacement tooth is the permanent, discontinuous dental lamina. The teeth denoted with black and white arrows represent a group of replacement teeth, with approximately 5 replacement teeth present in each group. Image taken at 20X magnification, scale bar = 0.2 mm.

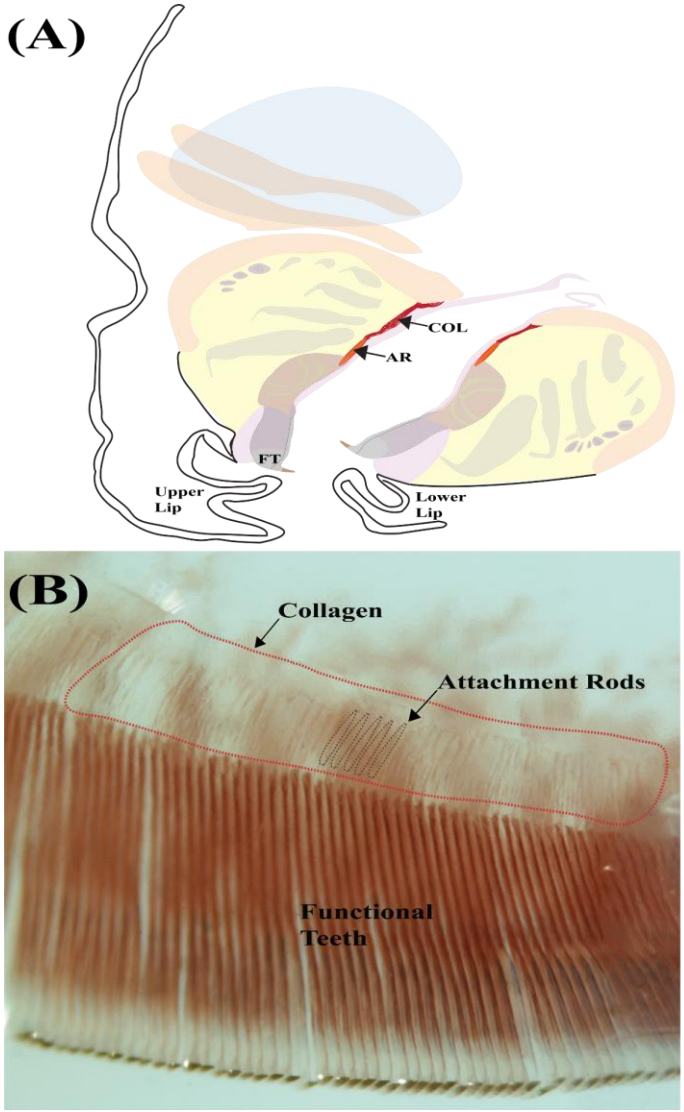

Figure 1.7. Primary tooth attachment in *Alticus arnoldorum*. **(A)** Illustration of oral jaw tissues, with only primary attachment structures highlighted. **(B)** Ventral view of cleared & stained premaxillary functional teeth and primary attachment tissues. Red-dashed outline indicates the band of collagen that connect the oral jaws to the attachment rods. Structures outlined in black dashes are attachment rods, which connect to the basses of the functional teeth and are surrounded by the band of collagen.

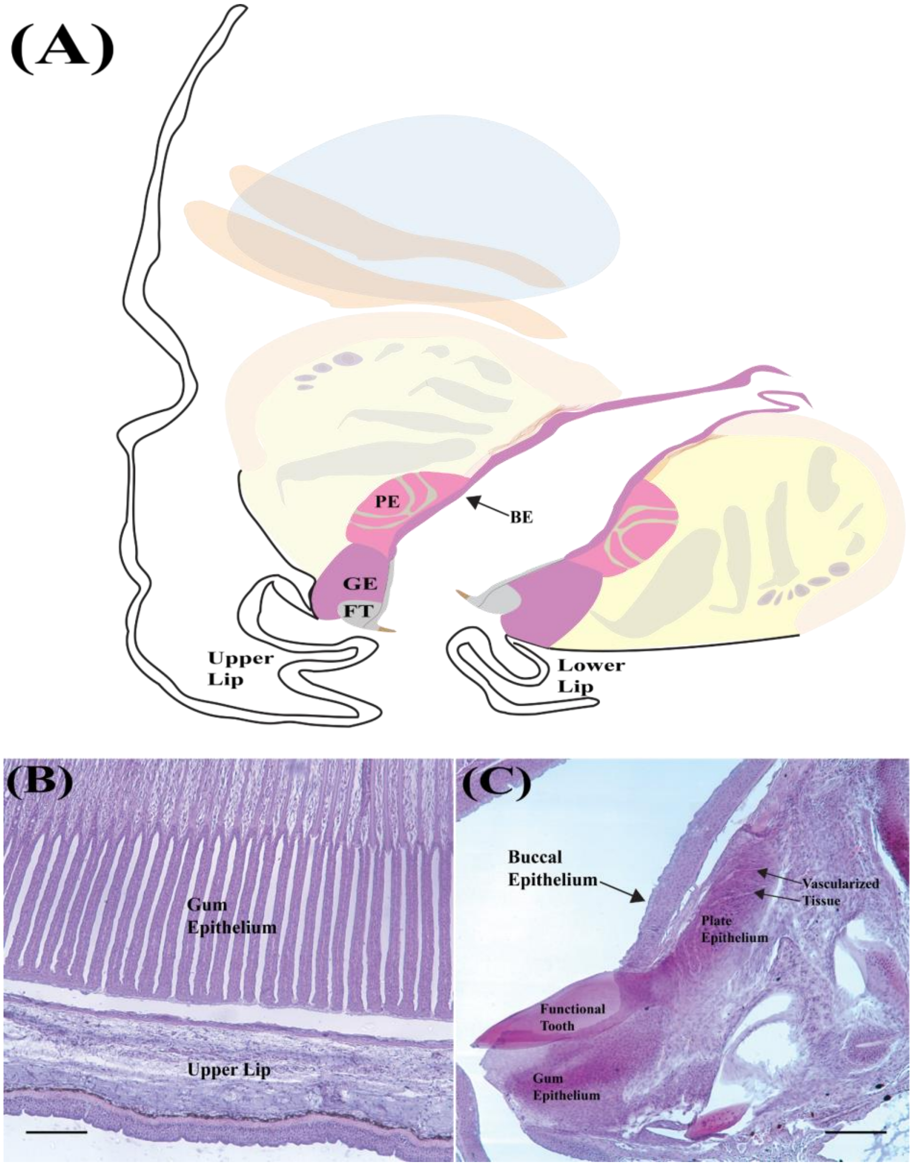

Figure 1.8. Secondary tooth attachment in *Alticus arnoldorum*. **(A)** Illustration of oral jaw tissues, with only secondary attachment structures highlighted. **(B)** Ventral orientation of upper jaw tissue, showing gum epithelium and upper lip tissue. Scale bar = 0.2 mm. **(C)** Left sagittal orientation of lower jaw tissue, showing secondary attachment modes of the functional tooth. Scale bar = 0.2 mm.

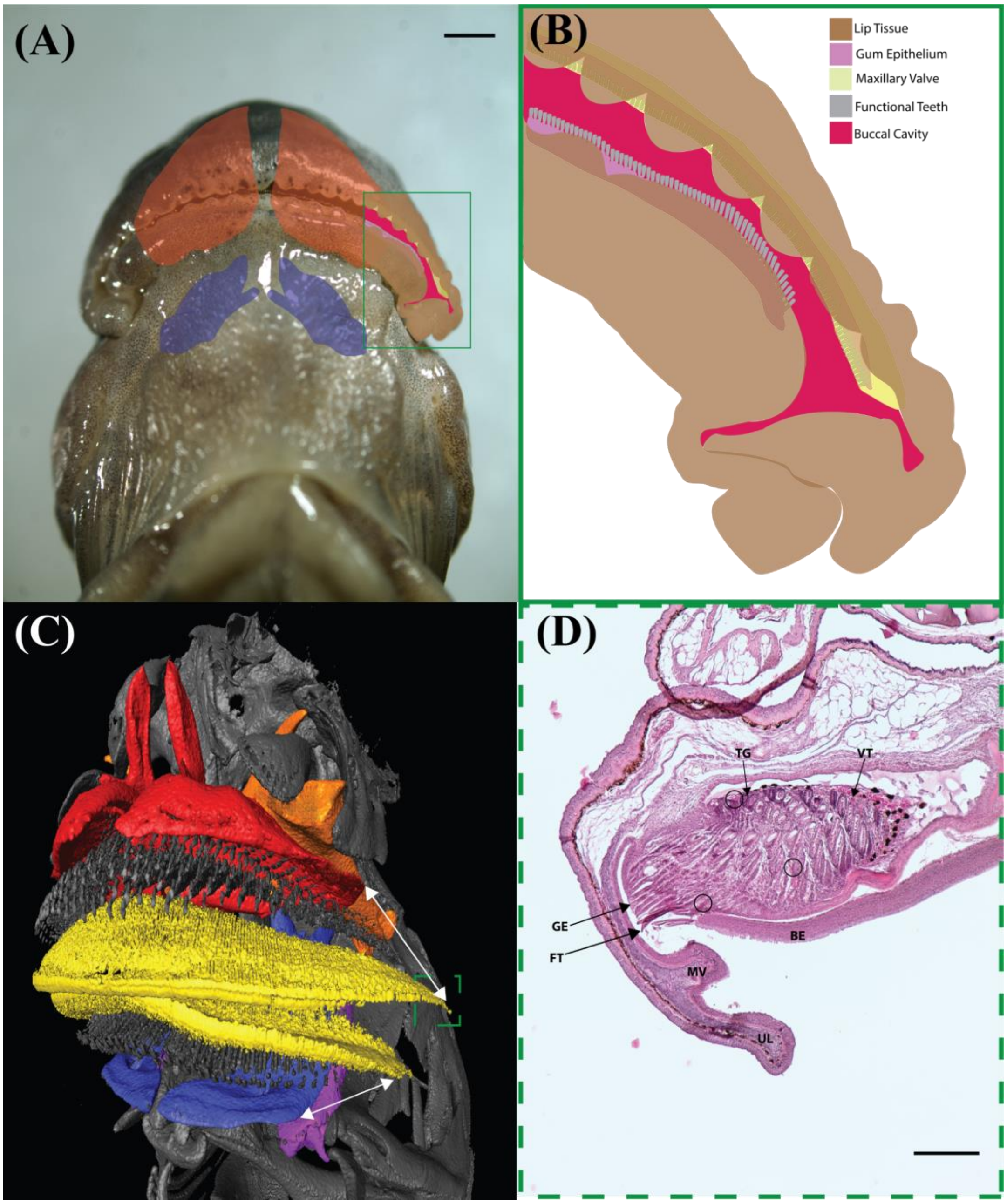

Figure 1.9. Distal lip tissue replacement in *Alticus arnoldorum*. **(A)** Ventral view of the head of *A. arnoldorum*. Positions of the premaxilla and dentary are illustrated in red and blue, respectively. Tissues outlined in the green box are illustrated. Scalebar = 1 mm. **(B)** Illustration of the tissues outlined in the green box in panel (A). **(C)** µCT scan of *A.*

arnoldorum in an anterior-lateral orientation. Color scheme of structures follows Figure 1B. The white arrows indicate the distance from the lateral edges of both the premaxilla and the dentary and the edges of the respective premaxilla and dentary distal lip tissues. **(D)** 10X magnified ventral orientation of a histology section through distal lip tissue. All components of tooth replacement are present. Black circles denote vascularized tissue. Scalebar = 0.2 mm. Abbreviations: TG = tooth germ, VT = vascular tissue, BE = buccal epithelium, UL = upper lip, MV = maxillary valve, FT = functional tooth, GE = gum epithelium.

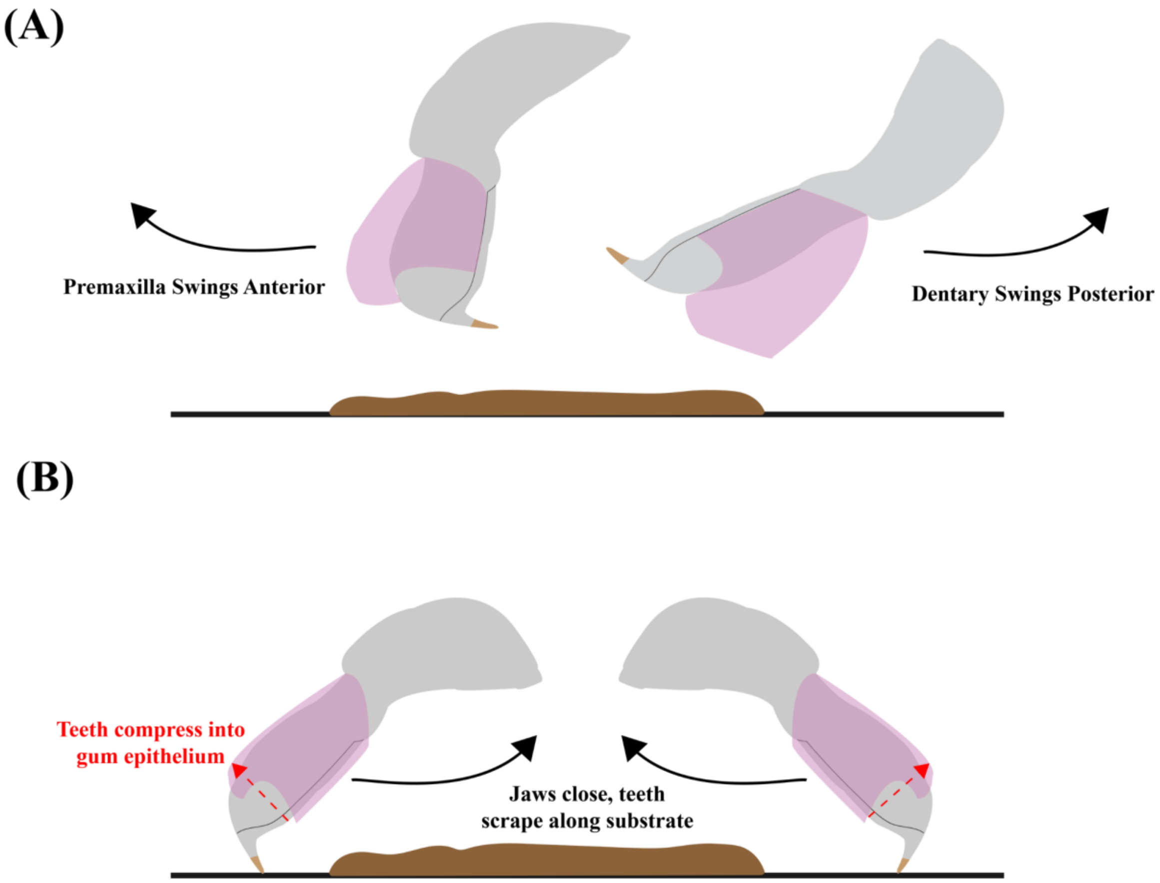

Figure 1.10. Hypothesis of *Alticus arnoldorum* tooth function. **(A)** Demonstrates tooth position prior to the oral jaws swinging open. **(B)** Demonstrates the point of contact on substrate, in which functional teeth will compress into the gum epithelium. Black arrows in both (A) and (B) illustrate the next kinematic motion to occur in the feeding process.

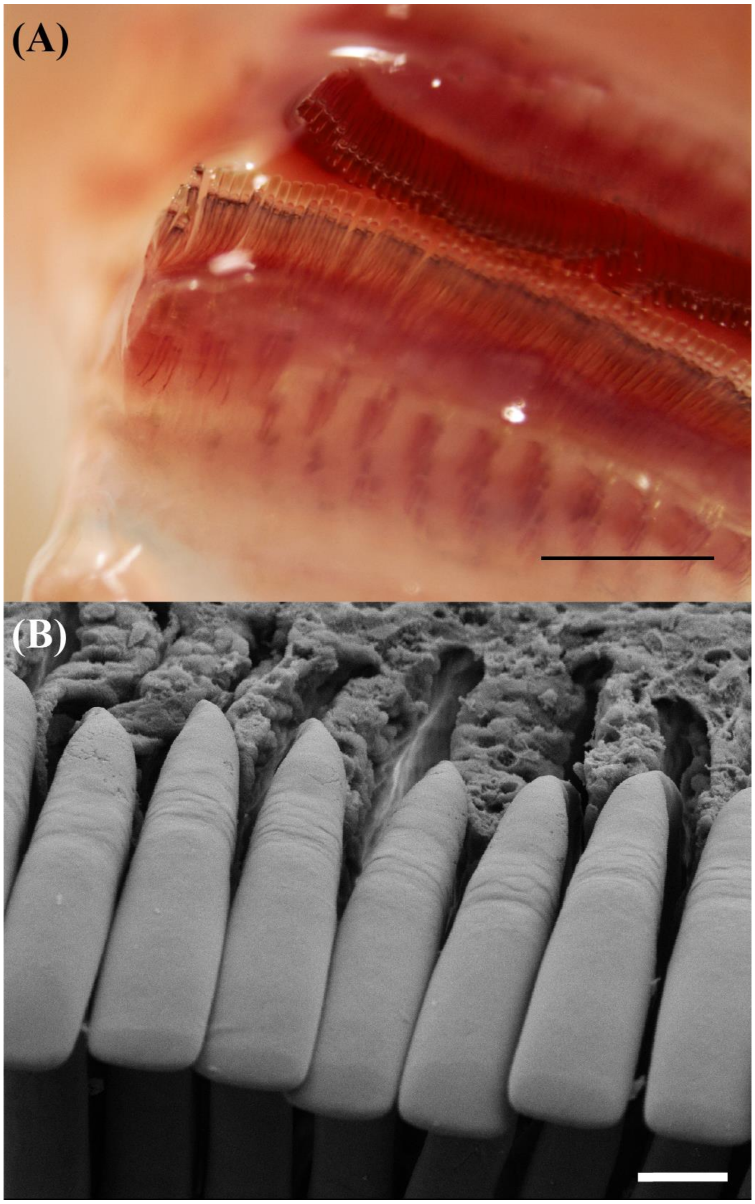

Figure 1.11. Morphological evidence of *Alticus arnoldorum* tooth function. **(A)** Anterior view of cleared & stained feeding teeth, showing mineralization at the tooth tips. Scale bar = 0.5 mm. **(B)** SEM of anterior tooth tips. Wrinkles at the base of each tooth tip could indicate region of soft dentin on the feeding tooth, allowing some degree of flexibility while feeding (Geerinckx et al, 2012). Scale bar = 20 µm.

Chapter 2. A re-evaluation of teleost tooth replacement using the dentally diverse combtooth blennies (Ovalentaria: Blenniidae).

Introduction

Continuous tooth replacement in teleost fishes is a long-studied trait relevant to understanding patterns of ecological diversification and evolutionary relationships among taxa (Fink, 1981; Trapani, 2001; Bemis et al., 2005; Bemis et al., 2019). However, little is known regarding the full extent of continuous tooth replacement pattern diversity across teleosts (Huyssuene & Witten, 2006). Modes of teleost tooth replacement were categorized by Trapani (2001) as one of two modes: extraosseous or intraosseous replacement. Extraosseous tooth replacement occurs in soft tissue outside the dentigerous (tooth bearing) bone, while intraosseously replaced teeth develop in sockets within the dentigerous bone. Trapani (2001) described extraosseous replacement as the plesiomorphic state for teleosts, while describing intraosseous replacement as a derived state and evolving at least three times. However, whether extraosseous replacement has re-evolved from an intraosseous state within teleost dental evolution remains unclear.

Recent work suggests that extra- and intraosseous modes of tooth replacement are likely opposite extremes on a continuum rather than discrete classifications (Conway et al., 2015; Chapter 1 of this thesis). In their study of clingfish (Gobiesocidae) dentition, Conway et al. (2015) found that developing replacement teeth were only partially enclosed within bone but showed a greater interaction with bone than what occurs in typical extraosseous replacement. The Pacific Leaping Blenny, *Alticus arnoldorum*, demonstrates an extreme case of extraosseous replacement in which teeth develop within soft distal lip tissue, and become loosely attached to the dentigerous bone via epithelium and collagen (Chapter 1 of this thesis). The intermediate nature of tooth replacement in the jaws of gobiesocids in concert with the extreme dissociation of tooth replacement from the jaw bones in *A. arnoldorum* suggests teleost tooth replacement is more complex than the extra- and intraosseous classification system and warrants further investigation.

The combtooth blennies (Blenniidae) are small, cryptobenthic fishes found in shallow, mostly marine communities in both tropical and temperate climates worldwide (Hastings & Springer, 2009). The common name "combtooth" is derived from the arrangement of their feeding teeth, which are positioned along the premaxilla and dentary in a single row, resembling a comb. The family consists of three major clades, Springerichthys, Salariini, and *Ecsenius* (Hundt et al., 2014), and dental patterns found throughout the family largely track with these evolutionary relationships (Hundt et al., 2018). Springerichthys is mostly comprised of lineages with feeding teeth that are fused to the bone of attachment, while salariin blennies possess feeding teeth that are not in direct contact with the jawbones, and instead are weakly attached via epithelium and connective tissue (Bath 2001, 2002; chapter 1 of this thesis). *Ecsenius* blennies possess dentition very similar to that of the salariin blennies, although they also possess anterior caniniform teeth positioned in the same row as the anterior feeding teeth along the dentary (Springer, 1968; Bath, 2001). However, anterior dentary canines will not be examined in this study.

Most blennies possess recurved canines positioned posteriorly on the dentary (Springer, 1968; Smith-Vaniz & Springer, 1971; Bath, 2001; Bath, 2002). These canines are thought to be used for intraspecific combat and interspecific defense, although their function has only been closely examined in a few Springerichthys lineages (Kotrschal & Goldschmid, 1992). Recurved canines are also located posteriorly on the premaxilla of most Springerichthys blennies, while the aforementioned anterior dentary canines are only present in *Ecsenius* lineages (Springer, 1968; Smith-Vaniz & Springer, 1971; Kotrschal & Goldschmid, 1992; Bath, 2001; Bath, 2002).

Despite the diversity in dental patterns, the mechanisms of tooth replacement and attachment in combtooth blennies has remained largely overlooked. Most prior studies have focused on the complex attachment and replacement of feeding teeth (Springer, 1968; Bath, 2001, 2002; Christiansen et al., 2010; Hundt et al., 2018; chapter 1 of this thesis), while canine attachment and replacement has received much less attention. Springer (1968) and Bath (2001) established general observations of canine replacement and attachment, although these studies focus primarily on feeding teeth to infer relationships among taxa. The question of how these canines are replaced, and whether they vary from the mode of feeding tooth replacement, has never been addressed. In addition, the evolution of tooth replacement modes across this group has never been examined. More broadly, it is also unknown across all teleosts whether extraosseous replacement has ever re-evolved from an intraosseous ancestral state. Therefore, this study will address the following questions using the diverse dentition of the combtooth blennies: (1) are canine teeth developmentally distinct from feeding teeth in combtooth blennies? (2) Is extraosseous tooth replacement derived from intraosseous tooth replacement in combtooth blennies? I expect that canines will exhibit an intraosseous mode of tooth replacement, and that intraosseous replacement will be the ancestral mode of tooth replacement in the combtooth blennies. Extraosseous replacement in blennies will therefore be a derived form of intraosseous replacement, furthering the necessity of understanding tooth replacement in teleosts as a continuum, rather than discrete modes of classification.

Materials and Methods

2.1 Terminology

The terminology in this study follows many of the definitions established in the first chapter of this thesis, including “replacement tooth”, “functional tooth”, “tooth locus”, and “tooth family”. These terms apply to the current study, although teeth are further specified as either “feeding” or “canine” teeth (i.e., “feeding replacement tooth” and “canine replacement tooth”). I use the term “feeding tooth” to describe the slender, elongate teeth located anteriorly in the jaws used to acquire food. Springer (1968) and Bath (2001) used the term “canine tooth” to describe the various recurved caniniform teeth that are found on both the premaxilla and dentary and their associated tissues. Bath (2001) further specifies canines based upon position in the jaw, i.e. anterior canines and posterior canines. I use similar terminology to describe canine teeth in this study, specifying canines first by position and then jaw. However, anterior canine replacement and attachment was not examined in this study.

The terms “fossa” and “foramen” have each been used to describe the openings in dentigerous bones in which feeding and canine teeth develop in combtooth blennies (Springer, 1968; Bath, 2001). Alternatively, Trapani (2001) uses the term “bony crypt” to describe the openings in which intraosseous tooth replacement occurs in teleosts. To simplify, I use the term “bony crypt” to describe the openings in which replacement teeth develop in cases of observed intraosseous replacement in this study.

2.2 Histology

Descriptions of canine and feeding tooth replacement and attachment in blennies are based on histological preparations. Samples in this study were prepared from formalin-fixed specimens deposited at the Bell Museum of Natural History. Blenny species examined include *Alticus arnoldorum* (n=5), *Andamia tetradactylus* (n=1), *Cirripectes castaneus* (n=1), *Entomacrodus striatus* (n=2), *Exallias brevis* (n=1), *Istiblennius edentulus* (n=1), *Rhabdoblennius snowi* (n=1), and *Salarias fasciatus* (n=1). Specimens ranged in size from 24.5-60.6 mm SL (Table 2.1). The oral jaws were removed via dissection, with incisions made around the premaxilla and dentary bones to remove the jaws. For longitudinal sections, upper and lower jaw tissues were kept intact, while in transverse sections upper and lower jaw tissues were separated to allow for individual examination of the tissues.

Samples were decalcified using a range of decalcifying agents including Decal® Overnight Bone Decalcifier, Formical-2000, and 10% ethylenediaminetetraacetic acid (EDTA). Decalcification time varied, both decalcifying agents and decalcification times are available in Table 2.1. Following decalcification, samples were dehydrated in an ethanol series to 100% ethanol, infiltrated with toluene, and subsequently embedded in paraffin. Paraffin sections were cut at six µm thickness using a Leica Jung 820 Histocut Rotary Microtome and mounted on slides. Sections were stained with Hematoxylin and Eosin and Alizarin Red stain using protocols modified from Humasson (1972).

2.3 Morphological Examination

A total of 111 specimens were examined for canine presence and replacement patterns in this study. 67 specimens were cleared & stained individuals available from the Bell Museum of Natural History (University of Minnesota, MN; Table 2.2). The other 54 specimens were microCT scanned, including two outgroup blennioid lineages (Table 2.3). Specimens for scanning were obtained from the Bell Museum of Natural History. Additional scans were acquired through the #ScanAllFish Open Science Framework initiative and included specimens from the Bell Museum and the Burke Museum (University of Washington, WA). Specimens were scanned both at the Karel F. Liem Bioimaging Center at Friday Harbor Laboratories with a Bruker Skyscan 1173 and at the X-Ray CT Laboratory in the University of Minnesota's Department of Earth and Environmental Sciences with an NSI X5000. Specimens were scanned at voxel sizes ranging from 8.5-35.5 µm (Table 2.3). Scans were reconstructed as .bmp stacks and extracted using Dataviewer software (2012-2017 Bruker microCT, version 1.5.6.2). Specimens were visualized using Bruker CTVox volume-render software (2012-2017

Bruker microCT, version 3.3.0) and *Amira* (1999-2019 Thermo Fisher Scientific, version 6.4.0), and segmented in *Amira*.

2.4 Comparative Methods

I used cleared & stained specimens along with microCT scanning to visualize and record dental traits in the jaws of combtooth blennies and outgroup specimens (Table 2.2; Table 2.3). The presence or absence of functional and replacement canines on the dentary and premaxilla were recorded, and the replacement mode of canine and feeding teeth was categorized as extraosseous, interosseous, or intraosseous replacement. Functional canines were considered present if a tooth was visible at the functional canine locus, and absent if no tooth was detected. If canines were not detected but are known in the literature to be a variable trait for a species, the absence was recorded but the trait was noted to vary within the species (Table 2.2; Table 2.3; Smith-Vaniz & Springer, 1971; Bath, 2001). In microCT scans, replacement canines could often be observed in the bony crypt beneath the functional canine locus in varying states of development. If present beneath one functional canine locus but not the other, the side undergoing replacement was recorded, and if both canines were undergoing replacement both sides were recorded. If no replacement canine was observed, replacement canines were recorded as absent (Table 2.3). Canine and feeding tooth replacement were recorded as derived extraosseous if teeth were replaced in soft tissue outside the dentigerous bone and considered intraosseous if replacement occurred within a bony crypt *sensu* Trapani (2001) (Table 2.2; Table 2.3). Tooth replacement was considered interosseous if the bony crypts in which teeth develop in either the premaxilla or dentary were only partially formed and if the replacement teeth exhibited closer association to the dentigerous bones.

I used the most recent combtooth blenny phylogeny (Hundt et al., 2014) to assess the evolution of feeding tooth replacement mode in blennies. I used an outgroup species from the blennioid family Labrisomidae (*Labrisomus nuchipinnis*). I trimmed the phylogeny using the [drop.tip] function *ape* (Paradis et al., 2004) to match specimens in my microCT dataset. I used stochastic character mapping (Bollback, 2006) to assess canine tooth evolution across taxa (0 = absence, 1 = presence, 2 = canine varies within species) and tooth replacement mode (0 = extraosseous, 1 = intermediate, 2 = intraosseous) with the [make.simmap] function in *phytools* (Revell, 2012). Ancestral states were reconstructed within a maximum likelihood framework to determine changes in traits across the phylogeny using the [ace] function in *geiger* (Harmon, Weir, Brock, Glor, & Challenger, 2007). Stochastic character mapping and ancestral state reconstruction simulations were performed with 100 replicates. All analyses were performed in the statistics software R (www.r-project.org).

Results

3.1 Caniniform tooth replacement and attachment modes in salariin blennies

Posterior dentary canines in salariin blennies are replaced intraosseously upon a direct dental lamina. Replacement canines develop one at a time within bony crypts ventral to the functional canine loci (Figure 2.1). The bony crypt is vascularized and consists of oral epithelium and associated tooth replacement cells (Figure 2.1B, C, E). As these replacement canines develop along the dental lamina, they erupt posteriorly to the functional canine loci via sub-epithelial replacement pores (Figure 2.1C). Replacement posterior dentary canines develop directly from the oral epithelium and are not associated with a previous tooth generation (Figure 2.1E, F), therefore fitting the definition of a direct dental lamina *sensu* Bemis & Bemis (2019). I found no obvious relationship between left and right canine replacement in any observed blenny taxa on either the premaxilla or dentary (Table 2.3, Figure 2.2). Individual canines appear to be replaced independently from one another, showing no signals of alternate or simultaneous replacement.

All canines exhibited tooth attachment closely resembling Fink's type 2 attachment mode (teeth attached via a band of unmineralized collagen; Fink, 1981). In all examined specimens, the band of collagen attaches at the base of the functional canines to the bone of attachment and is submerged under a layer of buccal epithelium (Figure 2.3B). The collagen band in most observed specimens is thin and resembles a fibrous dividing zone (Figure 2.1B, E, Figure 2.3B; Berkovitz & Shellis, 2019). This thin layer of collagen appears to thicken posteriorly, a region in close proximity to where replacement teeth erupt (Figure 2.3B).

3.2 Description of interosseous feeding replacement modes in *Rhabdoblennius snowi*

A partially formed dentary capsule is present in the species *Mimoblennius atrocinctus*, *Alloblennius pictus,* and *Rhabdoblennius snowi,* and is a deviation from the dentary morphology of other salariin blennies (Figure 2.4, Chapter 1 discussion in this thesis). I examined this morphology in *R. snowi* using histology and microCT. I found that tooth replacement is occurring within the partially formed dentary capsule and generally resembles the replacement patterns of other salariin blennies, although replacement teeth exhibit greater contact with the margins of the dentary (Figure 2.4, Chapter 1 discussion in this thesis). In contrast to the dentary, the premaxilla in *R. snowi* is comprised of two ventrally concave plates that are observed in most salariin blennies (Figure 2.4, Chapter 1 discussion in this thesis). Feeding tooth replacement in the premaxilla is also similar to the replacement of other salariin lineages, although replacement teeth exhibit a closer association to the premaxilla (Figure 2.4). I find that the premaxillary feeding teeth in *R. snowi* more closely resembles extraosseous replacement. However, functional feeding teeth in both the premaxilla and dentary demonstrate a tight collagen attachment to the jaws, rather than the loose collagen attachment found in other salariin lineages (Figure 2.4, Chapter 1 discussion of this thesis). Taking all of these traits into consideration, I find the lineages *M. atrocinctus*, *A. pictus*, and *R. snowi* exhibit an intermediate mode of tooth replacement with respect to extra- and intraosseous replacement. Given the intermediate morphology of this replacement mode, I describe this as an interosseous mode of replacement, and will make reference to this terminology throughout the remainder of the study.

3.3 Evolution of tooth replacement modes in combtooth blennies

The common ancestor of blennioids likely replaced oral jaw teeth intraosseously (Figure 2.5). An extraosseous feeding teeth replacement mode derived from an intraosseous ancestral state then evolved at least once at the common ancestor of combtooth blennies. This derived extraosseous state is largely conserved throughout both the *Ecsenius* and salariin clades (Figure 2.5). Two transitions to an interosseous replacement mode in dentary feeding teeth occurred in the common ancestor of *Rhabdoblennius* and again in the common ancestor of *Mimoblennius* and *Alloblennius* (Figure 2.5). An additional transition to an interosseously replaced dentary occurs in the common ancestor of *Plagiotremus* blennies. Derived extraosseous replacement reverted to intraosseous replacement at the common ancestor of Springerichthys blennies and remained the dominant mode of tooth replacement throughout this clade. When present, all posterior canines on both the premaxilla and dentary are replaced intraosseously (Figure 2.1).

Discussion

Teleost fishes exhibit a wide range of tooth replacement patterns (Huyssuene & Witten, 2006). These patterns have historically been categorized to fit into two discrete modes of tooth replacement, extraosseous and intraosseous replacement (Trapani, 2001). Trapani (2001) found that extraosseous replacement is the ancestral state for teleosts, with intraosseous serving as a derived state. However, recent work has challenged these dichotomous extra- and intraosseous modes to encompass a tooth replacement continuum, with both of these modes representing opposite ends of the continuum (Conway et al., 2015; chapter 1 of this thesis). In this current chapter, I further expand upon the extra- and intraosseous continuum concept by conducting a broad investigation of tooth replacement across the combtooth blennies. I first describe the morphology of caniniform tooth replacement and attachment in blennies and then compare it with feeding tooth replacement to determine if these tooth morphs are developmentally distinct. I find that posterior canines on both the premaxilla and dentary are replaced intraosseously upon a direct dental lamina, with no obvious pattern of coordination between either left or right-side canines across the family (Table 2.3; Figure 2.7). Developed canines attach via a thin band of unmineralized collagen that thickens posteriorly, and that closely resembles Fink's type 2 tooth attachment. I also find the morphology of posterior canine tooth development to differ markedly from the feeding tooth replacement found in most Salariini and *Ecsenius* blennies. In contrast, Springerichthys blennies demonstrate canine replacement similar to the morphology of feeding teeth replacement found throughout this group. I also find that numerous lineages exhibit an intermediate replacement mode in which teeth develop with a close association to bony particles dissociated from the jaw bones, while still developing within soft tissue. I describe this as interosseous replacement. Finally, I propose that the extraosseous feeding tooth replacement present throughout Salariini and *Ecsenius* blennies is derived from an intraosseous ancestral state. This proposed framework of interosseous replacement and the presence of a derived extraosseous replacement lends further support to the tooth replacement continuum concept proposed in Conway et al. (2015).

4.1 The complex dental lamina and salariin tooth replacement

Canine teeth in Salariins are intraosseously replaced upon a direct dental lamina (Figure 2.1). This dental lamina derives directly from the oral epithelium and is not associated with a previous tooth generation. The direct dental lamina is also documented across numerous lineages with intraosseous replacement (Bemis et al., 2005; Bemis & Bemis, 2015; Bemis et al., 2019). This classification contrasts with the successional lamina, which is associated with extraosseous replacement and occurs when a new tooth germ is formed from the epithelium of a preceding tooth generation (Huysseune, 2006). However, the dental lamina is a complex dental organ that was previously established as two non-mutual classifications of permanent/non-permanent, and continuous/discontinuous (Reif, 1982; Moriyama et al., 2010; Chapter 1 discussion in this thesis). I find the direct dental lamina classification established by Bemis et al. (2019) to most effectively describe canine tooth replacement in the Salariins. Tooth germs are seen developing within the bony crypt, deriving directly from the oral epithelium along sub-epithelial replacement pores independent from previous tooth generations (Figure 2.1B, 2.1F). However, this is not to say that the classifications of Reif (1982) are inaccurate; the dental lamina of salariin canines can also be accurately described as a non-permanent, discontinuous dental lamina. Rather, the direct dental lamina classification provides a simpler explanation of tooth generation in salariin canines. In the future, a synthesis of dental lamina classifications for teleosts would provide significant guidance for dental morphology research.

Intraosseous replacement in teleosts is frequently associated with coordination in tooth replacement of specific tooth loci. Bluefish and related scombrids display alternate replacement of teeth (Bemis et al. 2019), Triple tails exhibit grouped replacement (Hilton & Bemis, 2005), while serrasalmids replace all teeth on one side of the jaws simultaneously (Kolmann et al. 2019). In contrast, I found no consistent pattern of canine tooth replacement between either side of the dentary across microCT-scanned specimens (Table 2.3). Even in specimens in which replacement canines are visible on both sides of the jaw, often the developing canines differ in position and size, indicating a difference in developmental stages (Figure 2.2). Given that canines are used in agonistic interactions in some blennies (Kotrschal & Goldschmid, 1992), it is possible that these teeth are used infrequently relative to feeding teeth, and therefore require less regular replacement. In addition, my observations suggest that functional canine loss is initiated by the posterior eruption of replacement canines, and is intrinsically regulated, rather than extrinsically regulated by reaching a wear-induced threshold (Fraser et al., 2006; Kolmann et al., 2019). Experimental observations of tooth loss to determine tooth replacement rates of canine and feeding teeth in Salariins would provide further clarity into this apparent variation in tooth replacement regulation. Additionally, this group provides a useful framework to test whether extraosseous replacement confers higher rates of tooth replacement in comparison to intraosseous replacement among teleosts.

4.2 Expanding the teleost tooth replacement continuum

Extraosseous feeding tooth replacement is present across most of the Salariini and throughout all *Ecsenius* lineages, and it likely evolved at the common ancestor of all combtooth blennies (Figure 2.5). However, the ancestor of all blennioids likely replaced its teeth intraosseously, suggesting this extraosseous replacement is a derived form of extraosseous replacement originating from an intraosseous ancestor (Figure 2.5, Table 2.3). The presence of intraosseously replaced posterior canines along the premaxilla and dentary throughout extant blennies further supports this hypothesis of a derived extraosseous replacement mode (Figure 2.1, Table 2.2, Table 2.3). When considered outside of an evolutionary context and using the classic, dichotomous classification of extra- and intraosseous tooth replacement modes established by Trapani (2001), the dentition found throughout many Salariini and *Ecsenius* blennies presents a dental paradox. Extraosseous feeding teeth and intraosseous canine replacement within the same jaws seemingly presents a striking dichotomy of two different tooth replacement modes. However, when taking evolutionary relationships into account and interpreting teleost tooth replacement modes as a continuum rather than discrete categories (Conway et al., 2015; Chapter 1 of this thesis), we can interpret extraosseous tooth replacement in blennies as a highly derived form of intraosseous replacement (Figure 2.5).

Several hypothetical explanations exist to describe intraosseous feeding tooth replacement in the Springerichthys. Intraosseous tooth replacement in this group could indicate a reversion to the plesiomorphic condition of blennioid tooth replacement

(Figure 2.5). However, this would require reconstruction of the jaw bones lost through formation of the derived extraosseous state, in addition to a reconfiguration of the typically more robust Springerichthys teeth. Alternatively, feeding tooth replacement in *Ecsenius* might have convergently evolved with the Salariini, and Springerichthys instead have retained the ancestral blennioid replacement mode. While this is a possibility—the feeding teeth replacement in *Ecsenius* lineages exhibit numerous trait similarities with salariin tooth replacement, including teeth replaced in distal lip tissue, dissociation of the tooth germ from the dentigerous bone, and teeth attached via collagen and epithelium (chapter 1 of this thesis; unpublished data)—it remains unclear whether these traits are convergent or conserved from a derived extraosseous ancestor. Closer observations of *Ecsenius* tooth attachment in comparison to the Salariini will aid in resolving this question. A third explanation is inaccuracy of the phylogenetic hypothesis of combtooth blennies used in this study. It is possible that *Ecsenius* is actually a sister clade to Salariini, and Springerichthys is instead sister to all other blennies. A phylogenomic approach to establishing a phylogeny might provide further phylogenetic resolution of the clade, as the current phylogeny uses a multi-locus methodology (Hundt et al., 2014). However, a detailed comparison of feeding teeth attachment between Springerichthys and *Ecsenius* blennies will also provide crucial insight towards resolving this issue. Future studies following the methods established in chapter 1 of this thesis that examine species from both *Ecsenius* and Springerichthys will provide a reliable framework to test hypotheses and further resolve the evolution of tooth replacement modes in blennies.

The interpretation of teleost tooth replacement upon a continuum also provides a structural framework to understand intermediate states of tooth replacement. The interosseous replacement morphology within the dentaries of numerous blennies demonstrate that discrete modes of categorization do not account for all teleost dentitions. This idea was first expressed in Conway et al. (2015), wherein their analysis of gobiesocid tooth replacement morphology was found to be an intermediate mode between extra- and intraosseous replacement. The close phylogenetic relationship of Gobiesocidae to Blenniidae might suggest that interosseous dentitions are rare across teleosts and conserved only to this monophyletic clade. However, beyond broad surveys of taxa (Trapani et al., 2001; Bertrand, 2014) and a small collection of studies examining specific teleost lineages (Bemis & Bemis, 2015; Bemis et al., 2019; Hilton & Bemis, 2015; Bemis et al., 2005; Kolmann et al., 2019; Geerinckx et al., 2007) very little is still known regarding the full diversity of tooth replacement modes across teleosts. To understand the range and evolution of tooth replacement modes across teleosts, further studies of tooth replacement are needed. In conducting future studies, it will be important to maintain an understanding of phylogenetic history among taxa when describing tooth replacement modes, as transitions across different replacement modes might mirror relevant changes in ecology or functional morphology.

The disparity in replacement morphology between feeding and canine teeth likely diverged early in the combtooth blennies, as canines are found throughout the clade and are a plesiomorphic trait within the group (Table 2.2, Table 2.3; unpublished data). The divergence in tooth morphology and replacement modes between feeding and canine teeth may suggest a variation in selective pressures along the jaws. This selective variation appears to create two separate modules of tooth replacement between the feeding and canine teeth. The reduction of dentigerous bone and expansion of the feeding teeth germline away from dentigerous bone and into novel soft tissues likely relates to the variation of tooth replacement patterns across blennies. Literature on the interaction between bone, teeth, and expression of genes responsible for odontogenesis and tooth production in teleosts has largely focused on model systems, such as rainbow trout and cichlids (Fraser et al., 2004; Fraser et al., 2013). However, little focus has been directed at the developmental differences between intraosseous and extraosseously replaced dentitions. To better understand how posterior canines retain an intraosseous replacement mode while feeding teeth evolved into a derived extraosseous pattern, more understanding of the interactions between gene expression, bone and tooth development in blennies and other teleosts possessing with both intra- and extraosseously replaced dentitions alike is warranted.

Future work for publication

More data collection is required to address the questions proposed in this chapter. Question one will require further histological analysis of Springerichthys and *Ecsenius* blennies dentition to determine the nature of tooth replacement in these lineages. In specific, I need to determine if the dental lamina is successional or direct for both feeding and canine teeth in these lineages. I predict that posterior canines will exhibit a direct dental lamina that will reflect the pattern of canine replacement I found in salariin lineages (Figure 2.1). I predict that Springerichthys feeding teeth will also exhibit a direct dental lamina, as this type of dental lamina is known to occur in numerous other species with intraosseous oral jaw tooth replacement (Bemis et al., 2005; Bemis & Bemis, 2015; Thiery et al., 2017; Bemis & Bemis, 2019). I also need to determine the tooth attachment type in these lineages. I expect to find type 2 attachment modes for posterior canines on both the premaxillary and dentary, given that this is the attachment type found for salariin canines (Figure 2.2).

To answer question two, the relationship between replacement tooth development and dentigerous bone needs to be further investigated across all blennies. It is evident from salariin lineages with interosseous tooth replacement that the dentary is partially formed around replacement teeth, and functional teeth attach in much closer proximity to the dentigerous bones (Figure 2.4). Expansion of my sampling to include non-salariin lineages and additional outgroup specimens (*Gobiesox maeandricus*, Gobiesocidae; *Enneapterygius minutus*, Tripterygiidae) will provide a holistic comparison of the interaction of tooth and bone across combtooth blennies and their immediate relatives. In addition, my phylogenetic comparative analyses show that the ancestral mode of feeding tooth replacement for blennioids is likely intraosseous (Figure 2.5). Considering that all posterior canines are replaced intraosseously, these comparative analyses support my hypothesis that extraosseous replacement in combtooth blennies is derived from an ancestral intraosseous state. Additional histological sectioning of *Labrisomus nuchipinnis* (Labrisomidae) and *G. maeandricus* (Gobiesocidae) will provide comparative inference regarding what the ancestral state of tooth replacement in combtooth blennies could have looked like, and further support my derived extraosseous replacement hypothesis.

Species	Museum catalogue no.	Standard length (mm)	Decalcification solution	Time decalcified (hours)	Stain
Alticus arnoldorum	JFBM 46263	49.53	Cal-Ex	25	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	47.21	Cal-Ex	24	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	55.41	Formical 2000	66	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	51.37	Cal-Ex	24	Hematoxylin & Eosin
Alticus arnoldorum	JFBM 46263	43.56	10% EDTA	264	Hematoxylin & Eosin
Andamia tetradactylus	JFBM 46748	50.66	Cal-Ex	18	Hematoxylin & Eosin
Cirripectes castaneus	JFBM 47119	31.6	10% EDTA	288	Hematoxylin & Eosin
Entomacrodus striatus	JFBM 46350	60.6	Cal-Ex	48	Alizarin Red
Entomacrodus striatus	JFBM 46350	45.84	Cal-Ex	45	Hematoxylin & Eosin
Exallias brevis	JFBM 47007	24.49	10% EDTA	288	Hematoxylin & Eosin
Istiblennius edentulus	JFBM 47001	51.84	Cal-Ex	18	Hematoxylin & Eosin
Rhabdoblennius snowi	JFBM 49628	18.7	10% EDTA	288	Hematoxylin & Eosin
Salarias fasciatus	JFBM 47049	62.07	10% EDTA	288	Hematoxylin & Eosin

Table 2.1. Specimens used for histology, including sample decalcification time and stain used. All specimens were obtained through the Bell Museum of Natural History (JFBM).

Species	**Catalogue Number**	**Dentary Canines**	**Dentary Canine Replacement Mode**	**Premaxilla Canines**	**Premaxilla Canine Replacement Mode**	**Feeding Teeth Replacement Mode**
Alticus anjouanae	JFBM 47727	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Alticus arnoldorum	JFBM 46263	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Alticus sp. (saliens)	JFBM 47067	Absent	N/A	Absent	N/A	Derived Extraosseous
Andamia reyi	JFBM 47842	Absent	N/A	Absent	N/A	Derived Extraosseous
Andamia reyi	JFBM 47842	Absent	N/A	Absent	N/A	Derived Extraosseous
Andamia tetradactylus	JFBM 46748	Absent	N/A	Absent	N/A	Derived Extraosseous
Blenniella bilitonesis	JFBM 47058	Present	Intraosseous	Absent	N/A	Derived Extraosseous
Blenniella chrysospilos	JFBM 46372	Present	Intraosseous	Absent	N/A	Derived Extraosseous
Blenniella gibbifrons	JFBM 19203	Present	Intraosseous	Absent	N/A	Derived Extraosseous
Blenniella periopthalmus	JFBM 47048	Present	Intraosseous	Absent	N/A	Derived Extraosseous
Chasmodes saburrae	JFBM 46414	Absent	N/A	Absent	N/A	Intraosseous

Cirripectes obscurus	JFBM 19187	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Cirripectes variolosus	JFBM 19178	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Crossosalarias macrospilus	JFBM 47108	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius aroni	JFBM 47176	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius bicolor	JFBM 47044	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius bimaculatus	JFBM 48599	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius bimaculatus	JFBM 48599	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius namiyei	JFBM 46724	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Ecsenius opsifrontalis	JFBM 46380	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous

Ecsenius stictus	JFBM 47288	Anterior and Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus marmoratus	JFBM 19204	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus sealei	JFBM 46267	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus stellifer	JFBM 47149	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus striatus	JFBM 47854	posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus thalassinus	JFBM 47717	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Entomacrodus vermiculatus	JFBM 47713	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Exallias brevis	JFBM 46766	Absent	N/A	Absent	N/A	Derived Extraosseous
Glyptoparus delicatulus	JFBM 46369	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Hypsoblennius hentz	JFBM 46471	Absent	N/A	Absent	N/A	Intraosseous
Istiblennius lineatus	JFBM 46750	Absent	N/A	Absent	N/A	Derived Extraosseous
Lipophrys pholis	JFBM 48571	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Meiacanthus atrodorsalis	JFBM 47081	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Meiacanthus kamoharai	JFBM 46739	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous

Meiacanthus oualanensis	JFBM 48598	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus banditus	JFBM 37501	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus elongatus	JFBM 47709	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus fasciolatoceps	JFBM 47139	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus fasciolatoceps	JFBM 47139	Absent	N/A	Absent	N/A	Intraosseous
Omobranchus germaini	JFBM 47796	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus longispinis	JFBM 46756	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Omobranchus loxozonus	JFBM 46727	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Parablennius pilicornis	JFBM 37491	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Parablennius ruber	JFBM 47170	Posterior Present	Intraosseous	Absent	N/A	Intraosseous
Parablennius yatabei	JFBM 47005	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Petroscirtes breviceps	JFBM 47034	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Petroscirtes mitratus	JFBM 46355	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Plagiotremus rhinorhynchos	JFBM 47003	Posterior Present	Intraosseous	Absent	N/A	Interosseous
Plagiotremus tapeinosoma	JFBM 46389	Posterior Present	Intraosseous	Absent	N/A	Interosseous

Praealticus labrovittatus	JFBM 46353	Absent	N/A	Absent	N/A	Derived Extraosseous
Praealticus margaritarius	JFBM 46729	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Praealticus poptae	JFBM 46352	Absent	N/A	Absent	N/A	Derived Extraosseous
Praealticus striatus	JFBM 47805	Absent	N/A	Absent	N/A	Derived Extraosseous
Praealticus tanegasimae	JFBM 47102	Absent	N/A	Absent	N/A	Derived Extraosseous
Rhabdoblennius nitidus	JFBM 47130	Posterior Present	Intraosseous	Absent	N/A	Interosseous
Rhabdoblennius snowi	JFBM 46398	Posterior Present	Intraosseous	Absent	N/A	Interosseous
Salaria pavo	JFBM 48574	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous
Salarias alboguttatus	JFBM 47307	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Salarias fasciatus	JFBM 47093	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Salarias holomelas	JFBM 46706	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Salarias luctuosus	JFBM 46718	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Salarias ramosus	JFBM 48600	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Salarias sinuosus	JFBM 46697	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Scartella cristata	JFBM 46275	Posterior Present	Intraosseous	Absent	N/A	Intraosseous

Scartella emarginata	JFBM 47155	Posterior Present	Intraosseous	Absent	N/A	Intraosseous
Scartichthys viridis	JFBM 46849	Posterior Present	Intraosseous	Absent	N/A	Derived Extraosseous
Xiphasia setifer	JFBM 46996	Posterior Present	Intraosseous	Posterior Present	Intraosseous	Intraosseous

Table 2.2. Cleared & stained specimens and dental traits used for comparative analyses.

Species	Catalogue Number	Dentary Canines	Dentary Canine Replacement Position	Dentary Canine Replacement Mode	Premaxilla Canines	Premaxilla Canine Replacement Position	Premaxilla Canine Replacement Mode	Feeding Teeth Replacement Mode
Alloblennius pictus	JFBM 47174	Absent	N/A	N/A	Absent	N/A	N/A	Interosseous
Alticus arnoldorum	JFBM 46263	Posterior Present (varies)	Both sides visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Alticus arnoldorum	JFBM 49537	Posterior Present (varies)	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Alticus arnoldorum*	JFBM 46349	Posterior Present (varies)	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Alticus saliens *	JFBM 47823	Absent (varies)	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous
Andamia tetradactylus*	JFBM 47103	Absent	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous
Blenniella caudolineata	JFBM 46401	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Blenniella gibbifrons*	UW 115485	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Blenniella paula	JFBM 49615	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Blennius	JFBM 47164	Posterior Present	Both sides visible	Intraosseous	Posterior Present	Left side visible	Intraosseous	Intraosseous

ocellarius								
Chasmodes bosquinas*	UW 004083	Absent	N/A	N/A	Absent	N/A	N/A	Intraosseous
Chasmodes saburrae	JFBM 46414	Absent	N/A	Intraosseous	Absent	N/A	N/A	Intraosseous
Cirripectes castaneus	JFBM 46734	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Cirripectes quagga	JFBM 46373	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Cirripectes variolosus*	UW 015462	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Coryphoblennius galerita	JFBM 48573	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Intraosseous
Crossosalarias macrospilus	JFBM 47286	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Ecsenius bicolor	JFBM 47044	Anterior and Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Ecsenius lineatus	JFBM 47801	Anterior and Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous

Enchelyurus kraussi	JFBM 46759	Posterior Present	Not visible	Intraosseous	Posterior Present	Not visible	Intraosseous	Intraosseous
Entomacrodus decussatus	JFBM.47844	Posterior Present	Left side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Entomacrodus stellifer *	JFBM 47142	Posterior Present	Right side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Entomacrodus stellifer *	JFBM 47142	Posterior Present	Right side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Entomacrodus striatus	JFBM 49621	Posterior Present	Left side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Exallias brevis	JFBM 47090	Absent	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous
Gibbonsia elegans	JFBM 18402	Absent	N/A	N/A	Absent	N/A	N/A	Intraosseous
Glyptoparus delicatulus	JFBM 47016	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Hypleurochilus geminatus	JFBM 46839	Posterior Present	Both sides visible	Intraosseous	Posterior Present	Both sides visible	Intraosseous	Intraosseous
Istiblennius edentulus	JFBM 49548	Absent (varies)	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous

Istiblennius edentulus	JFBM 49619	Absent (varies)	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous
Istiblennius lineatus	JFBM 47071	Absent (varies)	N/A	N/A	Absent	N/A	N/A	Derived Extraosseous
Labrisomus nuchipinnis	JFBM 46255	Absent	N/A	N/A	Absent	N/A	N/A	Intraosseous
Lipophrys pholis	JFBM 48571	Posterior Present	Both sides visible	Intraosseous	Posterior Present	Both sides visible	Intraosseous	Intraosseous
Meiacanthus kamoharai	JFBM 47002	Posterior Present	Both sides visible	Intraosseous	Posterior Present	Not visible	Intraosseous	Intraosseous
Mimoblennius atrocinctus	JFBM 47795	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Interosseous
Nannosalarias nativitatus	JFBM 46732	Posterior Present	Both sides visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Omobranchus elegans	JFBM 47136	Posterior Present	Not visible	Intraosseous	Posterior Present	Not visible	N/A	Intraosseous
Omobranchus fasciolatoceps*	JFBM 47139	Posterior Present (varies)	Left side visible	Intraosseous	Posterior Present (varies)	Left side visible	Intraosseous	Intraosseous
Ophioblennius	JFBM 46840	Posterior Present	Both sides visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous

atlanticus								
Petroscirtes breviceps	JFBM 47793	Posterior Present	Both sides visible	Intraosseous	Posterior Present	Right side visible	Intraosseous	Intraosseous
Petroscirtes mitratus*	JFBM 46387	Posterior Present	Not visible	Intraosseous	Posterior Present	Not visible	Intraosseous	Intraosseous
Plagiotremus laudandus*	JFBM 47084	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Interosseous
Plagiotremus rhinorhynchos	JFBM 48515	Posterior Present	Both sides visible	Intraosseous	Absent	N/A	N/A	Interosseous
Praealticus margaritarius	JFBM 46753	Posterior Present (varies)	Right side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Praealticus poptae	JFBM 49622	Posterior Present (varies)	Left side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Rhabdoblennius snowi	JFBM 49558	Posterior Present	Both sides visible	Intraosseous	Absent	N/A	N/A	Interosseous
Salaria pavo	JFBM 48574	Posterior Present	Left side visible	Intraosseous	Posterior Present	Not visible	Intraosseous	Intraosseous
Salarias fasciatus	JFBM 49544	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Salarias	UW 012005	Posterior Present	Not visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous

fasciatus*								
Scartichthys variolatus	JFBM 46848	Posterior Present	Both sides visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Scartichthys viridis	JFBM 46846	Posterior Present	Left side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Stanulus talboti	JFBM 47098	Posterior Present	Left side visible	Intraosseous	Absent	N/A	N/A	Derived Extraosseous
Xiphasia setifer	JFBM 48984	Posterior Present	Left side visible	Intraosseous	Posterior Present	Both sides visible	Intraosseous	Intraosseous

Table 2.3. Specimens used for microCT scanning and dental traits used for comparative analyses. ‘*’ denotes a specimen acquired through the #ScanAllFish Open Science Framework initiative.

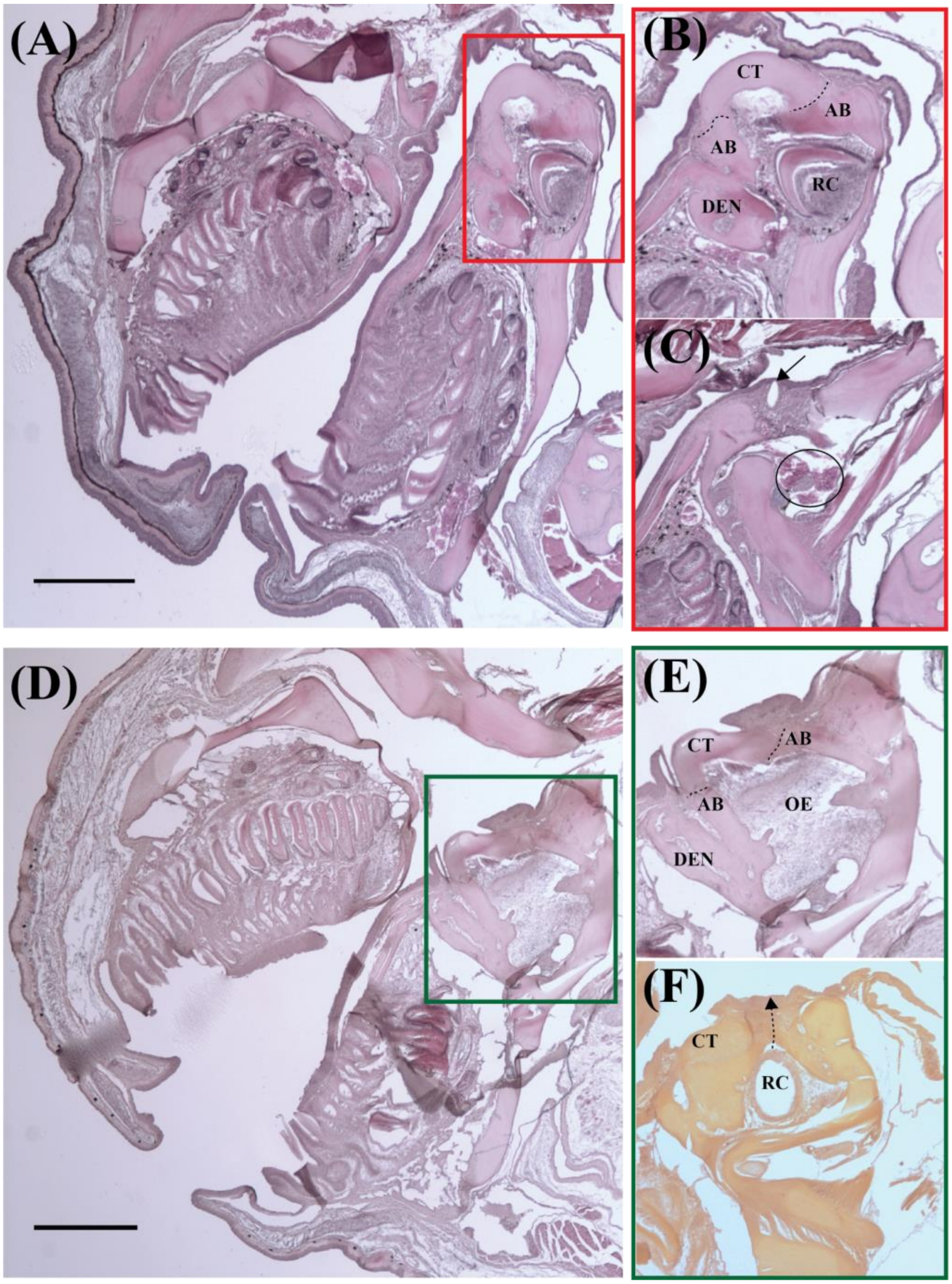

Figure 2.1. Dentary canine tooth replacement in salariin blennies. **(A)** Hematoxylin and eosin stained section of *A. arnoldorum* oral jaws. Scale bar = 0.5 mm. Red box indicates tissues

present in (B) and (C). **(B)** *A. arnoldorum* functional canine and canine replacement tissues. Dotted line indicates band of collagen between the functional canine and bone of attachment. **(C)** Canine replacement pore and vascularized tissue. Arrow indicates canine replacement pore, and vascularized tissue is indicated by a circle. This section occurs sequentially after the section present in (A) and (B). The replacement pore is associated with the replacement canine in (B). **(D)** Hematoxylin and eosin stained section of *E. striatus* oral jaws. Scale bar = 0.5 mm. Green box indicates tissues present in (E) and (F). **(E)** *E. striatus* functional canine and oral epithelium. Dotted line indicates band of collagen between the functional canine and bone of attachment. **(F)** Alizarin red stained section of a developing replacement canine posterior to the functional canine. Dotted arrow indicates direction of replacement canine development. This section occurs sequentially after the section present in (D) and (E). Abbreviations: CT = canine tooth, AB = attachment bone, RC = replacement canine, DEN = dentary, OE = oral epithelium.

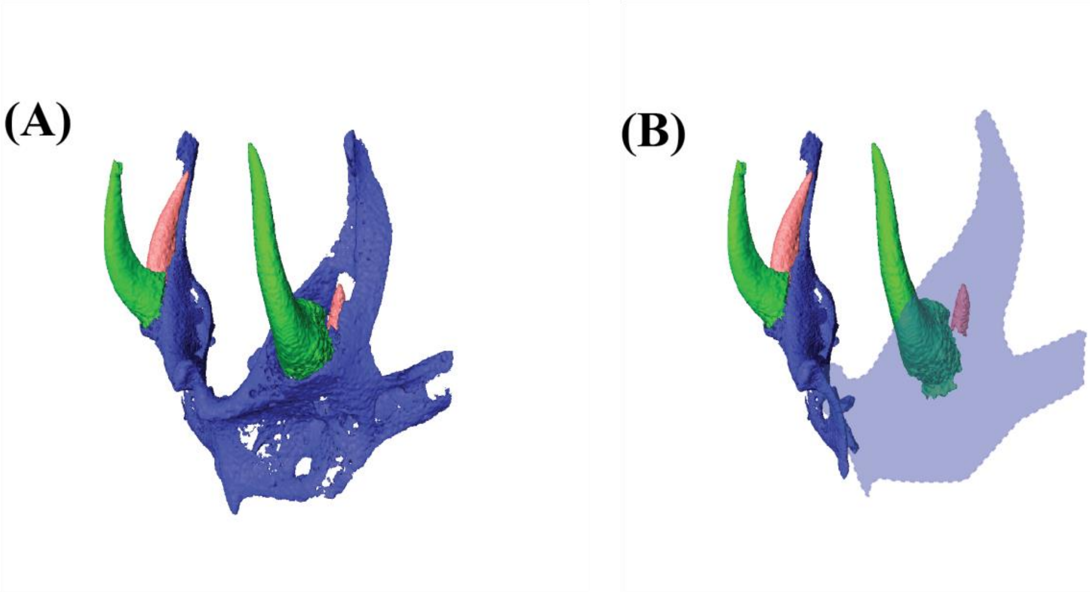

Figure 2.2. *Ophioblennius atlanticus* posterior canine replacement pattern. **(A)** Dentary with functional canines present, and incoming replacement canines posterior to the functional canines. **(B)** Left dentary plate removed to show relative size of the left-most developing replacement canine in comparison to the right-most. Color scheme: Blue = dentary, Green = functional canine tooth, Pink = replacement canine tooth.

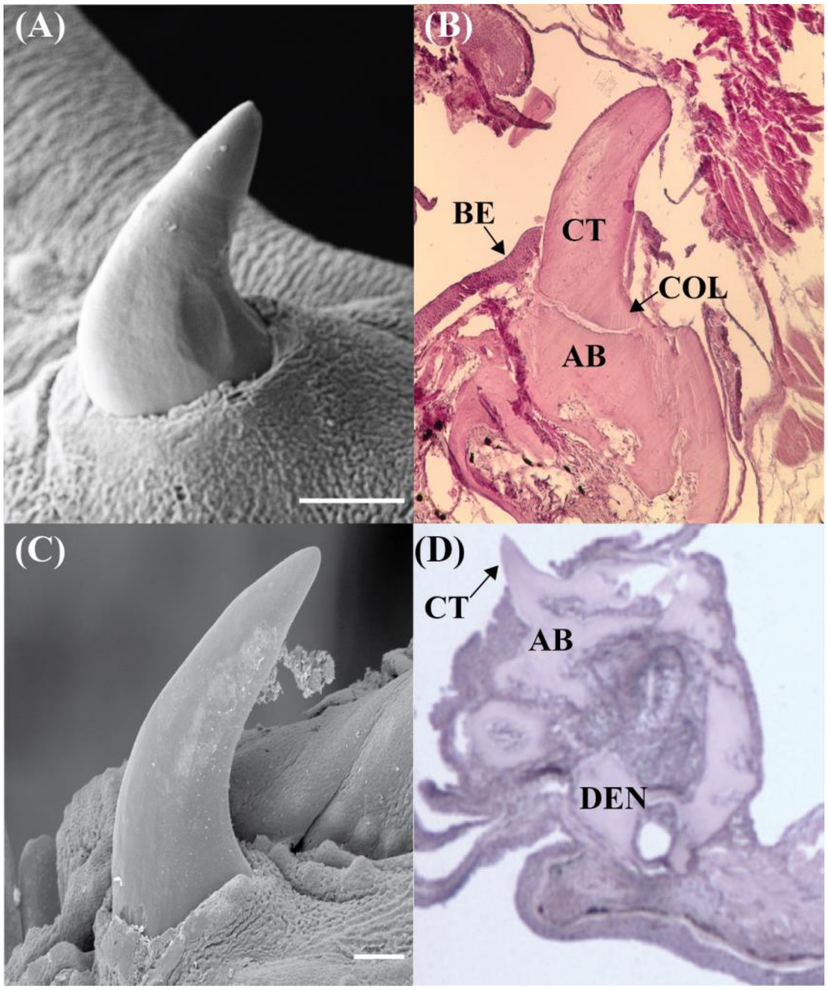

Figure 2.3. Dentary canine attachment in salariin blennies. **(A)** SEM lateral view of dentary canine in *A. arnoldorum*. Scale bar = 100 µm. **(B)** Hematoxylin and eosin stained section of *Alticus arnoldorum* dentary canine. Thin separation between the canine tooth and attachment bone is the collagenous attachment band. Note that the collagen band widens posteriorly. **(C)** SEM lateral view of dentary canine in *Rhabdoblennius nitidus*. Scale bar = 100 µm. **(D)** Hematoxylin and eosin stained section of *R. snowi* dentary canine and associated tissues. Abbreviations: BE = buccal epithelium, CT = canine tooth, COL = collagen, AB = attachment bone, DEN = dentary.

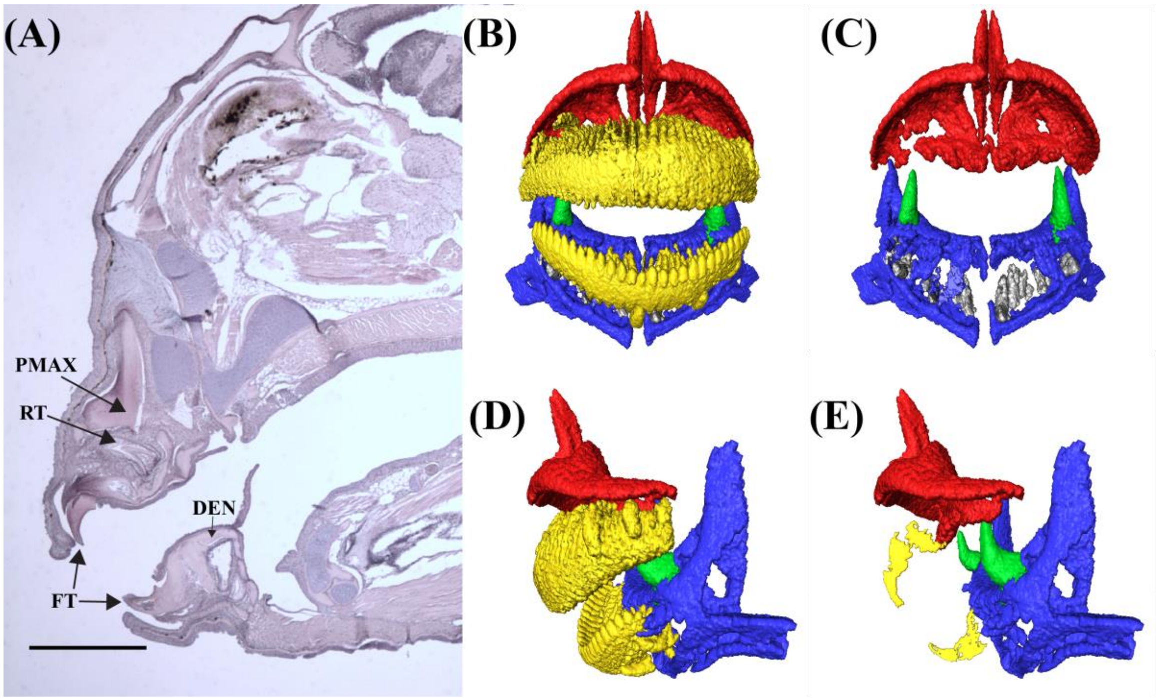

Figure 2.4. Interosseous tooth replacement in *R. snowi*. **(A)** Hematoxylin and eosin stained section of *R. snowi* oral jaws. The functional teeth exhibit a much closer association to the tooth-bearing bones in *R. snowi* than in most other salariin lineages. **(B)** MicroCT scan of *R. snowi* jaws, anterior view. **(C)** Anterior view of *R. snowi* jaws with functional feeding tooth rows removed, revealing the partially formed dentary capsule with replacement teeth penetrating through openings in the bone. **(D)** Left Lateral view of *R. snowi* jaws. **(E)** Left lateral view of jaws with only one functional feeding tooth present in the upper and lower jaws. Abbreviations: PMAX = premaxilla, RT = replacement tooth, DEN = dentary, FT = functional tooth. MicroCT color scheme: Red = premaxilla, Yellow = functional feeding teeth, Blue = dentary, Green = canine tooth, Grey = replacement feeding teeth.

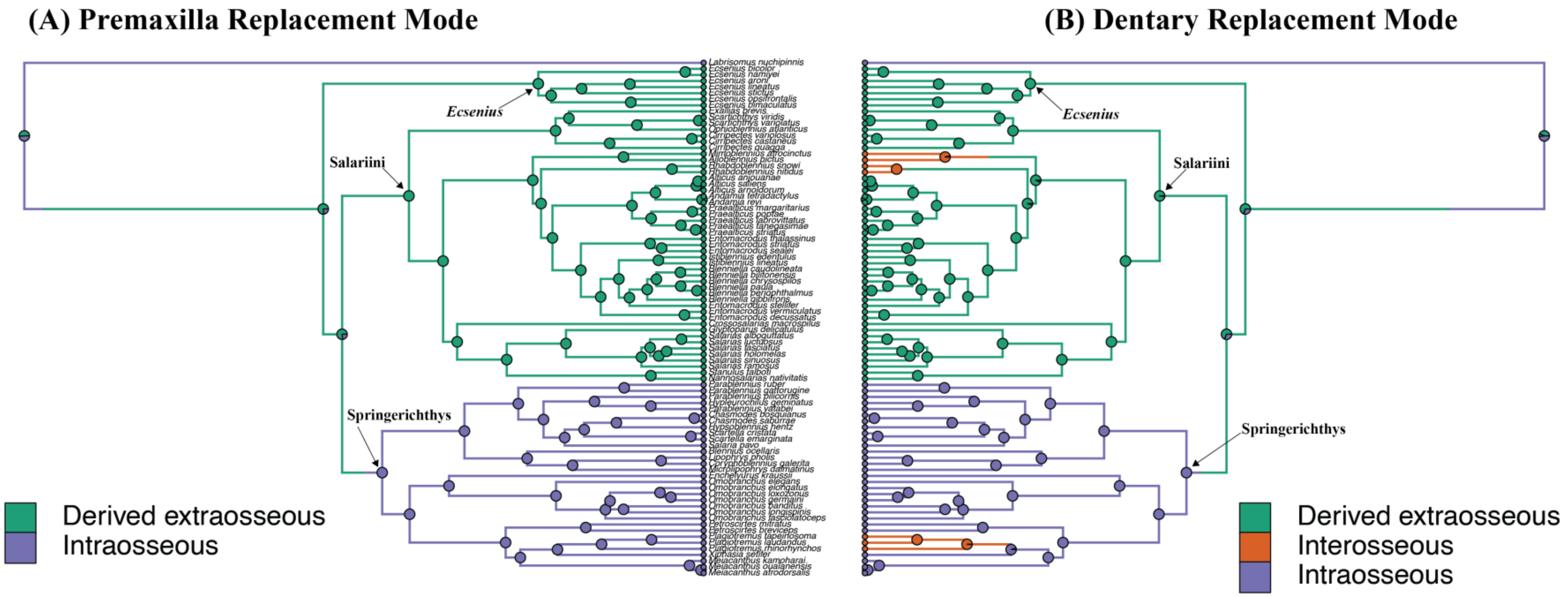

Figure 2.5. Evolution of feeding teeth replacement mode in blennies. Pie charts at each node show the likely ancestral state for feeding teeth replacement mode: derived extraosseous (green), intermediate (orange), intraosseous (purple). **(A)** Evolution of replacement mode for premaxilla feeding teeth. **(B)** Evolution of replacement mode for dentary feeding teeth.

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