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Teeth outside the jaw: Evolution and development of the toothed head clasper in chimaeras

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Chimaeras (*Holocephali*) are an understudied group of mostly deep-ocean cartilaginous fishes (*Chondrichthyes*) with unique characteristics that distinguish them from their distant relatives, sharks, skates, and rays. Unlike sharks, chimaeras lack scales and do not have serially replacing rows of serrated teeth crowned with enameloid. Instead, they possess a fused dentition of dentine tooth plates. Additionally, male chimaeras develop an articulated cartilaginous facial appendage, the tenaculum, which is covered in an arcade of tooth-like structures. These extraoral teeth remain poorly understood, and their evolutionary origin is unclear. We investigate the development of the tenaculum and its teeth throughout the ontogeny of the Spotted Ratfish, *Hydrolagus colliei*, to assess homology and convergence between this novel craniofacial feature and oral jaws. Our study aims to 1) describe the development of the tenaculum, 2) assess tenaculum tooth development in comparison to oral teeth and denticles, and 3) characterize the genes and tissues responsible for tenaculum tooth emergence. We found that juvenile male chimaeras develop a full tenaculum before tooth development is complete and that only mature males possess a fully toothed tenaculum. These extraoral teeth emerge from within the tenaculum rather than from the surrounding epithelium. We integrate our developmental data with fossil evidence of the tenacular dentition from the Carboniferous holocephalan *Helodus simplex*. Our findings show that the tenaculum is closely associated with the upper jaw and that tenacular dentition resembles separate shark-like oral tooth whorls more than modified dermal denticles.

tooth development | novelty | ghost sharks | craniofacial | head evolution

Significance

The development and evolutionary history of extraoral dentition in vertebrates remains largely unexplored. This study investigates the ontogeny of the male tenaculum, a unique feature of chimaeras, revealing a tooth development pathway similar to the oral dentition in sharks. By integrating fossil data and molecular techniques, we hypothesize that tenaculum teeth are homologous to oral teeth rather than modified skin denticles, providing key insights into the plasticity of odontogenesis and craniofacial diversity in vertebrates.

Living holocephalans are the remnants of a formerly diverse lineage that separated from the ancestry of modern sharks over 385 Mya (1, 2). As such, holocephalans represent a fundamental division of extant gnathostome diversity and provide unique insights into conditions among early vertebrates. Extant holocephalans are characterized by a distinctive suite of anatomical specializations vastly different from other chondrichthyans (3–7). They mostly lack denticles and do not have a replacing arcade of separated teeth, instead, they have a tooth plate composed of only dentine. Among their distinctive features, one standout is the tenaculum, a club-shaped, articulated, cartilaginous facial appendage studded with an arcade of tooth-like structures found only in male chimaeras (3, 4). The tooth-like structures are exposed and visible when the tenaculum is extended (raised out of the head), and when not in use, the unit sits in a central recess between the eyes (Fig. 1). The tenaculum is an unmistakable structure on the front of the male head, it is typically not pigmented compared to the surrounding head color pattern. The function of this sexually dimorphic structure is unknown, although video records of rare mating behaviors suggest the toothed tenaculum is used by males to grip female pectoral fins during copulation. Some of the first descriptions of this dimorphic behavior were noted by Dean (8) who described scars present on the dorsal surface of female *Hydrolagus colliei* caused by the scraping of male tenacular teeth during courtship.

A number of anatomical specializations in male chimaeras are directly tied to courtship and mating [(3), Fig. 1]. Males possess multiple grasping structures, including a pair of pelvic claspers covered in sharp denticles, a prepelvic tenaculum positioned just anterior to the pelvic fins, in addition to the cephalic tenaculum [(3, 9–11), Fig. 1]. These highly specialized and diverse structures are found across all extant holocephalans, underscoring the evolutionary investment in reinforced mating behaviors within the extended lineage. Beyond their reproductive morphology, chimaeras diverge from other chondrichthyans in possessing a beak-like arrangement of hypermineralized tooth plates rather than individual, replaceable teeth. This feeding apparatus consists of three paired plates—mandibular, vomerine, and palatine—each exhibiting distinct mineralized tissues, including trabecular dentine and hypermineralized tritors, which provide the durability needed for durophagy (7, 12, 13). Despite these departures from other cartilaginous fishes,

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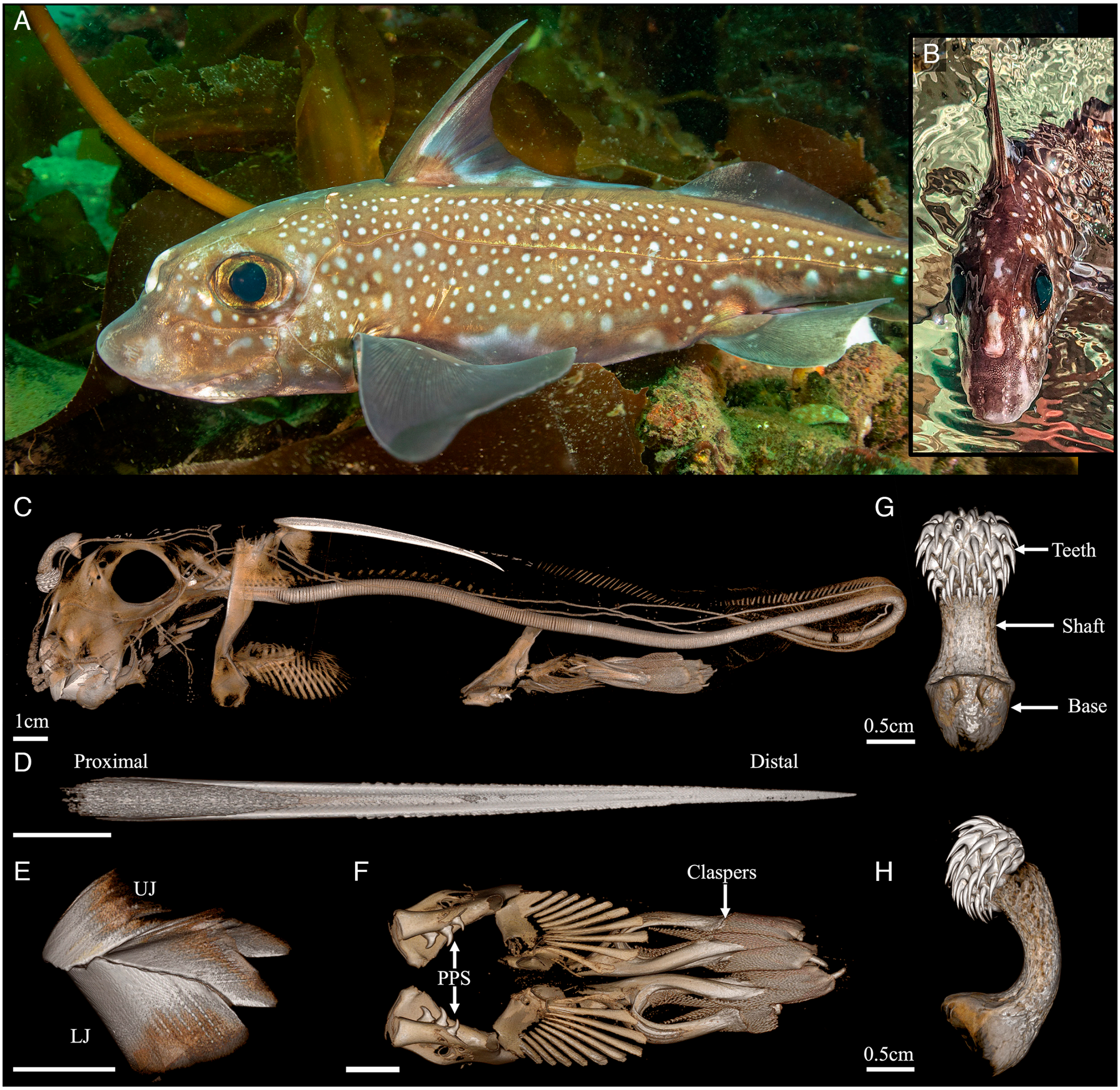

Fig. 1. Basic anatomy of *H. colliei*. (*A* and *B*) Photograph of adult, male *H. colliei* in Puget Sound. (Photograph used with permission from Tiare Boyes) (*C*) Micro-CT scan of male *H. colliei*. (*D*) Micro-CT scan volume of dorsal spine in ventral view, highlighting serrations along the spine's length. (*E*) Volume rendering of modified beak, hypermineralized regions visible as beaded columns. (*F*) Volume rendering of claspers and prepelvic anatomy. The pelvis is studded with six large denticles, and the claspers are covered in hundreds of small, rhomboid-shaped denticles. (*G* and *H*) Segmentation of the adult tenaculum, with teeth colored to highlight arrangement. Scale, 1 cm.

holocephalans have retained at least one deeply conserved feature: the dorsal fin spine. Present in all living species and often venomous, the rigid spine is a feature retained from the earliest members of the chondrichthyan clade, extending back for at least 436 My (14).

Fossil holocephalans exhibit diversity absent in modern lineages. For instance, some individuals possess oral dentitions with serially organized molariform teeth (15). It has long been suggested that the transformation and development of these individual tooth units eventually led to the tooth plate dentition of modern lineages (7, 10, 16). In addition, extinct chimaeras featured various extraoral structures adorned with teeth, including a median frontal clasper (10, 15). Patterson (10) suggested that toothed frontal claspers (tenacular) were originally present in both sexes, based on fossil evidence of early Chimaeriformes. Therefore, it was postulated a defensive function may have preceded the restriction to males for a copulatory function (10): an intriguing notion although unsupported thus far by the proliferation of Palaeozoic forms (15) evident at fossil localities such as Bear Gulch (17, 18).

In modern elasmobranchs (sharks, skates, and rays), dermal denticles are tooth-like in structure but do not form via a dental lamina and lack the coordinated replacement systems seen in oral dentitions (19–23). Tenacular teeth are presumably dermally derived, similar to the dermal denticles in other cartilaginous fishes. Yet, without early developmental or genetic data, it remains unclear whether the evolutionary origin of tenacular teeth lies in the evolution of oral teeth or whether the tenaculum represents convergent modification of body denticles. The broader debate surrounding odontodes—the mineralized structures composed of dentine found both externally and internally in vertebrates—adds complexity to this question. While teeth are often considered a specialized subset of odontodes, recent perspectives challenge the assumption that teeth evolved directly from dermal denticles, as early vertebrate scales differ substantially from those of modern sharks (21, 24–27). Instead, early vertebrate skeletal tissues exhibit a mosaic of structures that complicates straightforward homology between teeth and scales. The long-standing debate over whether teeth originated via an "outside-in" model—from dermal denticles migrating into the oral cavity—or via an "inside-out" model—from internal pharyngeal dentitions extending forward—remains unresolved, and emerging data suggest that elements of both models may be correct in different lineages. Indeed, pharyngeal and extraoral dentitions observed in jawless and early jawed vertebrates are increasingly interpreted not as aberrations, but as evidence of a deeper ancestral plasticity in odontode patterning. This view is further supported by fossil data indicating shifting boundaries of odontogenic competence in stem gnathostomes, including holocephalans, suggesting that the spatial deployment of dental tissues may have been more flexible in early vertebrate evolution than previously appreciated.

Chondrichthyans (shark, skates, rays, and chimaeras) have long been studied for their dental characteristics as they provide ample opportunity to investigate the evolutionary or developmental origin of teeth (25, 28–32). For example, the emergence of teeth in chondrichthyans is initiated through the formation of a dental lamina—an epithelial thickening that requires the coordinated activity of Sox2, a marker of epithelial stemness, and activated β-catenin, a key mediator of Wnt signaling. These factors not only demarcate the dental competence field but also play a conserved role in maintaining the proliferative niche necessary for sequential tooth generation, underscoring the deep developmental homology that exists despite striking anatomical disparity. If tenacular teeth are homologous to oral teeth, they may provide a model for reconstructing the evolution of mineralized dental structures outside of the oral cavity. Conversely, if they are instead modified body denticles, their independent evolution could highlight the plasticity of dermal odontogenic mechanisms in chondrichthyans.

In this study, we provide an account of complete tenaculum development and ontogeny in chimaeras. We take advantage of an uncommon set of ontogenetic stages from the Spotted Ratfish, *H. colliei*; one of approximately 50 species of extant Chimaera, across three families (3, 15, 32). Using histology and μCT scanning we assess how the tenaculum and its associated tooth set develop. We ask whether tenacular tooth development is more similar to that of the oral dentition or follows a pattern of development similar to the skin denticles seen in other elasmobranch lineages. Through developmental stages of tenaculum ontogeny and immunohistochemistry, we characterize the molecular signature of tenaculum tooth emergence and renewal, aiming to establish connections between modern and extinct dental features.

Results

Tenaculum Development. Tenaculum development initiates during embryonic development (in ovo) and appears to be a default morphological structure observed in both embryonic males (A&B) as well as recently hatched females (Fig. 2 *C* and *D*). In males, the tenaculum continues to grow through latter stages of juvenile development; from a small cellular condensation in embryos to a pimple-like structure situated at the midline between the orbits and the rostrum (Figs. 2*A* and 3 *A* and *B*). As the tenaculum continues to grow rostrally, the characteristic rod gradually takes shape, elongating into a pill form (Fig. 3 *C* and *D*). CT scans revealed that, at this stage, there are only minimal levels of mineralization and true cartilage surrounding the outer surface of the tenacular rod (Fig. 3 *C* and *D*). By way of comparison, we examined the earliest stages of development in *Callorhinchus milii* [the "elephant shark" chimaera, (33)] and observed that the tenaculum arises in a manner broadly analogous to that seen in *H. colliei.* Micro-CT scans and histological analysis reveal that the callorhinchid tenaculum originates as a modest epithelial thickening situated just anterior to the orbital region. This initial condensation enlarges and subsequently begins to mineralize, assuming the appearance of a pimple-like protrusion (*SI Appendix*, Fig. S1). Notably, the base or proximal end of the rod exhibits a more bulbous shape compared to the anterior or distal end. Furthermore, we observe the insertion of muscle fibers at the proximal end from both sides. Although the exact origin of these muscle fibers remains unclear, contrast CT imaging suggests recruitment from the adductor series.

At approximately 25 cm total body length (TL), the tenaculum undergoes elongation rostrally and widens, eventually erupting through the surface skin on the ratfish's forehead (Fig. 3 *C* and *D*). As the tenaculum widens, a central pocket forms around the distal end, which continues to accommodate the tenaculum through juvenile development. Through the whole of development, the front of the tenaculum is tightly adhered to the epithelial pocket and cannot be extended or removed. During this stage, we observe the differentiation and separation of epithelial tissue, creating a distinct line of tissue throughout the anterior bulb.

Tenaculum tooth development first becomes evident in specimens ranging from 30 to 50 cm total length (TL, Fig. 3 *E* and *F*). Teeth emerge in sequential rows, originating from lamina tissue formed during earlier ontogenetic stages. On average, seven rows develop, with the most proximal rows forming first and exhibiting more advanced tooth stages, while earlier-stage teeth appear in more distal rows. The base of each tooth overlaps with its adjacent neighbor; however, the tooth crowns do not overlap but rather interdigitate across rows. They are curved, conical in shape, retain a central pulp cavity, and have highly mineralized tips.

Teeth gradually erupt through the closed cartilaginous anterior bulb of the tenaculum (Fig. 3 *G* and *H*). The first two teeth appear at the midline of the initial row, followed by the sequential emergence of additional teeth. Tenacular teeth mineralize in a manner like true teeth in other chondrichthyans, with the crown forming before the base. These teeth retain a pulp cavity, containing multiple layers of dentin. This process significantly widens the epithelial pocket on the surface. As the teeth protrude, the tenaculum bulges outward. The tenaculum is now free from the surrounding epithelium and can either be erected or retracted. Once all teeth have erupted, individual recesses become visible in the pocket, allowing the teeth to slot into place when the tenaculum retracts. The cartilage surrounding the base of each tooth expands into a teardrop shape (Fig. 3 *G* and *H*).

Histological examination of juvenile through adult stages reveals that the tenaculum has a core of dense mesenchymal tissue surrounded by a layer of mineralized cartilage (Fig. 4). In early juveniles, the cells proliferate and organize to form a small cartilaginous nub (Fig. 4 *A* and *B*). This nub, when viewed from the surface, presents itself as a mineralized white spot or "pimple" on the fish's forehead. Histological sections indicate that the outer epithelium covering is sequestered and folded underneath the developing tenaculum (Fig. 4 *A* and *B*; IE). The developing

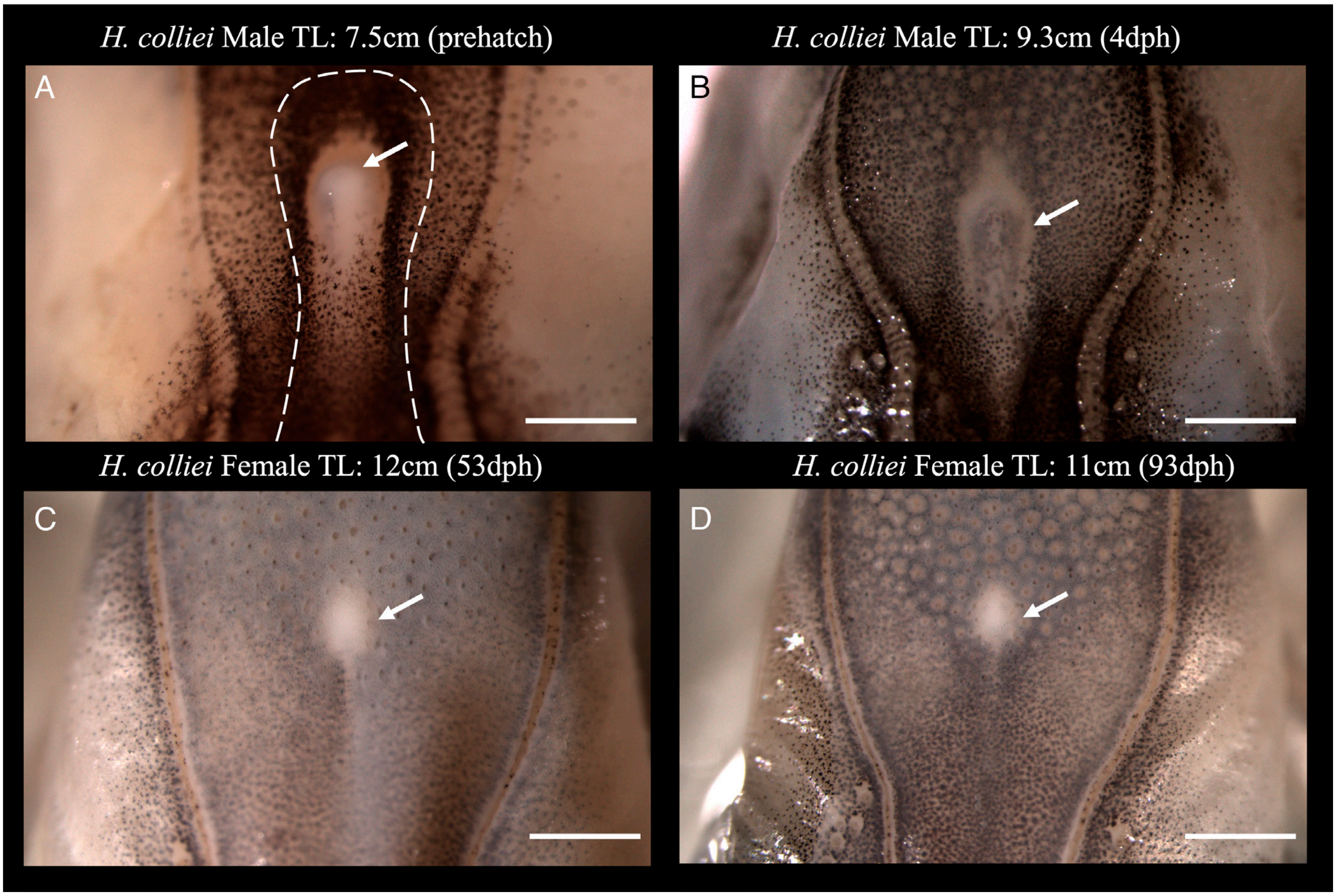

Fig. 2. The rudimentary tenaculum (pimple) is a morphological default present in both embryonic males and recently hatched females *H. colliei*, retained later only in males. Condensations of the rudimentary tenaculum are apparent as white thickenings (later cartilage, white arrow) in the superficial mid-interorbital region of embryonic Spotted Ratfish. (*A* and *B*) In males, this condensation becomes more pronounced and furrowed at the apical tip. (*C* and *D*) In embryonic females, the condensation is less pronounced and appears as a white spot in the region of the male tenacular tip. The dashed line represents approximate zone of growth for the tenaculum. Scale bar set to 500 mm.

tenaculum has a central core primarily composed of undifferentiated mesenchymal cell types (Fig. 4 *D* and *E*; M). Eventually, strings of epithelial condensations are found inside the anterior end of the tenaculum representative of a dental lamina (Fig. 4*E*; DL). As the tenaculum continues to grow, the inner core regulates into hyaline cartilage with a layer of mineralized tissue surrounding the entire rod (Fig. 4*G*: MC). The tooth-bearing anterior end of the tenaculum is less mineralized than the rest of the structure, with teeth extending from inside to outside, embedded in a dense matrix of mesenchyme (connective tissues) and cartilage (Fig. 4*G*). Protein expression analyses (immunohistochemistry) of early and late stage developing tenacular reveal expression of Sox2 and activated β-catenin in these streams of epithelial tissue. These signals continue to progress throughout the differentiation of the tenaculum and early stages of odontogenesis (Fig. 4 *C*, *F*, and *I*). This signal is observed in the earliest stages of tenaculum growth and development with the first signs of cellular condensation occurring on the cranium. Prior to tenaculum development we do not see such expression patterns.

Adult tenacula vary in the total number of teeth and rows, though the average tooth size within each row remains consistent. Teeth at the center of each row are larger than those at the lateral edges (Fig. 5 *A* and *B*). Additionally, smaller, unaligned teeth are present on the ventral surface of the anterior bulb. Newer teeth form closer to the posterior bulb base (Figs. 1 *G* and *H* and 5 *A* and *B*), and newer tooth bases overlap older bases (anterior to the tenacula bulb base). New tenacula teeth are present closest to the posterior bulb base (Fig. 5*A* and BS) initially without overlap. The adult tenaculum is a club-like structure composed of a dense cartilaginous core encased in a fibrous connective tissue sheath. The distal surface is embedded with numerous small, recurved denticles arranged in about seven rows. The teeth retain their pulp cavity even after eruption (Figs. 4*G* and 5*C*), and the outer surface of the tooth-bearing area is covered by a thick layer of sponge-like epithelial tissues devoid of goblet cells or tooth buds, allowing the teeth to flex slightly (Fig. 5*C*). Following each tooth into the tenaculum is the dental lamina that wraps around the base of each tooth (Fig. 5 *C*–*E*). The lamina surrounding the tenacular teeth resembles that of the lamina in the jaw (Fig. 5 *G* and *H*).

Tenaculum Growth vs. Clasper Growth. Unlike tenaculum teeth, there is no lamina-like structure associated with clasper denticles. Over ontogeny, claspers develop numerous small diamond-shaped denticles. Histology revealed that these denticles are mineralized with dentin and cells inside of the pulp cavity. Notably, no replacement denticles were observed beneath the surface of the odontode-bearing epithelium (*SI Appendix*, Fig. S2). In the earlier stages of clasper denticle development, we observed small placodes condensing and emerging from the skin as tissues differentiated and underwent mineralization. However, these did not show positive signs of Sox2 expression, and based on previous results, Sox2 is not expressed in the surrounding epithelia of developing skin denticles in chondrichthyans (Martin et al., 2016; (20)). We anticipated parallel growth between the tenaculum and body

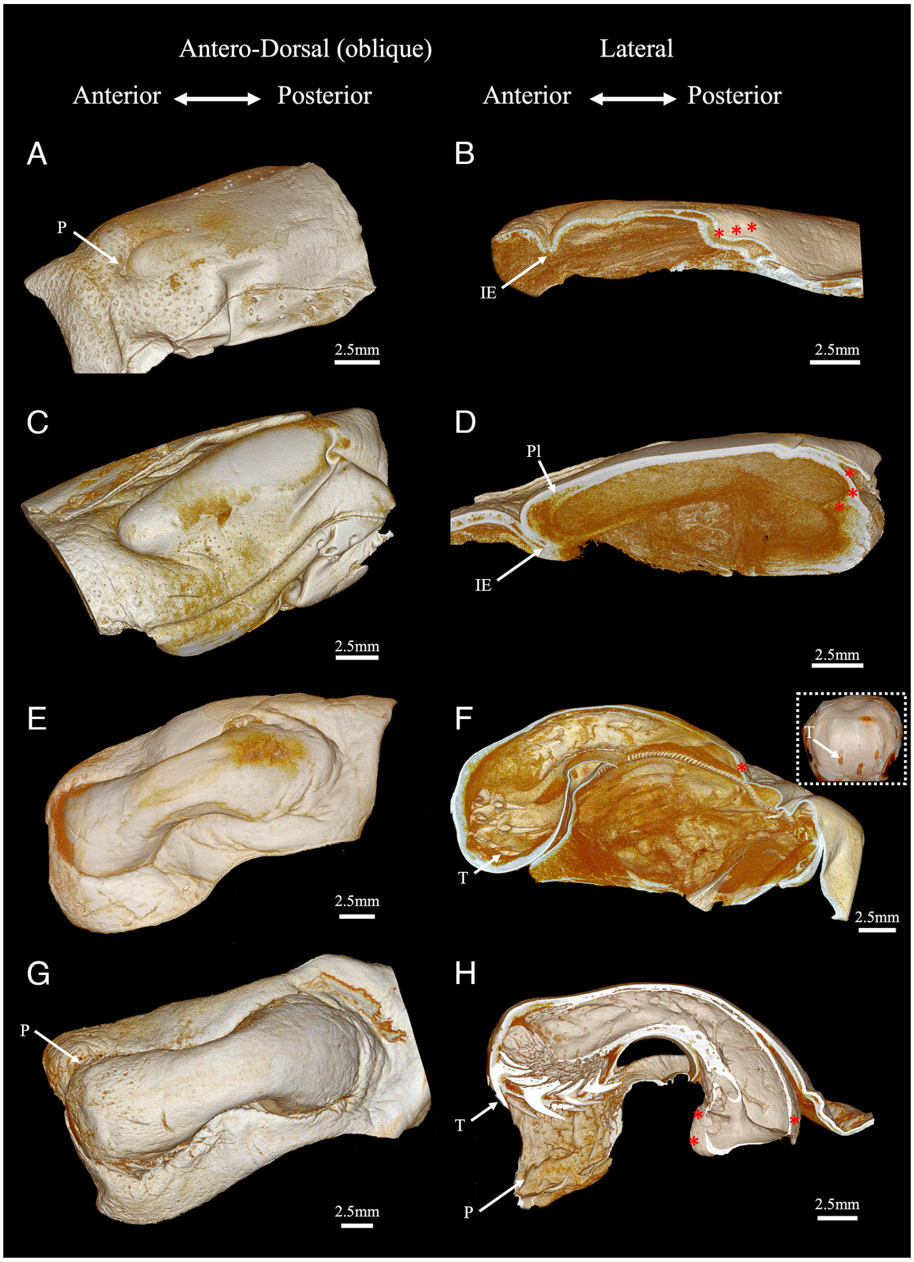

Fig. 3. Development of the tenaculum in *H. colliei*. (*A*–*G*) CT scans of the antero-dorsal oblique view of the developing tenaculum from 20 to 85 cm SL. (*B*–*H*) Lateral section revealing internal anatomy. The red asterisk indicates the location of attached muscle fibers. IE = invaginating epithelium; P = pocket; T = teeth.

length, however, our investigation revealed a distinct relationship between the tenaculum's size and the development of the pelvic claspers. Smaller males with fully developed pelvic claspers exhibited significantly larger tenacular than some of the longer males with less developed claspers.

Discussion

The process of tooth development and emergence is highly conserved across gnathostomes (jawed vertebrates), but the chimaeras present the first example of a dental lamina outside of the jaw and offer expanded insights into the possibilities of gnathostome dental diversity. The dental lamina begins as an invagination of oral epithelium, and its early establishment in gnathostome jaws is crucial for proper tooth emergence and regeneration [Fig. 5*F* arrowheads, (25, 34–36)]. In chondrichthyans, continuous dental replacement and the alternating pattern of tooth emergence are controlled by genetic expression through this ectodermally derived string of tissue (Cooper et al., 2023) (21, 30, 34, 37). Sharks and their relatives (elasmobranchs) are covered in tooth-like scales known as dermal denticles, but these do not emerge or replace by means of a dental lamina (22, 23). Rather, concentrated expression of an odontogenic network is responsible for sequential generations of denticle replacement. Unlike elasmobranchs, modern chimaeras lack extensive scale cover (10, 11, 15). Remaining denticles are limited to the pre- and postpelvic claspers, the dorsal spine in some very large males, and those ephemerally present on the cranium in hatchlings [separate to the tooth-like whorls present on the head tenaculum (3) (Finarelli & Coates, 2011)].

While teeth and denticles in elasmobranchs share several key genetic and physical traits, teeth uniquely develop from a dental lamina in the oral epithelium. The present study shows that the

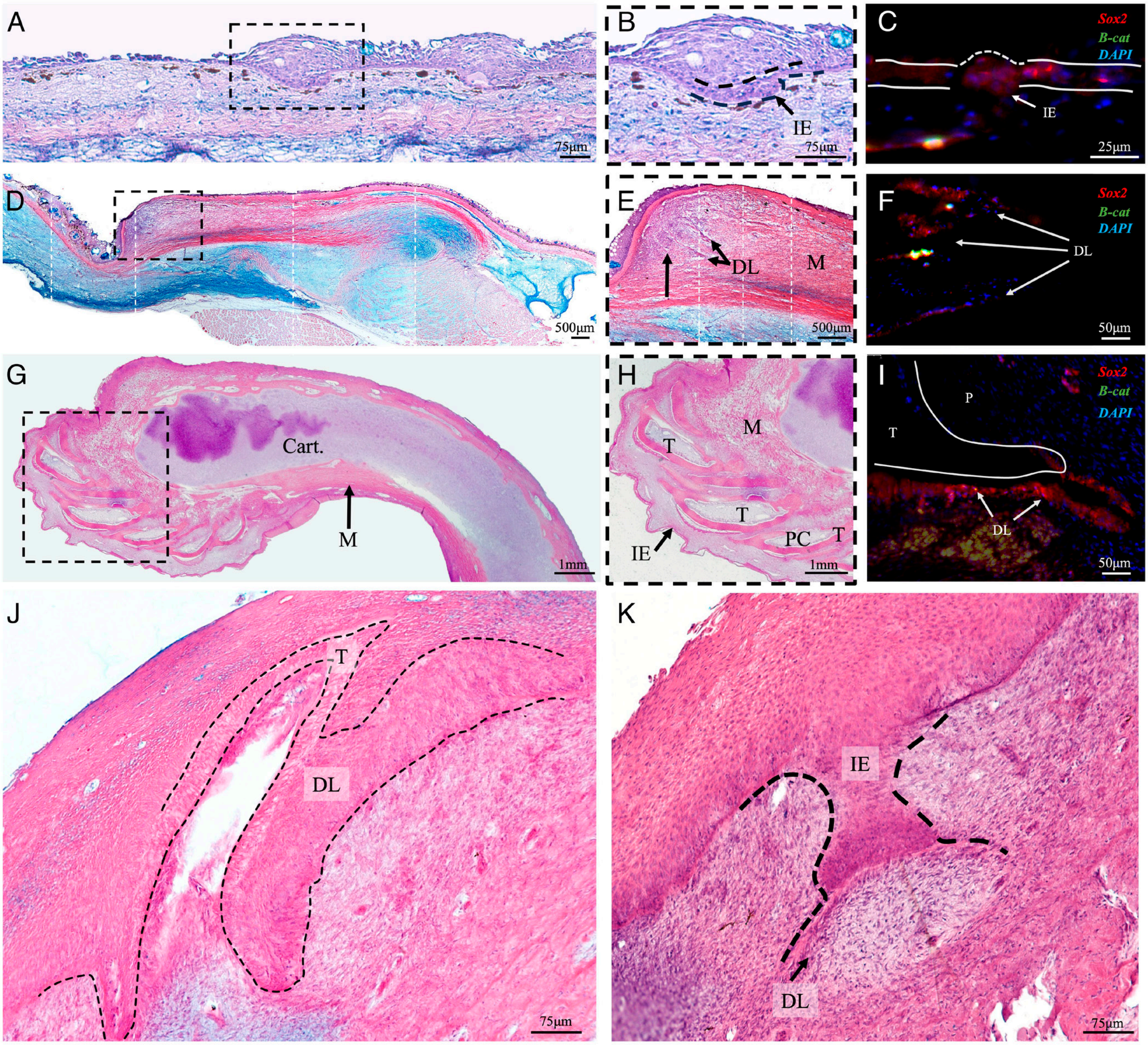

Fig. 4. Histology and gene expression of the developing tenaculum. (*A* and *B*) Cellular condensation marks the first signs of tenacular development as epithelial layers incorporate. (*C*) Tissue staining reveals gene expression patterns of Sox2 and activated β-catenin in the early developing tenaculum. (*D* and *E*) The tenaculum assumes its rod-like shape, and a dental lamina (DL) form (E, arrows). (*F*) Gene expression analysis indicates odontogenic potential in the juvenile tenaculum. (*G* and *H*) Adult tenaculum structure, showing serially arranged teeth embedded in mesenchyme and cartilage. (*I*) Gene expression of persistent odontogenic potential in the adult tenaculum. (*J and K*) Histology of the adult tenaculum highlighting dental lamina structure. IE: internal epithelium, DL: dental lamina, Cart: cartilage, T: tooth, PC: pulp cavity, M: mesenchymal cells. Panels *A*–*C* are sagittal sections through the region of the developing tenaculum, while panels *D*–*I* show sagittal sections along the length of the fully formed tenaculum. Panels *J* and *K* are sagittal sections within the tenaculum bulb. Panels *D* and *E* were generated by stitching individual .tiff images in ImageJ to produce a composite view as indicated by the white dashed lines.

tenaculum of *H. colliei* develops from an island of cells that could potentially be oral epithelial cells found on its forehead (further work will elucidate the cellular lineage origins of these cells), paleontological evidence has provided context for this hypothesis (see below). Expression of Sox2, activated β-catenin, and other key genetic markers is comparatively stronger in this thickened forehead epithelium, likely conferring odontogenic potential to the underlying dental lamina-like tissue. Teeth continue to develop along and in response to this string of epithelial tissue throughout development. Furthermore, in adults, this tissue extends basally around each of the tenacular teeth in a similar form to what is observed in the dentitions of sharks and batoids. The presence of this dental lamina in the tenaculum of *H. colliei* challenges traditional views on the spatial segregation of teeth from denticles (21, 38), (Cooperet al., 2023). We suggest that the origin of this diversification lies in extinct chimaeroid fishes where the tenaculum first evolved and adds substantial evidence to previous discussion about the evolutionary origin of holocephalan tenaculae first offered on the basis of morphological data alone (39).

The cranial skeleton of the Pennsylvanian (Moscovian; around 315 My old) holocephalan *Helodus simplex* bears the oldest and most primitive known example of a tenaculum [(39), Fig. 6 *A*–*C*]. The tenacular cartilage (the stem or rod) lies above the ethmoid region, which itself appears to represent an intermediate stage in

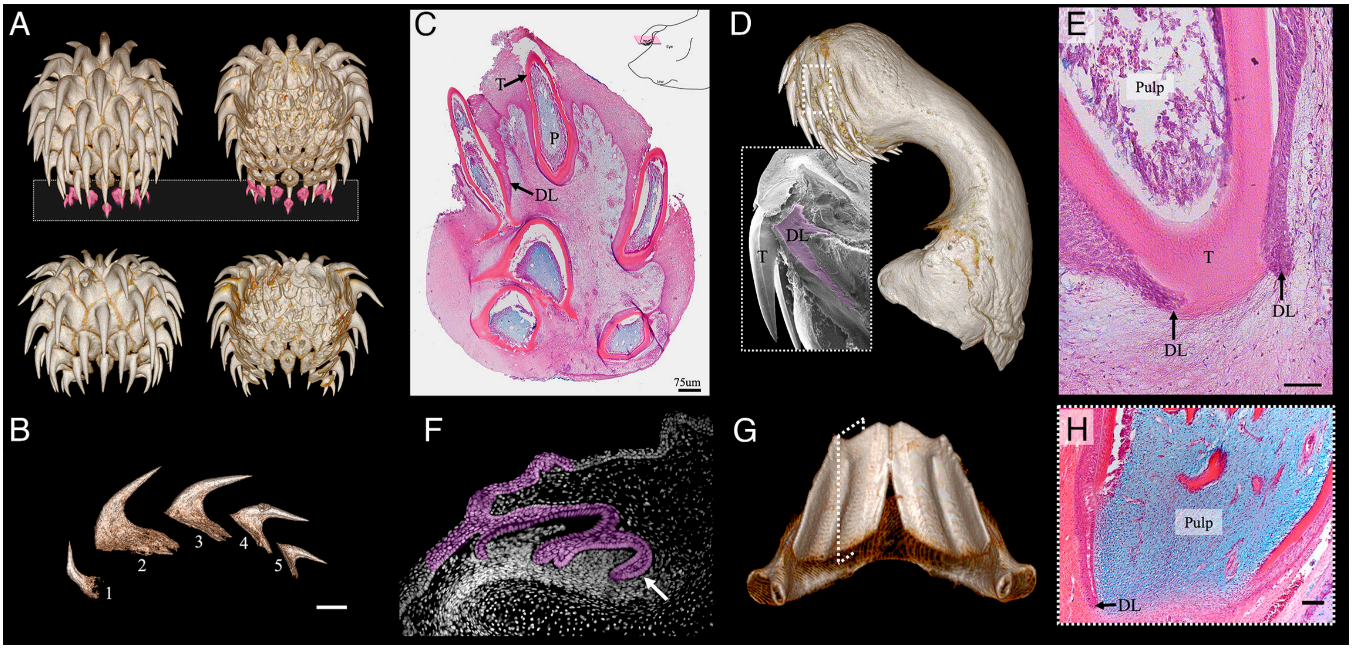

Fig. 5. Adult tenaculum morphology in *H. colliei.* (*A*) CT scan showing the frontal and interna views of the adult tenaculum. Teeth in pink represent a newer generation (*B*) Lateral view of tenaculum tooth whorl showing the relative size and patterning of adult tenacular teeth. (*C*) Coronal section through the adult tenaculum showing the cellular anatomy of multiple teeth including the dental lamina. (*D*) CT Image of the adult tenaculum with inset to SEM showing the exposed tooth and adult dental lamina wrapping around the individual teeth. (*E*) Histological section of adult tenacular tooth to show detail of the lamina around the basal surface of the tooth. (*F*) DAPI stained histological section showing the standard morphological condition of the dental lamina (arrowhead, false colored magenta) in the embryonic catshark. (*G*) CT scan of the lower tooth plate in *H. colliei* dashed line reveals the sectioning plane of (*H*) histological section through the *H. colliei* lower jaw tooth plate showing the positioning of the dental lamina, reminiscent of the lamina in tenacular teeth (*E*) and other chondrichthyan species, e.g., sharks. DL = Dental Lamina, T = Tooth, P = Pulp.

the evolution of the highly specialized preorbital region of modern chimaera crania [(3, 10, 11, 40), Fig. 6*D*]. The evolutionary origin of the tenacular rod is unclear, other than its proximity to the ethmoid roof of which it might be considered a subdivision or outgrowth. However, the denticles of the *Helodus* tenaculum are remarkably tooth-like (Fig. 6*C*). These teeth are arranged in a tightly packed whorl that coils through the front of the tenacular cartilage. Furthermore, these tenacular teeth have roots that resemble those of the mandibular teeth, although the bicuspid crowns are markedly different from the pillow shapes of the palatal and

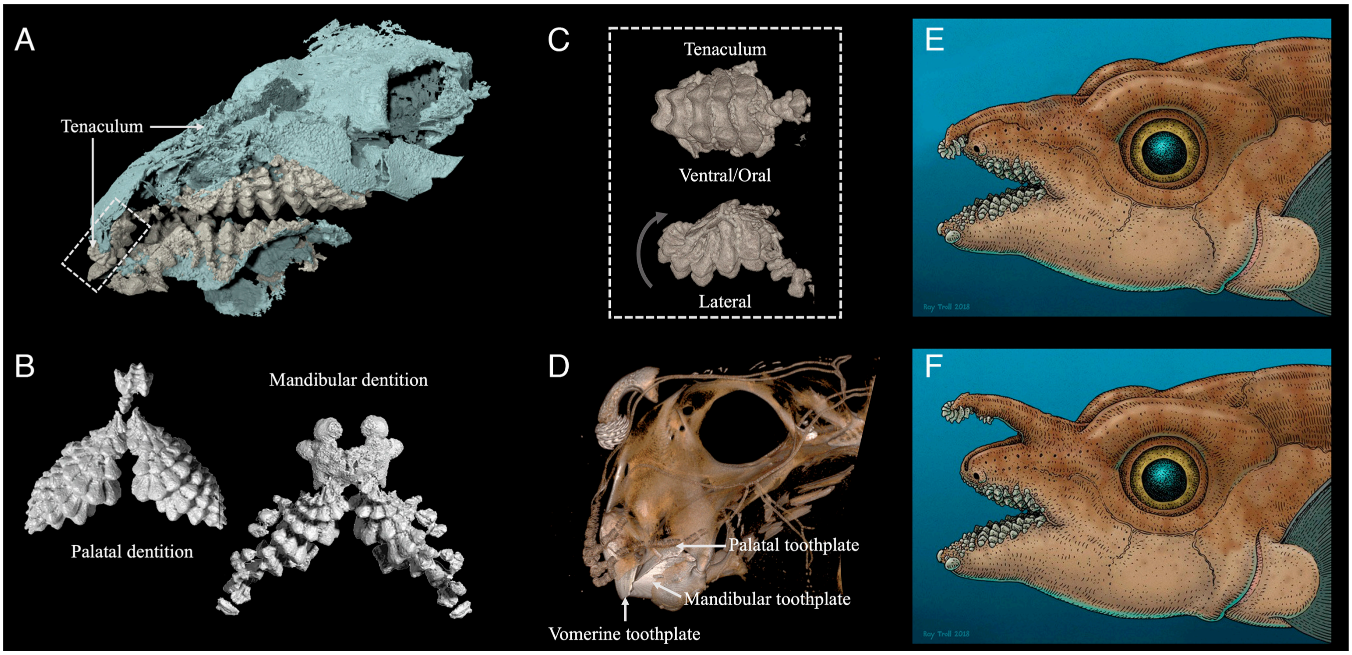

Fig. 6. Origin of the modern tenaculum. (*A*) The Carboniferous stem-holocephalan *Helodus simplex* (see ref. 39 for details) cranium, partly crushed, in anterolateral view showing elongate tenaculum with tenacular teeth positioned anterior to palatal dentition and (when depressed) seated in gap between anterior mandibular teeth. (*B*) *H. simplex* tenacular tooth whorls (large and small). (*C*) *H. simplex* dentition, palatal and mandibular, showing closely interdigitated tooth families with lingual to labial serial replacement akin to dental arcades present in modern sharks and rays. (*D*) *H. colliei*, the spotted ratfish, showing the short tenaculum of modern chimaeroids and tooth plate dentition. The tenaculum retains ancestral features such as individual tooth units, reflecting its origin in the oral jaw dentition rather than as a modification of dermal denticles. (*E* and *F*) Reconstruction art of *H. simplex* (used with permission from Ray Troll) showing likely elevated and depressed positions of elongate primitive tenaculum.

mandibular dentition (Fig. 6*B*). Here, it is worth mentioning that *Helodus* mandibular teeth exhibit preconditions for the dental plates of modern chimaeras (7, 11, 15), with separate teeth (cf. elasmobranch tooth sets) consolidated into massive batteries occupying each quarter of the gape (39).

The *Helodus* tenaculum extends over the entire length of the ethmoid region, and the apical tooth whorl (which is rather small compared to neighboring teeth) sits within a symphysial gap between the left and right upper jaw tooth batteries [Fig. 6*A*; (39)]. This unusually long tenaculum, relative to modern examples, is not unique and might, in fact, represent a primitive condition: similarly elongate tenaculae are known in Mesozoic holocephalans (10) including genera such as *Squaloraja*, *Acanthorhina*, and *Metopacanthus* (15). These, too, bear a wide variety of denticles, but detailed descriptions have not been completed.

Here, we suggest that in Palaeozoic taxa such as *Helodus*, the close proximity of the tenacular tip with the upper jaw dentition allowed for an epithelial connection, at least during development, promoting a transfer of dental competence onto the tenacular appendage. This, in turn, seems likely to be a legacy of these teeth originating, evolutionarily, from the front of the gape. Further, we argue that this retention and/or transfer, derived within the stem lineage of today's Chimaeriformes, led to the development of what we now see as oral teeth outside of the jaws in extant holocephalans. Subsequently, under differing selection regimes, the tenacular teeth and jaw teeth became increasingly dissociated and adopted new and separate functions. A curious result is that cyclical tooth replacement, widely considered to be a defining characteristic of chondrichthyans, in extant chimaeras is retained only in the tenaculae while distinctly absent in the mandibular dentition (7, 11, 15).

The tenaculae of extant chimaeras are morphologically diverse, and this probably reflects their as yet undefined roles in reproduction. In species like *Harriotta raleighana*, tenacular teeth are long and slender, whereas in *C. milii,* they are short and squat (3, 4, 41, 42). Notably, some chimaeras exhibit occluding tenacular teeth located outside, within the pocket housing the tenaculum (i.e., *C. milii*), in stark contrast to *H. colliei* where teeth are confined to only the tenacular bulb. This pattern indicates that odontogenic cells capable of tooth formation are distributed even further across the forehead and are not restricted to tenacular territory. Despite differences in tooth morphology, all tenaculae share the defining characteristic of a serially replacing dentition, a capability (as noted above) lost in the oral jaws of modern chimaeras. While a dental lamina is not always essential for the initial emergence of teeth (43), and several vertebrates generate odontic structures without one, it is required for sustaining a continuous dentition (44).

Chimaera dermal denticles are structurally and histologically distinct from tenacular teeth. Dermal denticles surrounding the dorsal spine lack a mineralized crown and consist solely of a base, while pelvic clasper denticles, like those in elasmobranchs, are embedded in the skin without lamina-like tissue. The mineralization pattern of tenacular teeth mirrors key dental characteristics of oral teeth in other chondrichthyans, with crown formation preceding base development. These teeth also retain a pulp cavity and are composed of multiple layers of dentin, consistent with true teeth in other chondrichthyans. Notably, they are capped with a distinct material that is histologically different from the underlying dentin. While we cannot confirm the presence of enameloid without further analysis, the capping material is structurally unlike what is observed in dermal denticles, including those found on the claspers. The presence of dentin and pulp, which are absent in the osteodont dermal denticles, suggests a structural and functional distinction. We argue that the tenaculum of extant chimaeroids may represent a retained expression of tooth-forming capacity outside the oral jaw, potentially linked to facial epithelial territories that were historically competent for odontode development. Rather than positioning the tenaculum as an evolutionary "missing link," we interpret it as a developmental relic—preserving evolutionary heritage in odontogenic patterning that has been lost or transformed in other lineages. This perspective is distinct from the more traditional search for a singular evolutionary origin of teeth and instead highlights the capacity for heterotopic retention of dental potential in early vertebrates. The tenaculum is considered a sexually dimorphic structure, but its development appears to be at least partially hormonally regulated. While males develop a fully formed tenaculum with functional teeth, females may form a rudimentary shaft that never develops teeth or resembles the adult male structure (8, 45). This pattern suggests that the tenaculum follows a default developmental pathway that matures only in males while regressing in females (Fig. 2). A similar trend is seen in other sexually dimorphic dermal structures in chimaeras, where denticle appearance is often linked to reproductive traits or body size.

In summary, our findings challenge the idea that the tooth-like denticles of the tenaculum are modified dermal denticles, and instead support the interpretation that they are true teeth—developing outside the jaws from facial epithelium that was once competent to initiate oral dentition. These findings contribute to a growing body of evidence that vertebrate dentitions are not strictly confined to the oral cavity and challenge the assumption that dental competence was limited to fixed axial regions early in evolution. Coupled with recently described material from fossils and phylogenies, these data shed light on the evolution of an extraordinary morphological innovation: a rare example of the kind of evolutionary developmental tinkering long theorized about (46) but rarely substantiated with experimental and comparative data. This insight reinforces the view that dental systems evolved not as a singular, linear pathway, but through the repeated reuse and redeployment of shared developmental modules across distinct anatomical regions.

Materials and Methods

Specimen Collection. Samples of adult and juvenile *H. colliei* were collected during trawls in the San Juan Channel, Friday Harbor Washington, USA. These trawls were deployed from April 2022–August of 2023. All samples collected with permission under a University of Washington animal care and use protocol (IACUC 4238-03).

Tenaculum and Body Ontogenetic Morphology. We used microcomputed tomography (μ-CT) to examine the morphology and development of the tenaculum in *H. colliei*, scanning both unstained and dye-enhanced specimens. In total, we analyzed 40 specimens ranging from 25 to 80 cm total length (TL). For whole-body morphometrics, we μ-CT scanned 16 specimens without contrast. These scans were conducted at the University of Florida using a Nano-CT GE V|TOME|X M 240 at 28 to 25 μm resolution, reconstructed in VG Studio, and exported as .tiff files for segmentation in 3D Slicer. To visualize nonmineralized tissues and developing tooth buds, we dissected 12 additional tenacular for dye-enhanced CT scanning. These were immersed in a 3% phosphotungstic acid and ethanol solution for 1 wk with continuous agitation to improve dye penetration. Scans were performed at the Friday Harbor Laboratories Karel F. Liem Bio-Imaging Center using a Bruker Skyscan 1173 with a voxel size of 6.9 to 12.1 μm, a voltage of 55 kV, an amperage of 133 μA, and an exposure time of 1.175 to 1.350 s. We processed all scans using 3D Slicer and the SlicerMorph toolkit for segmentation, visualization, and morphological measurements (47, 48).

Histology. Adult and juvenile samples (n = 7) were decalcified using either 0.5 M Ethylenediaminetetraacetic acid (EDTA) in water for 1 to 2 wk until softened or Cal-Ex for 12 h at 4 °C (for adult tissues). Following decalcification, samples were dehydrated in ethanol, cleared with xylene, and embedded in paraffin.

We sectioned sagittal paraffin-embedded samples (6 to 7 μm thick) using a Leica RM2145 microtome. Slides were stained with hematoxylin and eosin, mounted with DPX (Sigma), and imaged using a BX51 Olympus compound microscope equipped with an Olympus DP71 camera. Additional slides were prepared for immunohistochemistry and stained for Sox2, PCNA, and activated β-catenin. These sections were mounted and sealed with Fluoromount and imaged with a BX51 Olympus compound microscope fitted with an OlympusDP71 camera.

Statistical Analysis. We measured tenaculum length, tooth count, individual tooth length, volume, and surface area for all adult and subadult specimens. Total length and clasper length were also recorded, with the former measured in ImageJ from Digital single-lens reflex camera (DSLR) images (Canon Mark III, 180 mm macrolens) and the latter assessed in 3D Slicer. To evaluate tenaculum size variation among adults, we conducted a two-way ANOVA with clasper development and total length as independent variables. The analysis met assumptions of independence, homogeneity of variances, and normality of residuals. The significance threshold was set at $\alpha = 0.05$. Data and analysis code are available for download at https://github.com/karlycohen/ratfish.git.

Data, Materials, and Software Availability. Computed tomography, data, and analysis code data have been deposited in Morphosource.org and GitHub (https://www.morphosource.org/projects/000766503?locale=en, https://github.com/karlycohen/ratfish) (49, 50). All other data are included in the article and/or *SI Appendix*.

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