Investigating the features that facilitate sexual parasitism in male Anglerfishes (Teleostei, Ceratioidei)

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Abstract

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Deep-sea anglerfishes (Ceratioidei) remain some of the most enigmatic fishes in the world. The sexual dimorphism seen in this group is the most extreme example amongst vertebrates. Male anglerfishes develop a specialized jaw called the denticular apparatus that is used to attach to females for reproduction. There are many questions regarding the biological mechanisms that facilitate this type of reproduction. The goals of the present study are to (1) describe the origin and structure of the denticular apparatus in male ceratioids with emphasis on the family Melanocetidae; (2) examine the ontogeny of male melanocetids to better define the metamorphic patterns; and (3) document similarities or differences in the denticular teeth and dermal spinules between taxa. Our results illustrate how dermal spinules and tooth-like structures of the denticular apparatus should be classified as odontodes, which represents a sixth radiation of

odontodes in teleost fishes. Here we show that ceratioid anglerfishes develop odontodes that function as both body spines and as a secondary sexual trait, and we confirm that the denticular apparatus is a unique part of the cephalic skeleton not seen anywhere else in teleosts. Furthermore, we document male ontogeny within the family Melanocetidae to better understand the formative patterns of the denticular apparatus. The arrangement of male and female anatomies represents two different examples of the extreme phylogenetic economy of morphological parts, with females evolving lures from existing fin rays and males evolving secondary jaws from existing body spinules – both as apparent adaptions to life in the bathypelagic zone.

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INTRODUCTION

The deep-sea anglerfishes of the suborder Ceratioidei (Lophiiformes) comprise one of the most morphologically unique groups of fishes in the world. This group consists of 177 species in 35 genera and 11 families, nearly all of which are distributed circumglobally in the deep waters of the bathypelagic zone (Pietsch, 2009; Fricke et al, 2022). The most important character that sets the Ceratioidei apart from the remaining four suborders within the Lophiiformes is the extreme sexual dimorphism seen between male and female anatomies (Figure 1). Only the female ceratioid bears the bioluminescent lure, or illicial apparatus, for which the group is named. Male ceratioids are dwarfed in comparison, the most extreme example of which is seen in *Ceratias holboelli* where females can be 60 times as long and nearly half a million times as heavy as the fully matured males (Bertelsen, 1951; Pietsch, 1976). Instead of an illicial apparatus, dwarfed males usually possess large eyes and nostrils as well as a specialized set of bones anterior to the jaws known as the denticular apparatus (Bertelsen, 1951). This denticular apparatus consists of an upper and lower denticular bone, both of which bear varying numbers of hooked teeth depending on the species. The overall body plan is so drastically different between sexes that male ceratioids were originally thought to belong to an entirely separate family of lure-less females known as the Aceratiidae (Parr, 1930; Regan and Trewavas, 1932).

Ceratioids undergo a dramatic metamorphosis throughout their life history, beginning as globose, transparent larvae and developing into darkly pigmented adults with massive jaws and teeth. Larval ceratioids share many of the same characters between sexes including body shape, but the females are distinguished from the males by the presence of the escal bulb, which later develops into the characteristic lure, embedded in the skin of the forehead. This bulb is visible even at sizes as small as 2 to 3 mm total length (Pietsch, 2009). The females soon outgrow the males and develop large teeth on the jaws, a gaping mouth, and an elongated illicial apparatus. The males, however, initially retain the small teeth on the premaxilla and dentary and begin to develop enlarged nostrils as they mature. The juvenile metamorphosis begins around 10 mm SL and in males involves the loss of the jaw teeth, further enlargement of the nostrils, tightening of the skin around the body, pigmentation of the skin, and the development of the upper and lower denticular teeth (Bertelsen, 1951). This extreme sexual dimorphism is associated with the peculiar reproductive strategy known as sexual parasitism. This strategy is unique amongst all vertebrates and involves the male fish biting and attaching to the female to facilitate fertilization of buoyant eggs which are then dispersed into the water column. In some ceratioid taxa, this attachment is permanent and involves fusion of the male and female tissues where the male becomes a functional parasite that depends on the female for nutrients and simply supplies her with sperm during spawning (Parr, 1930; Regan and Trewavas, 1932; Bertelsen, 1951; Pietsch 1976; Munk, 2000). However, within the Melanocetidae, Himantolophidae, Diceratiidae, Gigantactinidae*,* and some genera of the Oneirodidae, males attach temporarily without evidence of tissue fusion (Bertelsen, 1951; Pietsch, 1976; Pietsch, 2005; Pietsch, 2009).

Attachment mode is reflected in the morphology of the male denticular apparatus. Species that attach only temporarily have numerous denticular teeth that appear suited for grasping onto females as well as feeding when not attached (Bertelsen, 1951; Pietsch, 1976; Pietsch and Van Duzer, 1980; Bertelsen and Krefft, 1988). In contrast, the morphology of the obligately parasitic taxa consists of only a few large denticular teeth and smaller denticular bones that do not seem well-suited for prey capture (Bertelsen, 1951; Pietsch 2005; Pietsch, 2009). There are two known examples of parasitized female melanocetids: a 75 mm female *Melanocetus johnsonii* with a 23.5 mm male (Figure 1A) and a 73 mm female *M. murrayi* with a 15 mm male. In both cases, the males are only loosely attached with the female tissue pinched between the denticular bones with no evidence of tissue fusion (Pietsch 2005). Additionally, there is one documented case of an erroneous attachment of a male *M. johnsonii* to a female *Centrophryne spinulosa*, but similarly without tissue fusion (Pietsch and Nafpaktitis, 1971). Much of the existing literature focuses on the female characteristics where interspecific differences are relatively well documented. Variation in the morphology of the esca, the tip of the illicium that houses the bioluminescent bacteria, is the most consistent and informative character used in identifying female ceratioids. Furthermore, the larger females are encountered more readily than males and as such are typically easier to study than their male counterparts. Generally, males and females possess the same bones in the skull, with the exception of the denticular apparatus that is not present in females, however the sexes have drastically altered bone structure (Figure 2). Females tend to have a greatly enlarged cephalic skeleton that makes up a large portion of the body and houses huge fang-like teeth on the jaws. Males, by comparison, tend to have much more elongated and slender bones in the head with a more depressed head shape. The drastic morphological differences observed between both species and sexes have generated taxonomic uncertainty throughout the history of studying this group. While intergeneric differences generally agree with distinctions based on female characters, many of the characters that allow for specific identification of male ceratioids are not well defined. Meristic characters, including fin ray and denticular teeth counts, overlap in many of the described species and there are few other diagnostic characters within any given genus (Bertelsen, 1951; Pietsch, 1976; Pietsch and Van Duzer, 1980; Pietsch 2009). In several cases, there have been no conspecific males or larvae documented for well described female species, lending further uncertainty to the taxonomy in this group.

Despite the significant body of research describing sexual parasitism across the Ceratioidei, the exact origin of the male denticular apparatus remains unconfirmed. Parr (1930) first suggested that the entire structure is homologous to the illicial apparatus of the female, and thus derived from the first dorsal fin spine, in what he described as “an example of extreme phylogenetic economy of morphological parts”. Later hypotheses supposed that the denticular apparatus is derived from the dermal spinules found embedded in the skin of some taxa (Bertelsen, 1951; Pietsch, 1976; Bertelsen and Krefft, 1988; Munk, 2000). The specialization of the dermal spinules seems the most likely explanation for this phenomenon and is the hypothesis we will explore here. We hypothesize that these dermal spinules belong to a class of structures called odontodes. Following the definitions given by Fraser et al (2010), odontodes are any mineralized epidermal structure formed from a single papilla consisting of attachment bone, dentine, and sometimes enamel (Figure 3). Traditionally, teeth are defined as existing only with in the oro-pharyngeal cavity and denticles may be dermal or oro-pharyngeal in location (Sire and Huysseune, 1998; Fraser et al, 2010). The term odontode is used to describe all tooth-like structures and includes both teeth and denticles. Odontodes have been described in several groups of fishes, namely chondrichthyans and siluriformes (Gillis et al, 2017; Schaefer and Buitrago-Suarez, 2002; Sire, 1993; Rivera-Rivera and Montoya-Burgos, 2017; Rivera-Rivera et al, 2021). The most well-known type of odontodes are the dermal denticles, or placoid scales, found in chondrichthyans, but odontodes can take on a variety of morphologies and functions. Historically, the terms denticles, spines, spinules, and teeth have been used somewhat interchangeably within this group when describing the male anatomy. To date there has been no direct comparison of the denticular teeth to the dermal spinules, nor has there been robust documentation to confirm the origin of the denticular apparatus. If the denticular apparatus does in fact derive from the dermal spinules found on the head and body, then it necessarily constitutes an entirely unique element of the cephalic skeleton and is distinctly separate from illicial apparatus of the female anglerfish. Furthermore, the denticular teeth represent a novel development of extra-oral tooth-like structures in teleosts.

The goal of the present study is to (1) describe the origin and structure of the denticular apparatus in male ceratioids with emphasis on the family Melanocetidae; (2) examine the ontogeny of male melanocetids to better define the metamorphic patterns; and (3) document any similarities or differences in the denticular teeth and dermal spinules between a few taxa. I examined male specimens primarily from the monogeneric family Melanocetidae (Günther, 1864), as well as a small number of female conspecifics and males of two additional families, the Himantolophidae (Gill, 1861) and Linophrynidae (Regan, 1926).

METHODS

Specimen Acquisition

A web-based search of all available specimens identified as *Melanocetus* on iDigBio, VertNet, and FishNet2 yielded 2045 hits from universities, museums, and other collections worldwide. I focused on collections in the United States with multiple lots of *Melanocetus johnsonii*, the most common species, and *Melanocetus sp*, the most abundant record. Unfortunately, the majority of these online records do not indicate sex or life history stage, which made focusing solely on a developmental series of males difficult. I visited Nova Southeasern University (NSU) in Fort Lauderdale, Florida, the Museum of Comparative Zoology (MCZ) at Harvard in Boston, Massachusetts, and the Marine Vertebrate Collection at Scripps Institute of Oceanography (SIO), San Diego, to verify the sex and life history stages of the available specimens. This yielded 73 males of various life history stages within the genus *Melanocetus* (Appendix). All specimens were preserved in 10% buffered formalin and stored in 70% ethanol or 50% isopropanol, with the exception of 6 specimens from NSU that were fixed in 95% ethanol. In addition to these 72 *Melanocetus* males, we selected two female *Melanocetus* specimens to compare between sexes. We also sampled two male *Linophryne sp.* and *Himantolophus sp.* to compare histological sections of the denticular bones and dermal spinules across multiple taxa. These 6 additional specimens all came from UW and are listed in the Appendix. All lengths given are standard length in millimeters unless otherwise stated.

Digital Photography

All images were captured with ZEN 2.3 Pro software using a Zeiss Discovery V20 microscope with an Axiocam 503 color and Achromat S 0.5x, 1.0x, and PlanApo 1.5x objective lenses. Images were composite Z-stacks of 25 slices across a narrow focal range (0.1-3.0 mm). These stacks were then aligned and merged in ZenPro and processed through the Extended Depth of Focus function (highest wavelet alignment). Images were post-processed in Adobe PhotoShop version 22.4.2.

Clearing and Staining

A total of 18 specimens were cleared and double stained following modifications to the protocol outlined in Smith, 2019. Specimens were initially in 70% or 95% ethanol (ETOH) or 50% isopropanol (see Appendix). All specimens were stepped up into 95% ETOH for initial dehydration. Fish were then transferred into a 30% glacial acetic acid alcian blue solution for 10-15 hours. Acid was neutralized and specimens destained in saturated sodium borate overnight. Specimens were bleached in 10% hydrogen peroxide for 15-60 minutes then transferred into buffered trypsin (35ml saturated sodium borate, 65 ml distilled water, ~1/4 teaspoon trypsin) until clear (15 hours mean). Bones were stained in a 1% KOH solution with alizarin red for a maximum of 12 hours. Specimens were then either returned to trypsin or placed into saturated sodium borate for destaining. Once cleared, specimens were moved into 10% glycerin-90% KOH and stepped up into 100% glycerin with thymol over the course of several days.

Histology

We used histological sectioning to explore differences in the development and form of the denticular apparatus, body spinules, and oral teeth of *Melanocetus* (n=3; 2 males, 1 female), *Linophryne sp.* (n=2 males), and *Himantolophus sp.* (n=2 males). Heads of each species were dissected away from the body and both halves were independently prepped to be infiltrated and embedded for sectioning. Specimens arrived in 70% ETOH and were postfixed in DMSO for 6hrs before being rehydrated to distilled water and decalcified in 10% EDTA following the protocol laid out in Histological Processing of Teeth and Periodontal Tissues for Light Microscopy Analysis (Silva, Moreira, & Alves, 2011). Tissues that were thoroughly decalcified were then dehydrated in stepwise series, starting from 25% ETOH until 95% ETOH. We embedded whole heads and bodies in JB-4 embedding media (Electron Microscopy Science JB-4 embedding media protocol, see table below for exact embedding details). Jaws and associated tissue were sectioned laterally and the bodies were sectioned axially at 2-3.5 μm. Sections were placed on glass slides, dried for 24 hrs, and then stained with Lee’s Basic Fuchsin and Methylene Blue stain and visualized using a Nikon eclipse E600 compound microscope and imaged with a MicroPublisher 5.0 rtv camera from Q imaging.

RESULTS

Results Summary

Our examination of the internal and external morphology and histology of developing male *Melanocetus* revealed the following: (i) the denticular ‘teeth’ of male ceratioid anglerfishes are extra-oral odontodes and not true teeth, and are similar in structure to the dermal spinules found on the body but differ somewhat in overall morphology; (ii) we supported the conclusions of Bertelsen (1951) that the male upper and lower denticular bones do not form from the same dorsal fin elements as the female illicial apparatus; (iii) we confirmed the underlying illicial musculature and documented previously undescribed muscles linked to the lower denticular bone; (iv) our data suggested the presence of roughly 4 distinct developmental stages in male *Melanocetus* (Figures 6-9) based on 9 informative morphological features (Figures 11-14); (v) both the denticular odontodes and dermal spinules are structurally homologous across taxa but may differ slightly in morphology.

Denticular apparatus and dermal spinules

Serial histological sectioning revealed that the tooth-like structures found on the snout, chin, and body of *Melanocetus* males are odontodes. Each odontode forms from a single papilla with a heavily mineralized layer of dentine surrounding an inner pulp cavity without an exterior enamel layer (Fig 4). This structure is rooted to an attachment bone, in this case the upper or lower denticular bone. These bones are formed from the basal fusion of the individual odontodes and contain a network of support struts called odontic channels (visible in Figure 4B). The base of each odontode contains unmineralized collagen that acts as an anchoring point to the attachment bone (Fig 4B). In *Melanocetus*, the upper denticular bone also contains a medio-rostral series of odontodes called rostral spines. These spines are homologous to the other denticular odontodes but are generally associated with an individual bony rostral plate. The lower denticular bone does not contain comparable bony plates but the odontodes are similar in size, shape, and structure to those of the upper odontodes and are rooted to a similar network of odontic channels. The epidermis is composed mainly of stratified squamous epithelium surrounding the oral cavity and the protruding odontodes. The fibrous connective tissue of the dermis is visible underneath the epidermal layer and contains numerous blood vessels (Figure 4).

In fully metamorphosed males, the upper denticular bone is connected to the illicial pterygiophore via a strut of cartilage, which allows the denticular to move anteriorly and posteriorly via extension and contraction of the extrinsic muscles of the dorsal fin (Fig 5). The inclinator dorsalis anterior (IDA) originates on the frontals and inserts laterally along the pterygiophore. The inclinator dorsalis posterior (IDP) originates on the parietals and inserts laterally on the pterygiophore near the attachment of the IDA. The supracarinalis anterior (SA) originates on the frontals, just posterior to the IDA, and inserts at the base of the pterygiophore above the supraoccipital. The insertion of the IDP is visible in Figure 5, along with a small sliver of the SA. Our examination also revealed previously undocumented musculature that controls the motion of the lower denticular bone in a similar manner to that of the upper. This muscle, likely an anterior extension of the adductor mandibulae section Aw, runs ventrally along the edge of the dentary and anchors near the symphyseal spine (Figure 5C).

The dermal spinules are similar in structure to the denticular odontodes found on the body, both consisting of a pulp cavity with a dentine covering and bone of attachment. The main differences between these odontodes are the terminal morphology and the extent of the attachment bone. The denticular odontodes are large and curved whereas the dermal spinules remain small and straight, only slightly protruding through the integument. The spinules are singular, isolated units placed sporadically across the body and attached to a small subdermal bony plate without odontic channels (Figure 3, 6). The bony plate is rooted within the epidermis and does not protrude through the skin but does create a small bump in the skin with the triangular spinule at its center. In contrast, the denticular odontodes form singularly but ultimately fuse together into the robust network of odontic channels that make up the upper and lower denticular bones. Spinules were most easily visible in cleared and stained specimens but were also visible in many preserved specimens. Some individuals had numerous spinules covering the entire head and body that were clearly visible under low magnification (0.5x objective), while others had only a few spinules that required higher magnifications or were lacking spinules entirely. Size and shape of spinules were generally consistent regardless of where they were found on the body when present.

Ontogeny of Melanocetus

After assessing the morphology of all specimens here, nine characters were determined to be useful indicators of developmental stage: counts of premaxilla teeth, dentary teeth, vomerine teeth, upper denticular odontodes, lower denticular odontodes, rostral spines, rostral plates, and fusion of the upper and lower denticular bones. The breakdown of these characters are given in Figures 11-14. While these characters in combination were useful in grouping developing males into roughly four stages, we saw some variation, and thus partial overlap between adjacent stages, in many characters throughout ontogeny. Overall size is not an accurate indicator of development in this group and overlaps between all four stages (Figure 15). The four stages are described below.

Stage 1

Stage 1 development is defined as individuals that have jaw and vomerine teeth present and no growth of the upper or lower denticular odontodes. 34% of our sample size (n = 23 specimens) were larvae of less than 10 mm in Stage 1. Specimens at this stage all have globose bodies with inflated and unpigmented skin. Subdermal pigment is visible in most specimens, concentrated on the dorsum, forehead, and sometimes the caudal peduncle. The eyes are large, spherical, and are directed more laterally than anteriorly. The skin of the nostrils is typically tight around the nasal rosettes but may be slightly inflated. The whole nostril is smaller than the orbit length. The interorbital space is concave and the snout blunt and smooth. In preserved specimens, there are no projections or raised bumps in the skin of the head or snout and the illicial pterygiophore is not visible. In cleared and stained specimens, we see the illicial pterygiophore sits dorsal to the frontal and supraoccipital and is rooted to the cranial bones via connective tissue and the illicial musculature. The first cephalic fin spine (illicial bone, D1) is positioned near the tip of the pterygiophore and is small, sharp, and pyramid shaped. The second dorsal-fin spine (D2) is relatively smaller and more blunt than the first and sits around the midpoint of the pterygiophore. There are no indications of budding denticular odontodes in the skin of the snout or lower jaw, even in specimens where dermal spinules are readily visible across the head and trunk. The maxilla is toothless, distinctly separate from the premaxilla, and has a small triangular tip at its base. The number and distribution of teeth on the jaws is highly variable across individuals, sometimes symmetrical and other times uneven across the left and right sides. The premaxilla holds 5 to 18 fang-like teeth, 2 to 9 per side, most of which are concentrated towards the center of the mouth. These teeth are found in two distinct rows with the smaller teeth sitting along the anterior edge of the premaxilla and larger teeth placed immediately posterior. The dentary bears 7 to 25 total teeth with 3 to 13 per side. These teeth are similar in size and shape to those on the premaxilla and typically occur in clusters of 2 or 3 teeth with space in between each cluster. There is consistently a single large tooth on the either side of the dentary deep inside of the mouth, posterior to the tip of the maxilla on the exterior surface. The symphysis of the lower jaw is not ossified and is usually small and rounded, though some specimens show a triangular projection ventrally on both sides. The vomer is broad and flat, nearly the same width as the buccal cavity, and holds anywhere from 2 to 9 large teeth with 6 evenly spaced teeth being the most common arrangement. It is joined basally to the parasphenoid, which curves upward to meet the neurocranium and the first (atlas) vertebra. The rest of the cephalic bones are either weakly ossified or are not visible because of poor alizarin uptake.

Stage 2

Stage 2 development involves the first indications of the denticular odontodes appearing in the skin of the snout and chin. We saw considerable size variation at this stage, the smallest measuring 11 mm and the largest 21 mm. In the smaller specimens, the skin is loose around the globose body and lightly pigmented, but the subdermal pigment remains clearly visible. The larger specimens have heavily pigmented black skin (dark brown in preservative) that is tight around a slender and elongated body. The head maintains its blunted profile in smaller specimens but is elongate and narrow in specimens greater than 17 mm. The nostrils begin to grow in both height and width and are shorter than orbit length in specimens under 17 mm and greater than or equal to orbit length in those larger than 17 mm. The nasal rosettes are greatly enlarged, becoming broad and flat in the larger specimens. The tip of the illicial pterygiophore may be visible in some preserved specimens as a small bump in the skin of the interorbital space. The pterygiophore itself begins to lengthen and ossify more heavily. The tip is broadly flattened into a spade or shovel shape, with two posteriorly curved projections laterally and a single projection anteriorly, all connected by thin bony plates. The first cephalic spine is more slender than in Stage 1 and may become incorporated into the tip of the pterygiophore. The second spine maintains its size, shape, and position. The upper denticular odontodes begin to bud out of the skin in the tip of the snout, immediately above the ascending process, and are cartilaginous at the base and mineralized distally. These buds are small, triangular, and similar in overall appearance to the dermal spinules seen on the body. Generally, the odontodes form in small clusters of 2 or 3 to as many as 12, although singular isolated odontodes were observed in some individuals. As the fish progress through Stage 2, the odontodes become long, curved, and fang-like. The lower denticular odontodes bud in similar fashion to the upper but tend to be more haphazardly placed around the tip of the lower jaw. The lower odontodes begin to form in three groups, one median and two lateral, which will ultimately fuse into the three projections of the lower denticular bone. There is no fusion between the odontodes at this stage. Many specimens showed simultaneous initiation of both upper and lower denticular growth, but others showed only upper or lower with no indications of the other and no discernible pattern of appearance. The surfaces of the dentary and premaxilla become notched and jagged on the interior surfaces and the oral teeth begin to break off, though how the mechanisms that cause this change are unclear. Vomerine teeth typically begin to break off at this stage as well. The dentary symphysis remains unossified, but the bones become longer and more pointed ventrally, developing into a sharp symphyseal spine. The triangular tips of the maxilla become larger and begin to project anteriorly. The premaxilla is reduced and nests almost flush with the anterior edge of the maxilla. Other cephalic bones maintain similar appearance and positions as described in Stage 1.

Stage 3

Stage 3 is characterized by the continued loss of oral teeth, clustering and fusion of the denticular odontodes, and protrusion of the illicial pterygiophore. Most of the jaw and vomerine teeth have been lost by this stage with n=5 specimens retaining 2 to 4 teeth on the dentary, n=3 with only 1 tooth on the premaxilla, and a single specimen with 3 vomerine teeth left. Observations showed that any remaining dentary teeth are found near the symphysis of the lower jaw where, if they do not fall out, they become more or less incorporated into the interior surface of the dentary. The skin inside of the mouth is drawn anteriorly over the dentary and the lower denticular, covering any of these retained teeth. The dentary and premaxilla may be smooth at this stage, but many individuals retain the jagged or notched appearance found in Stage 2. The maxilla remains similar in size to Stage 2 but the anterior projections are now more pronounced and fully wrap around the premaxilla where they will eventually join with the base of the upper denticular bone. The dentaries are not fused medially but have a strong symphyseal spine ventrally and the beginnings of a dorsal ridge curving anteriorly along the base of the lower denticular bone. The illicial pterygiophore projects strongly at this stage and forms a very distinct bump or knob in between the nostrils. The tip of the pterygiophore is flat, spade shaped, and angled ventrally, pointing nearly straight down in several cases. The first cephalic spine is now fused ventrally with the tip of the pterygiophore, but the dorsal point is visible in some specimens protruding slightly through the skin. The second cephalic fin spine remains unchanged. Rostral spines are present in most specimens just ventral to the tip of the pterygiophore but there is no formation of the underlying rostral plates that form the dorsal projection of the upper denticular bone. This means that there is not yet a solid connection between the illicial pterygiophore and the upper denticular bone. The upper odontodes continue to elongate and have a distinct lip between root and tip that is visible in cleared and stained specimens as well as some of the poorly preserved and damaged specimens. The root of the odontode remains under the skin of the snout while the tip protrudes and is generally the only portion visible in preserved specimens. Occasionally, one root bifurcates into two tips, which was counted as a single odontode when this was observed. The odontodes as a whole begin to cluster in the center of the snout and become fused at the roots to form the blocky base of the upper denticular bone. I counted 8 to 19 upper odontodes in the observed specimens (excluding 2 damaged specimens), with one individual having an additional cluster of 5 odontodes on the ventral surface of the upper denticular bone, nearly into the maxilla, that was not seen in any other specimens. In general, the odontodes protrude at an upward angle initially and will rotate anteriorly and ventrally as they fuse together into the completed denticular bone. Some of the more developed individuals have downward facing odontodes clustered together into a bony block/group. The lower denticular tends to mirror the patterns seen in the upper at this stage but is generally farther along in terms of fusion. Only a single specimen at this stage did not have the 3 distinct lobes, and this specimen also had the lowest number of odontodes overall (n=3). In general, the median group contains more odontodes than the lateral groups and there were anywhere from 14 to 35 total lower odontodes in the observed specimens. Of the 13 Stage 3 specimens: 5 showed indications of fusion in the upper denticular, 7 were unclear (probable) fusion, and 1 had no fusion; 10 specimens showed fusion in the lower denticular, 2 were unclear (probable), and 1 specimen was not fused (Figures 13, 14).

Stage 4

Stage 4 development is primarily characterized by the mineralization of the rostral plates and the connection of the denticular apparatus to the pterygiophore dorsally and upper jaw ventrally. The body is long and slender with uninflated, dark black skin (brown in preservative). Over half of the examined Stage 4 specimens were greater than 20mm (n=11), with the smallest observed 15.5mm and the largest 26mm. Overall, the head becomes greatly elongated and is generally around a 1/3 of the standard length at this stage. The size and shape of many of the cephalic bones remains relatively similar to the early stages with the main differences observed in the illicial pterygiophore, the denticular apparatus, and the jaw bones. The pterygiophore is distinctly visible in preserved specimens as a strong projection under the skin between the eyes and nostrils. The tip of the pterygiophore is elliptical, flattened anteriorly, and is joined to the upper denticular bone via a posteromedial extension of 4 to 8 enlarged, spine-bearing rostral plates. Generally, the 2 or 3 most dorsal plates are weakly ossified, but the spines are clearly visible. The odontodes in both upper and lower denticular bones are strongly curved and pointed. There were 10 to 20 odontodes on the upper denticular in the examined specimens. These odontodes are completely fused basally to one another and create a V-shaped posterior surface. This surface sits flush laterally with the anterior projections of the maxilla, but a small gap remains medially. There is no fusion between the upper denticular and maxilla. Instead, this junction acts as a pivot point for the motion of the upper denticular by means of moving the pterygiophore. The lower denticular bone is trifurcated into one large median projection and two smaller lateral projections. There were 16 to 32 lower odontodes in the examined specimens, with 8 to 18 in the median series and 4 to 8 in either lateral series. The odontodes in the lower denticular tend to be more haphazardly arranged than those in the upper with many examples directed ventrally or posteriorly. The lower denticular is positioned immediately anterior to the jaw and contacts the dentary along its posterolateral margins. The lower denticular has a small, curved gap along its posterior edge where it meets the lower jaw and appears to be anchored only by connective tissue. There is no evidence of fusion between the dentary and lower denticular bone. The dentary fused into a strong symphyseal spine ventrally as well as a curved dorsal ridge that sits above the posterior margin of the lower denticular bone. The premaxilla is greatly reduced and sits flush with the anterior edge of the maxilla. The maxilla looks relatively similar to previous stages except the anterior projections now wrap fully around the premaxilla to meet the base of the upper denticular bone. In general, the jaw and vomerine teeth are completely lost at this stage, with a few notable exceptions. There were 3 specimens that retained 2 to 4 teeth on the dentary, 2 of which also had a single tooth left on the premaxilla. No specimens at this stage retained any vomerine teeth. The jaws do not fully close at this stage and instead the upper and lower denticular bones come together like pincers via the swinging action of the pterygiophore and lower jaw musculature, respectively. In specimens where dermal spinules are present, the spinules are much larger and flatter than in previous stages, very similar in overall appearance to the weakly ossified rostral plates of the upper denticular.

Results across taxa

We compared the upper denticular odontodes in three taxa: *Melanocetus*, *Linophryne*, and *Himantolophus* (Figure 4). There is little variation in the histology of each odontode. All three contain the homogeneous and heavily mineralized dentine layer surrounding the inner pulp cavity. The odontodes are all rooted to the upper and lower denticular bones, with both *Melanocetus* and *Himantolophus* sharing similar networks of odontic channels. *Linophryne*, our only permanently parasitic taxa, do not develop the rostral plates or spines seen in *Melanocetus* and *Himantolophus* and have smaller denticular bones and a less extensive network of odontic channels. Our results also showed similar dermal spinule histology in *Melanocetus* and *Linophryne.* The dermal spinules in both taxa contain a small pulp cavity covered in heavily mineralized dentine. They are roughly triangular and are rooted to a small bone of attachment in the epidermis, not the dense network of bone seen in the denticular apparatus (Figure 10). We were unable to capture a dermal spinule in *Himantolophus* in our histology, but the spinules are clearly visible, even to the naked eye, in preserved specimens.

DISCUSSION

Homology of denticles and dermal spinules

Parr's (1930) description of the denticular apparatus as "an example of extreme phylogenetic economy of morphological parts" remains an elegant descriptor of ceratioid morphology. He originally referred to the denticular apparatus as entirely homologous to the female illicial apparatus, although he did state that he was not entirely convinced it was not a new element. Bertelsen (1951) proposed the unique rise of the denticular apparatus as a modification of the dermal spinules and refuted the homology between it and the female anatomy but did not further investigate any link between the denticular apparatus and any other existing morphological feature of anglerfishes. Here we show that the repurposing of the existing dermal spinules into the highly specialized organ that is the denticular apparatus surely represents another example of an "extreme phylogenetic economy of morphological parts", albeit one distinct from Parr's hypothesized example.

One of the most important results from our examination is that the denticular odontodes are not teeth. These odontodes have historically been described as teeth formed from the fusion of modified dermal spinules (Bertelsen, 1951; Pietsch, 2005; Pietsch 2009). While they resemble teeth both in their superficial morphology and potentially their function, the denticular odontodes are located outside the oro-pharyngeal cavity and lack some of the key characteristics of teeth, such as an exterior enamel layer and organized replacement. The dermal spinules differ from teeth in similar fashion and we found strong support for the homology of the dermal spinules to the denticular odontodes. However, further research on the underlying genetic pathways would help confirm a shared evolutionary origin between these two structures. Fraser et al (2010) described a set of genes called the odontode gene regulatory network (oGRN) that controls and regulates the growth of all odontode units. Several studies have documented this oGRN in extra-oral odontode development in chondrichthyans, siluriformes, and atherniformes (Fraser et al, 2010; Martin et al, 2016; Cooper et al, 2017; Mori and Nakamura, 2021; Rivera-Rivera et al, 2021). We expect that both the dermal spinules and denticular odontodes share the same developmental pathways controlled by a similar oGRN, but early phenotypic plasticity allows for the specialization of the denticular odontodes into the denticular apparatus. The denticular odontodes are structurally homologous across taxa regardless of the morphology of the whole denticular apparatus (Figure 6). We see similar morphology of the apparatus in *Melanocetus* and *Himantolophus*, two genera with males that attach to females only temporarily, and different morphology in the permanently attaching *Linophryne*. Only melanocetid males have been found with food items in the stomach and are known to increase in relative size while free-living (Bertelsen, 1951; Pietsch 1976; Pietsch and Van Duzer, 1980). Himantolophid males were initially thought to feed and grow while free-living in similar fashion to melanocetids, but the work of Bertelsen and Krefft (1988) suggests they may not feed after metamorphosis, even though the denticular bones seem well-suited for prey capture. We thus propose that the denticular apparatus, at least in *Melanocetus* and possibly *Himantolophus*, has become a new set of functional “jaws” and “teeth”. While we did not specifically examine stomach contents, several of the cleared and stained *Melanocetus* males had food items in the stomach, which lends further support to this conclusion.

Dermal spinules are highly variable across the Ceratioidei as a whole and can be found in males and/or females of the Centrophrynidae, Ceratiidae, Diceratiidae, Gigantactinidae, Himantolophidae, Melanocetidae, and some genera of the Oneirodidae, although the evolutionary origin of the spinules has not been explored. Within the Melanocetidae, dermal spinules may or may not be present in all species, except for *M. nudus* (*nomen dubium*, females and larvae unknown), where they are hypothesized to always be absent (Pietsch and Van Duzer 1980; Pietsch 2009). We found dermal spinules in 60% of examined males (n= 40 out of 67), nearly all of which were visible under weak magnification. Unfortunately, we were unable to capture a male spinule in histology and only have a representation from a female *Melanocetus johnsonii* (UW 46520), but we assume the spinules are homologous between sexes. We found the dermal spinules are structurally homologous between two of our three examined taxa. Previous research has documented similarities in female spinules across several families using scanning electron microscopy (SEM), but this technique has yet to be applied to any male taxa (Pietsch, 2009). According to the literature, neither males nor females within the Linophrynidae have dermal spinules, but here we present evidence to the contrary (Bertelsen, 1951; Pietsch, 1976; Pietsch, 2005). These spinules were not visible even under strong magnification, but the histology clearly captured spinules in a male (Fig 4B). We were unable to isolate spinules from *Himantolophus*, but both male and female himantolophids have some of the strongest and most abundant spinules of all ceratioids that are clearly visible to the naked eye.

Development of male deep-sea anglerfishes

Bertelsen (1951) identified three stages of ceratioid ontogeny as follows: 1) Larvae: skin inflated, only pigment is sub-dermal, nostrils small, no denticles; 2) Metamorphosis: skin becomes tighter, some pigment in the skin, sub-dermal pigment remains, denticles appearing in snout and chin, jaw teeth reducing, nostrils enlarging; and 3) Adolescent: skin brown or black, not inflated, sub-dermal pigment gone, eyes and nostrils enlarging, denticles usually fused basally, larval teeth lost. While our observations agree with the three stages of Bertelsen, we described 4 distinct stages based on the 9 characters given above. The patterns explained by these characters provide greater detail and consistency in our understanding of how the denticular odontodes and bones develop. These stages are not an attempt to redefine the work of Bertelsen, but rather serve to enhance our ability to discuss male ontogeny where many other taxonomic characters remain unclear. While our study focused primarily on Melanocetidae, the stages here apply to the

Himantolophidae as well, the only other family where males develop rostral plates and spines. All other male ceratioids, except those in the Ceratiidae, never develop a direct connection between the denticular apparatus and the pterygiophore, so the application of Stage 4 development to broader ceratioid ontogeny is unknown.

In general, larger specimens were more developed than smaller ones, but size is not a consistent indicator of age or development in this group (Figure 15). We saw considerable variation and overlap in size across all stages. For example, the largest Stage 2 individual was 21 mm while the smallest Stage 4 was 15.5 mm. Stage 1 saw the least amount of overlap with other stages where almost all larvae were under 10 mm. However, we had 6 individuals in this stage ranging from 11-17.4 mm that retained larval characteristics and showed no signs of denticular development. These 6 individuals all look very similar to one another and look like greatly enlarged versions of the standard larval form. This may indicate the presence of multiple species (some perhaps undescribed) with different rates of development in our sample but is more likely explained by intraspecifically variable growth rates, although the underlying mechanisms driving this are unknown in this group. Most adolescent non-parasitic male ceratioids that have been examined have had large testes, suggesting these males reach sexual maturity quite quickly (Pietsch, 2009). However, the relationship between gonad size and denticular development has not been explored.

Muscular control of denticular apparatus

While some observations have demonstrated the motion of the upper denticular bone via gross manipulation, this study represents the first detailed histology of the denticular apparatus and its associated muscles and connections. Previous histological examinations of male ceratioids have focused almost exclusively on the gonads and viscera or the fusion area between male and female tissues (Parr, 1930; Regan and Trewavas, 1926; Shoemaker, 1958; Olsson, 1974; Munk and Bertelsen, 1983; Munk, 2000). There are no previous observations of lower denticular motion, and the description of the muscle along the lower jaw is, to the best of our knowledge, entirely new. This muscle is most likely the Aw section of the adductor mandibulae complex. The Aw section has been documented laterally along the dentary in females of the genus *Gigantactis* (Gigantactinidae), but there are no comparable descriptions of males or other female species (Waterman 1948; Bertelsen et al, 1981). While our histology only captured the musculature in *Melanocetus*, previous work has demonstrated that parasitic males tend to have smaller and less developed muscles around the pterygiophore than non-parasitic males, and similar patterns may exist in the lower denticular musculature as well.

Distribution of odontodes in bony fishes

To date, odontodes have been described in five unrelated teleost lineages: the denticulate catfishes (suborder Loricarioidei), the denticle herring (*Denticeps clupeiodes*), the bearded silverside (*Atherion elymus*), swordfish and billfish (Xiphiidae), and spiny lumpsuckers (Cyclopteridae) (Carter, 1919; Schaefer and Buitrago-Suarez, 2002; Rivera-Rivera and Montoya-Burgos, 2017; Rivera-Rivera et al, 2021; Sire, 2001; Sire, Marin, and Allizard, 1998; Woodruff et al, 2022). The odontodes seen here in anglerfish represents a sixth radiation in teleosts and a novel application of the odontodes. While similar in structure and morphology to other teleost odontodes, the denticular odontodes of ceratioid anglerfishes lack an enamel cap and have been adapted to serve as a new set of functional 'jaws' and 'teeth', at least in some taxa. Furthermore, the modification of the attachment bone into the movable upper and lower denticular bones is unique to the ceratioids. Most other teleost odontodes are isolated (i.e. not fused) though they may densely cover certain areas of the head or body, as seen in *Denticeps*, *Atherion*, and some loricariids (Sire et al, 1998; Sire, 2001; Schaefer and Buitrago-Suarez, 2002). Some loricarioids may have the large networks of attachment bone in the form of large bony plates, but these are starkly different from the fused denticular bones of ceratioids (Mori and Nakamura, 2021). Ceratioid fishes remain one of the most uniquely adapted groups of fishes and further research is necessary to understand the intense selective pressures exerted in the deep-sea and explore the extreme diversity seen in this group. Further research should focus on examining the underlying genetic and developmental pathways to assess the evolutionary origin of these ceratioid odontodes and their relation to teleost odontodes.

FIGURES

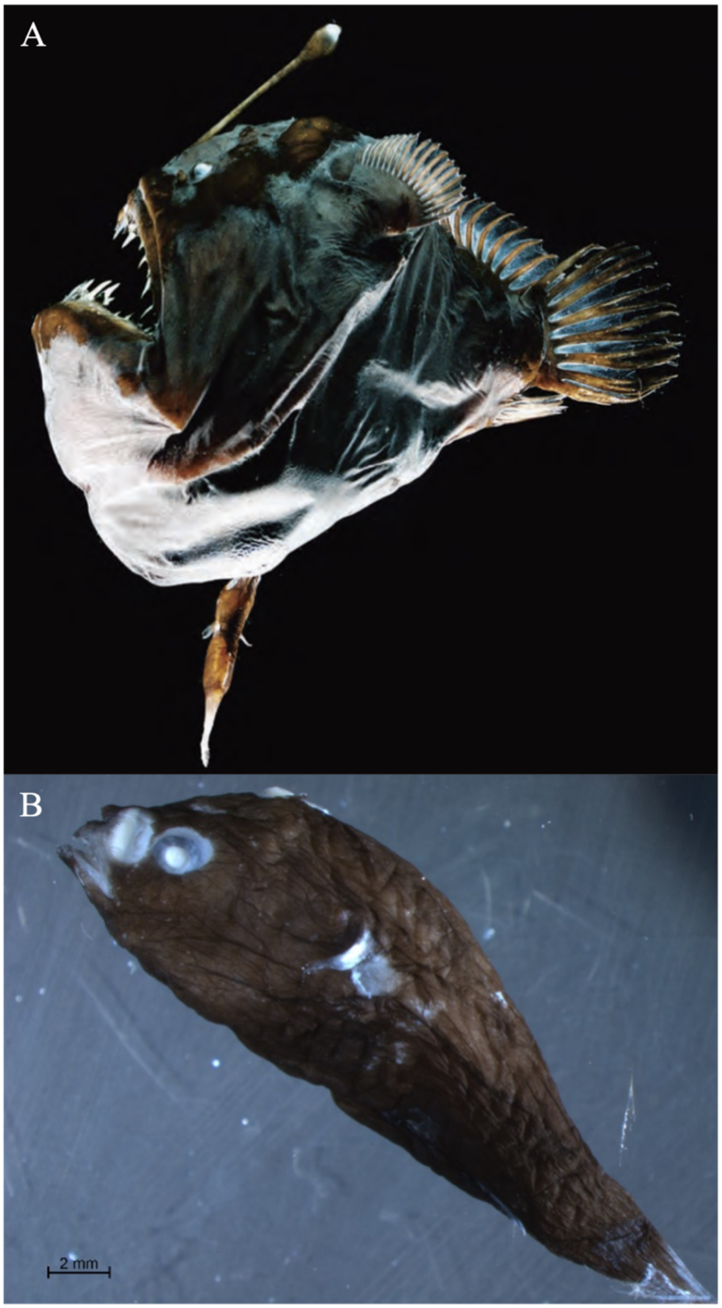


Figure 1 (A) *Melanocetus johnsonii*, a 75 mm female with a 23.5 mm male attached, BMNH 2004.6.3.2-3. Photo by Edith A. Widder. (B) *Melanocetus johnsonii*, a 23.5 mm free-living male (UW 158696).

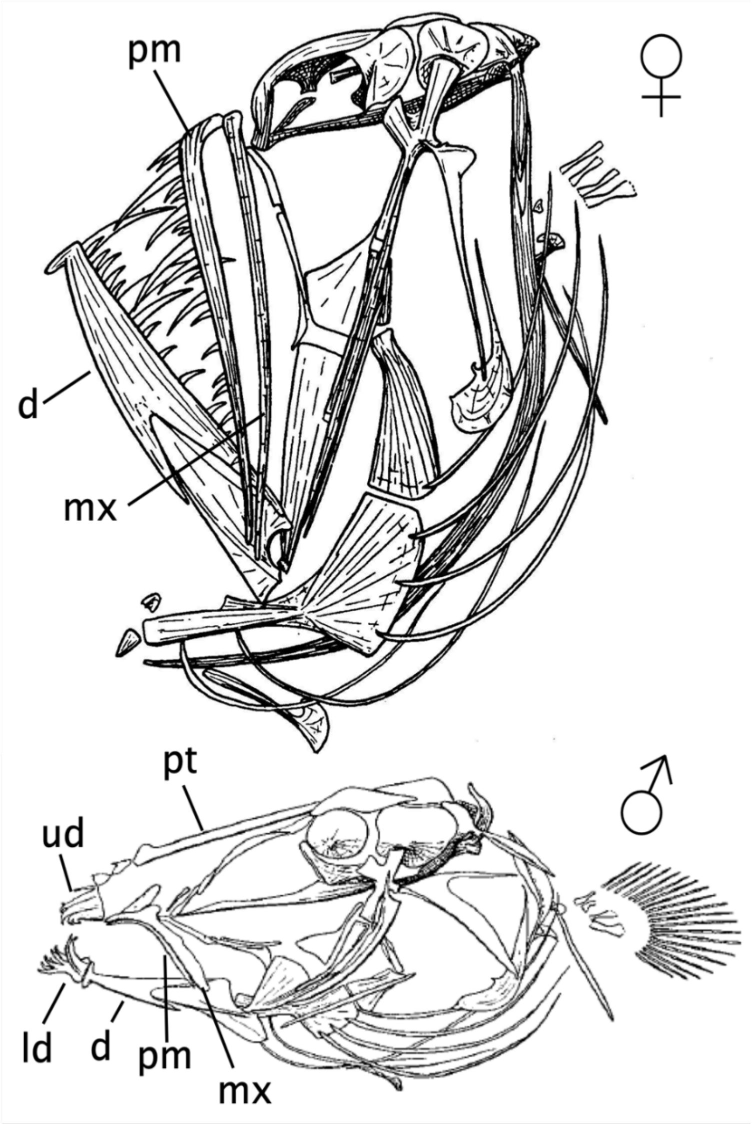


Figure 2 Comparison of the cephalic skeleton of an adult female (above) and an adult male (below) *Melanocetus johnsonii* showing the sexual dimorphism found in ceratioid anatomies. Modified from Regan and Trewavas, 1932.

Labels: d-dentary, ld-lower denticular, mx-maxilla, pm-premaxilla, pt-pterygiophore, ud-upper denticular

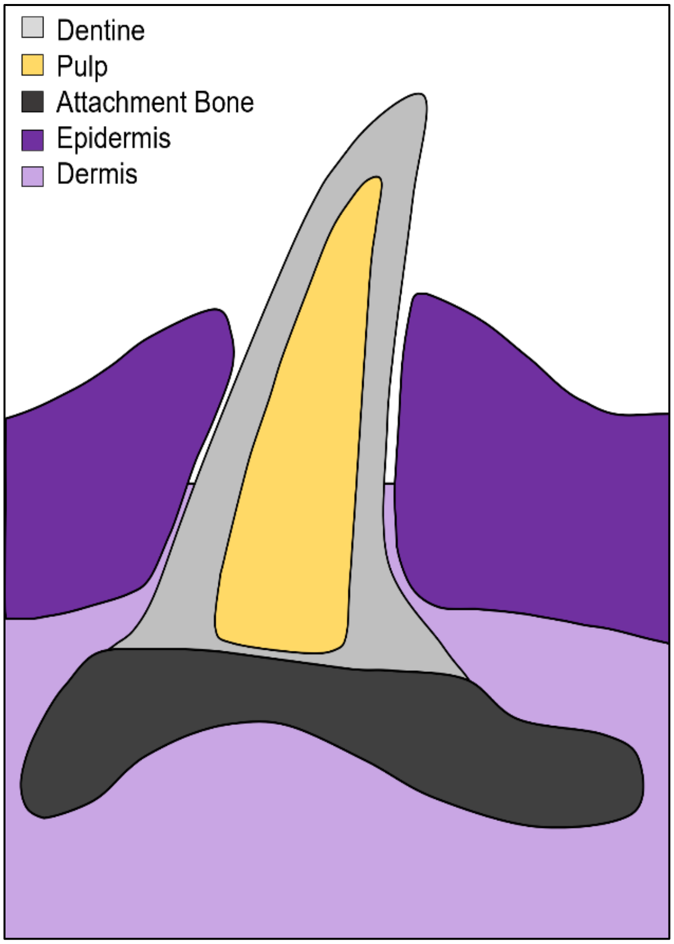


Figure 3 Generalized schematic of an odontode

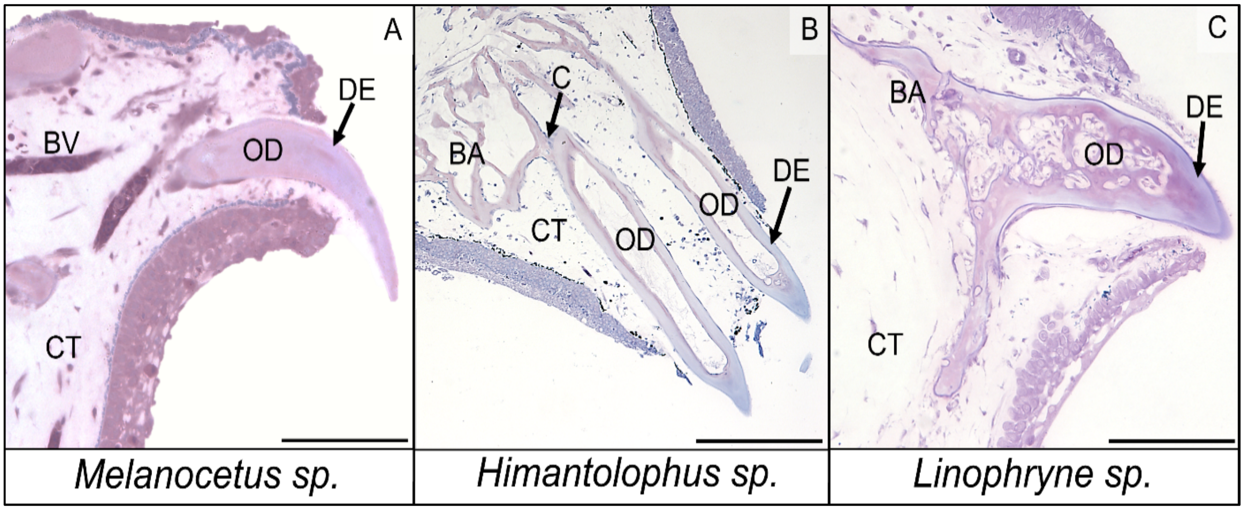


Figure 4 Variation in the morphology of denticular odontodes in male ceratioids.

Sagittal section at 3um through the upper denticular bone in (A) *Melanocetus*, (B) *Himantolophus*, and (C) *Linophryne.* Panel A shows vascularized tissue near the odontode root. The odontic channels (BA) can be seen in panel B posterior to the odontode roots. Scale set to 250um. BA = bone of attachment, BV = blood vessel, C = unmineralized collagen, DE = dentin, CT = connective tissue, OD = odontode.

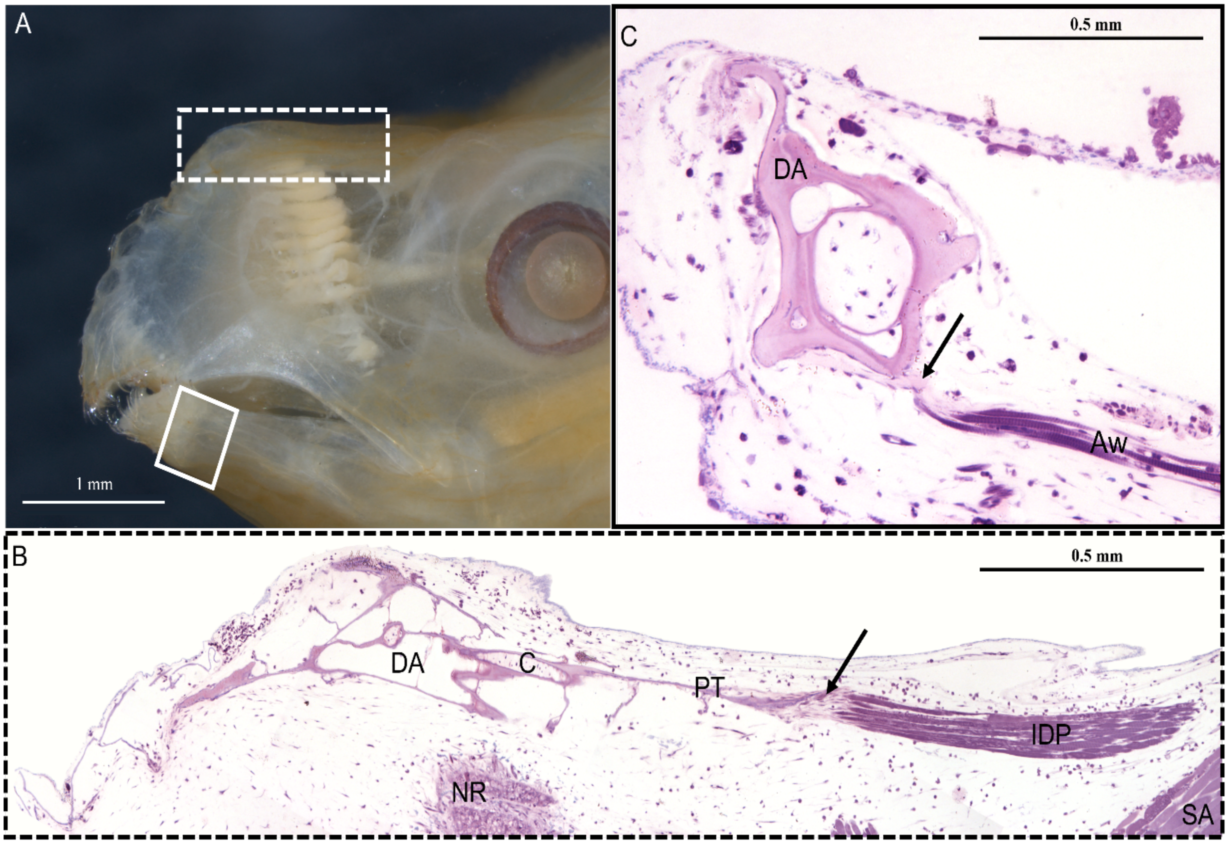


Figure 5 Muscle attachment to the denticular apparatus in *Melanocetus* sp. (UW 47111)

(A) Lateral profile showing the preserved specimen with the fully formed denticular apparatus. (B) Section through the junction between the upper denticular bone and the illicial pterygiophore. (C) Skeletal muscle along the ventral surface of the dentary inserts onto the distal edge of the lower denticular bone.

Arrows point to zones of muscle attachment. Aw = adductor mandibulae section Aw, C= cartilage, DA = denticular apparatus, IDP = inclinator dorsalis posterior, NR = nasal rosettes, PT= pterygiophore, SA = supracarinalis anterior. Sectioned at 3um

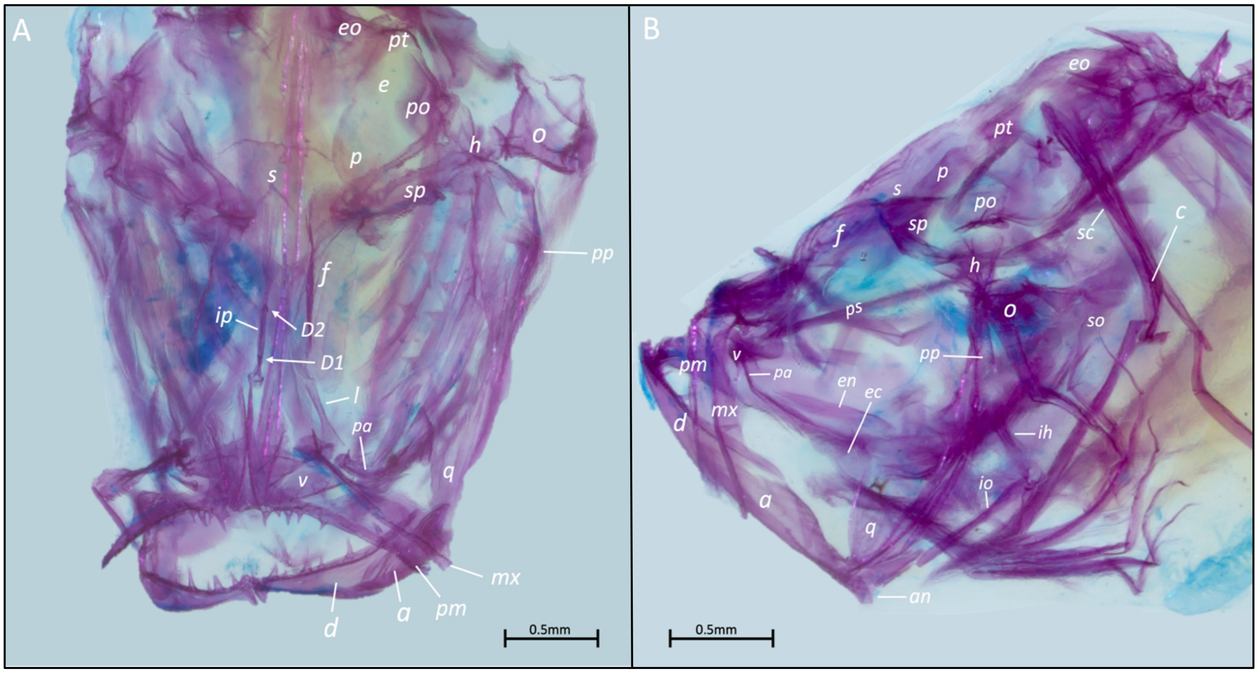


Figure 6 Stage 1 Cephalic Skeleton- *Melanocetus sp.* (MCZ 67989)

(A) Dorsal view. Many of the cranial bones are weakly ossified at this stage. (B) Left lateral view. Some bones here are slightly broken or displaced, namely the cleithrum and operculum. Labels: *a*- articular, *an*- angular, *c*- cleithrum, *ch*- ceratohyal, *d*- dentary, *D1*- dorsal fin spine 1, *D2*- dorsal fin spine 2, *e*- epiotic *ec*- ectopterygoid, *eh*- epihyal, *en*- entopterygoid, *eo*-exoccipital, *f*- frontal, *h*- hyomandibular, *ih*- interhyal, *io*- interoperculum, *ip*- illicial pterygiophore, *l*- lateral ethmoid, *m*- mesethmoid, *mx*- maxilla, *o*- operculum, *p*- parietal, *pa*-palatine, *pm*- premaxilla, *po*- pterotic, *pp*- preoperculum, *ps*- parasphenoid, *pt*- posttemporal, *q*-quadrate, *s*- supraoccipital, *sc*- supracleithrum, *so*- suboperculum, *sp*- sphenotic, *ud*- upper denticular, *v*- vomer

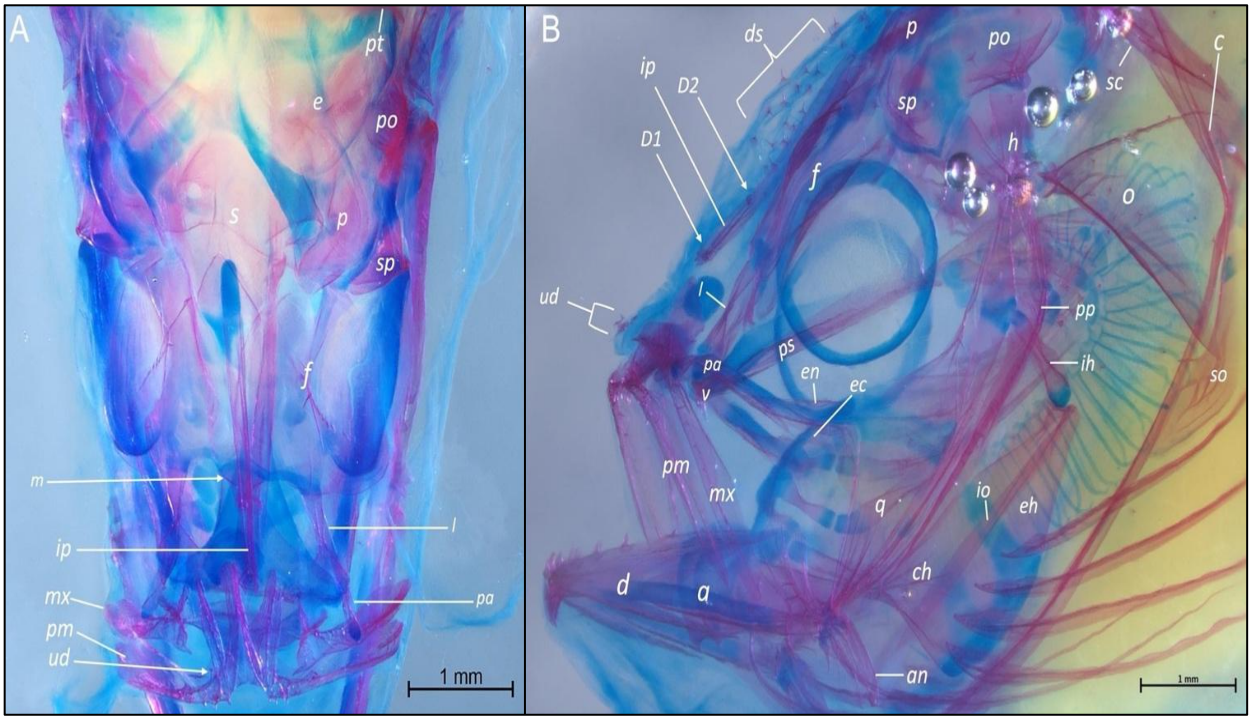


Figure 7 Stage 2 Cephalic Skeleton- ***Melanocetus johnsonii*** **(UW 158702)**

(A) Dorsal view with the skin dissected away for a clearer view of the bones. (B) Left lateral view with the skin still intact and bearing numerous dermal spinules.

Labels: *a*- articular, *an*- angular, *c*- cleithrum, *ch*- ceratohyal, *d*- dentary, *D1*- dorsal fin spine 1, *D2*- dorsal fin spine 2, *e*- epiotic *ec*- ectopterygoid, *eh*- epihyal, *en*- entopterygoid, *eo*- exoccipital, *f*- frontal, *h*- hyomandibular, *ih*- interhyal, *io*- interoperculum, *ip*- illicial pterygiophore, *l*- lateral ethmoid, *m*- mesethmoid, *mx*- maxilla, *o*- operculum, *p*- parietal, *pa*- palatine, *pm*- premaxilla, *po*- pterotic, *pp*- preoperculum, *ps*- parasphenoid, *pt*- posttemporal, *q*- quadrate, *s*- supraoccipital, *sc*- supracleithrum, *so*- suboperculum, *sp*- sphenotic, *ud*- upper denticular, *v*- vomer

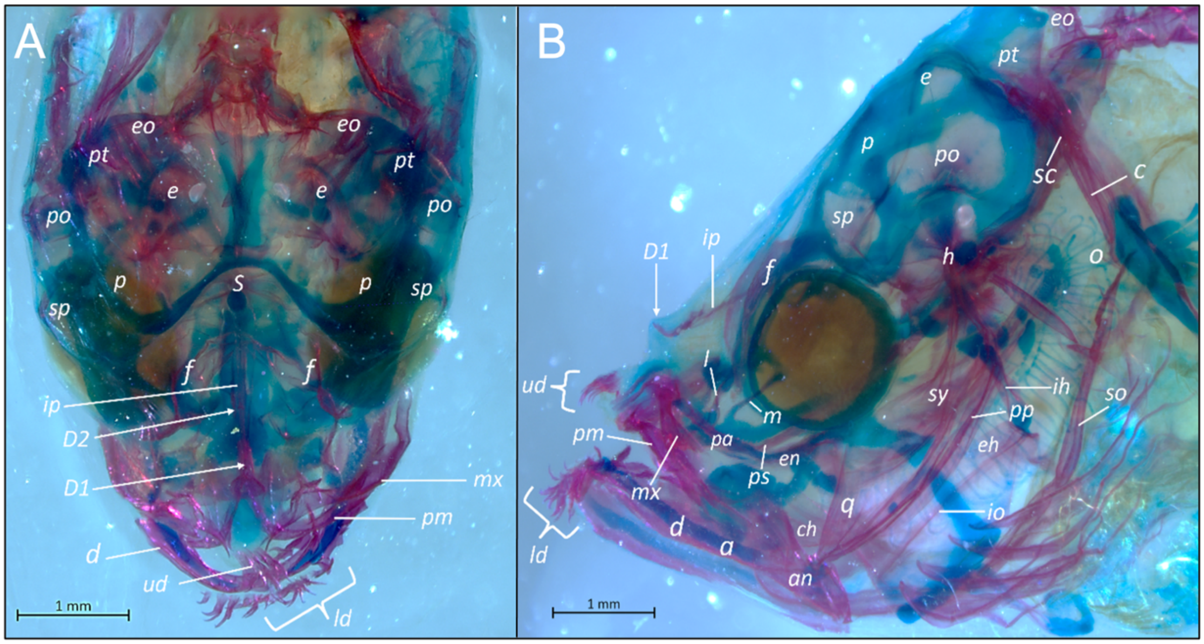


Figure 8 Stage 3 Cephalic Skeleton- *Melanocetus johnsonii* (UW 158699)

(A) Dorsal view. (B) Left lateral view showing the denticular odontodes in the early stages of fusion. The maxilla and dentary are partially broken.

Labels: *a*- articular, *an*- angular, *c*- cleithrum, *ch*- ceratohyal, *d*- dentary, *D1*- dorsal fin spine 1, *D2*- dorsal fin spine 2, *e*- epiotic, *eh*- epihyal, *en*- entopterygoid, *eo*- exoccipital, *f*- frontal, *h*- hyomandibular, *ih*- interhyal, *io*- interoperculum, *ip*- illicial pterygiophore, *l*- lateral ethmoid, *m*- mesethmoid, *mx*- maxilla, *o*- operculum, *p*- parietal, *pa*- palatine, *pm*- premaxilla, *po*- pterotic, *pp*- preoperculum, *ps*- parasphenoid, *pt*- posttemporal, *q*- quadrate, *s*- supraoccipital, *sc*- supracleithrum, *so*- suboperculum, *sp*- sphenotic, *sy*-symplectic, *ud*- upper denticular, *v*- vomer

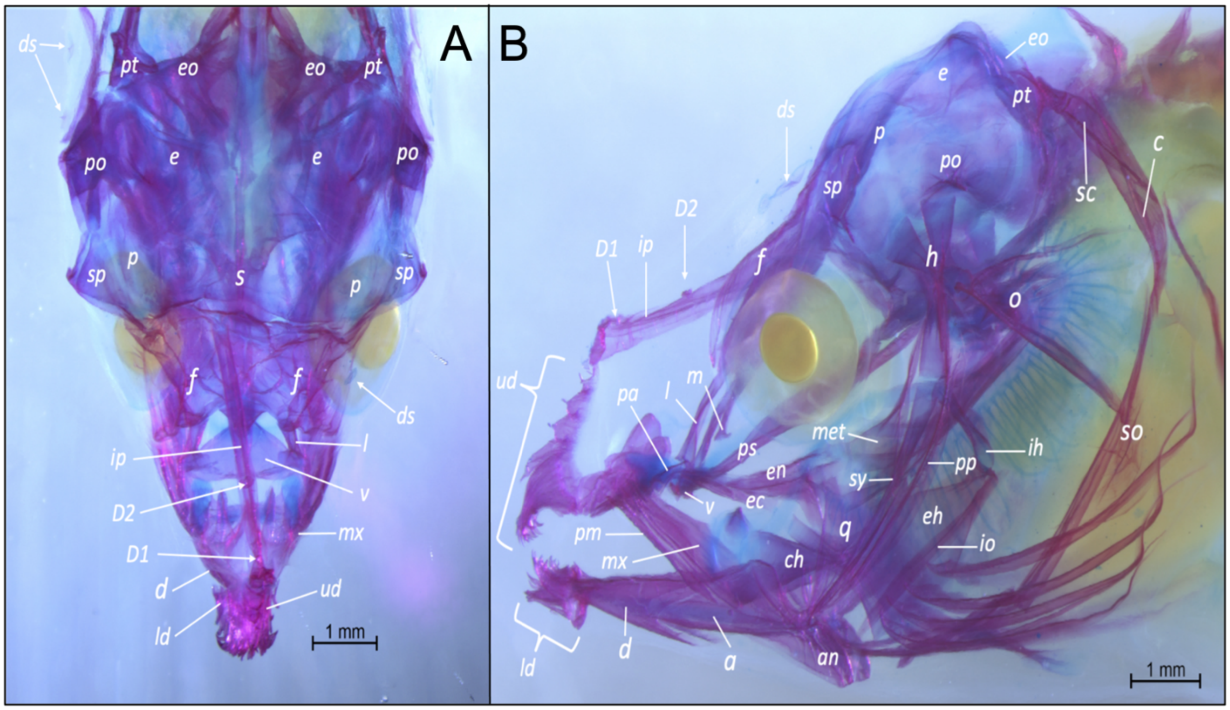


Figure 9 Stage 4 Cephalic Skeleton- *Melanocetus johnsonii* (UW 47110)

(A) Dorsal view. (B) Left lateral view showing the fully formed denticular apparatus. The upper denticular bone is joined to the tip of the pterygiophore and a faint dermal spinule is visible dorsal to the supraoccipital.

Labels (after Bertelsen, 1951): *a*- articular, *an*- angular, *c*- cleithrum, *ch*- ceratohyal, *d*- dentary, *D1*- dorsal fin spine 1, *D2*- dorsal fin spine 2, *ds*- dermal spinule, *e*- epiotic *ec*- ectopterygoid, *eh*- epihyal, *en*- entopterygoid, *eo*- exoccipital, *f*- frontal, *h*- hyomandibular, *ih*- interhyal, *io*- interoperculum, *ip*- illicial pterygiophore, *l*- lateral ethmoid, *m*- mesethmoid, *mx*- maxilla, *o*- operculum, *p*- parietal, *pa*- palatine, *pm*- premaxilla, *po*- pterotic, *pp*- preoperculum, *ps*- parasphenoid, *pt*- posttemporal, *q*- quadrate, *s*- supraoccipital, *sc*- supracleithrum, *so*- suboperculum, *sp*- sphenotic, *sy*-symplectic, *ud*- upper denticular, *v*- vomer

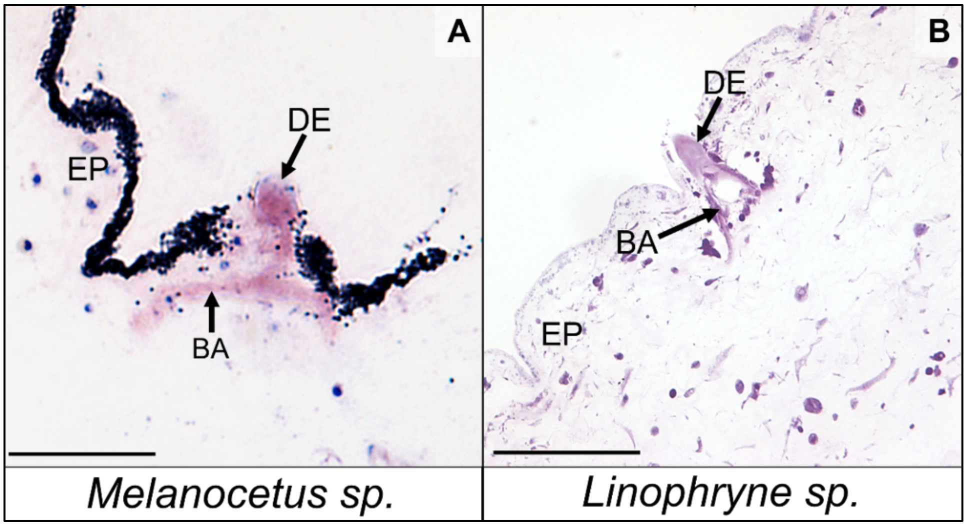


Figure 10 Histology of dermal spinules in Ceratioids. The dermal spinules share similar structure and morphology in both (A) female *Melanocetus*, and (B) male Linophryne. Scale set to 250um. BA = bone of attachment, DE = dentin, EP = epidermis

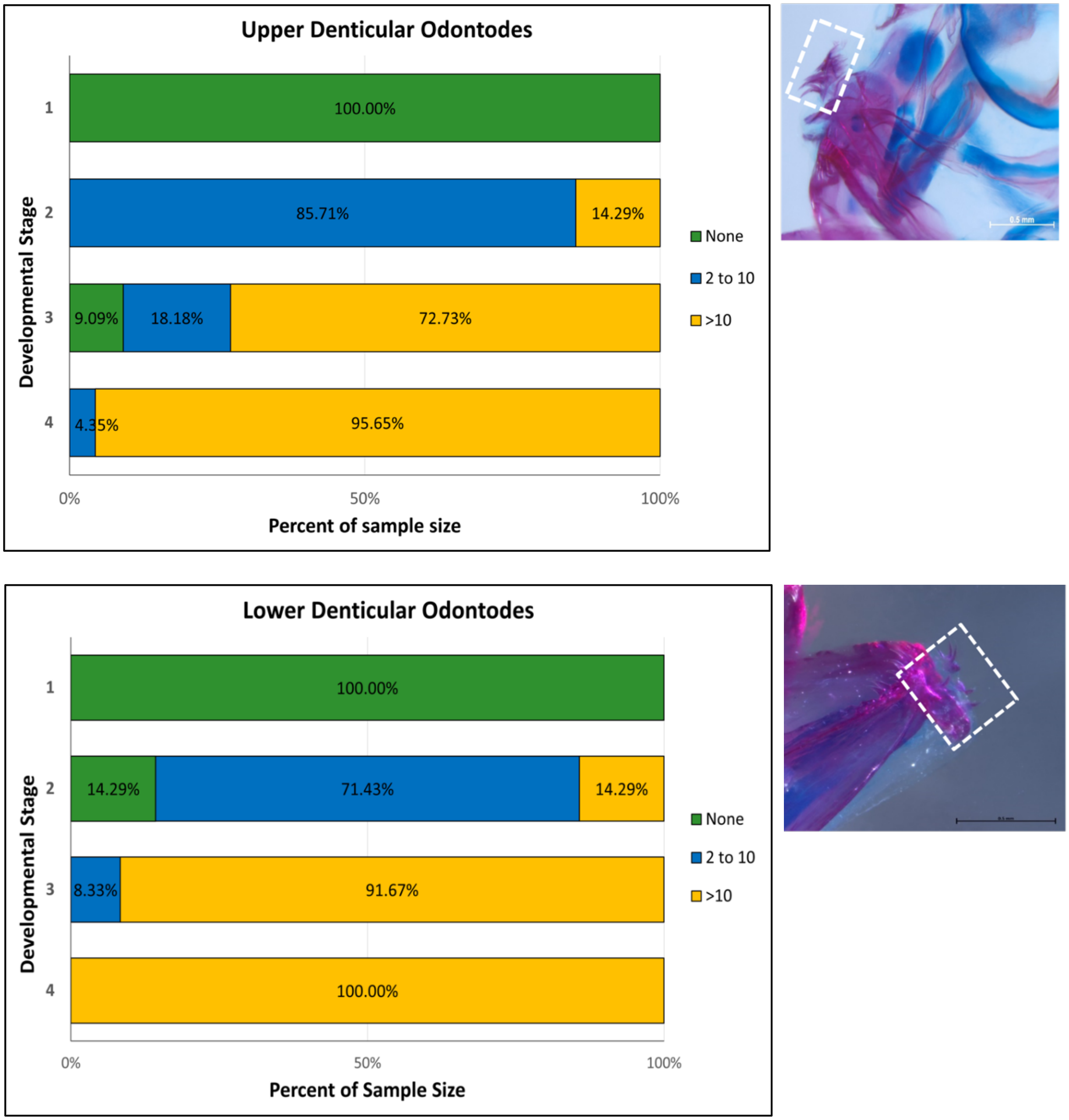
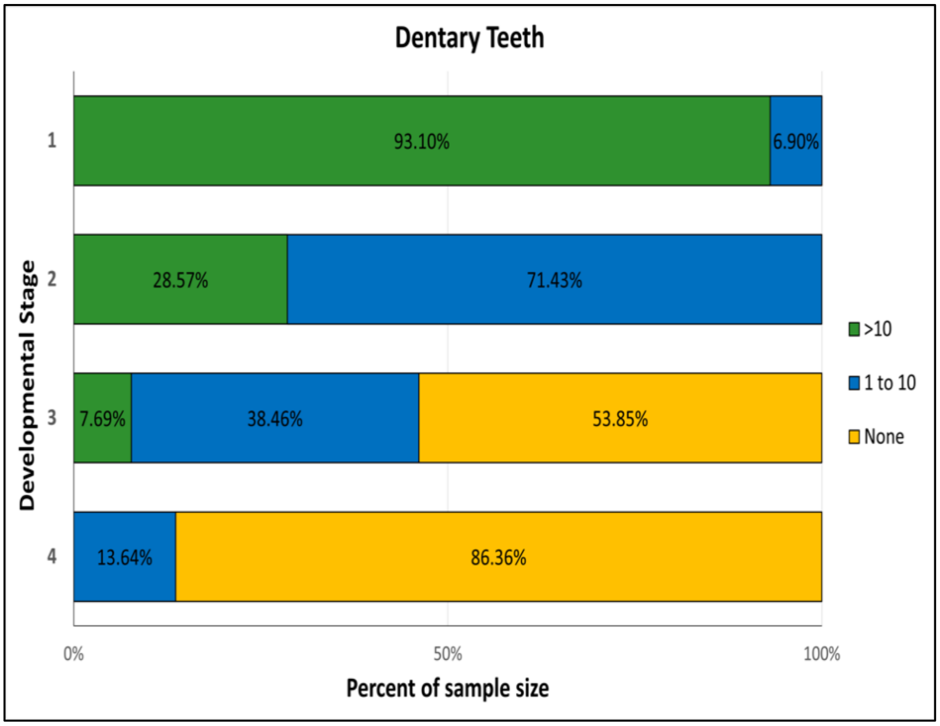
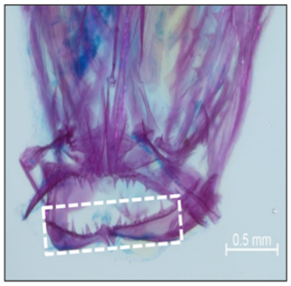
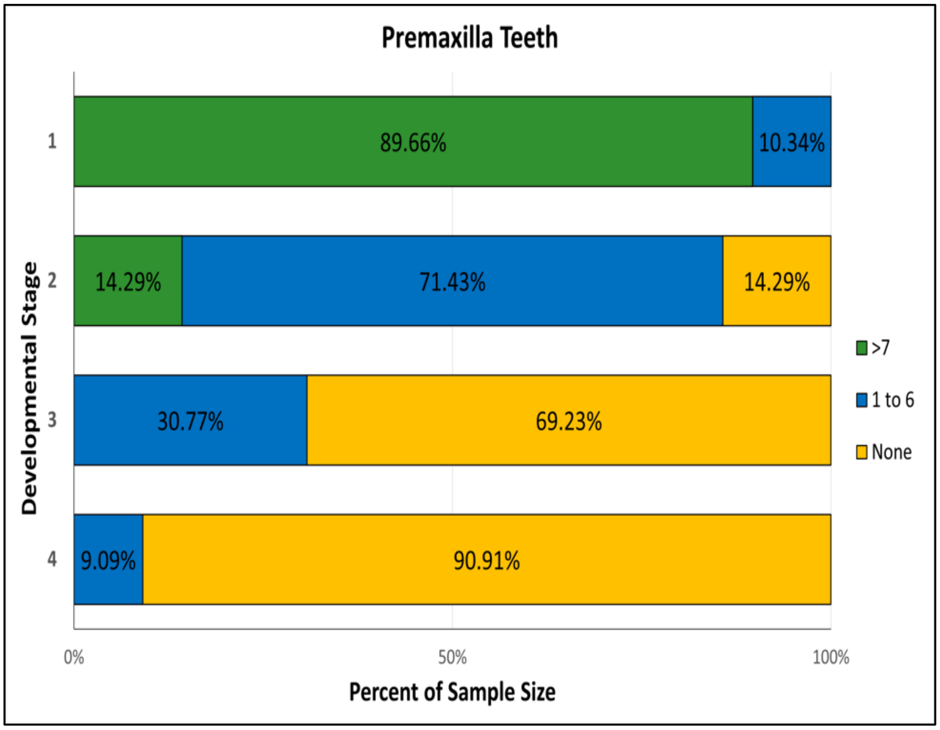
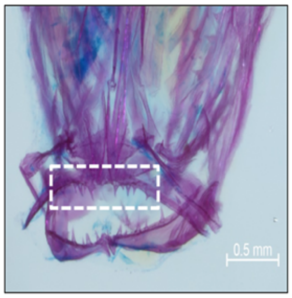


Figure 11 Distribution of upper and lower denticular odontodes in *Melanocetus*. Only a single Stage 4 fish had fewer than 10 odontodes in the upper denticular. Bifurcated odontodes were counted as a single unit.


Dentary Teeth
Developmental Stage
1
2
3
4
93.10%
6.90%
28.57%
71.43%
7.69%
38.46%
53.85%
13.64%
86.36%
🟩 >10
🟦 1 to 10
🟨 None
0%
50%
100%
Percent of sample size


0.5 mm


Premaxilla Teeth
Developmental Stage
1
2
3
4
89.66%
10.34%
14.29%
71.43%
14.29%
30.77%
69.23%
9.09%
90.91%
🟩 >7
🟦 1 to 6
🟨 None
0%
50%
100%
Percent of Sample Size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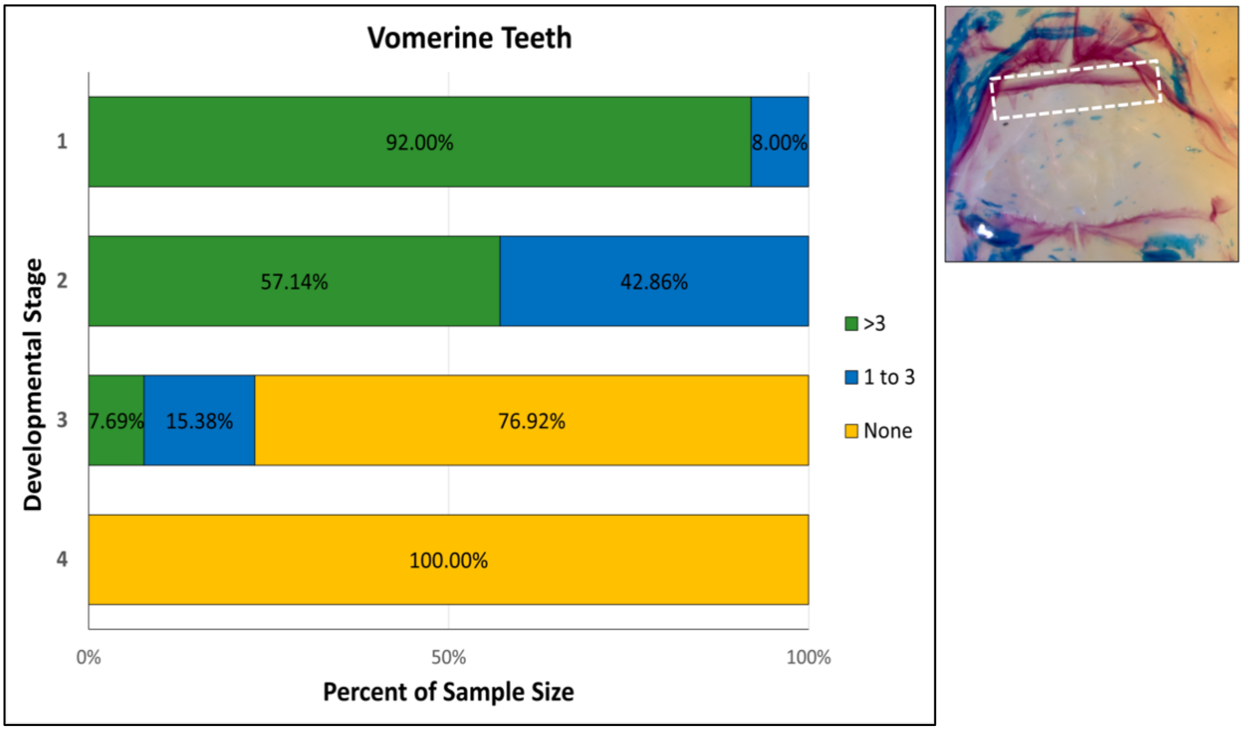

Figure 12 Distribution of oral teeth. Jaw teeth were lost in 20 of 23 Stage 4 individuals. Vomerine teeth were completely lost in all Stage 4 individuals.

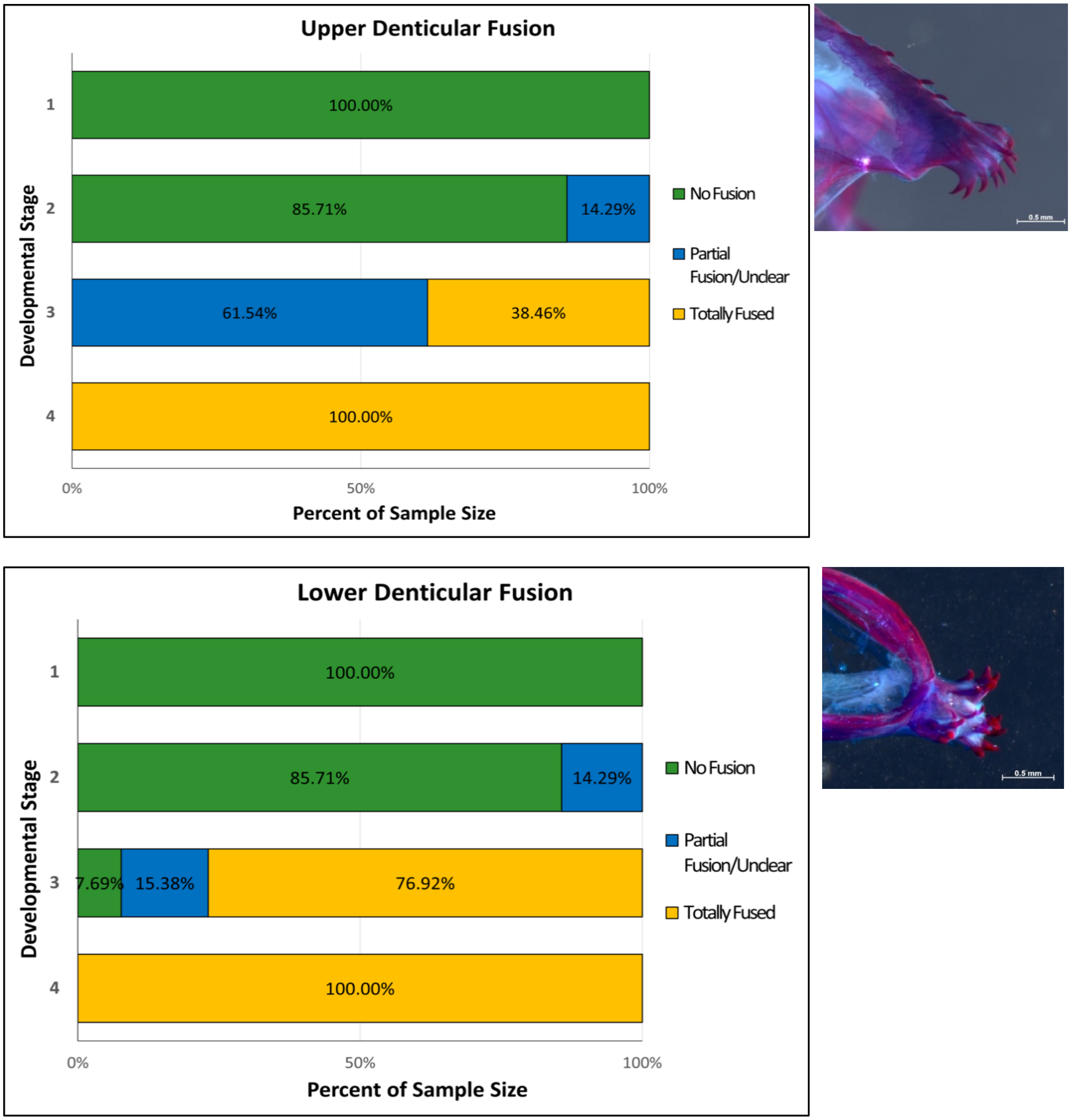

Figure 13 Frequency of denticular odontode fusion. The lower denticular bone fused before the upper denticular bone in 6 out of 13 Stage 3 specimens.

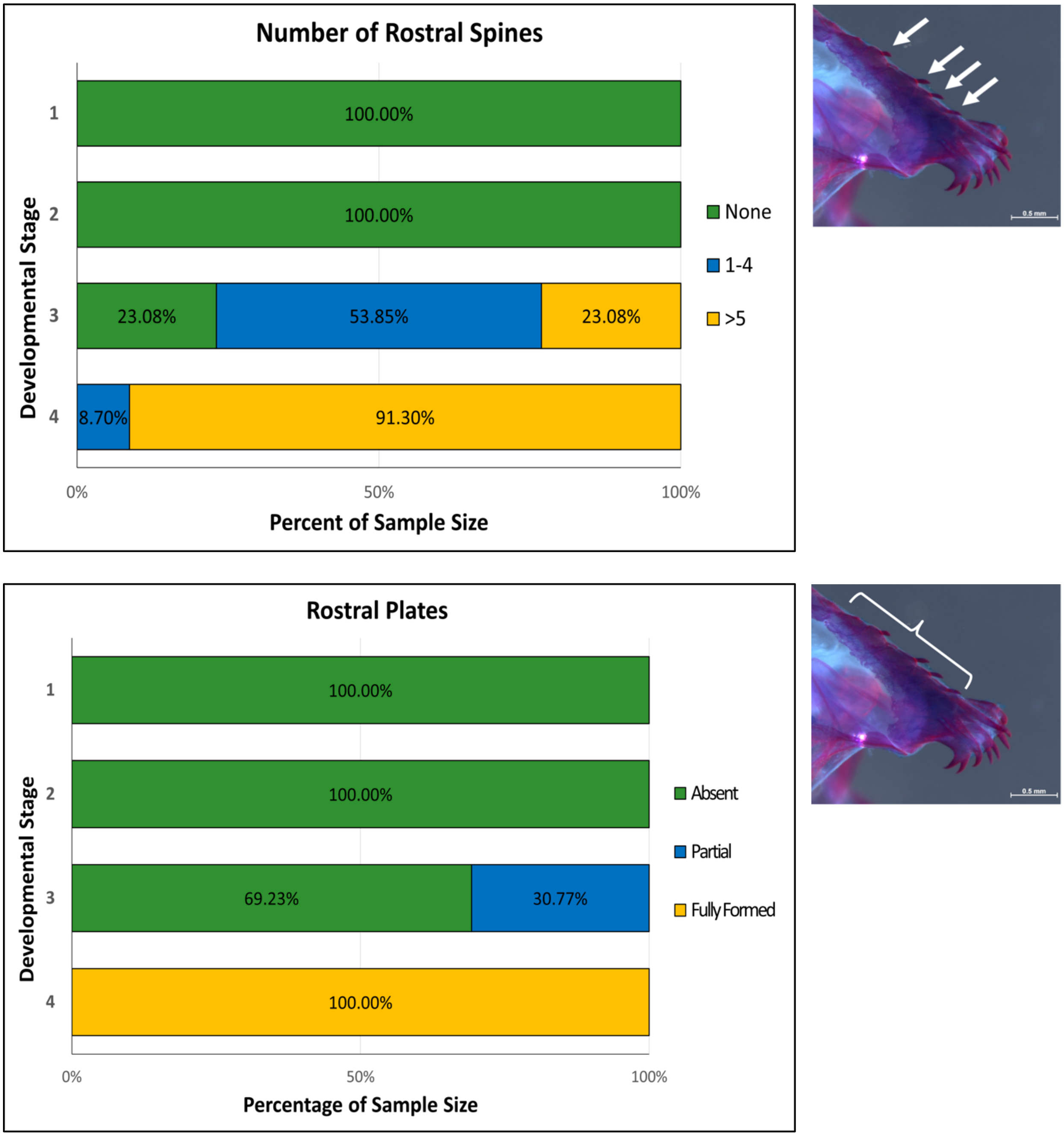

Figure 14 Count of rostral spines and distribution of rostral plate formation. Partial rostral plate formation was seen in 4 of the 13 examined Stage 3 fish. Only Stage 4 fish had fully formed rostral plates.

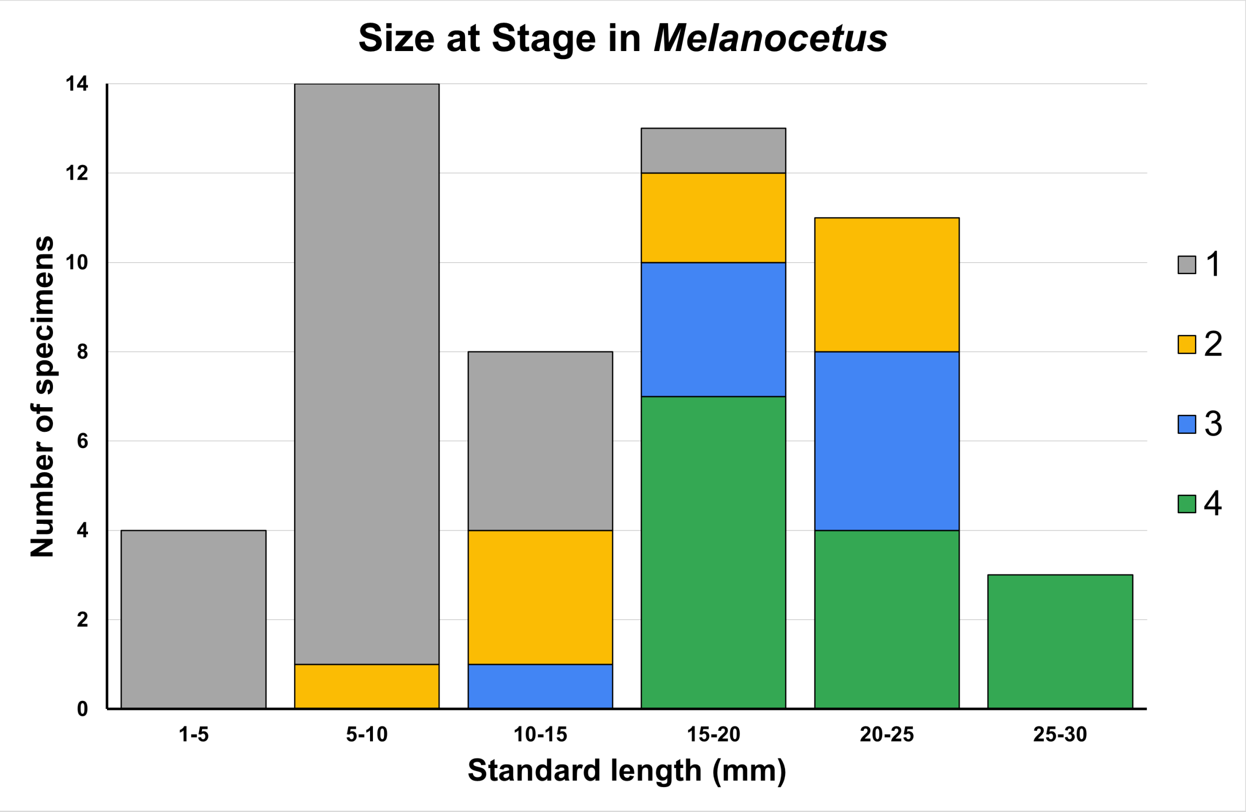

Figure 15 Breakdown of standard lengths across all four developmental stages in *Melanocetus*. Sizes were separated by increments of five millimeters from 1 to 30 mm SL. The largest Stage 1 individual (17.6mm) overlapped with the smallest Stage 4 individual (15.5mm).

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APPENDIX Summary of examined material.

Institution	**Catalog Number**	**Species**	**n**	**SL (mm)**	**Disposition**
MCZ	49942	*Melanocetus sp.*	1	1.25	70% ETOH
MCZ	50029	*Melanocetus sp.*	1	3.3	70% ETOH
MCZ	68005-2	*Melanocetus sp.*	1	3.5	C&S
MCZ	68005-1	*Melanocetus sp.*	1	4.8	70% ETOH
MCZ	67989	*Melanocetus sp.*	1	5.2	C&S
MCZ	161472	*Melanocetus sp.*	1	5.38	70% ETOH
MCZ	51190	*Melanocetus sp.*	1	5.5	70% ETOH
MCZ	68008	*Melanocetus johnsonii*	1	6.3	70% ETOH
MCZ	50037-2	*Melanocetus sp.*	1	6.3	C&S
MCZ	67993-2	*Melanocetus sp.*	1	6.4	70% ETOH
MCZ	68000	*Melanocetus sp.*	1	7.1	70% ETOH
MCZ	50037-1	*Melanocetus sp.*	1	7.1	70% ETOH
MCZ	51179	*Melanocetus sp.*	1	7.72	70% ETOH
MCZ	173822-1	*Melanocetus johnsonii*	2	7.9	70% ETOH
MCZ	67988	*Melanocetus sp.*	1	8.2	70% ETOH
MCZ	97103-2	*Melanocetus sp.*	1	8.24	70% ETOH
MCZ	67993-1	*Melanocetus sp.*	1	8.3	C&S
MCZ	173822-2	*Melanocetus johnsonii*	2	8.5	70% ETOH
MCZ	97103-1	*Melanocetus sp.*	1	8.56	70% ETOH
MCZ	50039	*Melanocetus johnsonii*	1	8.6	70% ETOH

MCZ	168806	*Melanocetus sp.*	1	8.7	70% ETOH
MCZ	76304	*Melanocetus johnsonii*	1	9.2	70% ETOH
MCZ	51188	*Melanocetus sp.*	1	9.22	70% ETOH
MCZ	65545-2	*Melanocetus sp.*	1	10	C&S
MCZ	51181-2	*Melanocetus sp.*	1	11	C&S
MCZ	51181-1	*Melanocetus sp.*	1	11.3	70% ETOH
MCZ	57807	*Melanocetus johnsonii*	1	12.3	70% ETOH
MCZ	164926	*Melanocetus johnsonii*	1	15.6	70% ETOH
MCZ	57495	*Melanocetus sp.*	1	17.4	70% ETOH
MCZ	65542-2	*Melanocetus sp.*	1	11.6	70% ETOH
MCZ	65545-1	*Melanocetus sp.*	1	10.7	70% ETOH
MCZ	65542-1	*Melanocetus sp.*	1	12.5	70% ETOH
MCZ	51198	*Melanocetus sp.*	1	15.2	70% ETOH
MCZ	134642	*Melanocetus sp.*	1	21	70% ETOH
MCZ	61197	*Melanocetus sp.*	1	25	70% ETOH
MCZ	51184	*Melanocetus sp.*	1	15.5	C&S
MCZ	51185-1	*Melanocetus sp.*	1	16.2	C&S
MCZ	144829	*Melanocetus johnsonii*	1	22.5	70% ETOH
MCZ	51182	*Melanocetus sp.*	1	23.2	70% ETOH
MCZ	51185-2	*Melanocetus sp.*	1	19.4	70% ETOH
SIO	63-905	*Melanocetus sp.*	1	13	50% ISOPROPANOL

SIO	70-311	Melanocetus sp.	1	14	50% ISOPROPANOL
SIO	73-156	Melanocetus sp.	1	14.4	50% ISOPROPANOL
SIO	71-304	*Melanocetus murrayi*	1	12.1	C&S
SIO	97-159-2	*Melanocetus johnsonii*	1	16	C&S
SIO	87- 38	Melanocetus johnsonii	1	19	50% ISOPROPANOL
SIO	60-215	*Melanocetus murrayi*	1	19.5	50% ISOPROPANOL
SIO	60-241-2	*Melanocetus johnsonii*	2	20.7	50% ISOPROPANOL
SIO	69-497	*Melanocetus sp.*	1	21	50% ISOPROPANOL
SIO	70-341	*Melanocetus sp.*	1	21	50% ISOPROPANOL
SIO	60-241	*Melanocetus murrayi*	1	21.1	50% ISOPROPANOL
SIO	60-241-1	*Melanocetus johnsonii*	2	21.2	50% ISOPROPANOL
SIO	61-46	*Melanocetus murrayi*	1	22	50% ISOPROPANOL

SIO	65-243	*Melanocetus sp.*	1	22	50% ISOPROPANOL
SIO	68-490	*Melanocetus sp.*	1	22	50% ISOPROPANOL
SIO	77-165	*Melanocetus johnsonii*	1	24	50% ISOPROPANOL
SIO	77-167	*Melanocetus sp.*	1	24	50% ISOPROPANOL
SIO	73-140	*Melanocetus sp.*	1	24.6	50% ISOPROPANOL
SIO	55-221	*Melanocetus sp.*	3	18-22	50% ISOPROPANOL
SIO	69-351	*Melanocetus sp.*	2	20-22.4	50% ISOPROPANOL
SIO	52-363	Melanocetus sp.	2	10-18.5	50% ISOPROPANOL
SIO	97-159-1	*Melanocetus johnsonii*	1	17	50% ISOPROPANOL
SIO	68-535	*Melanocetus sp.*	1	20	C&S
UW	47114	*Melanocetus sp.*	1	12.5	C&S
UW	158702	*Melanocetus johnsonii*	1	17.2	C&S
UW	158698	*Melanocetus johnsonii*	1	18.9	C&S
UW	158697	*Melanocetus johnsonii*	1	20.3	C&S

UW	158695	*Melanocetus johnsonii*	1	21.2	C&S
UW	158700	*Melanocetus johnsonii*	1	21.9	HISTOLOGY
UW	158699	*Melanocetus johnsonii*	1	18.4	95% ETOH
UW	158701	*Melanocetus johnsonii*	1	20.2	70% ETOH
UW	158696	*Melanocetus johnsonii*	1	23.5	95% ETOH
UW	47109	*Melanocetus sp.*	1	16	C&S
UW	47111	*Melanocetus sp.*	1	16	HISTOLOGY
UW	47112	*Melanocetus sp.*	1	16	HISTOLOGY
UW	158703	*Melanocetus johnsonii*	1	24	70% ETOH
UW	21189	*Melanocetus sp.*	1	25	C&S
UW	47110	*Melanocetus sp.*	1	26	C&S
UW	47113	*Melanocetus sp.*	1	23.5	70% ETOH
UW	47128	*Linophryne sp*	1	14	70% ETOH
UW	46520	*Melanocetus johnsonii*	1	17	70% ETOH
UW	47117	*Himantolophus sp*	1	17	70% ETOH
UW	47124	*Linophryne sp*	1	20.5	70% ETOH
UW	48242	*Himantolophus sp*	1	24	70% ETOH
UW	47540	*Melanocetus murrayi*	1	26	70% ETOH