

Highlights of Cetacean Embryology

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Abstract

The embryology of cetaceans documents features that elucidate the interaction between form and function in modern species, as well as their evolution from extinct ones. Prenatal specimens of the dolphin *Stenella attenuata* demonstrate critical aspects of the embryonic and fetal development of cetaceans, the pharyngeal arches, ear ossicles, nasal opening, teeth, forelimb, hind limb, and flukes. In addition, specimens of the bowhead whale (*Balaena mysticetus*) illustrate the development of baleen and hair.

Key Words: embryology, pharyngeal arch, ear development, tooth development, limb development, baleen development, hair, Delphinidae, *Stenella attenuata*, Balaenidae, *Balaena mysticetus*

Introduction

Cetacean embryos have features that they share with all other vertebrates, features they share with just mammals, and traits that are unique and best explained as developing adaptations to life in water. In this article, I highlight salient aspects of cetacean development that fall into these categories as I believe these traits can be used as striking examples to teach anatomy and embryology

against an evolutionary background. While most of the organ systems discussed herein have been published in the scientific literature, the illustrations in those papers are often technical, are in older publications that are often hard to find, and the text can be difficult to interpret by those who lack background knowledge in embryology. This article attempts to make cetacean embryology accessible as it has implications far beyond answering narrow embryological and developmental biological questions.

Embryological development of cetaceans is tightly linked to the evolution of cetaceans. The early part of cetacean evolution is one of the best-documented examples of major morphological evolution in mammals with a well-understood ecological context (Gatesy et al., 2013; Thewissen, 2014; Pyenson, 2017; Figure 1). Embryological evidence synergizes with it, showing evidence from the time when vertebrates emerged from the water, to the origin of mammals, to the eventual return of early whales back into first fresh water and then sea water. Furthermore, understanding cetacean embryology is important for understanding form and function of living whales and dolphins as much of cetacean behavior and ecology are constrained by their morphology, and because cetacean morphology is a direct consequence of prenatal development.

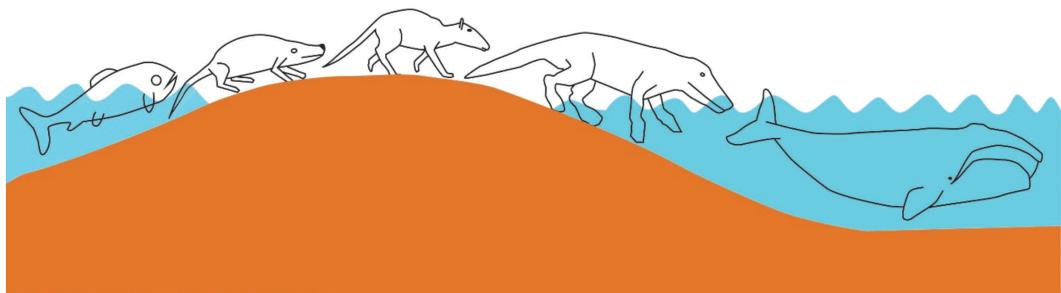


Figure 1. Diagrammatic representation of cetacean evolution, showing, respectively, from left to right, a bony fish (the ancestors of cetaceans left the water around 370 million years ago [mya]); an early mammal (the ancestors of which lived around 220 mya); the even-toed ungulate *Indohyus* (the sister group to cetaceans; it lived from 50-42 mya); the early whale *Ambulocetus*, which lived around 48 mya; and a modern bowhead whale (cetaceans became fully aquatic around 42 mya)

Methods

Most of the following discussion is based on an ontogenetic series of the *Stenella attenuata* (Odontoceti, Delphinidae), the pantropical spotted dolphin. The best description of the ontogeny of this species was published by Štěrba et al. (2000), who also made inferences about the gestational period of this species (Figure 2). A shorter description with a more conventional staging system and specific to the particular embryos used here is by Thewissen & Heyning (2007). These specimens are part of the collection of the Los Angeles Museum of Natural History (LACM) and were retrieved as part of monitoring activities of the Pacific Ocean tuna industry, an effort led by the National Oceanic and Atmospheric Administration (NOAA) of the United States.

In addition to dolphins, we also discuss some aspects of the embryology of the bowhead whale (*Balaena mysticetus*) and the beluga whale (*Delphinapterus leucas*). These specimens were retrieved from whales harvested in the Inupiat subsistence hunt in northern Alaska. They are part of the collections of the North Slope Borough, Department of Wildlife Management (NSB-DWM).

Results

Pharyngeal Arches

Pharyngeal arches, also called branchial arches or gill arches, are a row of tissue swellings on the left and right side of the throat area of an embryo (Graham, 2001; Figure 3). On the outside of the embryo, the pharyngeal arches are separated from each other by vertical grooves called pharyngeal clefts. The clefts are met by vertical grooves on the throat side (inside the embryo) called pharyngeal pouches. Arches, clefts, and pouches can be seen in a section through the head (Figure 4)

and are present in all vertebrates. The pharyngeal arches are a classic example of a structure that is present in all vertebrates in early development but modified beyond recognition in vertebrates that live on land. Even though they are transitory in mammals, they play an important role in the formation of the head and neck in all vertebrates and underlie congenitally many malformations of these areas (Frisdal & Trainor, 2014).

In fish embryos, clefts and pouches eventually form an open connection, creating a row of slits that can channel water from the throat of the animal to the outside: its system of gills (Wilson & Laurent, 2002). Clefts and pouches also form in mammalian embryos, but an open connection does not form in these. There are four pharyngeal arches in mammals, and they form in order: the first arch forms before the second, and so on. As development proceeds, they disappear again, leaving a smooth neck region. However, the arches, clefts, and pouches contribute to the formation of many postnatal structures, from aorta to thymus, and from mandible to ear bones. The retention in cetacean and other mammalian embryos of the embryonic structures that develop into gills in fish is a reminder of evolution that took place 370 million years ago (mya) when a bony fish evolved into an air-breathing tetrapod with lungs (Shubin, 2008).

Ear Ossicles

The ear ossicles are tiny bones located in an air-filled space (the middle ear cavity) in the skull. All mammals, including cetaceans, have three ear ossicles: (1) malleus (hammer), (2) incus (anvil), and (3) stapes (stirrup). They are arranged in a chain with joints between them. In general, the ossicles transmit sound, but their function in cetaceans is not clear, and their shape is very different from that of other mammals (Nummela et al., 2007).

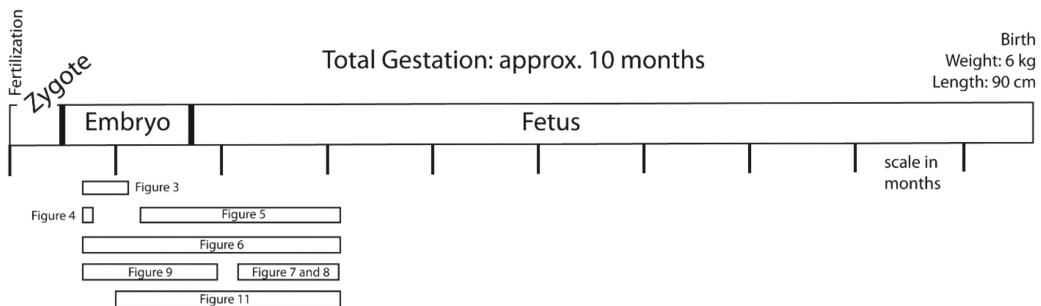


Figure 2. Time line for the prenatal development of the dolphin *Stenella attenuata*, showing the timing of zygotic, embryonic, and fetal period; boxes reference the timing of the prenatal stages used in the illustrations that follow, thereby indicating when major change occurs in different parts of ontogeny.

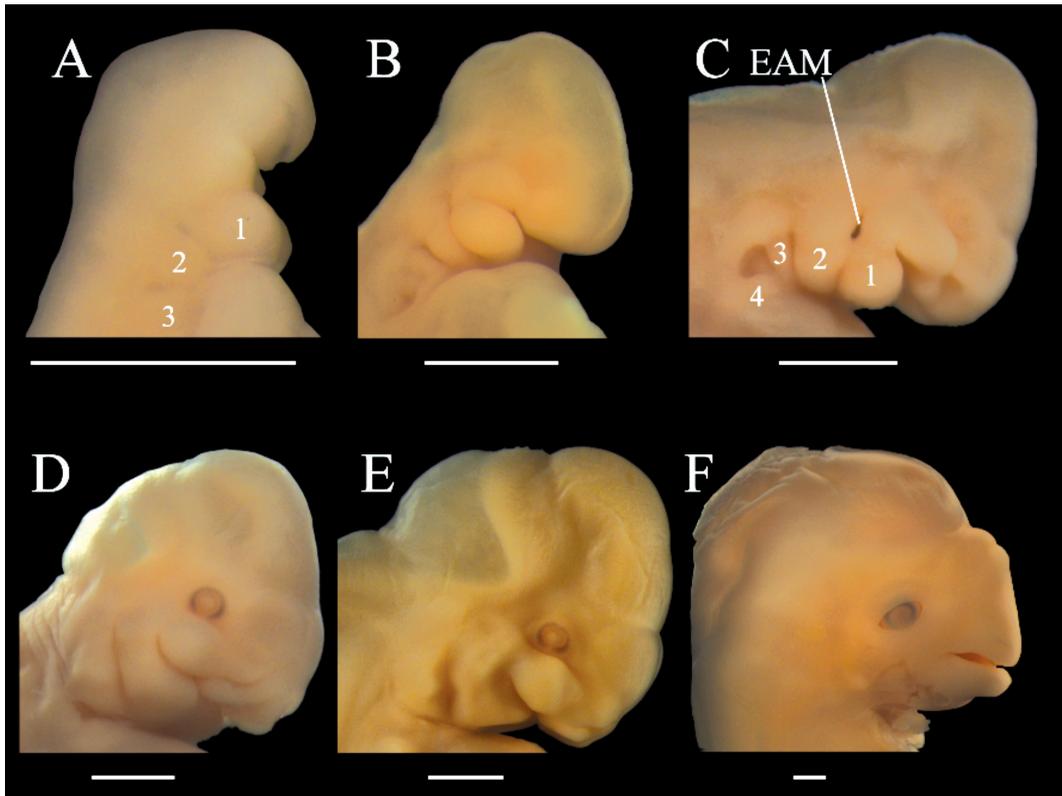


Figure 3. Faces of *S. attenuata* embryos showing the development of pharyngeal arches 1 through 4 (labeled with numbers in A and C) and clefts. Embryos are arranged by increasing gestational age: (A) LACM 94783, (B) LACM 94656, (C) LACM 94819, (D) LACM 94808, (E) LACM 94722, and (F) LACM 94742. In (A), only three pharyngeal arches have formed; in (F), all arches have disappeared. EAM = external auditory meatus. All scale bars are 1 mm, and the range of gestational ages of these specimens is shown in Figure 2.

Birds, reptiles, and the ancestors of mammals only have a single ear ossicle in their middle ear. The homologues of the mammalian malleus and incus in these vertebrates are not located in the middle ear cavity (Tucker, 2017); instead, they form the joint between the lower jaw and skull. In mammalian evolution, a series of fossils show transitional forms in which these two ossicles migrate posteriorly, detach from the jaw, make contact with the stapes, and form a chain with the stapes (Tucker, 2017). This evolutionary sequence is repeated in the ontogeny of all mammals, including cetaceans (Moran et al., 2011; Figure 5).

In embryology, the ear ossicles are derived from skeletal elements inside the pharyngeal arches. In young embryos, these skeletal elements are bars of cartilage but, later in development, they ossify. The malleus and incus develop from the first pharyngeal arch and stapes from the second. The skeleton of the first arch also includes a long bar of cartilage that extends most

of the length of the lower jaw and is continuous with the malleus: Meckel's cartilage. The malleus shares a joint with the incus and that joint is thus the original jaw joint of evolution and is retained as the jaw joint in birds and reptiles. As development proceeds, the bone of the lower jaw forms around Meckel's cartilage, and the cartilage disappears. In cetaceans (Moran et al., 2011) as well as in other mammals, the malleus and incus release from Meckel's cartilage and move posteriorly to make contact with the stapes, thus forming the chain of middle ear bones.

Nasal Opening

In all mammals, the left and right sides of the nasal cavity are divided by a wall that consists of bone and cartilage. In most mammals, these cavities open separately to the surface by means of openings in the soft tissue of the nose that are called the external nares. In the bony tissues underneath, there is only a single opening. The external nares

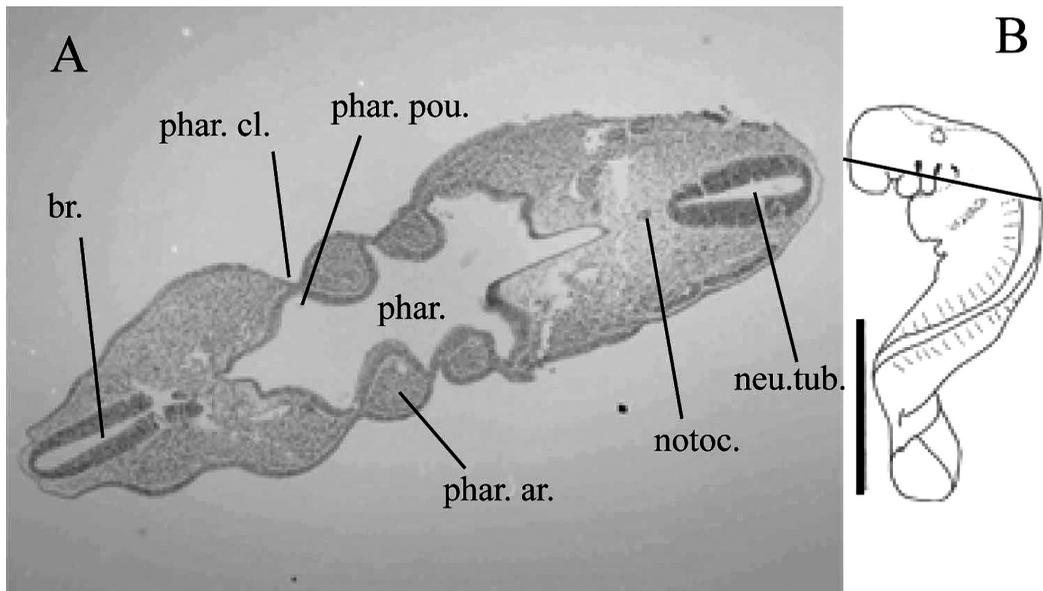


Figure 4. Histological section through the head of an *S. attenuata* embryo (A) (LACM 94674) with outline drawing of entire embryo (B) (slide 17; line shows position of section A; scale bar is 1 mm). Section shows inside of the oral cavity and pharynx (phar.), with pharyngeal arches (phar. ar.), pouches (phar. pou.), and clefts (phar. cl.). Also visible are the notochord (notoc.), neural tube (neu.tub), and developing brain (br.).

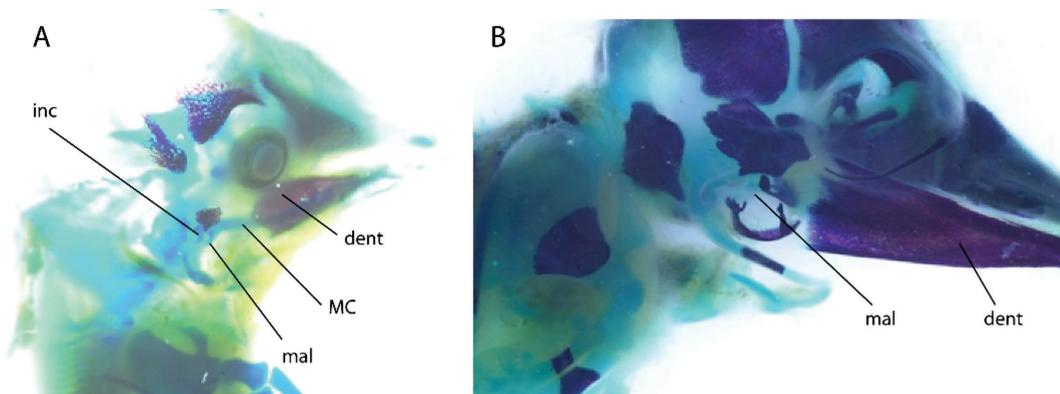


Figure 5. Heads of a young (A) (LACM 94671) and older (B) (LACM 94310) *S. attenuata* fetus for which soft tissues have been made transparent, bone dyed purple, and cartilage dyed green, a technique called clear-and-stain. Dent = dentary (bone of the lower jaw), inc = incus, MC = Meckel's cartilage, and mal = malleus.

are located at the tip of the snout in most mammals; but in most modern cetaceans, the external nares form the blowhole. The blowhole is located on the forehead just anterior to the brain (an exception is sperm whales [*Physeter macrocephalus*], where the blowhole is at the tip of the enormous snout; Klima, 1987, 1990).

In mysticetes, there are distinct left and right blowholes (external nares), but the external nares of odontocetes are fused to form a single midline structure. In the earliest cetaceans, the nasal opening

was at the tip of the snout (Bajpai et al., 2011); and during subsequent evolution, the nares shifted caudal onto the forehead (Thewissen et al., 2009).

In early mammalian embryos, including those of cetaceans, the external nares form as two depressions called the nasal pits. These are located far lateral to the midline (Figure 6A). As development proceeds, the nasal pits deepen to form the nasal cavity and, eventually, the nasal cavity will connect to the pharynx. In addition (Figure 6C-E), the external nares shift onto the forehead to form the

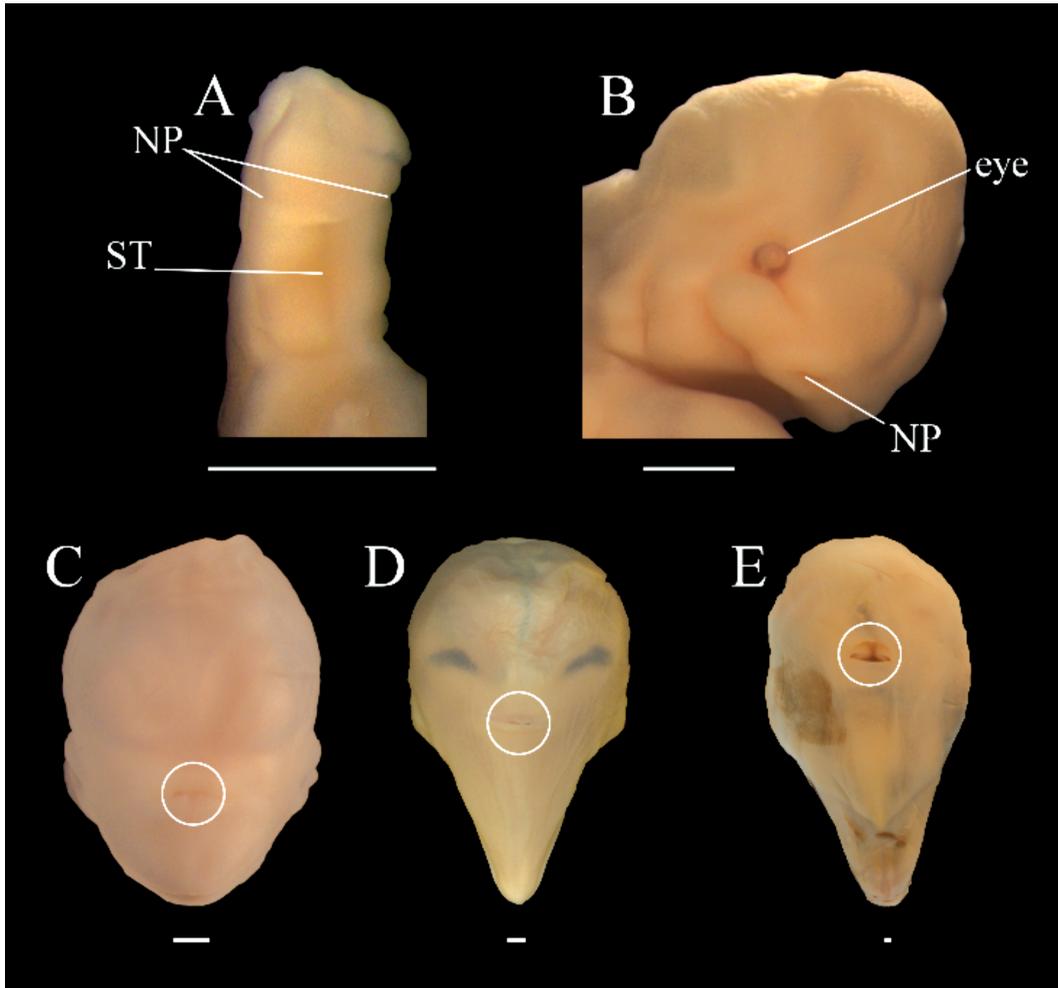


Figure 6. Heads of *S. attenuata* embryos—(A) LACM 94783, (B) LACM 94722, (C) LACM 94742, (D) LACM 94676, and (E) LACM 94931—arranged by increasing gestational age. Left and right nasal pit (NP) are initially to the side of the stomodeum (ST) (the depression that will form the mouth) laterally on the head in (A). As the eye forms, nasal pits move toward the midline in (B). (C) to (E) represent rostral (top) views of the head where the left and right external nares have fused to make a blowhole (circled) and document the move, in embryology, to their postnatal position on the forehead, just rostral to the brain. All scale bars are 1 mm, and the range of gestational ages of these specimens is shown in Figure 2.

blowhole. This shift of the nares caudally mimics the evolutionary shift that took place in evolution and is related to the origin of aquatic life.

Hair

In addition to having three ear ossicles, mammals are also characterized by the presence of hair. Hair consists of long filamentous strands that are made primarily of a protein called keratin. Feathers in birds and scales in reptiles also consist of keratin, and there are developmental similarities between these tissues (Dhouailly, 2009). However, the filamentous shape of hairs occurs only in mammals.

In cetacean evolution, hair was lost secondarily; functions related to insulation are taken on by the blubber underneath their skin. At no stage of development does any modern cetacean have a full hair coat, but some cetaceans have sparse hairs on their face. The face of right whales (*Balaenidae*) has three patches of hair, and these hairs serve as whiskers (Reeb et al., 2007; Drake et al., 2015). Prenatally, most or all cetaceans have some hairs on their rostrum as fetuses. In *Stenella* fetuses, a row of hairs occurs along the left and right side of the upper jaw (Figure 7), but these disappear before birth.

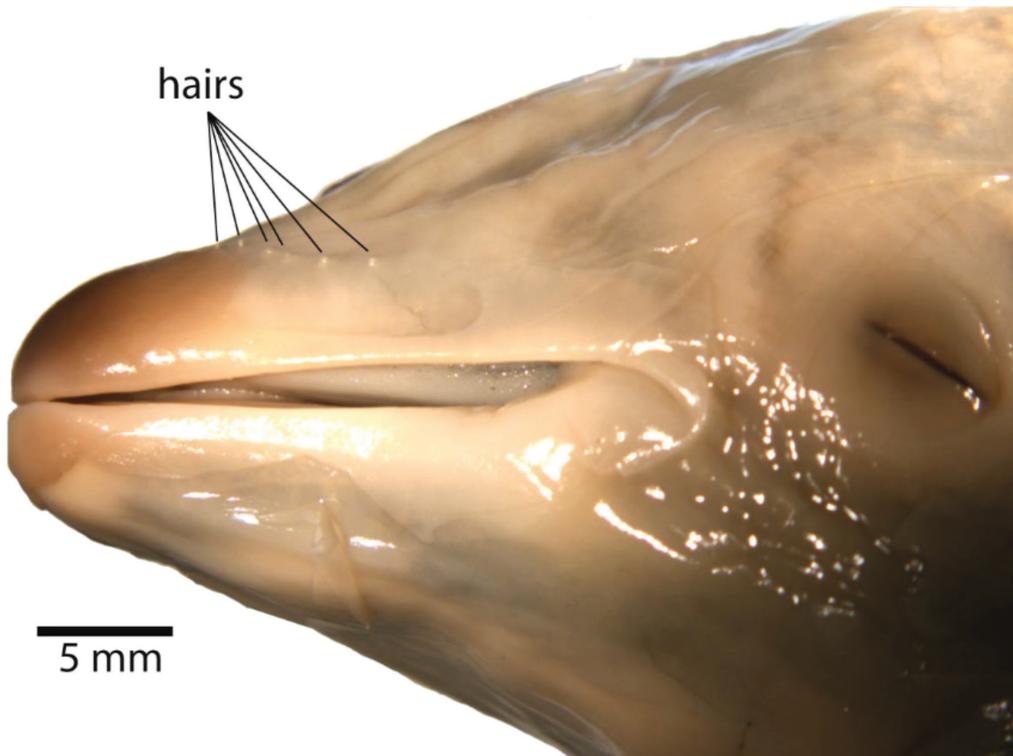


Figure 7. Head of *S. attenuata* fetus (LACM 94310), showing a row of hairs on the snout (image from Thewissen et al., 2009)

Baleen

In addition to hair, baleen also consists of keratin. Keratin structures are not unusual in the vertebrate mouths (e.g., beaks in birds and horny teeth in lampreys), but baleen occurs only in mysticetes. The ancestors of baleen whales had teeth but no baleen, and the earliest mysticetes retained teeth (Demèré et al., 2008). Then, around 23 mya, all baleen whales lost postnatal teeth and used baleen racks to capture prey.

In the embryology of baleen whales, teeth are initially formed (Figure 8); and at least in some species, they are mineralized (Dissel-Scherft & Vervoort, 1954). As development proceeds, teeth are fully resorbed before birth (Karlsen, 1962). Baleen begins to form in fetal mysticetes after the teeth are resorbed.

Prenatal tooth formation in baleen whales is often considered to be a nonfunctional rudiment, and it has been thought that baleen is a homologue of the (keratinized) transverse ridges that many mammals have on their palate. However, there is evidence that the embryonic teeth have a role in baleen development (Thewissen et al., 2017). Some of the genes involved in the formation of baleen are actually genes that are involved in tooth development—not in the development of other keratin structures. In mammal tooth development,

the cascade of gene expression that leads to the formation of a tooth germ is switched on, silenced, and then switched on again—first to make deciduous (milk) teeth, and subsequently to make the permanent teeth. In the bowhead whales that were investigated (Thewissen et al., 2017), only one generation of tooth germs is formed, but then the tooth genes are expressed another time, now as part of the expression sequence that makes baleen.

Teeth

Primitively, placental mammals have 44 teeth—11 on each side of the upper and lower jaw (Gregory, 1922). The complexity of the shape of the crown of those teeth changes gradually from anterior to posterior, with simple crowns in the front and more complicated ones in the back. In most mammals, the front eight teeth (i.e., incisors, canines, and premolars) are replaced once in life when a permanent tooth replaces a deciduous (milk) tooth. The three teeth at the end of the tooth row, the molars, have only a single tooth generation. Tooth number, shape, and replacement together lead to a highly organized system that allows for accurate occlusion between upper and lower teeth, and this is necessary because mammals chew their food unlike other vertebrates. Such chewing is

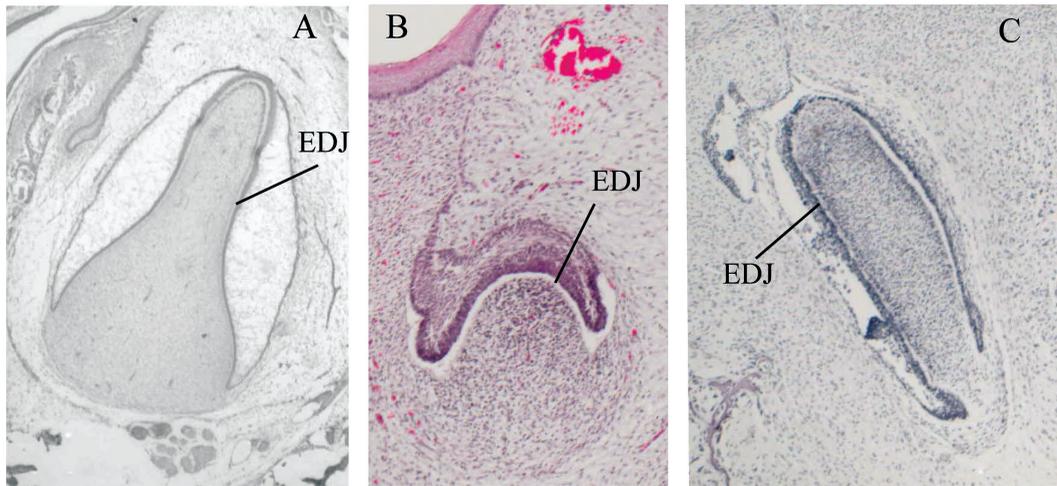


Figure 8. Tooth buds of pig (A) (P180 sl. 460), beluga whale (B) (NSB-DWM 2012BDL1F, sl. 180), and bowhead whale (C) (NSB-DWM 2003B3F bl. C sl. 31c). The enamel-dentin junction (EDJ) forms the eventual contact layer between enamel and dentin after mineralization. Bowhead tooth buds do not reach that stage of development.

necessary because mammals have a high metabolic rate and need efficient food processing.

Unlike most mammals, odontocetes do not chew their food; they usually have more than 11 teeth per half jaw, all of which have simple crowns; and there is only a single tooth generation. It is likely that the absence of rhythmic chewing is evolutionarily related to the morphological changes in the dentition (Werth, 2000).

Gene expression and protein signaling pathways related to tooth development have been studied in detail in laboratory mammals (Jernvall, 2000). When the embryo is very small, and long before any tooth germs are formed (a stage similar to Figure 3F), two genes are expressed in the jaw that set up gradients of protein that dictate the subsequent differentiation from incisors to molars (Cobourne & Sharpe, 2003; Catón & Tucker, 2009). The highest concentration of BMP-4 protein is anterior, where the incisors will form; and for FGF-8 protein, it is posterior, where the molars will form. If the system is perturbed and the *bmp-4* gene is overexpressed in the back of the jaw of mice, the resulting molars will have simpler crowns, more similar to incisors, and there will be supernumerary teeth (Murashima-Suginami et al., 2007; Munne et al., 2010). Together, the *bmp-4* and *fgf-8* genes appear to have the basic information for the differentiation of the tooth classes.

Such experiments cannot be done with cetacean embryos, but the presence of these proteins can be investigated. Armfield et al. (2013) determined that BMP-4 in *Stenella* occurs in areas well posterior to where it is in other mammals, possibly resulting in the similarity in crown shape along

the jaw (homodonty) as well as in excess tooth numbers. In several ways, the odontocete morphology is reminiscent of an evolutionary process that took place 220 mya when mammals evolved (Luo, 2007). Indeed, it has been suggested that the evolution of complex crown morphologies and the reduction in tooth numbers in early mammal evolution was driven by *bmp-4* gene expression (Ohazama et al., 2010).

Forelimb

The forelimb of mammals forms as a small limb bud that grows out from the body wall (Figure 9B). Gene expression and signaling related to limb growth is well understood and was reviewed by Zuniga (2015). As the limb bud elongates, the tip flattens into a hand plate (Figure 9E). Initially, growth is controlled by a crest of cells at the tip of the limb bud, the apical ectodermal ridge (AER), and a cluster of cells embedded into the posterior part of the bud, the zone of polarizing ability (ZPA). The AER and ZPA secrete proteins that regulate the development of the hand, including the formation of five rays that will eventually develop into five fingers (Cooper et al., 2011; Figure 9F). Initially, these fingers are embedded into a single hand plate as if the hand were webbed. In most mammals, the tissue between the fingers dies by means of apoptosis, resulting in independently movable fingers (Pajni-Underwood et al., 2007). In contrast, the fingers of cetaceans remain tied together; interdigital apoptosis is absent or very limited (Cooper et al., 2017), similar to bats (Sears et al., 2006). Therefore, one might say that the hands of cetaceans resemble those of the embryos of all



Figure 9. *S. attenuata* embryos and fetuses: (A) LACM 94783, (B) LACM 94656, (C) LACM 94819, (D) LACM 94808, (E) LACM 94722, (F) LACM 94820, and (G) LACM 94646. Fore- and hind limb bud are absent in (A), but they are present in (B) (forelimb bud) and (C) (hind limb bud). In (E), the hand plate has developed, followed by individual finger rays (F), and multiple phalanges (G). The hind limb bud is circled in (C) and has been lost in (G). In (F), both hind limb bud (HL) and nipple (N) are visible. All scale bars are 1 mm, and the gestational ages of these specimens are shown in Figure 2.



Figure 10. Bottlenose dolphin (*Tursiops truncatus*) with hind limbs (flippers) projecting from its body. It represents a congenital malformation that shows that the genetic pattern that codes for hind limbs is still present in this dolphin. This photo was taken at the Taiji Whale Museum in Taiji, Japan, where the animal has since died.

mammals and that cetaceans retain this morphology throughout life (Amasaki et al., 1989; Calzada & Aguilar, 1996; Sedmera et al., 1997). Not only is webbing preserved throughout life, but the entire hand remains encased in a single sheet of tissue that is so thick that individual finger rays are not recognizable on the outside.

While a hand with nondistinct fingers occurs in all mammalian embryos, a second feature of cetacean hands is a developmental novelty for mammals: hyperphalangy, which is the presence of a greater than primitive number of phalanges per finger (Richardson & Oelschläger, 2002; Fedak & Hall, 2004). As the digital rays form in a mammal embryo, joints form inside each ray, breaking it up into what will become several bones (Figure 9G). The fingers of mammals have

four bones with three joints linking them: between the metacarpal and first phalanx, between the first and second phalanx, and between the second and third phalanx. The thumb has one fewer segment and, thus, one fewer joint. The genes that drive the process of joint formation are well known, and the developmental cascade of gene expression is repeated for each joint but stops when the third joint is formed. Cetaceans often have more than three joints per finger, however, resulting in hyperphalangy. Developmental evidence suggests that proteins secreted by the interdigital areas are responsible for the additional repetitions of the expression of genes that cause joint formation in the digital rays (Cooper et al., 2017). Therefore, it appears that the retention of interdigital tissue and hyperphalangy are developmentally linked.

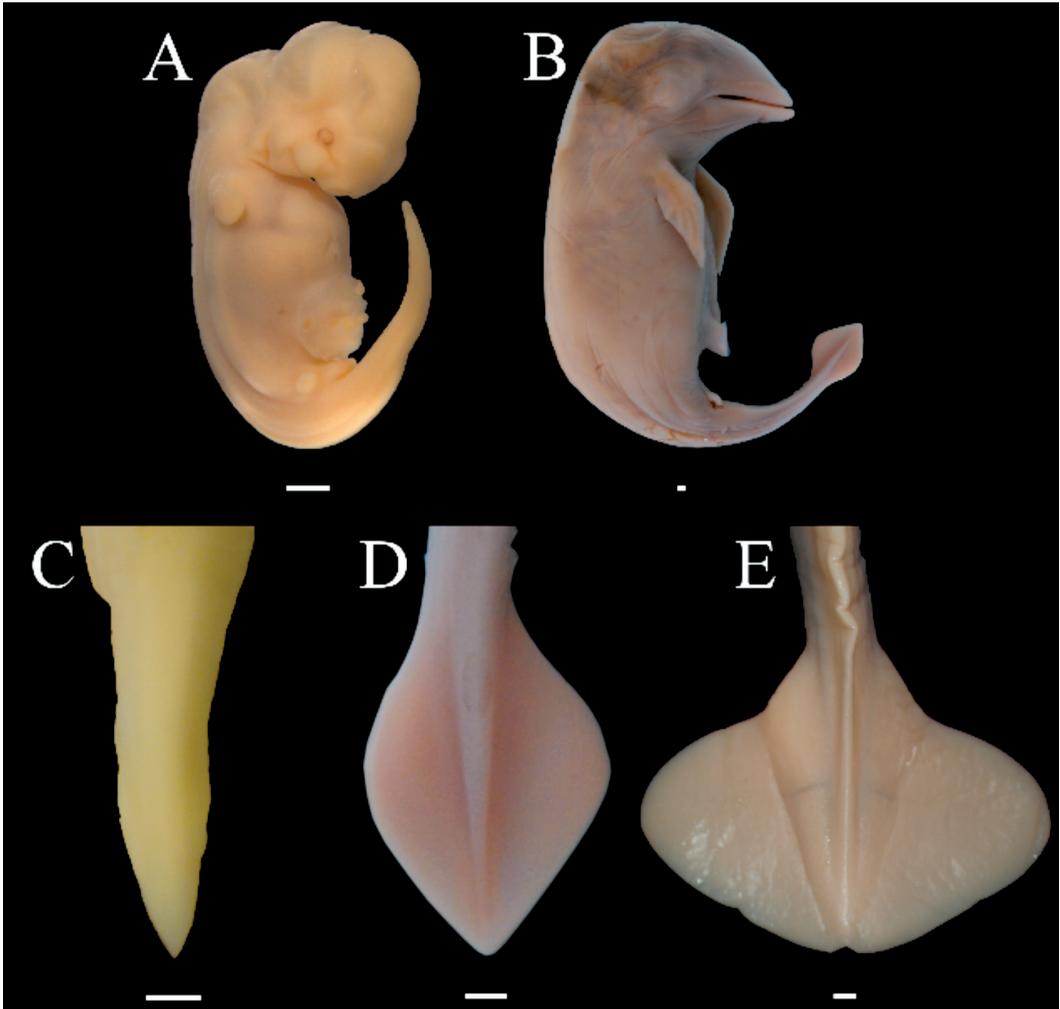


Figure 11. Tail ontogeny in an *S. attenuata* dolphin arranged from young to old: (A) LACM 94722, (B) LACM 94646, (C) LACM 94650, (D) LACM 94646, and (E) LACM 94413. The tail changes from being circular in cross section (A and C), to diamond-shaped (B, D, and E), and eventually to triangular.

Hind Limb

Just like the forelimb, the mammalian hind limb also initially forms as a small projection from the lateral abdominal wall, and the AER and ZPA regulate its development. Similar to other mammals, cetaceans also form a hind limb bud (Figure 9). However, soon after its formation and still in the embryonic period (Figure 2), the hind limb bud ceases to grow; and with the embryo growing fast at this time, the hind limb bud becomes insignificant and disappears from the surface of the body. An AER is clearly present in cetacean embryos (Thewissen et al., 2006), but the protein SHH, which is produced by the ZPA in other mammals as well as in cetacean forelimb buds, appears to be absent in the cetacean

hind limb. This absence disrupts the signaling cascade that forms the hind limb.

While it is still present, the hind limb bud is located just lateral to the genital slit; and while the former is shrinking in size, a second outgrowth forms, located between the hind limb bud and the genital slit. This is the embryonic precursor of the nipple (Anderssen, 1918). Sometimes, a cetacean is born with remnants of external hind limbs protruding from its abdomen. In the best-known case, a specimen of *Tursiops truncatus* had small triangular flippers projecting from its body (Ito et al., 2011; Figure 10). This shows that the gene expression patterns that regulate lower extremity formation are still present in the DNA of delphinids

but that they are switched off in normal dolphin development.

While the process of ontogenetic hind limb reduction is well documented in dolphins (Delphinidae), there are no embryos of the appropriate age described for other cetaceans. Thus, it is possible that hind limb development proceeds further in other cetaceans, and there are some indications that that is the case. Postnatal dolphins have a bony remnant of their pelvis but none of the bones of their hind limb as hind limb development was interrupted before precursors of these bones started to form. In contrast, right whales (Balaenidae) have an internal bony pelvis, femur, often a tibia, and sometimes even a metatarsal (Struthers, 1893; Thewissen, 2014). This suggests that embryonic hind limb development in at least right whales proceeds well beyond the stage where it is interrupted in delphinids.

Fluke

In the embryo, the tail of a mammal forms as an extension of the vertebral column that is circular in cross section, and, in most mammals, the tail retains that shape throughout life. In cetacean embryos, too, the tail is circular in cross section but that changes in the fetal period (Figure 11). At that time, the left and right sides of the tail grow out to initially make a flattened tail that is diamond-shaped (Ogawa, 1953). Later in the fetal period, the left and right sides of the fluke expand to make the entire structure triangular. Currently, nothing is known about the protein signaling involved in this process, but it is likely that some genes are involved that are also involved in the patterning of paired limbs and paired parts of the genitals (Infante et al., 2018).

Discussion

Cetacean ontogeny has been studied for centuries. Soon after the *Origin of Species* was published, embryology became an early source of support for Darwin's theory, with, for instance, the recognition that processes on the abdomen of embryos were remnants of the hind limbs of their ancestors (Kükenthal, 1895; Guldberg, 1899). In modern times, cetacean ontogeny is rarely studied because embryos and early fetuses are nearly impossible to acquire. Prenatal specimens from stranded pregnant cetaceans are usually in poor shape as decomposition commences soon after death. Museum specimens are usually preserved in such a way that modern molecular techniques cannot be applied to them. This is a pity since cetaceans have pushed the boundaries of mammalian design to their limit and could elucidate what the constraints on that design are (Thewissen et al., 2012).

In this article, I discussed some examples of cetacean organ systems that push those boundaries. For instance, the genetic background of the remarkable reduction of the hair coat in cetaceans goes to the root of what defines a mammal and is, in laboratory animals, the subject of much research effort (e.g., Plikus & Chuong, 2008). The significant changes in the forelimbs mimic, at a developmental level, those of bats (Cooper et al., 2017) and reflect both a retention of ancestral morphologies (webbing) as well as a break with basic mammalian design (hyperphalangy). The hind limbs disappear soon after forming in a cetacean embryo, but the gene expression cascade that forms limbs in terrestrial mammals is preserved though latent in the hind limb, even if occasionally activated in some individuals. The loss of regionalization of the dentition appears to be related to the loss of masticatory function in evolution (Thewissen et al., 2017). Odontocetes typically have more teeth than archaic placentals, more precaudal vertebrae (Thewissen et al., 2012), and more manual phalanges than most mammals, suggesting some fundamental deviation from traditional mammalian patterns of design. Finally, the formation of the fluke, a paired locomotor organ, challenges the orthodoxy that amniotes only have two pairs of locomotor organs.

While the regulation of gene expression and transcription of some of these ontogenetic patterns is reasonably well understood in developmental lab models, such as mice, very little about this is known in cetaceans. Studying the developmental biology of cetaceans offers great promise for understanding mammalian design and the path of their evolution.

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Literature Cited

- Amasaki, H., Ishikawa, H., & Daigo, M. (1989). Developmental changes in the fore- and hind-limbs in the fetuses of the southern minke whale, *Balaenoptera acutorostrata*. *Anatomischer Anzeiger*, 169(2), 145-148.
- Anderssen, M. S. (1918). Studier over mammarorganernes utvikling hos *Phocaena communis* [Studies on the development of mammary organs of *Phocaena communis*]. *Bergens Museum Aarbok, Naturvidenskap*, 3(1917-1918), 1-45.

- Armfield, B. A., Zheng, Z., Bajpai, S., Vinyard, C. J., & Thewissen, J. G. M. (2013). Development and evolution of the unique cetacean dentition. *PeerJ*, 24(1). <https://doi.org/10.7717/peerj.24>
- Bajpai, S., Thewissen, J. G. M., & Conley, R. W. (2011). Cranial anatomy of middle Eocene *Remingtonocetus* (Cetacea, Mammalia). *Journal of Paleontology*, 85(4), 703-718. <https://doi.org/10.1666/10-128.1>
- Calzada, N., & Aguilar, A. (1996). Flipper development in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *Anatomical Record*, 245(4), 708-714. [https://doi.org/10.1002/\(SICI\)1097-0185\(199608\)245:4<708::AID-AR11>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1097-0185(199608)245:4<708::AID-AR11>3.0.CO;2-T)
- Catón, J., & Tucker, A. S. (2009). Current knowledge of tooth development: Patterning and mineralization of the murine dentition. *Journal of Anatomy*, 214(4), 502-515. <https://doi.org/10.1111/j.1469-7580.2008.01014.x>
- Cobourne, M. T., & Sharpe, P. T. (2003). Tooth and jaw: Molecular mechanisms of patterning the first branchial arch. *Archives of Oral Biology*, 48(1), 1-14. [https://doi.org/10.1016/S0003-9969\(02\)00208-X](https://doi.org/10.1016/S0003-9969(02)00208-X)
- Cooper, K. L., Hu, J. K-H., ten Berge, D., Fernandez-Teran, M., Ros, M. A., & Tabin, C. J. (2011). Initiation of proximal-distal patterning in the vertebrate limb by signals and growth. *Science*, 332(6033), 1083-1086. <https://doi.org/10.1126/science.1199499>
- Cooper, L. N., Sears, K. E., Armfield, B. A., Kala, B., Hubler, M., & Thewissen, J. G. M. (2017). Review and experimental evaluation of the embryonic development and evolutionary history of flipper development and hyperphalangy in dolphins (Cetacea: Mammalia). *Genesis*, 56(1). <https://doi.org/10.1002/dvg.23076>
- Deméré, T. A., McGowen, M. R., Berta, A., & Gatesy, J. (2008). Morphological and molecular evidence for a stepwise evolutionary transition from teeth to baleen in mysticete whales. *Systematic Biology*, 57(1), 15-37. <https://doi.org/10.1080/10635150701884632>
- Dhouailly, D. (2009). A new scenario for the evolutionary origin of hair, feather, and avian scales. *Journal of Anatomy*, 214(4), 587-606. <https://doi.org/10.1111/j.1469-7580.2008.01041.x>
- Dissel-Scherft, M. C. V., & Vervoort, W. (1954). Development of the teeth in fetal *Balaenoptera physalus* (L.) (Cetacea, Mysticoceti). *Proceedings of the Nederlandse Akademie van Wetenschappen, Serie C*, 57, 196-210.
- Drake, S. E., Crish, S. D., George, J. C., Stimmelmayer, R., & Thewissen, J. G. M. (2015). Sensory hairs in the bowhead whale, *Balaena mysticetus* (Cetacea, Mammalia). *Anatomical Record*, 298(7), 1327-1335. <https://doi.org/10.1002/ar.23163>
- Fedak, T. J., & Hall, B. K. (2004). Perspectives on hyperphalangy: Patterns and processes. *Journal of Anatomy*, 204(3), 151-163. <https://doi.org/10.1111/j.0021-8782.2004.00278.x>
- Frisdal, A., & Trainor, P. A. (2014). Development and evolution of the pharyngeal apparatus. *Wiley Interdisciplinary Review of Developmental Biology*, 3(6), 403-418. <https://doi.org/10.1002/wdev.147>
- Gatesy, J., Geisler, J. H., Chang, J., Buell, C., Berta, A., Meredith, R. W., . . . McGowen, M. R. (2013). A phylogenetic blueprint for a modern whale. *Molecular Phylogenetics and Evolution*, 66(2), 479-506. <https://doi.org/10.1016/j.ympev.2012.10>
- Graham, A. (2001). The development and evolution of the pharyngeal arches. *Journal of Anatomy*, 199, 133-141. <https://doi.org/10.1046/j.1469-7580.2001.19910133.x>
- Gregory, W. K. (1922). *The origin of the human dentition*. Baltimore, MD: Williams & Wilkins Co.
- Guldberg, G. (1899). Neue untersuchungen über die rudimente von hinterflossen und die milchdrüsenanlage bei jungen delphinembryonen [Recent studies on the rudiments of backfins and the mammary glands in young dolphin embryos]. *Internationales Monatschrift für: Anatomie und Physiologie*, 4, 301-320.
- Infante, C. R., Rasys, A. M., & Menke, D. B. (2018). Appendages and gene regulatory networks: Lessons from the limbless. *Genesis*, 56. <https://doi.org/10.1002/dvg.23078>
- Ito, H., Koizumi, K., Ichishima, H., Uchida, S., Hayashi, K., Ueda, K., . . . Kato, H. (2011, November). Inner structure of the fin-shaped hind limbs of a bottlenose dolphin (*Tursiops truncatus*). *Abstracts of the Biennial Conference on the Biology of Marine Mammals*, Tampa, FL.
- Jernvall, J. (2000). Linking development with generation of novelty in mammalian teeth. *Proceedings of the National Academy of Sciences, U.S.A.*, 97(6), 2641-2645. <https://doi.org/10.1073/pnas.050586297>
- Karlsen, K. (1962). Development of the tooth germs and adjacent structures in the whalebone whale (*Balaenoptera physalus* [L.]) with a contribution to the theories of the mammalian dentition. *Hvalrådets Skrifter*, 45, 1-56.
- Klima, M. (1987). Morphogenesis of the nasal structures of the skull in toothed whales (Odontoceti). In H. J. Kuhn & U. Zeller (Eds.), *Morphogenesis of the mammalian skull* (pp. 105-122). Hamburg, Germany: Verlag Paul Parey.
- Klima, M. (1990). Histologische Untersuchungen an Knorpelstrukturen im Vorderkopf des Pottwals, *Physeter macrocephalus* [Histological examination of cartilaginous structures in the forehead of the sperm whale, *Physeter macrocephalus*]. *Gegenbaur's Morphologisches Jahrbuch*, 136(1), 1-16.
- Kükenthal, W. (1895). Über rudimente von hinterflosse bei embryonen von walen [About rudiments of hind flippers in embryos of whales]. *Anatomischer Anzeiger*, 534-537.
- Luo, Z-X. (2007). Transformation and diversification in early mammal evolution. *Nature*, 450, 1011-1019. <https://doi.org/10.1038/nature06277>
- Moran, M. M., Nummela, S., & Thewissen, J. G. M. (2011). Development of the skull of the pantropical spotted dolphin (*Stenella attenuata*). *Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology*, 294(10), 1743-1756. <https://doi.org/10.1002/ar.21388>
- Munne, P. M., Felszeghy, S., Jussila, M., Suomalainen, M., Thesleff, I., & Jernvall, J. (2010). Splitting placodes: Effects of bone morphogenetic protein and activin on the patterning and identity of mouse incisors. *Evolution*

- & *Development*, 12(4), 383-392. <https://doi.org/10.1111/j.1525-142X.2010.00425.x>
- Murashima-Suginami, A., Takahashi, K., Kawabata, T., Sakata, T., Tsukamoto, H., Sugai, M., . . . Bessho, K. (2007). Rudiment incisors survive and erupt as supernumerary teeth as a result of USAG-1 abrogation. *Biochemical and Biophysical Research Communications*, 359(3), 549-555. <https://doi.org/10.1016/j.bbrc.2007.05.148>
- Nummela, S., Thewissen, J. G. M., Bajpai, S., Hussain, S. T., & Kumar, K. (2007). Sound transmission in archaic and modern whales: Anatomical adaptations for underwater hearing. *Anatomical Record*, 290(6), 716-733. <https://doi.org/10.1002/ar.20528>
- Ogawa, T. (1953). On the presence and disappearance of the hind limb in the cetacean embryos. *Scientific Reports of the Whales Research Institute*, 8, 127-132.
- Ohazama, A., Blackburn, J., Pomtaveetus, T., Ota, M. S., Choi, H. Y., Johnson, E. B., . . . Sharpe, P. T. (2010). A role for suppressed incisor cuspal morphogenesis in the evolution of mammalian heterodont dentition. *Proceedings of the National Academy of Sciences, U.S.A.*, 107(1), 92-97. <https://doi.org/10.1073/pnas.0907236107>
- Pajni-Underwood, S., Wilson, C., Elder, C., Mishina, Y., & Lewandoski, M. (2007). BMP signals control limb bud interdigital programmed cell death by regulating FGF signaling. *Development*, 134(12), 2359-2368. <https://doi.org/10.1242/dev.001677>
- Plikus, M. V., & Chuong, C-M. (2008). Complex hair cycle domain patterns and regenerative hair waves in living rodents. *Journal of Investigative Dermatology*, 128(5), 1071-1080. <https://doi.org/10.1038/sj.jid.5701180>
- Pyenson, N. D. (2017). The ecological rise of whales chronicled by the fossil record. *Current Biology*, 27(11), R558-R564. <https://doi.org/10.1016/j.cub.2017.05.001>
- Reeb, D., Best, P. B., & Kidson, S. H. (2007). Structure of the integument of southern right whales, *Eubalaena australis*. *Anatomical Record*, 290(6), 596-613. <https://doi.org/10.1002/ar.20535>
- Richardson, M. K., & Oelschläger, H. H. A. (2002). Time, pattern, and heterochrony: A study of hyperphalangy in the dolphin embryo flipper. *Evolution & Development*, 4(6), 435-444. <https://doi.org/10.1046/j.1525-142X.2002.02032.x>
- Sears, K. E., Behringer, R. R., Rasweiler, J. J., & Niswander, L. A. (2006). Development of bat flight: Morphologic and molecular evolution of bat wing digits. *Proceedings of the National Academy of Sciences, U.S.A.*, 103(17), 6581-6586. <https://doi.org/10.1073/pnas.0509716103>
- Sedmera, D., Mříšek, I., & Klima, M. (1997). On the development of cetacean extremities. II. Morphogenesis and histogenesis of the flippers in the spotted dolphin (*Stenella attenuata*). *European Journal of Morphology*, 35(1), 117-123. <https://doi.org/10.1076/ejom.35.2.117.13067>
- Shubin, N. (2008). *Your inner fish: A journey into the 3.5 billion-year history of the human body*. New York: Random House.
- Stërba, O., Klima, M., & Schlidger, B. (2000). Embryology of dolphins: Staging and ageing of embryos and fetuses of some cetaceans. *Advances in Anatomy, Embryology, and Cell Biology*, 157, 1-133.
- Struthers, M. D. (1893). On the rudimentary hind limb of the great fin-whale (*Balaenoptera musculus*) in comparison with those of the humpback whale and the Greenland right whale. *Journal of Anatomy and Physiology*, 27(3), 291-335.
- Thewissen, J. G. M. (2014). *The walking whales, from land to water in eight million years*. Berkeley: University of California Press.
- Thewissen, J. G. M., & Heyning, J. (2007). Embryogenesis and development in *Stenella attenuata* and other cetaceans. In D. L. Miller (Ed.), *Reproductive biology and phylogeny of Cetacea* (pp. 307-330). Enfield, NH: Science Publishers Ltd. <https://doi.org/10.1201/b11001-12>
- Thewissen, J. G. M., Cooper, L. N., & Behringer, R. R. (2012). Developmental biology enriches paleontology. *Journal of Vertebrate Paleontology*, 32(6), 1223-1234. <https://doi.org/10.1080/02724634.2012.707717>
- Thewissen, J. G. M., Cooper, L. N., George, J. C., & Bajpai, S. (2009). From land to water: The origin of whales, dolphins, and porpoises. *Evolution: Education and Outreach*, 2, 272-288. Retrieved from <https://link.springer.com/article/10.1007%2F12052-009-0135-2>
- Thewissen, J. G. M., Cohn, M. J., Stevens, L. S., Bajpai, S., Heyning, J., & Horton, W. E., Jr. (2006). Developmental basis for hind limb loss in dolphins and the origin of the cetacean bodyplan. *Proceedings of the National Academy of Sciences, U.S.A.*, 103(22), 8414-8418. <https://doi.org/10.1073/pnas.0602920103>
- Thewissen, J. G. M., Hieronymus, T. L., George, J. C., Suydam, R., Stimmelmayer, R., & McBurney, D. (2017). Evolutionary aspects of the development of teeth and baleen in the bowhead whale. *Journal of Anatomy*, 230(4), 549-566. <https://doi.org/10.1111/joa.12579>
- Tucker, A. S. (2017). Major evolutionary transitions and innovations: The tympanic middle ear. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1713). <https://doi.org/10.1098/rstb.2015.0483>
- Werth, A. (2000). Feeding in marine mammals. In K. Schwenk (Ed.), *Feeding, form, function, and evolution in tetrapod vertebrates* (pp. 487-526). San Diego, CA: Academic Press.
- Wilson, J. M., & Laurent, P. (2002). Fish gill morphology: Inside out. *Journal of Experimental Zoology*, 293(3), 192-213. <https://doi.org/10.1002/jez.10124>
- Zuniga, A. (2015). Next generation limb development and evolution: Old questions, new perspectives. *Development*, 142(22), 3810-3820. <https://doi.org/10.1080/02724634.2012.707717>