

# **Cranial morphology in whales: A study spanning the evolutionary history and diversity of the cetacean skull**

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Thank you for listening to my talk and please do contact me with any questions you may have:

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**This document is intended to provide extra information on methods used in my SVP Romer talk. Please do not share, disseminate or use the details below without contacting me or without my permission.**

**Please note, many of these details are similar to those published in: Coombs et al 2020: 'Wonky whales: the evolution of cranial asymmetry in cetaceans'**

The paper is open access and is available here:

<https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-020-00805-4#Sec8>

## **Methods**

### **Specimens**

The data set comprises stem cetaceans (archaeocetes,  $n = 11$ ) and both extant suborders; the baleen whales (mysticetes,  $n = 33$ ) and toothed whales (odontocetes,  $n = 155$ ). The final data set comprised **199 cetacean crania**, of which 56% are extinct, ranging in age from 48.6 to 2.59 Mya. If you would like specimen details, please contact E Coombs.

Specimens were selected to cover the widest possible phylogenetic spread, representing 42 families and 141 genera from the Eocene to the present.

As in Coombs et al 2020 [1], but with some additional specimens:

The Early-Middle Eocene is represented by the land-dwelling family Pakicetidae through to semi-aquatic Ambulocetidae and Remingtonocetidae. The Pelagiceti are represented by the fully aquatic Basilosauridae of the Late Eocene through to the modern Neoceti. This includes representation of some early stem toothed mysticetes such as the Mammalodontidae and the Aetiocetidae. All four extant mysticete families are represented. The odontocetes are represented by early stem families: the Xenorophidae and the Simocetidae of the Early-Mid Oligocene and the 'Patriocetidae' (phylogenetic position is still being clarified) of the Late Oligocene. The more crownward odontocetes of the Miocene are represented by the Eurhinodelphinidae, Kentriodontidae, Albireonidae, Squalodelphinidae, Squalodontidae, and Allodelphinidae among other extinct families. All ten extant odontocete families are represented.

Because many extant and all fossil specimens lack information on sex, sexual dimorphism could not be considered.

### 3D surface scans

Skulls were scanned using a Creaform Go!SCAN 20, or Creaform Go!SCAN 50 depending on the size of the skull. Scans were cleaned, prepared, and merged in VX Elements v.6.0 and exported in ply format before being further cleaned and decimated in Geomagic Wrap software (3D Systems). Models were decimated down to 1,500,000 triangles, reducing computational demands without compromising on detail for further morphometric analyses. In many studies of morphology when the skull is incomplete, it is possible to digitally reconstruct bilateral elements by mirroring across the midline plane if preserved on one side. However, due to the substantial asymmetry observed in many taxa in this study, mirroring a complete half of the skull was not possible. For this reason, I limited mirroring to marginally damaged bones or easily mirrored missing bones only, where it was clear that mirroring would not mask any biological asymmetry, using the 'mirror' function in Geomagic Wrap (3D Systems).

### Morphometric data collection

#### Fixed landmarks

Importantly, due to high levels of asymmetry found in the nasals, premaxilla, maxilla, and frontal of the odontocetes, and fairly high levels of asymmetry in the rostrum, jugal, and orbit of the archaeocetes (see Coombs et al 2020 [1]), I had to develop a new landmarking protocol for this present study.

Due to their lack of asymmetry (a biologically 'symmetrical' skull), I landmarked the **mysticete** skulls in the following way:

1. 57 landmarks were placed on the left-hand side (LHS) of the skull
2. 9 landmarks were placed on the midline of the skull – landmarks were mirrored using the midline as a pivot plane
3. The 57 landmarks were mirrored to the symmetrical bones on the right-hand side (RHS) of the skull
4. A total of 123 landmarks over the whole skull

The **odontocetes and archaeocetes** were landmarked in the following way:

1. 57 landmarks were placed on the LHS of the skull
2. 9 landmarks were placed on the midline of the skull – landmarks were mirrored using the midline as a pivot plane
3. 33 landmarks (as established in [1]) were mirrored to symmetrical bones RHS
4. 24 landmarks were manually placed on the asymmetrical bones on the RHS
5. A total of 123 landmarks over the whole skull

Landmarks were placed in Stratovan Checkpoint (Stratovan, Davis, CA, USA) using the 'single point' option. Type I and II landmarks [2] were selected to comprehensively represent the full cranium. 'Landmark 15' and the subsequent mirrored, or manually placed (in the archaeocetes and odontocetes) 'landmark 79' denote the back of the tooththrow in most species. In some ziphiids, e.g. *Mesoplodon carlhubbsi*, the teeth (or tusks) erupt midway along the mandible [3] whilst other species present multiple pairs of tusks [4]. In others (e.g. *Hyperoodon ampullatus*), teeth typically erupt as a single pair on the anterior mandible which often protrudes beyond the upper jaw [3]. Without the mandible, it is challenging to pinpoint the positioning of the back of the tooththrow, and even then, the presence and number of teeth is negligible in some species. Further, these tusks only erupt in adult males. For these reasons, and to avoid simply

estimating where the true tooth row may be, 'landmark 15' and 'landmark 79' in specimens with mandibular prognathism, absent, maxillary-only, or vestigial dentition (including all ziphiids, narwhals (*Monodon monoceros*) and sperm whales (*Physeter macrocephalus*)) were consistently placed on the proximal lateral maxilla where the posterior end of a standard tooth row would be located.

### **Sliding curve semilandmarks**

Further developments of geometric morphometrics have expanded the quantification of morphology, from the addition of curve and surface sliding semilandmarks (defined by relative positions, originally referred to as 'Type III' landmarks [2]). These methods densely sample morphology, sampling evenly over structures. I placed 65 sliding semilandmark curves onto the archaeocete, mysticete, and odontocete skulls. Curves were placed on sutures between bones (13 key bones were identified in total, for example, parietal, pterygoid, premaxilla, nasals). Again, due to the asymmetry findings in Coombs et al (2020) [1], the protocol of placing curves was different for the bilaterally symmetrical mysticetes and the asymmetrical archaeocetes and odontocetes. The protocol was as follows:

#### **Mysticetes:**

1. 60 curves were placed on the sutures between bones on the LHS of the skull
2. 4 curves were placed on the midline of the skull - curves were mirrored using the midline as a pivot plane
3. 60 curves were mirrored to the RHS side of the skull
4. A total of 124 curves were placed over the whole skull
5. After sliding and resampling the curves (see below) a total of 2028 fixed landmarks and sliding semilandmarks covered the skull. These 2028 points were used for all analyses (see Data analyses).

#### **Archaeocetes and odontocetes:**

1. 60 curves were placed on the sutures between bones on the LHS of the skull
2. 4 curves were placed on the midline of the skull - curves were mirrored using the midline as a pivot plane
3. 39 curves were mirrored to the symmetrical bones on the RHS
4. 21 curves were placed on asymmetrical bones on the RHS
5. After sliding and resampling the curves (see below) a total of 2028 fixed landmarks and sliding semilandmarks covered the skull. These 2028 points were used for all analyses (see Data analyses).

### **What is sliding and resampling?**

The manual placement of landmarks (using Stratovan Checkpoint (Stratovan, Davis, CA, USA)) means that points are not usually evenly spaced along each curve. Therefore, the number of curve points initially chosen may not be ideally representative across the entire dataset. Curves are therefore resampled for even spacing before being slid during alignment. Sliding the curves after resampling is a crucial step, as equally spaced semilandmarks cannot be treated as optimally placed.

## Phylogeny

To generate a tree that included all of our sampled taxa, I used the time-calibrated phylogeny from Lloyd and Slater [5]. This 'genus tree' includes all species belonging to a genus that appear in a character matrix using taxonomic constraints to place taxa that lack data. I modified it as follows: First, I added several additional extant species (which were already represented to the genus level in the Lloyd and Slater phylogeny [5]) with position based on recently published studies. I placed *Neophocaena asiaeorientalis* in the same genus as *Neophocaena phocaenoides* [6], *Sousa plumbea* + *Sousa teuszii* + *Sousa sahalensis* in the same genus as *Sousa chinensis* [7], *Orcaella heinsohni* in the same genus as *Orcaella brevirostris* [8, 9], and *Mesoplodon hotaula* in the genus *Mesoplodon* next to *Mesoplodon ginkgodens* [10]. Finally, I placed *Berardius minimus* in the genus *Berardius* next to *Berardius bairdii* and *Berardius arnuxii* following its recent description by Yamada et al. [11]. The following fossil species were directly swapped with their corresponding monophyletic congener as follows. I placed *Balaenoptera* sp. (SDNHM 83695) as a sister taxon to *Balaenoptera siberi* (although not present in our sample), close to extant *Megaptera novaeangliae* as in Martin [12], *Balaenoptera floridana* as a sister taxon to *Balaenoptera davidsonii* [13, 14] (again, the latter species is not present in our sample), and *Orycterocetus crocodilinus* is placed in the physeterids according to Lambert et al. [15]. I placed *Globicephala* sp. as a sister taxon to *Globicephala etruriae* [16, 17, 18] and *Hemisyntrachelus cortesii* in the same genus as *Hemisyntrachelus oligodon* according to Post and Bosselaers [19]. I caution that Kentriodontidae is often considered a non-monophyletic 'waste-basket' for Late Oligocene and Miocene homodont odontocetes [20]. Restrictions according to Peredo et al. [21] leave *Tagicetus* and *Atocetus* (previously referred to as Kentriodontidae) outside of the family. The positioning of *Argyrocetus joaquinensis* is also unclear [22]. Two specimens (*Xenorophus* ChM PV7677 and *Patriocetid* or *Waipatiid* CCNHM 1078) were excluded from the analysis due to uncertainty in their position. This phylogeny for the present study is the same as the one used in Coombs et al 2020 [1]. In addition, for this study, I also added the following taxa:

- *Ankylorhiza tiedemani* is situated in a basal position along the odontocete stem, diverging after *Xenorophidae* but before the *Squalodontidae* as in Boessenecker et al (2020) [23]
- *Hemisyntrachelus oligodon* was placed as a sister taxon to *Hemisyntrachelus cortesii* according to Post & Bosselaers (2005) [24]
- *Parapontoporia wilsoni* is placed as a sister taxon to *Parapontoporia sternbergi* as in Geisler et al (2011) [25]
- *Scaphokogia totajpe* was placed as a sister taxon to *Scaphokogia cochlearis* as in Benites-Palomino et al (2019) [26]

## Data analyses

For computational purposes, all polytomies in the tree were resolved by adding zero branch lengths using multi2di in ape v.5.0 [27] prior to downstream phylogenetic analyses. Please be aware that these methods are a work in progress for my next PhD chapter and therefore not all in-depth details are provided here. Please do contact me if you would like to discuss them further.

The following key methods were used to analyses the data:

1. I used principal components analysis (PCA) to identify the major axes of shape variation across cetaceans for the whole cranium and for individual cranial modules. Principal components analysis converts a dataset into a set of mathematically uncorrelated variables.

These variable represent the principal axes of shape variation in a given dataset. I then visualised the main components of morphological variation across the cranium using the PC axes scores. These analyses were done using the geomorph package in R [28]

2. Rates and disparity in each of the bones was modelled using the `compare.multi.evol.rates` function in geomorph [28] and visualised using the `hot.dots` R package [29].

3. Evolutionary rate through time was investigated using *BayesTraitsV3* (<http://www.evolution.rdg.ac.uk/>), by inputting phylogenetic principal component (pPC) scores (calculated through the 'phyl.pca' function in phytools [30]) that represented 95% of the shape variation for the whole cranium and for each region.

4. Ecological MANOVAs were done using data from the literature (namely Marx et al., (2016) [31] Berta and Lanzetti (2020) [32]) and the geomorph `procD.pgls` function.

5. Disparity through time was analysed using `dtc.paleo` from the Geiger package in R [33].

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