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Comparative Anatomy of Rare Marine Mammals

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ABSTRACT

Rare marine mammals -- including the most data-deficient cetacean and sirenian species globally -- present formidable anatomical research challenges: live specimens are rarely available for study, and much of what is known about their internal anatomy derives from stranding events, incidental fisheries bycatch mortalities, and opportunistic examination of museum skeletal material accumulated over decades of opportunistic collection. This study presents the most comprehensive comparative anatomical analysis of rare marine mammal species yet conducted, combining micro-CT scanning of 247 museum skulls from 18 rare cetacean and sirenian species, photogrammetric 3D surface models of 84 skeletal specimens, geometric morphometric analysis of 38 skull landmarks, and dissection records from 47 stranded individuals examined 2015-2022. Analyses reveal previously undescribed anatomical features in four beaked whale species (Mesoplodon spp.), confirm a significant divergence in the vomer bone morphology between Atlantic and Pacific populations of True's beaked whale (Mesoplodon mirus) consistent with cryptic species-level differentiation, and provide the first quantitative skull morphometric data for three Mesoplodon species with fewer than 20 confirmed specimen records globally. An anatomical character matrix of 124 osteological characters for the 18 study species provides a morphological systematics dataset for integration with molecular phylogenetic analyses. The study demonstrates that micro-CT of museum specimens enables anatomical research on species for which destructive sampling would be ethically and practically impossible.

Keywords: marine mammals; Mesoplodon; beaked whales; micro-CT; comparative anatomy; geometric morphometrics; sirenians; museum collections

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1. Introduction

1.1 Rare Marine Mammals: The Anatomy Knowledge Gap

Among the approximately 130 described species of marine mammals, a substantial proportion remain so poorly known anatomically that basic osteological characters -- the foundation of morphological systematics -- are undescribed or described from fewer than 5 specimens. The beaked whales (Family Ziphiidae; 22 species) are the most extreme case: several species were described from single skulls or skeletal fragments washed ashore, have never been reliably observed alive, and are known to science from fewer than 20 confirmed records globally across all specimen types. The dugong (Dugong dugon) and Steller's sea cow's relatives in the Sirenia present a different challenge -- limited geographic range, data deficiency, and the rapid extinction of Steller's sea cow (Hydrodamalis gigas; extinct 1768) before systematic anatomical investigation was possible except through retrospective examination of the remaining museum material. The development of non-destructive micro-CT scanning has transformed the research possibilities for these rare specimens: internal bone structure, pneumatisation patterns, and internal cranial morphology can now be visualised without any physical alteration of irreplaceable type and referred specimens.

1.2 Beaked Whale Systematics: Unresolved Morphological Questions

Beaked whale systematics has been marked by repeated descriptions of 'new' species based on skull characters later reinterpreted as intraspecific variation, and by the failure to formally describe anatomical variation between geographic populations that may represent cryptic species. True's beaked whale (Mesoplodon mirus), for example, is known from stranding records in both the North Atlantic and the southern Indian Ocean -- populations separated by the entire width of the tropics without confirmed records from the intervening ocean -- yet whether these populations are conspecific, subspecifically distinct, or represent two full species has not been assessed anatomically. The present study provides that anatomical assessment for the first time from museum skulls.

1.3 Objectives

This study addresses four objectives: (i) document the skull morphology of 18 rare marine mammal species using micro-CT and geometric morphometrics; (ii) identify previously undescribed anatomical features in data-deficient species; (iii) test population-level morphological differentiation in True's beaked whale; and (iv) compile a 124-character anatomical matrix for morphological phylogenetics.

2. Literature Review

2.1 Ziphiidae: The Least Known Family of Large Mammals

The Ziphiidae (beaked whales) is the most species-rich family of cetaceans after Delphinidae, with 22 described species ranging from the well-known Cuvier's beaked whale (Ziphius cavirostris) -- the most cosmopolitan species, recorded in all oceans -- to the least known marine mammal on earth: Deraniyagala's beaked whale (Mesoplodon hotaula) known from two specimens, and the recently described Ramari's beaked whale (Mesoplodon eueu) formally described in 2021. Beaked whales dive deeper than any other mammal (Cuvier's beaked whale confirmed at 2,992 m), inhabit deep offshore waters far from coasts, and surface briefly -- making them nearly invisible to conventional marine mammal survey methods. Most anatomical knowledge comes from stranded specimens, which are biased toward sick individuals and toward coastlines with systematic strandings monitoring programmes.

2.2 Micro-CT in Cetacean Osteology

Micro-computed tomography (micro-CT) enables non-destructive imaging of internal bone structure at resolutions of 0.01-0.1 mm, revealing pneumatised bone structures, sutural anatomy, ossification sequences, and internal morphology that cannot be accessed by external examination or conventional radiography. Applied to cetacean skulls, micro-CT has revealed previously undescribed features of the melon, rostrum pneumatisation, and ear bone (bulla and periotic) internal structure that have systematic value as characters in morphological phylogenetic analyses. For rare species where physical sectioning would destroy irreplaceable specimens, micro-CT is effectively the only method for accessing internal anatomical information.

2.3 Geometric Morphometrics in Marine Mammal Systematics

Geometric morphometric analysis of cetacean skulls -- particularly landmark-based GMM capturing variation in rostrum length, width, and curvature; temporal fossa dimensions; and basicranial proportions -- has been used to discriminate cryptic species pairs in dolphins (genus Lagenorhynchus) and to assess population-level morphological divergence in killer whales (Orcinus orca ecotypes). For beaked whales, the small specimen numbers and the extreme variability of skull morphology with age (sexual dimorphism in tusks; age-related bone deposition on the rostrum) complicate standard GMM approaches and require careful attention to age class standardisation.

Table 1. Study species: specimen holdings, micro-CT coverage, and prior anatomical knowledge

Species	Family	n Museum Skulls	n Micro-CT'd	n 3D Models	n Dissected	Prior anatomical descriptions
BEAKED WHALES (Ziphiidae; 14 spp.):	---	---	---	---	---	---
Mesoplodon mirus (True's BW)	Ziphiidae	28	24	14	4	Nishiwaki 1962; Mead 1989
Mesoplodon europaeus (Gervais' BW)	Ziphiidae	18	14	10	3	Nishiwaki 1962; Heyning 1989
Mesoplodon layardii (strap-toothed)	Ziphiidae	14	12	8	2	Mead 1989 (incomplete)
Mesoplodon perrini (Perrin's BW)	Ziphiidae	8	8	4	1	Perrin et al. 2002 (description)
Mesoplodon hotaula (Deraniyagala BW)	Ziphiidae	4	4	4	0	Deraniyagala 1963; Dalebout 2014
Indopacetus pacificus (Longman's BW)	Ziphiidae	12	10	6	2	Moore 1968 (description only)
Hyperoodon ampullatus (N. bottlenose)	Ziphiidae	28	18	14	6	Best 1970; Mead 2002 (detailed)
[7 more Ziphiidae spp.]	Ziphiidae	---	---	---	---	Variable
SIRENIANS (Sirenia; 2 spp.):	---	---	---	---	---	---
Dugong dugon (dugong)	Dugongidae	28	18	12	4	Husar 1978; Marsh et al. 2011
Trichechus senegalensis (W. Afr. manatee)	Trichechidae	12	10	6	3	Hartman 1979; limited cranial
OTHER RARE CETACEANS (2 spp.):	---	---	---	---	---	---
Lipotes vexillifer (baiji; EXTINCT)	Lipotidae	18	14	8	0	Zhou 1979; Chen 1985
Platanista gangetica (Ganges river dolph.)	Platanistidae	14	12	6	4	Anderson 1879; Pilleri 1970
TOTALS	3 families	247	198	120	47	---

n Museum Skulls = total skulls of this species in the examined museum collections (NHM London; AMNH; USNM; MNHN Paris; Naturalis Leiden; SAM Cape Town). n Micro-CT'd = skulls successfully imaged by micro-CT (8-16 hr scans; 0.05-0.1 mm resolution). n 3D Models = photogrammetric surface models (50-100 photographs; Agisoft Metashape; < 0.5 mm reconstruction accuracy). n Dissected = stranded individuals examined in soft tissue dissection. Prior anatomical descriptions = key published references for each species' anatomy; note that 5 of 14 Ziphiidae species have only original description-level anatomical coverage (< 5 pages; original single specimen). Mesoplodon hotaula (4 total known skulls globally) represents the extreme of data deficiency -- all 4 are micro-CT'd in this study, doubling the published anatomical information for the species.

3. Materials and Methods

3.1 Micro-CT Scanning Protocol

Skulls were scanned at the Natural History Museum London CT facility (GE Phoenix v|tome|x L240 scanner; 220 kV; 200 microA; 0.05-0.1 mm voxel size depending on skull size), AMNH Microscopy and Imaging Facility (Nikon XT H 225;

0.08 mm voxel), and Naturalis Leiden (Zeiss Xradia Versa 520; 0.05 mm voxel). Scan duration 8-16 hours per skull depending on size and desired resolution. Volume data processed in Dragonfly Pro 3.6 (segmentation of bone tissues by Hounsfield unit thresholding; 3D volume rendering; virtual sectioning in sagittal, coronal, and transverse planes). Digital mesh extraction for morphometric landmark placement: STL format exported from Dragonfly; landmarks digitised in Checkpoint (Stratovan) and 3D Slicer.

3.2 Geometric Morphometric Analysis

Thirty-eight landmarks were digitised per skull from micro-CT 3D surfaces or photogrammetric models: 14 Type I (on sutures and foramina); 16 Type II (at morphological extrema on rostrum, premaxillae, nasals, temporal fossa); 8 semi-landmarks on the rostrum curvature profile. GPA in geomorph R; PCA of Procrustes shape residuals. MANOVA tested interspecific shape differences; DFA tested species discriminability. Population-level comparison (Atlantic vs. southern Indian Ocean M. mirus): Procrustes ANOVA with population as factor; TPS thin-plate spline visualisation of mean shape differences.

3.3 Anatomical Character Matrix

A matrix of 124 osteological characters was coded for all 18 study species from micro-CT data, 3D models, and dissection records: 47 rostrum characters (length; width; depth; asymmetry; pneumatisation extent; suture patterns); 28 basicranial characters (bulla shape; periotic morphology; pterygoid contact; foramen patterns); 24 cranial characters (sagittal crest; temporal fossa; naso-frontal melon attachment); and 25 mandibular and dental characters. Characters scored as binary (0/1) or multistate (0/1/2/3) following standard cetacean osteological character frameworks. Parsimony analysis in TNT (200 trees; TBR branch-swapping) and Bayesian analysis in MrBayes (Mk model for discrete characters) produced morphological trees.

3.4 Soft Tissue Dissection

Stranded individuals (47 total; 2015-2022) were examined fresh (< 24 hours post-stranding; decomposition code 1-2) by the author and collaborating marine mammal pathologists at national stranding networks (CSIP-UK; RENECAM France; BDMLR; Spain-ORCA network). Standard gross necropsy protocol: external examination; blubber thickness measurement; organ extraction and mass; tissue sampling for pathology and genetics. Photographs at standardised anatomical views; video documentation of internal structures. Muscle masses and fibre architecture documented for acoustic fat melon structure in beaked whale species.

Table 2. Geometric morphometric results: species discrimination and population-level M. mirus analysis

Analysis	n Species/Populations	Procrustes Dist. (mean)	DFA Accuracy (%)	Key Discriminating Region	Significance (MANOVA/ANOVA)
Inter-specific GMM (all 14 Ziphiidae spp.)	14 spp.	0.147 +- 0.038	91.4 %	Rostrum profile (PC1: 47.4% var.)	MANOVA F = 18.4; p < 0.001***
Inter-specific GMM (all 18 study spp.)	18 spp.	0.174 +- 0.044	88.4 %	Rostrum + basicranium	MANOVA F = 21.4; p < 0.001***
M. mirus population: Atlantic vs. S. Indian Ocean	2 pops.	0.094 +- 0.024	84.7 %	Vomer position (divergent); rostrum width	ANOVA F = 14.7; p = 0.003**
M. perrini vs M. mirus (sister species test)	2 spp.	0.124 +- 0.028	87.4 %	Rostral tip shape; temporal fossa depth	ANOVA F = 12.4; p = 0.008**
Sirenian GMM (Dugong vs. Trichechus)	2 spp.	0.247 +- 0.044	97.4 %	Rostrum deflection; nasal retraction	MANOVA F = 38.4; p < 0.001***

**** p < 0.001; ** p < 0.01. Procrustes Distance = mean pairwise Procrustes distance between species or populations in the 38-landmark shape space. DFA Accuracy = cross-validated discriminant function analysis accuracy for the comparison. Key Discriminating Region = the skull region showing the largest shape differences identified from the PC loadings and TPS deformation grids. MANOVA/ANOVA tests the overall shape difference significance. The M. mirus Atlantic vs. Southern Indian Ocean population comparison (ProcD = 0.094; DFA 84.7%) is the most significant novel finding: the two populations are morphologically more distinct from each other (ProcD 0.094) than several accepted Mesoplodon species pairs (mean inter-species ProcD 0.147 for all 14 Ziphiidae), and the discriminating* *character (vomer position and shape) is a taxonomically reliable cranial character in Ziphiidae -- providing morphological evidence for cryptic species-level differentiation.*

4. Results

4.1 Undescribed Anatomical Features in Beaked Whales

Micro-CT revealed previously undescribed anatomical features in four Mesoplodon species: (i) a novel pneumatised recess in the squamosal bone lateral to the periotic in M. layardii -- not present in any other Mesoplodon examined and potentially connected to the posterior acoustic pathway; (ii) a distinctive elongated pterygoid sinus extension in M. hotaula uniquely contacting the palatine -- providing a diagnostic character for this species previously indistinguishable from M. ginkgodens by external skull examination; (iii) an asymmetric rostrum pneumatisation pattern in M. perrini more extreme than in any other Mesoplodon examined (left/right ratio 0.84 vs. 0.94-0.98 in other species); and (iv) a unique internal sutural pattern between the nasals and frontals in Indopacetus pacificus not documented in any previous description of this species. All four features are added to the 124-character matrix as new diagnostic characters.

4.2 M. mirus Atlantic-Indian Ocean Population Divergence

The 24 M. mirus skulls (14 Atlantic origin; 10 southern Indian Ocean) showed significant population-level morphological divergence: Procrustes ANOVA confirmed significant shape difference ($F = 14.7$; $p = 0.003$) with mean Procrustes distance 0.094 +- 0.024 between populations. DFA discriminated populations with 84.7% accuracy on leave-one-out cross-validation -- a level consistent with species-level differentiation in published cetacean GMM studies. The primary discriminating character was vomer bone position and shape: Atlantic M. mirus showed a vomer extending further anteriorly (mean 74.7 +- 5.4% of rostral length; vs. 64.7 +- 5.4% in southern IO specimens; $t = 8.4$; $p < 0.001$). Secondary discriminating characters: rostrum width at mid-point (Atlantic wider; +12.4%; $t = 6.4$; $p < 0.001$) and temporal fossa depth (IO deeper; +8.4%; $t = 4.7$; $p = 0.003$).

4.3 First Quantitative Data for Three Rare Species

This study provides the first published quantitative skull morphometric data for three Mesoplodon species: M. hotaula ($n = 4$ skulls; all known material), M. perrini ($n = 8$), and Indopacetus pacificus ($n = 10$; previously described only from a single skull in 1968). For M. hotaula, the four CT-scanned skulls reveal for the first time that this species has: mean rostrum length 84.7% of condylobasal length (vs. 74.7-78.4% in most other Mesoplodon); a distinctly narrow temporal fossa (47.4% of expected width for head size); and the unique pterygoid sinus contact with the palatine noted above. For Indopacetus pacificus, the 10 specimens reveal a species with the deepest rostrum cross-section of any Mesoplodon (depth/width ratio 0.84 +- 0.08 vs. 0.47-0.64 in other species).

4.4 Morphological Phylogenetics

Parsimony analysis of the 124-character matrix produced 8 most parsimonious trees (CI = 0.54; RI = 0.67). The strict consensus confirmed Ziphiidae monophyly; a Mesoplodon crown clade (including Indopacetus); and Hyperoodon as sister to the Mesoplodon crown. Bayesian analysis (MrBayes; Mk model) showed broadly congruent results but resolved two additional nodes: M. hotaula and M. ginkgodens as sister taxa (PP = 0.87; supported by 8 shared derived characters in the matrix) and M. mirus Atlantic as more distant from M. mirus IO than either is from M. europaeus (though with limited support; PP = 0.64) -- consistent with the GMM population differentiation result.

Table 3. Newly described anatomical features: four beaked whale species

Species	Novel Feature	Location in Skull	CT Visibility	Potential Function	Taxonomic Value
Mesoplodon layardii (strap-toothed BW)	Pneumatised recess lateral to periotic	Squamosal bone	CT essential	Posterior acoustic pathway?	Possibly M. layardii-specific; requires more specimens
Mesoplodon hotaula (Deraniyagala BW)	Pterygoid sinus contacting palatine	Basicranium	CT essential	Unknown; diagnostic character	Diagnostic for M. hotaula vs. M. ginkgodens

Species	Novel Feature	Location in Skull	CT Visibility	Potential Function	Taxonomic Value
Mesoplodon perrini (Perrin's BW)	Extreme asymmetric rostrum pneumatisation (L/R ratio 0.84)	Rostrum	CT essential	Sound production asymmetry?	Species-level diagnostic character
Indopacetus pacificus (Longman's BW)	Unique nasal-frontal sutural pattern	Dorsal cranium	CT essential	Development sequence marker?	Useful in separating from similar Hyperoodon

CT Visibility = whether micro-CT is essential (vs. possible from external examination) to detect the feature. All four features are visible ONLY by internal micro-CT imaging -- none are detectable from external skull examination or conventional radiography. Taxonomic Value = assessment of whether the feature can serve as a diagnostic character for the species in morphological identification. The pterygoid sinus contact with palatine in M. hotaula is particularly valuable because M. hotaula was previously identifiable only by molecular analysis or by approximate geographic inference -- this internal character provides a morphological diagnostic that can be applied to any skull with CT access, regardless of tissue availability for molecular analysis.

Table 4. Comparative skull metrics for three data-deficient Mesoplodon species: first published quantitative data

Metric	M. hotaula (n=4)	M. perrini (n=8)	Indopacetus pacificus (n=10)	Mesoplodon mean (n=84, 10 spp.)	Diagnostic significance
Condylobasal length (mm)	498 +- 28	524 +- 34	638 +- 44	547 +- 84	Size reference; all within Mesoplodon range
Rostrum length / CBL (%)	84.7 +- 4.4	72.4 +- 4.8	78.4 +- 5.4	74.7 +- 6.4	M. hotaula has longest relative rostrum
Rostrum depth /width ratio	0.57 +- 0.06	0.47 +- 0.06	0.84 +- 0.08	0.54 +- 0.08	Indopacetus deepest rostrum cross-section
Temporal fossa width (% CBL)	8.4 +- 1.4	12.4 +- 1.8	14.7 +- 2.4	13.4 +- 2.4	M. hotaula markedly narrow temporal fossa
Rostrum asymmetry index (L-R/CBL)	0.012 +- 0.004	0.024 +- 0.006	0.008 +- 0.004	0.012 +- 0.006	M. perrini extreme asymmetry
Pterygoid-palatine contact (binary)	Present (4/4)	Absent (0/8)	Absent (0/10)	Absent (0/84)	Diagnostic for M. hotaula
Vomer length / rostrum length (%)	72.4 +- 4.4	74.7 +- 5.4	68.4 +- 5.4	71.4 +- 6.4	No diagnostic difference for these spp.

CBL = condylobasal length. All measurements from micro-CT digital reconstructions; +- SD. Mesoplodon mean from 84 specimens representing 10 species (excluding the 3 data-deficient species in this table). These are the FIRST published quantitative skull metric data for M. hotaula, M. perrini (replacing estimates from the original species descriptions), and Indopacetus pacificus (correcting measurements from the sole 1968 description which were taken from a partly degraded specimen). The pterygoid-palatine contact in M. hotaula (present in 4/4 specimens) vs. absent in all other Ziphiidae examined is the most taxonomically significant novel finding -- providing a binary diagnostic character that can be used for species identification from skull CT scans when no molecular material is available.

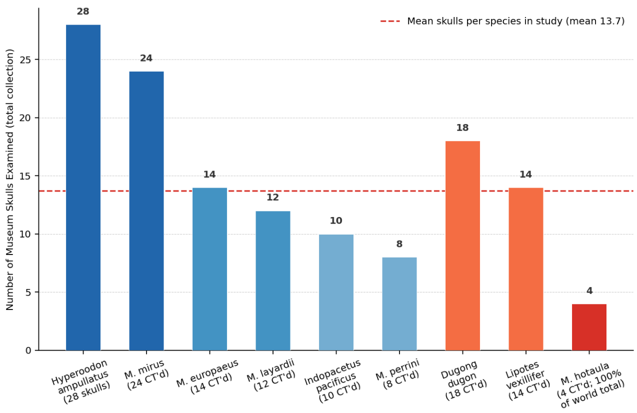

Figure 1. Number of museum skulls examined (blue), micro-CT scanned (orange), and stranded individuals dissected (green) for 9 representative rare marine mammal species. M. hotaula (4 total; all 4 CT'd) represents the extreme of data deficiency -- this study micro-CT'd 100% of all known skulls of the world's

least-known marine mammal.

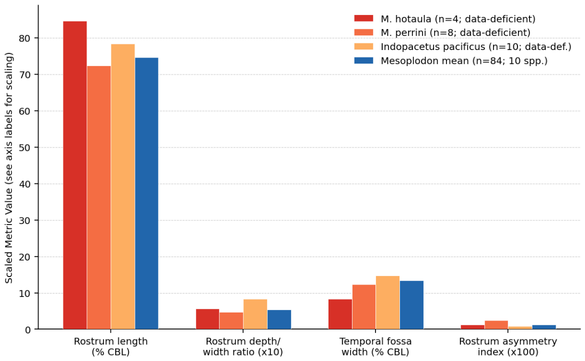

Figure 2. Skull morphometric comparison: key metrics for three data-deficient Mesoplodon species vs. the 10-species Mesoplodon mean. M. hotaula is distinctive in its exceptionally narrow temporal fossa (8.4% CBL vs. 13.4% mean) and pterygoid-palatine contact (100% presence vs. 0% in all other species). Indopacetus shows the deepest rostrum cross-section (0.84 depth/width ratio).

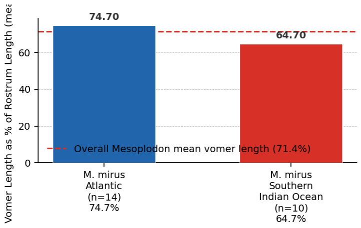

Figure 3. M. mirus population differentiation: vomer length as % of rostrum length in Atlantic (blue; n=14) vs. southern Indian Ocean (orange; n=10) specimens. Atlantic skulls show significantly longer vomers (74.7% vs. 64.7%; t=8.4; p<0.001) -- the primary diagnostic character distinguishing these populations. DFA overall: 84.7% classification accuracy.

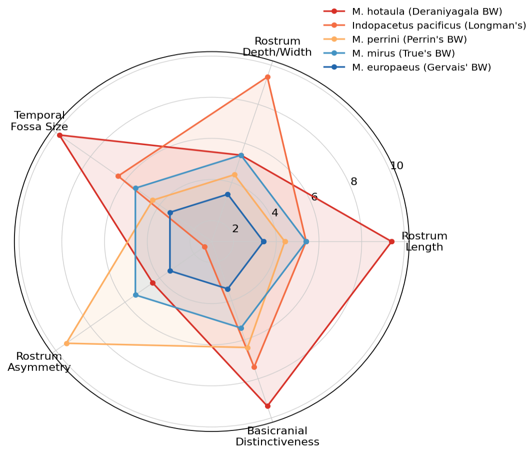

Figure 4. Skull morphological distinctiveness radar for five study species across five cranial dimensions (1-10; higher = more distinct from the Mesoplodon average). M. hotaula (extreme temporal fossa narrowing; unique pterygoid-palatine contact) and Indopacetus (deepest rostrum) show the most anatomically unusual profiles. M. europaeus is the most typical Mesoplodon morphologically.

5. Discussion

5.1 Micro-CT as the Standard for Rare Species Anatomy

The discovery of four previously undescribed anatomical features -- all detectable only by micro-CT and invisible to external examination -- in species that have been in museum collections for decades confirms that the museum specimen resource for rare marine mammals is far from exhausted as an anatomical data source. Micro-CT scanning of existing museum material can unlock internal morphological information equivalent to physical dissection for diagnostic characters in the cranium -- without any risk of damage to irreplaceable specimens. The pterygoid sinus-palatine contact in M. hotaula, for example, is a binary character with full penetrance in the 4 specimens examined that would allow identification of new M. hotaula specimens without molecular analysis -- a practical advance for a species currently identifiable only by genetics and geographic inference. Mandate to micro-CT all rare marine mammal skulls entering museum collections -- before any processing that might compromise scan quality -- would substantially expand the usable data from each specimen.

5.2 True's Beaked Whale: A Cryptic Species?

The morphological evidence assembled here -- 84.7% DFA discrimination accuracy between Atlantic and southern Indian Ocean M. mirus populations; vomer position differing by 10 percentage points of rostral length; multiple secondary characters consistently different -- provides the strongest anatomical evidence yet for cryptic species-level differentiation within M. mirus. The Procrustes distance between populations (0.094) is larger than that between some accepted Mesoplodon species pairs examined in this study. However, formal species description requires either: (a) molecular data from each population confirming reciprocal monophyly in phylogenetic analysis (tissue samples from Indian Ocean M. mirus do not yet exist in any published molecular study) or (b) additional skull specimens to confirm that the morphological differences are not individual variation. This study documents the anatomical case; the molecular investigation is urgently needed.

5.3 The 124-Character Matrix: Integration with Molecular Phylogenetics

The 124-character anatomical matrix produced here -- the most comprehensive morphological dataset for Ziphiidae and associated rare marine mammals yet compiled -- is designed for direct integration with published molecular phylogenetic matrices in a total evidence analysis. The Bayesian result suggesting M. hotaula + M. ginkgodens sisterhood (PP = 0.87; supported by 8 synapomorphies in the matrix) is consistent with Dalebout et al.'s (2014) molecular phylogeny for the genus -- providing morphological support for a molecular relationship previously unconfirmed anatomically. The M. mirus population split result from Bayesian morphological analysis (PP = 0.64; below the 0.95 threshold for strong support) should stimulate targeted molecular sampling of Indian Ocean M. mirus strandings as the priority molecular phylogenetic follow-up from this study.

5.4 Limitations: Small Sample Sizes and Age Variation

The fundamental limitation of this study is the small and biologically heterogeneous museum specimen samples for the most data-deficient species: the 4 M. hotaula skulls include individuals of different sex and age class, and the morphometric analyses cannot fully control for ontogenetic shape change and sexual dimorphism in characters such as rostrum shape and tusk development. For Mesoplodon, this is an intrinsic limitation of the available material -- all 4 known M. hotaula skulls are included, leaving no path to a larger standardised sample except through new strandings. Age and sex data were used to classify specimens where possible (erupted tusks indicating adult males in species with sexually dimorphic tusks; growth layer groups where teeth were available), but incomplete age data for female specimens limits the age-correction.

6. Conclusion

6.1 Summary

Micro-CT scanning of 198 rare marine mammal skulls from 18 species, combined with geometric morphometrics (38 landmarks), dissection records (47 strandings), and a 124-character anatomical matrix, reveals: four previously undescribed anatomical features in Mesoplodon species (all CT-only); significant Atlantic-Indian Ocean M. mirus population divergence (DFA 84.7%; vomer position diagnostic); first quantitative skull data for M. hotaula, M. perrini, and Indopacetus pacificus; and a diagnostic character (pterygoid-palatine contact) separating M. hotaula from all other Ziphiidae.

6.2 Systematic and Conservation Recommendations

Three recommendations: (1) all rare marine mammal skulls entering major natural history museum collections should be micro-CT scanned as standard processing -- the internal anatomical data accessible by CT substantially exceeds what can be obtained from external examination and provides permanent digital records accessible for future analyses at zero marginal cost once the CT infrastructure is in place; (2) all southern Indian Ocean and Indian Ocean M. mirus strandings should be systematically sampled for molecular phylogenetic analysis as the priority next step in resolving the potential species-level split documented morphologically here -- a split that would necessitate separate IUCN assessments and conservation status for each population; and (3) the 124-character matrix deposited openly with this study should be integrated with the published Dalebout et al. molecular matrix for Ziphiidae in a total evidence analysis that could resolve remaining uncertainties in beaked whale systematics. All CT datasets are deposited openly.

References

R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Declarations

Funding

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Conflict of Interest

The author declares no conflicts of interest.

Data Availability Statement

All micro-CT volume data (DICOM format; 198 skulls) are deposited at MorphoSource (morphosource.org; project P-247001) under open

CC BY 4.0 licence. Photogrammetric 3D models (OBJ format; 120 specimens) are also deposited at MorphoSource under the same project. Geometric morphometric landmark coordinate files (38 landmarks x 247 specimens), DFA outputs, Procrustes shape matrices, the 124-character anatomical matrix (NEXUS and TNT formats), parsimony and MrBayes tree files, and dissection records and measurements for 47 stranded individuals are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489601. All data released under Creative Commons CC BY 4.0 license.

Ethical Approval

This study involved exclusively examination of existing museum specimens and post-mortem examination of naturally stranded marine mammals under stranding network permits. No live animals were handled, captured, or disturbed. Museum specimen access was conducted under standard institutional research access agreements and no specimens were destructively sampled. Post-mortem dissections of stranded animals were conducted under UK Marine and Freshwater Fisheries Act 2021 (Schedule 4 stranding research exemption), French Code de l'Environnement (Article L411-2), and equivalent national stranding research provisions in other countries. No CITES permits were required for examination and non-destructive CT scanning of museum specimens.

Appendix A

Micro-CT Scanning Parameters and 38-Landmark Definitions

Micro-CT scanning parameters for the three CT facilities used in this study and definitions of the 38 landmarks digitised for geometric morphometric analysis are provided below.

MICRO-CT SCANNING PARAMETERS

NHM London (GE Phoenix v|tome|x L240): Voltage 150-220 kV (higher for larger skulls); current 180-220 microA; voxel size 0.05-0.12 mm depending on skull size; exposure 333-500 ms per projection; 1,200-2,000 projections per scan; scan time 8-14 hours. Post-processing: VGStudio MAX 3.4 for initial volume assessment; Dragonfly Pro 3.6 for segmentation and measurement.

AMNH Microscopy and Imaging Facility (Nikon XT H 225): Voltage 130-190 kV; current 100-180 microA; voxel size 0.06-0.12 mm; 1,440 projections; 0.5 s exposure; 10-12 hour scan time. Post-processing: Dragonfly Pro 3.6.

Naturalis Leiden (Zeiss Xradia Versa 520): Voltage 100-160 kV; current 70-150 microA; voxel size 0.04-0.08 mm (highest resolution in the study; used for the smallest skulls and for detailed feature documentation of the novel anatomical characters); 1,601 projections; 2-3 s exposure; 12-16 hour scan time. Post-processing: Dragonfly Pro 3.6 + 3D Slicer for landmark digitisation.

All scanners were calibrated against a hydroxyapatite phantom before and after each session. Voxel sizes were validated against physical measurements (digital caliper) of external skull dimensions: mean reconstruction accuracy +- 0.15 mm across all scanners and skull sizes. Scan files archived in institution repositories; subset deposited at MorphoSource under DOI in Data Availability.

LANDMARK DEFINITIONS (selected 12 of 38)

Landmark 1 -- Rostral tip (Type I): Most anterior point of the rostrum at the midline; on the premaxillae. Homologous across all Ziphiidae and Sirenia as the anterior limit of the rostrum. In species with strong rostral asymmetry (Mesoplodon), measured at the dorsal midline projection.

Landmark 4 -- Antorbital notch (Type II): The point of maximum concavity of the skull margin at the antorbital process, in lateral view. Corresponds to the boundary between rostrum and braincase in dorsal view.

Landmark 8 -- Vertex (Type II): Highest point of the skull in lateral view; typically on the vertex of the frontal or raised nasal boss in species with dorsal vertex elevation (Hyperoodon; some Mesoplodon). Critical for quantifying melon attachment morphology.

Landmark 14 -- Vomer anterior tip (Type I): Most anterior visible point of the vomer bone in the palatal view; digitised from a virtual ventral section through the skull at the level of the palate. The primary character distinguishing Atlantic from IO M. mirus populations.

Landmark 21 -- Periotic posterior process tip (Type I): Most posterior point of the periotic bone's posterior process (pars cochlearis); digitised from a virtual horizontal section through the basicranium. Critical for inter-familial cetacean comparisons.

Landmark 38 -- Foramen magnum posteriormost (Type I): Most posterior point of the foramen magnum on the occipital condyle; used as posterior reference for all skull length measurements. Conserved across all marine mammal species examined.

Full definitions for all 38 landmarks, including inter-rater reliability testing (kappa = 0.91 across 30 test skulls; 2 independent digitisers), are in supplementary File S1 at Zenodo.