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Review of the doctoral thesis entitled: "Adaptive skull shape changes in bottlenose dolphins (Tursiops spp.): inference from 3D morphological analyses" authored by Morgane Dromby, MSc

Review of Chapter 1

Chapter 1 offers a thorough introduction to the field of geometric morphometrics (GM), presenting the fundamental principles of the method, the different types of landmarks employed, and the rationale for its use in morphological research. In addition to this overview, the chapter provides **a comprehensive and systematic literature review** that clearly illustrates the contribution of GM to the study of skull diversity in odontocetes. The review covers publications from 1990 to 2021, with 1993 marking the year in which the term GM was first introduced into the scientific literature.

Altogether, 32 studies on odontocete skull morphology are cited, encompassing approaches based on 2D, 3D, and GM analyses. These studies address a wide taxonomic range, including the families Delphinidae, Iniidae, Phocoenidae, and Pontoporidae, as well as the genera Tursiops, Delphinus, Stenella, Phocoena, Lagenorhynchus, Pontoporia, Cephalorhynchus, Sousa, Sotalia, Globicephala, Orcinus, Lissodelphis, and Inia. From a geographic perspective, the published research has been conducted in nine regions: the North Atlantic, Mediterranean Sea, North Sea, South Pacific, South Atlantic, Indian Ocean, North Pacific, Black Sea, Amazon River, and the Baltic Sea.

The data presented in the literature demonstrate that GM is a powerful and versatile analytical tool, applicable to a broad range of biological questions. In odontocete research, it has provided valuable insights into evolution, ecology, ontogeny, sexual dimorphism, taxonomy, phylogeny, functional morphology, and population structure. **Mrs. Dromby also identifies the current limitations of this field. In particular, she highlights the absence of standardized landmarking protocols, the constraints associated with manual landmarking, and the scarcity of comprehensive studies focused on individual species. She further notes the lack of published research that systematically compares cranial shape differences with environmental parameters and functional interpretations.**

The chapter concludes with a clear presentation of the **objectives of the thesis. These are: (1) to quantify 3D skull shape differences in bottlenose dolphins on a global scale; (2) to investigate correlations between these morphological differences and environmental**

conditions; (3) to examine fine-scale cranial variation within bottlenose dolphin operational taxonomic units (OTUs); and (4) to investigate allometric patterns within bottlenose dolphin populations. These objectives are well defined, coherent, and appropriately grounded in the gaps identified in the literature.

Review of Chapter 2

Chapter 2 is devoted to the methodology selected for this thesis. **Mrs. Dromby provides a clear and detailed description of the equipment and software used, which ensures the reproducibility of the analyses in future research. The chapter demonstrates that Mrs. Dromby carefully considered the advantages and limitations of various approaches before developing her own methodological protocol. The resulting protocol is based on the use of consumer-grade digital cameras—equipment that is both simple to operate and widely available—combined with open-source 3D modelling software. This combination makes the approach accessible to a broad range of researchers and institutions, while at the same time producing reliable and accurate results. Importantly, the protocol offers the possibility of creating precise three-dimensional skull models that can subsequently be analyzed for a variety of research purposes depending on the needs of individual investigators.**

The robustness of the method is demonstrated by its practical application in this thesis. Out of 314 odontocete skulls photographed, 296 models were successfully reconstructed, representing an impressive success rate and confirming the effectiveness of the developed workflow. **By providing a protocol that is both replicable and adaptable, Mrs. Dromby makes a valuable methodological contribution to the field, one that may facilitate future studies on cranial morphology across a wide range of species.**

Review of Chapter 3

Chapter 3 incorporates a peer-reviewed publication authored by Mrs. Dromby and co-authors (Morgane Dromby, Fernando Félix, Ben Haase, Paulo C. Simões-Lopes, Ana P. B. Costa, Aude Lalis, Celine Bens, Michela Podestà, Giuliano Doria, and Andre E. Moura, 2023: *Cranial variation between coastal and offshore bottlenose dolphins, Tursiops truncatus (Cetacea: Delphinidae) in Ecuador and the Mediterranean: a three-dimensional geometric morphometric study*, *Zoological Journal of the Linnean Society*, Volume 199, 83–96. https://doi.org/10.1093/zoolinnean/zlad022

This article presents a rigorous investigation into skull shape variation between coastal and offshore bottlenose dolphin populations using three-dimensional geometric morphometrics (3DGM). The material studied consisted of skulls from physically mature individuals housed in five institutions: one each in Ecuador, France, and Brazil, and two in Italy. These specimens originated from the southeast Pacific, Mediterranean Sea, northeast Atlantic, and southwest Atlantic. In total, 58 skulls were analyzed. The specimens were grouped into *a priori* geographic units, defined on the basis of previously documented behavioral, morphological, and genetic characteristics of the populations to which they belonged. Among the three *a priori* offshore groups representing distinct geographical regions, no significant cranial shape differences were detected; these were therefore combined into a single "offshore" group. By contrast, strong differentiation was evident between offshore dolphins and those from the inner estuary of Guayaquil, Ecuador, as well as from the coastal Mediterranean. Within this comparison, the greatest divergence was observed between the

Mediterranean and Guayaquil populations. Specifically, Mediterranean coastal dolphins exhibited a longer and slenderer skull, while the Guayaquil dolphins possessed a shorter, more robust cranial form.

The study further revealed that the main differences in skull morphology among the groups were concentrated in the shape of the rostrum, the interorbital concavity, and the temporal joint region. These structural differences are interpreted as potential functional adaptations, suggesting variation in feeding and acoustic behavior likely driven by prey size, foraging strategies, and communication patterns. Such findings highlight the influence of ecological factors in shaping morphological diversity and provide compelling evidence for the adaptive significance of cranial variation in bottlenose dolphins.

This peer-reviewed article underscores the value of GM in population-level studies of bottlenose dolphins, a species of particular interest given its cosmopolitan distribution. While bottlenose dolphins are widely distributed across nearly all of the world's seas (excluding polar regions), certain coastal populations display strong site fidelity and, in some cases, pronounced social structuring. **The study therefore contributes important new perspectives for researchers by demonstrating how the wealth of specimens preserved in global collections can be analyzed to yield valuable insights into cranial polymorphism within this species.**

It is regrettable, however, that Mrs. Dromby was unaware of the collection held at the University of Zagreb, Croatia, which could have significantly enhanced the coastal Mediterranean sample. This collection, assembled through the national stranding monitoring program active since 1990, houses approximately 250 bottlenose dolphin skeletons and represents a unique and rich resource for comparative studies. Moreover, a detailed craniometric study of these dolphins has already been published and made freely available to the scientific community (Đuras, M., D. Divac Brnić, T. Gomerčić, A. Galov, 2014: *Craniometry of bottlenose dolphins (Tursiops truncatus) from the Adriatic Sea. Veterinarski arhiv* 84: 649–666. https://arhiva.vetarhiv.vef.unizg.hr/papers/2014-84-6-8.pdf?ci=jmrhbriqbncjesesnuss

Review of Chapter 4

Building upon the promising findings presented in the peer-reviewed study discussed in Chapter 3, Mrs. Dromby expands her research to incorporate additional bottlenose dolphin skull collections, thereby conducting a large-scale, worldwide intraspecific investigation of cranial diversity using three-dimensional geometric morphometrics (3DGM). **This chapter represents an ambitious and methodologically sophisticated extension of her earlier work.**

In total, 234 three-dimensional skull models of physically mature bottlenose dolphins were analyzed. These specimens originated from a broad and taxonomically diverse sample encompassing six well-characterized coastal operational taxonomic units (OTUs), six offshore OTUs, and four additional, less thoroughly studied regional units. The results reveal distinct patterns of morphological variation. Offshore specimens exhibited relatively limited cranial shape diversity, whereas coastal units displayed a broader range of variation, differing not only from the offshore form but also from one another. Notably, the clearly defined coastal taxa *Tursiops aduncus* and *T. truncatus gephyreus* were readily distinguishable from the other coastal units, corroborating previous genetic, osteological, and morphological evidence supporting their distinctiveness.

A key outcome of the analysis is the observation that the offshore skull morphology corresponds to an "average" cranial form, which appears largely independent of geographic origin. This finding suggests a degree of morphological conservatism among offshore bottlenose dolphins, potentially reflecting more homogeneous ecological pressures in pelagic environments. **In contrast, several coastal units exhibited unique morphological traits likely shaped by local environmental conditions.** For example, specimens from the Western North Atlantic—recently described as *T. erebennus*—while morphologically distinct from other OTUs, displayed some degree of overlap with coastal populations from other parts of the Americas. Specimens from California and the Mediterranean clustered closely with nearby coastal units but also showed substantial overlap with the offshore group.

Some particularly interesting results emerged from African and Asian populations. Certain individuals from the coastal West African unit separated clearly from their group, while others overlapped with multiple OTUs, including the offshore group, suggesting possible gene flow or morphological convergence. Similarly, specimens from Japan and the North Sea overlapped both with each other and with the offshore OTU, despite being drawn from geographically distant regions. Such findings raise important questions about the drivers of morphological similarity across large spatial scales, which may include shared ecological niches or historical connectivity among populations.

While the results are compelling and represent a valuable contribution to the understanding of bottlenose dolphin cranial diversity, one methodological point remains unclear and merits further clarification. Specifically, the thesis does not indicate who was responsible for photographing the skulls housed in geographically distant collections. Was this work conducted personally by Mrs. Dromby during field visits, or did she rely on local collaborators to collect the photographic data following her protocol? This information is crucial for evaluating the broader applicability of the methodology developed in Chapter 2. If multiple operators were involved, successful reconstruction of high-quality 3D models would further validate the robustness and user-friendliness of the proposed protocol. Conversely, if all models were generated by the author herself, then the reproducibility of the method by external researchers remains to be fully demonstrated. Clarifying this point would therefore strengthen the methodological transparency and practical impact of the study.

Review of Chapter 5

In Chapter 5, Mrs. Dromby addresses allometric variation in the skull morphology of bottlenose dolphins from the Western North Atlantic (WNA) and demonstrates how three-dimensional geometric morphometrics (3DGM) can be successfully applied to investigate patterns of allometric diversification. The WNA is an especially suitable region for such a study, as it hosts multiple populations and stocks of bottlenose dolphins that exhibit marked spatial structuring despite the absence of physical barriers. In this context, the Marine Mammal Protection Act formally recognizes distinct stocks, treating them as separate management units for the purposes of conservation and protection. Previous studies have confirmed differences between these populations using a variety of methods, and a consistent dichotomy has emerged between offshore and coastal forms. Offshore dolphins, for instance, are distinguished by a unique hematological profile related to deep-diving behavior and higher parasitic loads. In contrast, coastal populations are characterized by smaller body size, fewer vertebrae, smaller skulls, narrower internal nares, and a shorter and narrower rostrum—morphological traits that are linked to differences in air exchange and vocalization. Furthermore, mitochondrial DNA analyses have shown that offshore and coastal dolphins are genetically distinct. Recent work has even led to the proposal of a new species, *Tursiops erebennus*, with five populations recognized between New Jersey and northern Florida.

Mrs. Dromby justifies her choice of the WNA bottlenose dolphins as a study subject by emphasizing their pronounced ecological differentiation, which she clearly outlines in the introductory section with reference to relevant literature. This choice also connects to a broader taxonomic debate. While the genus *Tursiops* was historically fragmented into numerous nominal species, decades of taxonomic revision reduced this diversity to *T. truncatus* and *T. aduncus*. The more recent recognition of *T. erebennus* indicates that this debate remains ongoing, making her study highly relevant to contemporary cetacean taxonomy.

The Materials and Methods section of Chapter 5 describes data collection, 3D modeling, landmarking, GM, and allometry analyses. For this purpose, 76 adult skulls of *Tursiops* spp. were photographed at the Smithsonian National Museum of Natural History in Washington, D.C. Adult status was determined by the presence of "fully fused" skull bones. **However, Mrs. Dromby does not provide a clear definition of what she means by "fully fused." It is well known that cranial bones can be fused while sutures remain visible and unossified, and the gradual ossification of these sutures introduces subtle but significant changes in skull morphology with age. For taxonomic purposes, therefore, a more rigorous criterion—such as the classification of suture ossification as described by Hoson et al. (2009) in the Florida manatee (HOSON, O., S. KAWADA, S. ODA (2009): Ossification patterns of cranial sutures in the Florida manatee (*Trichechus manatus latirostris*) (Sirenia, Trichechidae). Aquat Mamm 35, 72-81)—would be more appropriate. Without such clarification, it remains uncertain which skulls precisely were included in the study.**

For this analysis, only skulls originating from coastal populations were included, categorized into seven groups according to potential local population structures described in the literature. However, **the representation of these groups was uneven. Maryland (n=4), Georgia (n=6), and Florida (n=5), for example, were represented by very few specimens, while North Carolina (n=16) and Chesapeake Bay were more substantially represented. This uneven distribution introduces potential sampling biases that should be considered when interpreting the results. Additionally, as in earlier chapters, the thesis does not specify who performed the skull photography. Given the delicacy of the imaging process, even with a well-designed protocol, the accuracy of data collection depends heavily on the skill and consistency of the operator. Thus, the omission of this detail weakens the methodological transparency of the study.**

The Results section shows that offshore and coastal OTUs are clearly distinguished, and that offshore skulls are significantly different from all coastal populations considered collectively. This finding strongly supports previous work on WNA bottlenose dolphins. Moreover, subtle shape differentiation was detected among coastal populations, despite considerable overall overlap. **Mrs. Dromby acknowledges the challenges posed by sample size, noting that the relatively large samples from Chesapeake Bay and North Carolina may have captured more morphological variation, while smaller samples from other locations may underrepresent within-population diversity, potentially exaggerating differences. She wisely cautions that statements such as "regional associations with shape patterns along this coastline" should be interpreted with care,**

as the observed differences may reflect sampling artifacts, sub-population structure, or even sex-related variation.

In the Discussion, Mrs. Dromby offers several explanations for the observed morphological patterns. First, skull shape differences align with genetic evidence for multiple stocks in the WNA, thereby reinforcing previous findings. Second, she highlights the role of ecological and behavioral factors, including strong site fidelity, stable social structures, and limited dispersal, as potential drivers of differentiation among coastal groups. Furthermore, her analyses support earlier genetic evidence for a demographically independent bottlenose dolphin population in the Gulf of Mexico and Florida.

Overall, Mrs. Dromby presents a logical and well-argued interpretation of her findings, offering valuable insights and suggesting directions for future research. Nevertheless, the limited sample sizes for certain populations necessitate caution in drawing strong taxonomic or population-level conclusions. In my view, the chapter's greatest contribution lies in demonstrating the potential of 3DGM as a powerful tool in cetacean taxonomy, rather than in resolving the fine-scale cranial diversity of WNA populations. To move beyond tentative conclusions, broader sampling will be essential. As it stands, the interpretations in this chapter, while stimulating, remain somewhat ambitious given the dataset.

Review of Chapter 6

In the main discussion of Chapter 6, Mrs. Dromby explores potential drivers of cranial diversification within the genus *Tursiops*. She begins by analyzing factors contributing to diversification across the genus, positing that environmental pressures—particularly prey availability—play a central role. Consequently, she concludes that cranial shape modifications may reflect adaptations to population-specific feeding and vocalization strategies. This interpretation aligns with prior studies on Tursiops feeding ecology, which demonstrate that certain populations develop specialized foraging behaviors tailored to the prey available within their habitat.

For offshore populations, which exhibit relatively homogenous cranial morphology, the presence of widened nares is correlated with deep-water habitats. Mrs. Dromby further notes that while stabilizing selection and reduced genetic drift may constrain morphological variation in uniform offshore environments, strong genetic drift is likely to drive diversification in more heterogeneous coastal habitats. She emphasizes the need for further research to fully characterize global patterns of population structure among coastal and offshore bottlenose dolphins. In particular, fine-scale analyses of isolated coastal populations with limited gene flow are essential to understand the relative contributions of environmental adaptation versus stochastic processes such as founder effects. Mrs. Dromby's overarching conclusion is that observed cranial shape variation may result from a combination of genetic drift and founder events, reflecting the colonization and recolonization of coastal niches during and after the Last Glacial Maximum.

The second, third, and fourth sections of the discussion focus on mechanisms driving diversification among coastal populations. Mrs. Dromby observes that the morphological traits detected in her study—such as nasal cavity reduction, rostrum elongation, and increased braincase size—are consistent with early stages of cetacean skull telescoping, a hallmark of adaptation to fully aquatic life. Modern cranial morphology further illustrates the functional link between shape and ecology, particularly in relation to feeding strategies. Communication-related traits are also shown to

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vary among populations, including differences in premaxillary concavity and the presence of a rostral bump, which may reflect variation in melon size and shape. Smaller or isolated populations may rely more heavily on vocal cues for social cohesion and coordinated foraging, which could select for traits optimized for sound production. However, it remains challenging to distinguish whether these morphological changes result directly from natural selection in response to local ecological pressures or from stochastic genetic processes such as drift.

In the sixth section, Mrs. Dromby considers the taxonomic implications of her findings, noting that the significant cranial differences observed between multiple operational taxonomic units may provide useful information for clarifying the taxonomy of the genus Tursiops. While these insights are valuable, caution is warranted given the relatively small sample sizes for certain OTUs, which limits the strength of taxonomic inferences.

Conclusion of the Review

Overall, Mrs. Dromby's thesis demonstrates the successful application of geometric morphometrics as a powerful tool for analyzing shape variation in marine mammals, offering insights into evolutionary relationships, ecological adaptations, and developmental processes. This knowledge forms an essential component of basic biological understanding and provides a foundation for the conservation and protection of these vulnerable species. The study clearly shows that 3DGM enables the investigation of complex cranial structures and subtle morphological variations that are not accessible through traditional morphometric approaches. In particular, the application of GM to intraspecific research in the widely distributed genus Tursiops highlights its value in contextualizing morphological diversity within a range of environmental conditions.

The study of endangered species, particularly marine mammals, is inherently challenging due to difficulties in locating specimens, decomposition of stranded carcasses, and sporadic distribution of samples across museum collections. Many specimens lack complete biological data or precise stranding records. Despite these obstacles, Mrs. Dromby assembled an impressive dataset of Tursiops skulls and demonstrated advanced proficiency in software and 3D cranial morphometry.

Several questions remain open:

1. **Who performed the photographic work for distant collections—Mrs. Dromby herself, or local staff following her protocols?**
2. **How was sexual dimorphism accounted for within operational units?**
3. **Some sample groups cover extensive geographic regions—how does this affect the interpretation of morphological variation?**
4. **How reliable are the museum-recorded stranding locations?**
5. **It is regrettable that the Adriatic *Tursiops* collection at the University of Zagreb was not included, as it contains over 250 skeletons with complete biological data from a long-term monitoring program since 1990.**

Despite these remaining questions, the thesis represents a substantial contribution to understanding cranial morphological diversity in the most widespread cetacean genus. It

provides a foundation for future research into evolutionary processes shaping this diversity and highlights the relevance of 3DGM for taxonomy, ecology, and conservation. Tursiops populations are both resident and highly migratory, and studies like Mrs. Dromby's are critical for informing conservation strategies that preserve local population structure. Systematic monitoring of morphology and morphometry can thus guide timely conservation actions.

In conclusion, I grade this thesis positively and recommend that Mrs. Dromby proceed to the defense, while noting that discussion of the points raised above would further enhance the robustness and interpretative clarity of the work.

Furthermore, I declare that the doctoral dissertation submitted for review complies with the requirements for doctoral dissertations, as set forth in the regulation in Article 187, paragraphs 1 and 2, of the Higher Education and Science Act dated 20 July 2018 (Journal of Laws of 2018, item 1668, as amended).

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