Mammal Research Institute
Polish Academy of Sciences
Białowieża

ul. Stoczek 1, 17-230 Białowieża, Poland
tel. 48 (85) 682 77 50, fax 48 (85) 682 77 52
NIP 543-12-25-396
www.ibs.bialowieza.pl

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Prof. dr hab. Krzysztof Schmidt

Head of the Population Ecology Unit MRI PAS

REVIEW

of doctoral dissertation by Morgane Dromby, MSc:

Adaptive skull shape changes in bottlenose dolphins (Tursiops spp.): inference from 3D morphological analyses

The subject of this dissertation is a profound analysis of a worldwide skull shape variation in a bottlenose dolphin with an attempt of understanding if ecological and environmental diversity may explain the differentiation among populations of these mammals. The leading topic of the PhD student's research is the differentiation of species into two ecotypes – coastal and offshore – of animals that, although they belong to the same species, live in clear separation and are potentially adapted to completely different environments. Therefore, the major question tackled in this dissertation refers to the evolutionary processes and conditions that may affect the shape of the skull through morphological and functional adaptations to different foods and local habitat characteristics. This topic is not new. In recent decades, a number of studies have been conducted (also cited by Morgane Dromby in her dissertation) that have identified the existence of the two dolphin ecotypes through a variety of methodological approaches, using both traditional linear morphological measurements and geometric morphometrics, as well as molecular methods. The association of morphological differentiation of dolphins' skulls with its functional roles and living in different environments have also been previously proposed by various authors. However, there are several aspects covered by the research of Ms. Dromby, which bring an additional, broader and more detailed perspective on the issue of bottlenose dolphin diversity. I think that, firstly, it is thanks to the use of a very detailed approach to studying the shape of the skull using 3D geometric morphometrics and automatic determination of very many measurement points that brings this new quality to previously conducted and published studies. Next, combining the

3DGM methods with environmental data analyses may allow for a better understanding of the ecological and evolutionary basis of the formation of variability in this genus. A new approach in her study is also the use of allometric analyses, which have provided deeper insights into the variation in dolphin skull shape yet within the coastal ecotype.

The work is organized into six chapters, three of which are those presenting the original research results. The remaining three chapters include an introduction, a general discussion, and an additional section with the methodological details describing the point by point procedure of taking skull images and preparing 3D models. From among the three chapters containing the research results one is the multi-author paper already published in Zoological Journal of the Linnean Society, where Morgane Dromby is the first author, and the two other chapters are in the form of manuscripts prepared for publication in scientific journals.

In **the first of them (chapter #3)**, Ms. Dromby, together with co-authors, analysed the shape variation in bottlenose dolphin skulls between two coastal (Gulf of Guayaquil, and the Mediterranean Sea) and three offshore populations. Unfortunately, the material studied was not abundant with a total of only 58 skulls, so that particular populations were represented by very few specimens (e.g. those from North and South Atlantic N=3 and 6, respectively). This low sample size is probably farther diminished if we consider a potential effect of sexual dimorphism (which occurs in the bottlenose dolphins and the effect of which is considered in the chapter 5). However, the effect of sex was not accounted for (and not acknowledged) in this study and could potentially introduce some bias into the obtained patterns. Realizing these limitations, the researchers approached the analysis of these materials with great meticulousness, using several different methods both to obtain morphological data and analysing them statistically. First, they created 3D digital models of each skull based on hundreds of images obtained by a photogrammetric technique and established the measurement points (landmarks) both manually and automatically, and also measured the numbers of tooth alveoli. They then applied a variety of statistical tests, including linear discriminant analysis (LDA) and hierarchical cluster analysis (HCA), to yield a set of comparative results aimed at increasing the reliability of the analyses and their interpretation. Following this, the authors obtained interesting results conforming to previous morphological studies of this species. They found that both coastal populations are significantly differentiated from the offshore ones. But, what was specifically new, while the two coastal populations clearly differed from each other, the three offshore ones were very similar, despite that they originated from distant locations (even different oceans). The detailed

analysis of the landmarks' vector displacement graphs allowed to translate the shape differentiation into possible functional meaning of the changes, which suggest its relevance to feeding and communication. It is however likely that the low sample size could have contributed to some discrepancies in results of different tests. While LDA assigned all individuals to predefined populations based on both manual and automatic landmarking, the HCA was not able to assign all specimens correctly and yielded different results for the two landmarking methods. This, unfortunately, was not specifically addressed in the discussion.

The **second chapter containing research results (chapter #4)** presents a significant extension of the research conducted on the variability of dolphin skull shapes shown in the chapter 3 with similar methodological approach. This study was, however, based on much larger material covering 11 populations distributed widely across the globe (in total 234 specimens) and each population was represented by a reasonable sample size (minimum 11). Another major advantage of this study is that it includes an attempt to comprehensively analyse the relationship between dolphin skull shape and many environmental variables. Therefore, it has a good basis for drawing stronger conclusions about the functional significance of the skull shape variability in the genus Tursiops.

The major achievement of this research (presented in chapter 4) is that a consistent pattern of the bottlenose dolphin skull shape variation was found across all studied populations with clear distinctness of all the coastal dolphins from those living offshore. A significant addition to the conclusions of previous chapter seems to be the finding that the degree of differentiation between the units varied, which means that not all coastal units equally clearly differed from the offshore ones. At this point, however, it is worth asking whether this result may be a consequence of the fact that there are no clear criteria for classifying dolphin specimens into offshore or coastal populations (as Ms. Dromby herself admits in the discussion). Unfortunately, how the specimens were classified into one population or the other in this particular study was also not disclosed. I think, this problem can farther affect the results of both classification analysis methods – the Random Forest (RF) and the Hierarchical Cluster Analysis (HCA), which in fact provided quite dissimilar results, specifically between the automatic (Surface-Semi Landmarking, SSL) and manual (Homologous Landmarking, HL) determination of measurement points. Please, note the offshore population was found to have the highest diversity of misclassifications, which may only confirm that difficulties in correctly categorizing skulls into one of the two groups may seriously affect the results. While Ms. Dromby briefly noted in the discussion the possible influence in relation to the two

populations (Japan and North Sea), this issue requires much more attention. Interestingly, although she devoted an entire section of results to comparing the classification with use of both landmarking methods, in the discussion she did not address the fact that in both tests (RF and HCA) they gave completely different results. I think, this is something very important that should be considered before possible submission of this manuscript for publication.

I appreciate very much the attempt of analysing the influence of environmental conditions on the skull shape variability. To my knowledge it is perhaps the first such a study trying to match multiple environmental variables to dolphin skull shape to investigate the potential relationship between habitat variation and the adaptive selection and evolution of feeding and communication organs in dolphins. Ms. Dromby found that while coastal populations were associated with such variables as silicate, temperature, chlorophyll and dissolved O2, the offshore ones correlated with bathymetry, mixed layer depth, slope, and salinity. Since the former environmental features can be considered as indicators of nutrient concentration and primary productivity, and are also subject to high variability in the coastal zone, they may indeed create diverse ecological conditions conducive to the eventual differentiation of skull shapes found in coastal dolphins. In contrast, features of the offshore environment, with which dolphins classified as offshores were associated, can be indicative of low nutrient abundance, deep water and wide temperature gradients, and may appear more stable over large oceanic areas. As the PhD candidate suggested, this is likely to exert uniform selective pressure stabilizing the morphological diversity in animals living in these harsh conditions of the open oceans.

Although, as I mentioned, I greatly appreciate this achievement, I must also add a critical comment. I mean that the array of environmental variables considered in the analyses by Ms. Dromby have a very general character and she did not explain why these particular variables were chosen. In result, while the analyses proved some correlative associations of various environmental characters with different populations, it is unlikely to associate these relationships with adaptive processes and resulting physical shape differences among dolphins' skulls. Fortunately, Ms. Dromby seems to fully understand this shortcoming, as she expressed her doubt about possibility of "directly associating skull shape to biological processes driven by salinity range" in case of the two populations "Gephyreus" and "Aduncus". I believe, this statement has a more universal application to the whole material. While the environmental variables considered are indeed reflecting the diversity of living conditions, including the general productivity of marine ecosystems, by no means they can be

directly translated into the mechanisms that may drive the evolution of skulls, muscles and ligaments. In fact, even none of the variables analysed was a direct index of food abundance. Although food abundance alone should not be considered as a factor directly capable of explaining the shape of specific cranial structures, I would expect that at least, instead of indirect indicators used (such as chlorophyll), the researcher should examine more direct measures, such as those provided by Hatton et al. (2021) (The global ocean size spectrum from bacteria to whales. Sci. Adv.7,eabh3732). There one can find a large database of biomass estimates of various organisms worldwide. Moreover, there is perhaps ample literature on the bottlenose dolphins diet across various worldwide populations and habitats, which could provide deeper insight into the relationship between the actual type of food consumed by dolphins in different locations and their skull morphology. Interestingly, Ms. Dromby provided a short overview of selected dietary studies (p. 128), based on which she concluded that: “distinct skull shapes are likely influenced by the diversity in diet and foraging strategies among coastal operational units”. It seems to suggest that a simple literature search provided more insightful conclusions regarding the direct mechanism of shaping skull shape than sophisticated analyses, which resulted in only indirect clues.

And last but not least, regarding the chapter 4, I must draw attention to the great similarity between the research topic, results and conclusions presented therein and the work of Oxford-Smith et al. published in 2024 in the Journal od Zoology. I admit that both this chapter and the published paper are also remarkably different, as they studied materials from different populations and used somewhat different methodological approaches (Oxford-Smith et al. used only manual landmarking and linear measurements). In addition, as I discussed above, Ms. Dromby's work contains very valuable analysis of the relationship between skull shape and environmental factors, which is not found in Oxford-Smith et al. paper. However, what I want to raise here is that major conclusions from research presented in the chapter 4 are almost identical as those already published (including differentiation among populations and possible functional role of skull shape changes). Therefore, it appears that the application of detailed measurements using automatic landmarking and sophisticated statistics would not, in practice, result in a significant improvement in the knowledge of dolphin skull shape variability. Although the work by Oxford-Smith et al. has been cited by Ms. Dromby, I strongly suggest that in case of submission of this manuscript for potential publication, this issue requires more attention and the manuscript should be adjusted accordingly.

In **the third substantive chapter (containing research results) (chapter #5)** the PhD candidate focused on one geographical area of bottlenose dolphin distribution – the West North Atlantic (WNA), to look for the signs of variation in skull shape patterns within a single population and possible ecological drivers behind their differentiation. An interesting and novel approach of this study is that along with "traditional" 3DGM skull shape analyses she applied allometric analysis that takes into account changes in skull proportions along with its size and potential sex differences. On the other hand, the subject of these investigations is a bit challenging, because the WNA coastal population harbors bottlenose dolphins, which have recently been recognized as a separate species – *Tursiops erebennus*. As such, the level of expected variability can be low, considering the fact of its very limited geographic distribution. Nevertheless, the new value of this research findings is that despite of this relatively small range, both the shape and the allometric analyses revealed some variability existing among these costal dolphins. Moreover, allometric analysis exposed different pattern of population differentiation as compared to that based on shape only. It appeared that while the offshore dolphins differ from the coastal ones mainly by skull shape, the populations within the coastal area (actually all belonging to *T. erebennus*) are additionally differentiated by size-shape relationships. From populations represented by largest sample size at least one (North Carolina) showed different allometric trajectory as compared to dolphins from Cheasapeake and Delaware Bays. This, indeed may possibly signify some local variation in response to different ecological conditions. In addition, Ms. Dromby found that some of this variability can be due to variation in allometric processes related to sexual dimorphism, because the allometric trajectories were clearly different for males and females in some populations. Altogether, these results along with patterns of skull landmarks displacement among populations (showing largest changes in the rostrum and squamosal area), allowed her to speculate about possible link between this morphological differentiation and the foraging habits of dolphins.

Although the general idea of this study is very interesting, I am again afraid (as in case of the chapter 3), that the sample size (n= 76) is small enough to significantly bias the results. Three (out of 7) coastal populations (Maryland, Florida and Georgia) were represented by only 4, 5 and 6 specimens, respectively. Farther, due to sexual dimorphism, which is one of the topics of this chapter, this effect can be yet stronger. The sex is either unequally represented, as in case of Maryland sample consisting of 3 males and just one female or largely unknown, as in Mexico Gulf. In fact, the Mexico Gulf dolphins were found by both analyses as one of the

most distinct populations. While the distinction of this population seems reasonable following its partial isolation from the remaining populations of *T. erebennus* by the Florida peninsula (and thus possibly limited gene flow) and relatively high sample size (n=9), in case of Maryland and Florida, it is questionable. Remarkably, following the results of the allometric analyses, Ms. Dromby noticed that Maryland and Florida populations are farther subdivided into two or three clusters, suggesting that "*morphological diversity within these populations (is) potentially driven by size differences*". Considering the n=4 or 5 specimens constituting these groups, this conclusion is really ungrounded. I am aware that just in the next sentence she herself noticed that a small sample size could have influenced these results, but this issue should be more thoroughly addressed in the discussion section. Therefore, I would suggest that, in case of preparing the final version of this manuscript for publication, the results should be presented and interpreted with greater caution. In particular, statements such as the one concluding the results section ("*Despite these limitations, the results presented here are informative and useful within the context of the subtle intraspecific variations explored in this study, and therefore they can be considered as biologically meaningful*") should not be forcibly made. Such formulations do not really help convince reviewers about the credibility of the obtained results.

The difference in the patterns of morphological differentiation among populations based on the shape and allometric analyses on the one hand seem to elucidate additional (ecological) background for this differentiation (next to the influence of gene flow), but on the other hand the two results are a bit confusing. This is mainly because the allometric analysis revealed the intra-population distinctiveness, which in fact (as I mentioned above) was largely generated by very small sample size and not by real differentiation.

The remaining three **chapters, which do not contain research results** include an introduction (**chapter #1**), a description of the workflow (methodology for digitizing skulls to obtain 3D models) as **chapter 2**, and a general discussion (**chapter 6**). Of these three chapters, I would like to spend a few words on **Chapter 2**. Here, in 16 pages, the PhD student describes in great detail the intricacies of creating a 3D model of a dolphin skull. This description is done in a very professional manner showing point by point the whole procedure of taking pictures, starting with setting up the objects on display, lighting conditions, selecting parameters, and then combining them into one three-dimensional model using specialized software. The intention for having this protocol is to ensure replicability and consistency across different sampling environments in case if similar studies should be repeated.

However, I wonder how useful the information in this chapter is, and I am not quite sure how to understand its role in this dissertation. And, above all, I ask myself whether this chapter would have a chance to be published in some scientific journal, as a methodological article showing an innovative approach to the analysis of research materials (or in other words, can it be treated as a separate, original author's work). As such, it would have to present a self-designed original method, or a modification of an existing technique. On the one hand, this chapter could meet such criteria, since it shows a certain compilation of various techniques and computer programs tested by Ms. Dromby on specific research material and combined into one coherent protocol. On the other hand, however, I have the conviction that in essence it simply presents an elaborate instruction manual for using the particular devices or programs compiled based on the user's experience. Moreover, it includes basic information which can be easily available elsewhere. For instance, much space is devoted to explain the difference between JPG and RAW formats of images or the rules for taking photos by adjusting the brightness, contrast or sharpness of the image. While the intention of this chapter was to provide a standard protocol for making 3D skull models, I believe that creating such a universal protocol for use in all conditions may be difficult. The author herself emphasized in Chapter 3 that camera settings depend on the lighting conditions in a given room. Therefore, I believe that this protocol does not necessarily is to be exactly replicated by other studies as the settings can always vary depending on museum conditions and possibly the size of the objects.

The **chapter 6** is a very broad discussion presenting a lot of interesting knowledge. However, it mostly repeats interpretation, discussion and conclusions provided in other, substantive chapters. As a result, it is unclear why these findings are being presented and discussed yet again, especially since many of the examples given in support of them do not directly refer to dolphins, and include taxa from fish to various mammals, including those as diverse as bats and large predators. This main discussion seems to be too broad and too verbose, giving the impression that it is somewhat forced, where the same information is processed and transformed in different ways in a bit disorderly way, so that it eventually leads to rather trivial conclusions in places, such as this one: "*This suggests that the coastal unit relies on nearshore resources and shallow waters, whereas the Offshore unit exploits pelagic prey and navigates more dynamic and open ocean environments*" or this: "*Within the Erebennus operational unit, bottlenose dolphins from different locations exhibit variations in both skull shape and allometric pattern, suggesting a considerable role for allometry in driving skull*

shape differences over time." (p. 187). Instead of such a long and very general discussion, I would have expected a concise summary and conclusions of the most important achievements of the entire doctoral dissertation, which is missing here. For instance, I miss a clear discussion of the findings regarding the effect of environmental factors on dolphin skull shape, which seems to be most innovative contribution of this dissertation to science. Unfortunately, although there are sections in the chapter that mention the role of the environment, they do not directly refer to the results obtained, but are very general considerations about the impact of the environment on the distribution of ecological niches supported by very distant comparisons (like giant pandas of parrotfish). Moreover, in the final paragraph, summarizing, it would seem, the entire thesis, but nevertheless referring to the results concerning only one of the studied populations (Southeast Pacific, SEP), Ms. Dromby draws the rather obvious conclusion that "*divergence processes in SEP may still be ongoing*".

Despite my criticism expressed above, I want to clearly emphasize that the entire dissertation made an overall **very positive impression** on me. The way in which she prepared and presented her dissertation indicates that Ms. Dromby is the type of very solid scientist. She carried out the task of preparing three scientific articles with extraordinary precision. The presentation of the research problem and its subsequent discussion by the doctoral student testifies to her deep and extensive knowledge, which is not limited to a good acquaintance with the narrow topic of dolphin skull shape variation, but goes far beyond this taxon, demonstrating her deep understanding of mechanisms of evolution based on morphological, ecological and genetic variation of animals. A very important asset of the doctoral student is also her perfect mastery of the research methodology involving a combination of photogrammetry and three-dimensional geometric morphometry. This gives her a powerful and versatile tool useful for scientific research, which she can conduct on any natural objects in the topics of ecology and evolution in future. Ms. Dromby also demonstrated in her thesis that she is also very knowledgeable about the statistical tests, which she uses with R, a software that is now widely recommended in the scientific community worldwide. All of this is something specifically required from PhD candidates based on the article 187.1 of the Law on Higher Education and Science (Law), so that I am sure Ms. Dromby has fulfilled this completely without reservation.

Following the art. 187.2 of the Law, the subject of a doctoral dissertation should be an original solution to a scientific problem. Also in this respect there is absolutely no doubt that Ms. Morgane Dromby's doctoral dissertation meets this criterion. Although, as I mentioned

above, the topic of skull shape variability in bottlenose dolphin populations is not new, Ms. Dromby has developed it to include the use of additional methods (automated landmarking, allometry), as well as an attempt to gain a broader understanding of the mechanisms determining this variability by including environmental variables in the analyses. All this testifies to an original approach to the scientific problem in search of answers to extended research questions, in this case concerning the mechanisms shaping intraspecific variability, environmental adaptations, divergence and evolution of dolphins.

In view of the above, I evaluate the dissertation of Ms. Morgane Dromby **positively** and concurrently state that the dissertation meets all the requirements of the Law of July 20, 2018 - Law on Higher Education and Science. Therefore, I request the Council of the Discipline of Biological Sciences of the University of Gdańsk to admit Ms. Morgane Dromby to the further stages of the doctoral proceedings in order to grant her a doctoral degree in the field of exact and natural sciences in the discipline of biological sciences.

Prof. dr hab. Krzysztof Schmidt