Marine Mammal Society 2023

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Project Information

Title Deeping into Delphinidae ear: insight into anatomy and phylogeny from 3D geometric

morphometric data

Location Puerto Madryn, Chubut Argentine

Applicant Information

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x[Student		Early Career		Experienced Investigator (> 5 y post degree or	equivalent)
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Biography

Introduce yourself. I am a biology PhD student at National University of Patagonia San Juan Bosco (UNPSJB), in Puerto Madryn Chubut Argentina. I am also the mother of a 7-year-old girl and a secondary school teacher. During my development as a student, I worked in the laboratory of marine mammals of the CESIMAR CCT CENPAT CONICET, developing activities such as studies of abundance of marine mammals through aerial, terrestrial and nautical censuses. Anatomical dissections and osteological conditioning of dead stranded specimens for their incorporation into the CESIMAR osteological collection. In my degree thesis, I studied the abundance and population structure of sea lions *Otaria flavescens* and *Arctocephalus australis* in Fuegian Archipelago, Patagonia Argentina. I have a scholarship from CONICET and this is my third year. As part of my PhD thesis, I have focused on the anatomical and phylogenetic study of the tympanic-periotic complex of Delphinidae, to interpret the morphological drivers of the ear region that promotes the success of one of the most diverse odontocetes group today.

Overview

What do you want to do? Cetaceans are currently the dominant group of aquatic mammals, both in terms of taxonomic and ecological diversity, with approximately 90 named species (1). The pattern of morphological and ecological diversity observed today is the result of a series of adaptive radiations that occurred over 50 million years, the moment in which the group originated (2). The second radiation (late Eocene-early Oligocene) marked the origin and diversification of modern

cetaceans or Neoceti (2) that include two clades: the Mysticeti or baleen whales and the Odontoceti or toothed cetaceans. In particular, the odontocetes had a rapid radiation in the early Oligocene, which was promoted by the acquisition of key innovations (3) linked to the acquisition of the echolocation system (emission and reception of high-frequency sounds for the location of prey and topographical characteristics of the environment). Odontoceti are structurally very disparate, in terms of their body size, morphology (the shape, size and composition of the feeding apparatus and teeth, the anatomy of the facial region, different specializations in the acoustic system and in the air sinus system) and feeding strategies (predators, suckers, or a combination of both). This disparity suggests that specialization occurred in many directions, making it difficult to interpret how this structural diversity originated and flourished. The Delphinidae family was originate in 11-12 Ma (Middle-Late Miocene) and they experienced explosive diversification. This family comprises more than 17 genera that can be divided into a 37 species. Many aspects of the phylogeny and evolution history remain unresolved, in particularly, exits a marked disparity between phylogenetic hypotheses based on morphological and molecular dates. On the other hand, the molecular markers (nuclear and mitochondrial) used in phylogenetic analysis also do not coincide in the division of the subfamilies and in the relationships of these with other species of dolphins. There are studies integrating multiple genetic databases in superarrays (4), which can resolve many relationships between basal taxa. However, many early diversification events and relationships within subfamilies (Delphininae, Globicephalinae, and Lissodelphininae) remain conflicting. We considered very important incorporated and create new data set and analysis, to review existing data and resolved conflictive relationships. Hearing was one of the most modified sensory systems in the evolution of cetaceans, as a result of the physical constraints imposed by the aquatic environment. In Odontoceti the ear inner is locked inside the tympanic-periotic bones, and this structure is acoustically isolated from the skull, held in place by ligamentous attachments and surrounded by a complex of air sinuses that aid in acoustic isolation. The tympanic-periotic complex presents a morphological disparity between the different groups and contains a high potential in taxonomical, phylogenetic and morphological studies. Numerical methods such as geometric morphometry (5), propose to study the changes in size and shape from the displacement in the plane (2D) or in space (3D) of a set of morphometric points or landmarks. This type of analysis makes it possible to quantify structural changes in shape, which has had multiple applications in the field of functional morphology, comparative anatomy, ontogenetic studies, as well as taxonomic studies, with several examples of applications in cetaceans (6). The use of landmarks as a source of characters for phylogenetic studies is a relatively recent field (7), but with very good results. In this context, the development of this methodology, unprecedented in the taxonomic study of cetaceans, is a completely novel tool with great potential to expand the morphological dataset used in phylogenetic analysis. Likewise, the detailed morphogeometric analysis of the auditory region (both bony and soft structures) may provide a better description of the morphological variability present in these structures and therefore improve the performance of this data source in the analysis of the phylogenetic relationships of this group. This may ultimately contribute to resolving the observed inconsistencies between morphological and molecular hypotheses of the phylogenetic relationships of Delphinidae.

As part of my PhD thesis and the general objective of is to achieve a deeper understanding of the evolutionary history of dolphins, through an exhaustive anatomical and phylogenetic study of the tympanic-periotic complex, in order to interpret the patterns of diversification and the morphological specializations that characterize the most diverse group of odontocetes today. The specific objectives are visit the collections of the three most important museums of Argentine. Photograph the ears of specimens that have little or no representation in the CESIMAR collection (198 complete skulls of 12 species of dolphins) and carry out the 3D reconstruction, to then carry out the morphometric analysis, and finally, generate a new database of morphological data of a highly taxonomically informative region such as the auditory region of Delphinidae.

Explain the background, rationale, and purpose of the study. Summarize current knowledge of the topic, identify knowledge gaps, and state the specific research question or hypothesis you will address. You may include essential numbered references [1]. 700 words max.

Approach

How will you do it? The methodology proposed is based on **three** main aspects:

- 1. Description and study specimens: The proposed collection is complete the sample of underrepresented species in the study area (coastal area of Argentine Patagonia, between the mouth of the Rio Negro and the south of Santa Cruz), the following will be visited: the Acantushun Museum (Ushuaia), the Bernardino Rivadavia Museum of Natural Sciences and the museum of La Plata (both in Buenos Aires). The analysis of the variation in the shape and size of the tympanic-periotic complex, in order to expand the morphological dataset for phylogenetic analyses. Each tympanic-periotic will be digitized using the technique of Photogrammetry.
- 2. Phylogenetic analysis: First, we will explore the phylogenetic signal in the tympanic-periotic characters of delphinids through the implementation of the Picante package (function: Phylosignal) in the R program (8). Phylogenetic studies will then be carried out following the methodology of phylogenetic systematics or clades. The resulting matrix will be analyzed using parsimony using parsimony algorithms in the free program TNT.
- 3. Morpho-functional and environmental: To determine whether there is a relationship between the morphology of the tympanic-periotic complex of the Delphinidae with the type of environment, a multivariate canonical analysis of variance will be performed in R (59) programs, using the environment occupied by each species, will be carried out.

Briefly explain the methods you will use to collect, analyze, and share data. You may include essential numbered references [1]. 700 words max.

Significance

Why is it important? Nowadays toothed whales inhabit the entire world's oceans and seas, as well as occupying a variety of habitats (coastal and pelagic marine, estuarine, and freshwater). Its wide distribution and the obstacles of collecting stranded specimens on the coasts make it tricky to access comprehensive taxonomic samples. For that reason, one of the main challenges when working with this group of marine mammals is access to the bone material in the collections.

Large-scale 3D morphometric analysis (i.e. Coombs et al 2022(10)) has been demonstrated the potential of this methodology to address phylogenetical and morpho-functional questions in cetacean evolution. In this sense, the information collected during my visit to the museums and the specimens that are included the cetacean collection from my institution (CESIMAR-CENPAT), which has spent more than 30 years of effort collecting a variety of species, will allow us to construct a broad morphological database of a highly informative region of delphinid anatomy as the tympanic-periotic. This will us to face the goals presented in this project but also has the potential to provide the bases for future analysis (i.e. including fossil specimens).

The results so far of the 3D reconstructions of the periotic tympanic complex of the specimens that are part of the CESIMAR- CONICET collection have been successful, allowing me to

advance with the setting of landmarks in each model, obtaining preliminary results that will soon be analyzed. That is why I consider it relevant to have a more comprehensive sample of the Delphinidae family. Besides, the construction of a 3D model via photogrammetry of specimens will make it accessible to any researcher in the future, being an important reward for the host institution.

Explain the anticipated outcomes of your proposed study and their significance to our understanding of the biology and/or conservation of marine mammals. You may include essential numbered references [1]. 400 words max.

Timeline

What is your timeline? This project is to be completed during 2022-2023, according to the following schedule:

- -October 2022: visit museum Bernardino Rivadavia and museum of La Plata- Buenos Aires (1 month, taking photographs for 3D reconstructions)
- -November: 2022: visit museum Acantushun –Tierra del Fuego (1 month, taking photographs for 3D reconstructions)
- -January- February 2023: Data analyzing, reconstruction 3D models
- -March- June 2023: Papers writing and submission

Give a brief timeline of expected research milestones. Funds should be used within 12 months of the award. A short summary report will be requested at the end of the year. An extension may be requested and granted at that time if needed. 200 words max.

Expenses

What support is needed and how will it be used? Society for Marine Mammalogy (required grant):

Trip to Buenos Aires (Museo de Ciencias Naturales Bernardino Rivadavia and Museo de La Plata)

-Roundtrip airfare: US\$ 400 (Puerto Madryn- Buenos Aires- Puerto Madryn)

-Food and daily rate (30 days).......US\$150

Trip to Ushuaia (Museo Acantushun)

- -Roundtrip airfare: US\$ 500 (Puerto Madryn- Ushuaia- Puerto Madryn)
- -Food and daily rate: (30 days)......US\$200

Provide a brief project budget and justify the expenses (breakdown by category and explain requested costs within each category). A maximum of 25% of the budget can be requested as a stipend. As these awards are modest, please describe other available funding or explain how the project can be completed with available resources. Grants are awarded to individuals, therefore no grant fees or overhead should be included. Budget not to exceed US \$2000.

Preparation

I have a degree in biological sciences, for more than 10 years I have specialized in the study of marine mammals, within the marine mammal laboratory of CESIMAR CCT CENPAT CONICET Argentine. For more than 30 years, the laboratory has been dedicated to the study and collection of marine mammals along the entire Patagonian coast, generating an extremely valuable osteological collection for CESIMAR and external researchers. For this particular study, there are already 198 complete skulls (with tympanic-periotic complexes) of 12 species of delphinids deposited in this collection. I have taken courses on morphogeometry, 3D reconstructions and their analysis with R software. In 2019 I presented, with my directors Dra Monica Buono and Dra Ma Florencia Grandi, at the XXXII Argentine Mammalogy Conference, showing the scope of the technique 3D reconstruction by Photogrammetry in different organisms through 2D images.

Tell us about your preparation or qualification for this study. If working with a mentor and/or team, briefly describe. 200 words max.

Animal Welfare Considerations

What are the legislative requirements for this study in this region and have the necessary permits been applied for or received?

Are there additional animal welfare requirements and does this study have the necessary approvals applied for or received?

If applicable, does this study comply with the <u>Guidelines of Treatment of Marine Mammals in Field</u>
Research? (yes/no) YES

Societal and Research Ethics Considerations

The Society for Marine Mammalogy recognizes the importance of advancing diversity, equity, and inclusion (DEI), and its essential role in generating novel research and conservation practices that truly reflect local needs.

Does this study comply with the Ethical Standards of the Society for Marine Mammalogy? (yes/no)

I agree with the Ethical Standards of the Society for Marine Mammalogy.

Please affirm that you have reviewed these important standards and best practices by stating so here. 30 words max.

Please provide a short statement that highlights how throughout your project you will be contributing to the Society's DEI goals. We recognize that this section might not be applicable to everyone. However, we would appreciate it if you could envision ways in which you can advance diversity, equity, and inclusion (DEI) in the country where your research will take place. 100 words max.

I believe that my work could contribute to the Society's DEI goals, showing that the construction of a 3D model using the photogrammetry technique is a viable and accessible tool for any researcher. On the

other side, the reconstruction of 3D models of delphinid tympano-periotic complex will be an important output for the CESIMAR collection, allowing digital access for worldwide researchers and preventing excessing handling of rare specimens. Furthermore, the digitization of collections enriches the scientific community, promotes their replication and modernizes the collections.

Citations

- (1) Perrin WF 2018. World Cetacea Database. Accessed at http://www.marinespecies.org/cetacea on 2018-06-14.
- (2) Fordyce RE 2018. Cetacean evolution. In: Würsig, et al. (Eds). Encyclopedia of Marine Mammals. pp 180-185.
- (3) Hunter JP 1998. Key innovations and the ecology of macroevolution. Trends Ecol Evol 13:31-36.
- **(4)** McGowen MR. 2011. Toward the resolution of an explosive radiation-A multilocus phylogeny of oceanic dolphins (Delphinidae). Mol Phylogenet Evol 60:345-357.
- **(5)** Bookstein FL 1991. Morphometric tools for landmark data. Cambridge University Press, Cambridge
- **(6)** Nicolosi P, Loy A. 2010. Landmark based morphometric variation in Common dolphin (*Delphinus delphis* L.,1758) In: Nimis PL, Vignes Lebbe R (Eds). Tools for Identifying Biodiversity: Progress and Problems. pp 263-268.
- (7) Catalano S, et al. 2015. The more, the better: the use of multiple landmark configurations to solve the phylogenetic relationships in musteloids. Syst Biol 64:294-306
- (8) Keck F, et al. 2016. Phylosignal: an R package to measure, test, and explore the phylogenetic signal. Ecol Evol 6:2774-2780
- **(9)** R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
- (10) Coombs et al., The tempo of cetacean cranial evolution, Current Biology (2022), https://doi.org/10.1016/j.cub.2022.04.06

Please provide the references cited above in numbered format. Limit citations to those most important to your work. Max 10.

¹ The Society's International Relations Committee and Committee of Scientific Advisors have defined all countries except the following as eligible countries: Applicants cannot be from Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Netherlands, New Zealand, Norway, Poland, Portugal, Singapore, Slovenia, Spain, Sweden, Switzerland, Taiwan, United Arab Emirates, United Kingdom, and United States. See https://marinemammalscience.org/awards-funding/awards-and-scholarships/grants-in-aid-of-research-information/