SOCIETY FOR MARINE MAMMALOGY: SMALL GRAND IN AID OF RESEARCH (2022)

Project Title

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

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Project Description:

Cetaceans are currently the dominant group of aquatic mammals, both in terms of taxonomic and ecological diversity, with approximately 90 named species (Perrin 2018). 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 (Fordyce 2018). In particular, the odontocetes had a rapid radiation in the early Oligocene, which was promoted by the acquisition of key innovations 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, Hunter 1998). 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 and air sinus systems) and feeding strategies (predators, suckers, or a combination of both) (Johnson & Berta, 2011). This disparity suggests that specialization occurred in many directions, making it difficult to interpret how this structural diversity originated and flourished (Fordyce 2018). Many aspects of the phylogeny and evolution history remain unresolved; particularly a marked disparity exists between phylogenetic hypotheses based on morphological and molecular dates (Kasuya T 1973). Moreover, 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 (Mead JG 1975). It is very important to incorporate and create a new data set and analysis, to review existing data and resolve conflictive relationships (Geisler JH, Sanders AE 2003). 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 (Ketten, 1994). 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 (Reidenberg & Laitman, 2008). The tympanic-periotic complex presents a morphological disparity between the different groups and contains a high potential for taxonomical, phylogenetic and morphological studies (Tsai & Fordyce, 2016; Gunstein et al 2014).

Numerical methods such as geometric morphometry, 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. The use of landmarks as a source of characters for phylogenetic studies is a relatively recent field, 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. This project aims to generate a new morphological database from a highly informative taxonomic perspective, such as the auditory region in odontocetes, and therefore improve the performance of this data source in the analysis of the phylogenetic relationships of this group. This could ultimately help resolve the inconsistencies observed between morphological and molecular hypotheses of phylogenetic relationships.

Result of the project:

To expand the dataset of odontocete tympanic-periotic complexes (TPCs) belonging to the marine mammal bone collection of the CESIMAR-CONICET Institute, between August and November 2022 I visited the collections at the Museum of Natural Sciences Bernardino Rivadavia (MACN) in Buenos Aires and the private collection of the Acatushun Museum in Ushuaia, Tierra del Fuego,. During these visits, I photographed a total of 90 ear bones, including bullae, periotic bones, and tympanic-periotic complexes, from 32 species (with 2-5 individuals *per* species) belonging to five families of odontocetes: Delphinidae, Pontoporiidae, Phocoenidae, Physeteridae, and Ziphiidae. I took 22,400 photographs using a Nikon D3100 digital single-lens reflex camera with an 18-55 mm lens, mounted on a tripod. Each specimen were fixed vertically on a rotating table, and 100–150 photographs were taken at 15-degree intervals horizontally and at three different angles vertically (90°, 45°, 15°).

A 3D model of the periotic bone for each specimen was reconstructed from a set of photographs taken and imported into Agisoft PhotoScan Professional (Agisoft 2018). The photos from each chunk/orientation were aligned to generate dense point clouds, which were merged together (Durão AF, et al. 2018; Agisoft 2018). The 3D models with texture were exported as PLY files and scaled by dividing the scaling factor identified in Meshlab by the scale measurements taken of the specimen bone (in mm). After obtaining the periotic bone model for each specimen, a template with 138 landmarks was created: 28 landmarks, 30 semilandmarks along 6 curves, and 80 surface semilandmarks. This template was designed using VIEWBOX 4.0 software (www.dhal.com) and was then applied to the rest of the sample. Currently, only the periotic dataset is being used to study shape variation, analyzed in terms of association and differentiation among taxonomic groups in sequential steps. In the nearly future, bullae and articulated tympanic-periotic complexes will be analyzed following the same methodology than periotics. The entire dataset underwent a generalized Procrustes analysis (GPA) to remove any variation unrelated to shape (Rohlf & Slice, 1990). Principal Component Analysis (PCA) was applied on Procrustes coordinates to describe shape variation and then used model-based clustering on the Procrustes coordinates to analyze the association between shape variation and taxonomic variables such as family, subfamily, and genera (Fraley & Raftery 2002). This method objectively classifies shape variation into groups and determines if these clusters are influenced by taxonomic variables. The best model and number of clusters were selected using Bayesian Information Criteria (Scrucca et al., 2016). As a third step, Leave-one-out cross-validated Canonical Variate Analysis (CVA) was applied on the PCA axes that explained at least 80% of the total variation, using the family as the classification category (Efron & Gong, 1983; Courtenay, 2023). Additionally, a Procrustes ANOVA on shape variables was performed, using the family and the logarithm of centroid size as linear predictors to capture the proportion of shape variation due to allometry (Goodall, 1991; Anderson, 2001; Anderson & Braak, 2003, Collyer et al., 2015). Finally, a pairwise comparison of mean and Procrustes variance between families was conducted (Collyer et al., 2015), enabling us to analyze differences between families in terms of mean and variance of shapes. Also a matrix of several ecological variables for each species was constructed from literature. A phylogenetic comparative analyses was conducted to assess the influence of ecological variables on periotic shape variation within a phylogenetic framework (Felsenstein, 1985; Adams & Collyer, 2019). For this analysis, a phylogenetic tree that encompassed our entire dataset was used (McGowen et al., 2009). To estimate and visualize the evolutionary history of periotic shape, a phylogenetic Principal Component Analysis (pPCA, Revell, 2009) and Phylogenetically Aligned Component Analysis (PACA, Collyer & Adams, 2021) was employed. Furthermore, a phylogenetic Procrustes ANOVA on shape as a response variable was performed, using the logarithm of centroid size and ecological variables as additive explanatory variables on shape (Adams & Collyer 2015). This model was referred to as independent variables PGLS. Additionally, an independent phylogenetic Procrustes ANOVA for each ecological variable (also adding logarithm of centroid size) was conducted to evaluate their effects independently of each other. These latter models were referred to as non-independent variables PGLS. The statistical analyses were performed using packages such as geomorph, Morpho, and RRPP (Schlager, 2017; Collyer & Adams, 2018, 2023; Baken et al., 2021; Adams et al., 2023) for morphometric analyses, and mclust (Scrucca et al., 2016) for cluster analysis in R statistical software (R core team 2022).

These preliminary results obtained were presented at the V Argentine Meeting of Evolutionary Biology 2023 (RABE, http://sedici.unlp.edu.ar/handle/10915/160435). Additionally, a scientific publication is being written. Furthermore, in the nearly future it is expected to publish other papers analyzing the bone morphological variability of the bulla and the tympanic-periotic complex, and the contribution of this variation in the phylogenetic relationships of Delphinidae.

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