

# PHYLOGENETIC AND ECOLOGICAL SIGNIFICANCE IN THE EVOLUTION OF TOOTHED WHALES COMMUNICATIVE ACOUSTIC SIGNALS

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## Summary

The goal of this doctoral study is to provide an innovative hypothesis about the evolution of toothed whales communicative acoustic signals (whistles) and determine the relative significance of the environment and phylogenetic relationships on these signals. This study consists of two parts (1) examination of the factors proposed to explain interspecific whistle variation (sociality, phylogeny, morphology, environment, and zoogeography), using a phylogenetic approach by reconstructing of a robust phylogeny where the evolutionary history of standard acoustical parameters (e.g., maximum and minimum frequencies, duration) will be reconstructed; (2) examination of the role of ambient noise and habitat structure in whistle intraspecific variation. To approach this second goal I will study the dolphin *Sotalia fluviatilis*. *Sotalia fluviatilis* is one of three dolphin species with both river and marine populations. It has two "ecotypes" a riverine and a marine form. This unusual situation in cetaceans represents a great opportunity to study geographical and local variations in vocal structure and the role of the environment in their communicative and non-communicative vocalizations. The study sites are located in rivers and marine protected and non-protected areas of Nicaragua, Costa Rica, Panama, Guyana, and Ecuador.

## Background

Several issues of Cetacean phylogenetics have been intensely debated, as a result of independent datasets (morphology, nuclear DNA, and mitochondrial DNA) suggesting conflicting hypotheses. These debates include the phylogenetic placement of Cetacea as sister to Artiodactyla<sup>e.g.1,2</sup> or embedded within Artiodactyla, a clade called Cetartiodactyla<sup>e.g.3,4</sup>, the relationship between toothed whales and baleen whales<sup>e.g.5,6</sup>, and the relationships among toothed whales<sup>e.g.7,8</sup>. However, some of these hypothesis such as the placement of Cetacea within Artiodactyla, and the unexpected hypothesis of the sister relationship of Cetacea and Hippopotamidae have now received support from studies based on new independent datasets. Using the entire mitochondrial genome reversed the earlier mitochondrial hypothesis and recovered the monophyly of Odontoceti<sup>9</sup>. These previously controversial clades can now be labeled as 'benchmark' clades, i.e. to be likely true: *Odontoceti*, *Cetartiodactyla*, and *Cetancodonta* (*Cetacea* + *Hippopotamidae*). Therefore, our understanding of deeper level cetacean phylogeny has grown strong. However, the strong focus of most recent studies, aiming specifically to resolve these higher level conflicts by adding mostly characters rather than taxa, has left our understanding of lower level relationships among whale species lagging behind. This lack of species level phylogenetic work hinders our understanding of other aspects of cetacean biology, such as the evolution of acoustic signals

Mammals are perhaps one of the most morphologically (e.g., bats, primates, cetaceans) and ecologically (e.g., fossorial, arboreal, aquatic, aerial) diverse vertebrate groups<sup>10</sup>. They have colonized greatly contrasting habitats and therefore evolved various ways to communicate and monitor these environments by using visual, olfactory, tactile (e.g., seismic), and acoustic senses. All mammals to some extent use all these senses, but exclusively aquatic mammals like cetaceans, rely almost entirely on sound. This dependence on sound stems from light limitations in aquatic environments<sup>11</sup>. Light attenuates rapidly with depth, limiting visual interactions

between sender and receiver. Olfactory senses are also less developed in cetaceans than in land mammals, limiting their use for communication purposes<sup>12</sup>. Sound, however, has the advantage of having low attenuation, allowing for effective communication and monitoring of aquatic environments. For instance, baleen whales produce low frequency sounds (<100 Hz), with very small absorption losses; some whales are able to communicate over hundreds of kilometers<sup>11</sup>.

The use of sound for underwater communication plays an important role in the life of any cetacean. Cetaceans are probably among the noisiest taxa in aquatic environments. They produce acoustic signals for communication (e.g., songs, whistles, social-pulsed sounds), prey location, navigation, and predator avoidance (e.g., echolocation-clicks)<sup>11</sup>. One of the most studied categories of communicative sounds in cetaceans is whistles. Whistles are narrowband and frequency modulated signals that vary in duration and contour shape<sup>11</sup>. Whistles variation in modulation and duration is more pronounced within species, whereas whistle frequency parameters vary across species. Several factors have been proposed to explain intra and interspecific whistle variation but few of these have been directly evaluated<sup>13</sup>. Despite the great amount on whistle work, there is an empty space in aspects related to the evolution of these signals such as How did whistles evolve? Did whistles arise de novo in delphinids as suggested by some studies? Which of the proposed factors or combination of factors may have played a major role in the evolution of these acoustic signals at the intra and interspecific level? The goal of my dissertation is to cast light on the evolutionary history of whistles.

### **Objectives**

1. To reconstruct a robust species cetacean phylogeny using cytochrome b.
2. Evaluate for phylogenetic signal of each whistle acoustic component.
3. Using the above phylogeny reconstruct the evolutionary history of whistles and each whistle acoustic component in relation to habitat and sociality.
4. Evaluate if body size is negatively correlated with frequency using a phylogenetic comparative approach.
5. Examine how these acoustic characters, defined as either phylogenetic or environmental, interplay with the acoustic properties of the environment.
6. Characterize habitat in terms of acoustic properties (e.g., sound speed, sound propagation and ambient noise) to determine its influence on whistle acoustic structure.
7. Determine the degree of dissimilarity in whistle acoustic structure between sympatric populations and non-sympatric populations of three dolphin species.

### **Materials and Methods**

#### *Phylogenetic Analysis*

Cytochrome data were compiled from GenBank for 66 cetaceans representing 63 species. For a stronger test of Cetartiodactyla monophyly and deeper level relationships we sampled 24 outgroup taxa using the recent mammalian phylogeny of Murphy et al. (2001)<sup>14</sup> as a guide to outgroup choice. Murphy's et al. (2001)<sup>14</sup> phylogeny, based on 18 gene segments, suggested the following relationships (Carnivora (Perissodactyla + Cetartiodactyla)). Outgroups therefore include non-cetacean cetartiodactylans (16 species), Perissodactyla (six species), and two carnivores chosen as primary outgroups. To minimize potential missing data problems in an already difficult phylogenetic problem, I chose to exclude cetacean taxa when the following two conditions applied: 1) only small partial Cytochrome b sequences were available (less than 50% of the entire sequence), and 2) congeners with longer sequences were already present in the matrix.

The molecular matrices were matched and aligned using the Needleman-Wunsch algorithm (gap cost=10, mismatch=1) in MacClade 4.07<sup>15</sup>. The data were analyzed using Bayesian, and parsimony methods. The appropriate model for the Bayesian analyses was selected

with Modeltest<sup>16</sup>, using the AIC criterion<sup>17</sup> with a parsimony tree chosen as the basis for Modeltest. Bayesian analysis was performed using MrBayes V3.0<sup>18</sup> with the following settings. The maximum likelihood model employed 6 substitution types ("nst=6"), with base frequencies estimated from the data. As substitution frequencies differ starkly between first, second and third positions in Cytochrome b<sup>19</sup>, each codon position was treated separately (charset 1st\_pos = 1-1140\3; charset 2nd\_pos = 2-1140\3; charset 3rd\_pos = 3-1140\3; partition by codon = 3: 1st\_pos; 2nd\_pos; 3rd\_pos; set partition = by codon). Rate variation across sites was modeled using a gamma distribution (rates="gamma"). The Markov chain Monte Carlo search was run with 4 chains for 5,000,000 generations, sampling the Markov chain every 1000 generations, and the discarding sample points of the first 70,000 generations as "burn-in".

Parsimony analyses were done in PAUP\* and NONA through the WINCLADA shell. In each of the analyses, heuristic searches were done with 1000 random stepwise additions, and subtree-pruning and regrafting branch swapping algorithm. As transitions (Ti) are much more common than transversions (Tv) and different codon positions show different levels of Ti saturations (third position showing the highest), I used some of the many weightings schemes suggested by previous authors. Node support for the parsimony analyses was estimated using Bootstrapping.<sup>20</sup> Each analysis ran for 200 Bootstrap replicates, with 10 random addition sequences, and holding a maximum of 100 trees, per replicate. To examine the effect of sparse taxon sampling on the Bayesian analysis (numerous previous studies have analyzed smaller Cytochrome b datasets using parsimony) I analyzed two, rather arbitrarily chosen subset of the data. First, I pruned the dataset to contain a comparable taxon sampling to that of Messenger and McGuire (1998)<sup>6</sup>; second, I used the pruned ingroup dataset, but added all the outgroups from the main data matrix.

Commonly used acoustic variables such as minimum and maximum frequency, inflection points, and duration will be tested for phylogenetic signal using the Test for Sequential Independence for continuous data and the Run Test for discrete data. If phylogenetic signal is significant the Independent Contrast Test<sup>20</sup> will be used to correct for phylogenetic relationships in order to evaluate the significance of phylogeny and morphological constraints in the evolution of whistles.

## **Ecological Significance**

### *Study Sites*

The proposed study sites are: (1) Cayos Miskitos, Marine Biological Reserve (RAMSAR) located along the Atlantic coast of Nicaragua protecting marine ecosystems like offshore islands, shoals, wetlands, riverine systems, and estuaries; (2) Gandoca-Manzanillo Wildlife Reserve (RAMSAR) located along the Atlantic coast of Costa Rica, close to Bocas del Toro, Panama; (3) Archipelago of Bocas del Toro located on the Atlantic coast of Panama; (4) Yasuni National Park and Cuyabeno Reserve located between the Napo and Curaray rivers in Ecuador; (5) Guyana's Rivers: the Waini, Cuyuni, Mazaruni, Demerram, and Essequibo rivers.

### *Habitat Characterization*

*Sotalia* habitat will be characterized based on acoustic and non-acoustic environmental variables. Non-acoustic environmental variables such as depth (m), water transparency, superficial temperature and salinity, will be obtained at the moment of dolphin encounters and during habitat acoustic assessments. The acoustic properties of each study site will be described in terms of ambient noise, sound speed profiles, and sound propagation characteristics.

*Ambient noise* will be measured by recording background noise and identifying (as possible) sources of the noise in each site (e.g. outboard boats, shrimps, fishes) and by recording boats (fishing, whale-watching, speed, and transport boats) as they move away from the hydrophone. Captains will be also asked to drive around the boat simulating the research boat is a group of dolphins. These recordings will be used to identify (a) the range of frequencies

produced by these boats, (b) their overall contribution to the ambient noise, (c) their sound attenuation along a propagation path (as the boat moves away distances will be taken using a range-finder), and (d) potential distance range at which boat noise may affect dolphin behavior.

*Sound propagation* will be assessed using a calibrated sound projector stepping through 8 to 10 frequencies and measuring the frequency-dependent attenuation along the propagation path at 0, 10, 20, 50, 100, 200 and 400 meters from the source using an ITC-1001 projector and recording equipment described below. *Sound speed* will be estimated with temperature-depth profiles and salinity measurements.

#### *Dolphins Vocalizations*

Study sites will be boat-surveyed during a 2 week period in each proposed study site 0630h to 1730h following strip transects. A number of transects will be randomly selected every day. This method maximizes the coverage of each study areas. Once a group of dolphins is encountered the boat will approach slowly and parallel reaching a front position at about 20-50 m distance to the group, then the engine will be turned off to initiate acoustic and behavioral recordings. A group is defined as a "cohesive collection of conspecifics in a limited area which maintain a body distance of maximum 10 m from each other. The following data will be collected: group size (minimum, maximum, and best estimation), group composition (age and gender if possible), photo-ID (unique marks in dolphin dorsal fin will be photograph for individual identification of group members), GPS location, behavioral state (see below) before and during acoustical recording sessions, and presence/absence of other boats, as well as their proximity to the dolphins.

#### *Acoustic Recording Methods*

Dolphin acoustic signals will be recorded in the field using a broadband system: a RESON hydrophone (-203 dB re 1V/ $\mu$ Pa, 1 Hz to 140 kHz) connected to AVISOFT recorder and Ultra Sound Gate 116 (sampling rate 400-500 kHz 16 bit) which sends the signals to the laptop. The acoustic data will be analyzed using the software Raven 1.1<sup>R</sup>. Dolphin signals will be classified as whistles, pulsed sounds, and echolocation clicks (Richardson et al. 1995). The number of randomly selected whistles per group is twice the group size, and the minimum interval between two selected whistles will be one minute. A total of 14 contour parameters will be measured following Barzua-Duran and Au (2003)<sup>21</sup>.

#### *Behavioral Data*

Behavioral data will be collected from onboard. The occurrence of social activities, travel, resting, milling, foraging-feeding events, and diving will be measured continuously during 2 min periods in synchrony with sound recordings every 3 min intervals to estimate proportions of time dedicated to vocalizations and to elucidate the context in which such vocalizations are produced. Groups will be followed in order to obtain recordings of their different behavioral states, a minimum of 20 min to 2h is consider as an optimal time to observe most behavioral states (based on preliminary fieldwork). Animals will be photo-ID to keep track of the individuals recorded during a specific survey using a digital camera Canon EOS Rebel, 8.0 Megapixel to allow for animal ID in the field (this a common technique to identify individual dolphins using scars and other marks in their dorsal fin and dorsum, e.g., see photos-below) simultaneously while recording vocalizations.

#### *Statistical Analysis*

Test for normality, independence, and inequality of variance. Overall geographical variation of the 12 whistle parameters for each dolphin will be evaluated using ANOVA and a posteriori test Tukey-Kramer. To classify all whistle components based on species and locality a Multivariate discriminant function will be used. The classification method Jackknife will be used to calculate percentage of correct classification within species and location. Association patterns between whistles and behavioral states will be evaluated using a Correspondence Analysis approach.

Effect of the Environment and Behavior among populations will be evaluated using a nested ANOVA to estimate the magnitude of variation attributed to location, behavior, and environment. Effect of ambient noise on whistle modulation (COFM) will be evaluated using an ANOVA. To estimate the magnitude of variation attributed to different type of ambient noise sources I will use a nested ANOVA.

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