

# Global Phylogeography of Delphinine Dolphins Using Mitogenomics

Madeleine Becker<sup>1,2</sup>, Katherine Murphy<sup>1</sup>, Charles Potter<sup>1</sup>, Ibrahima Ndong<sup>3</sup>, Lucy Keith-Diagne<sup>3</sup>, Michael McGowen<sup>1</sup>

<sup>1</sup>Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA

<sup>2</sup>University of Southern California, Los Angeles, CA, USA <sup>3</sup>African Aquatic Conservation Fund, Mbour, Senegal

## Objectives

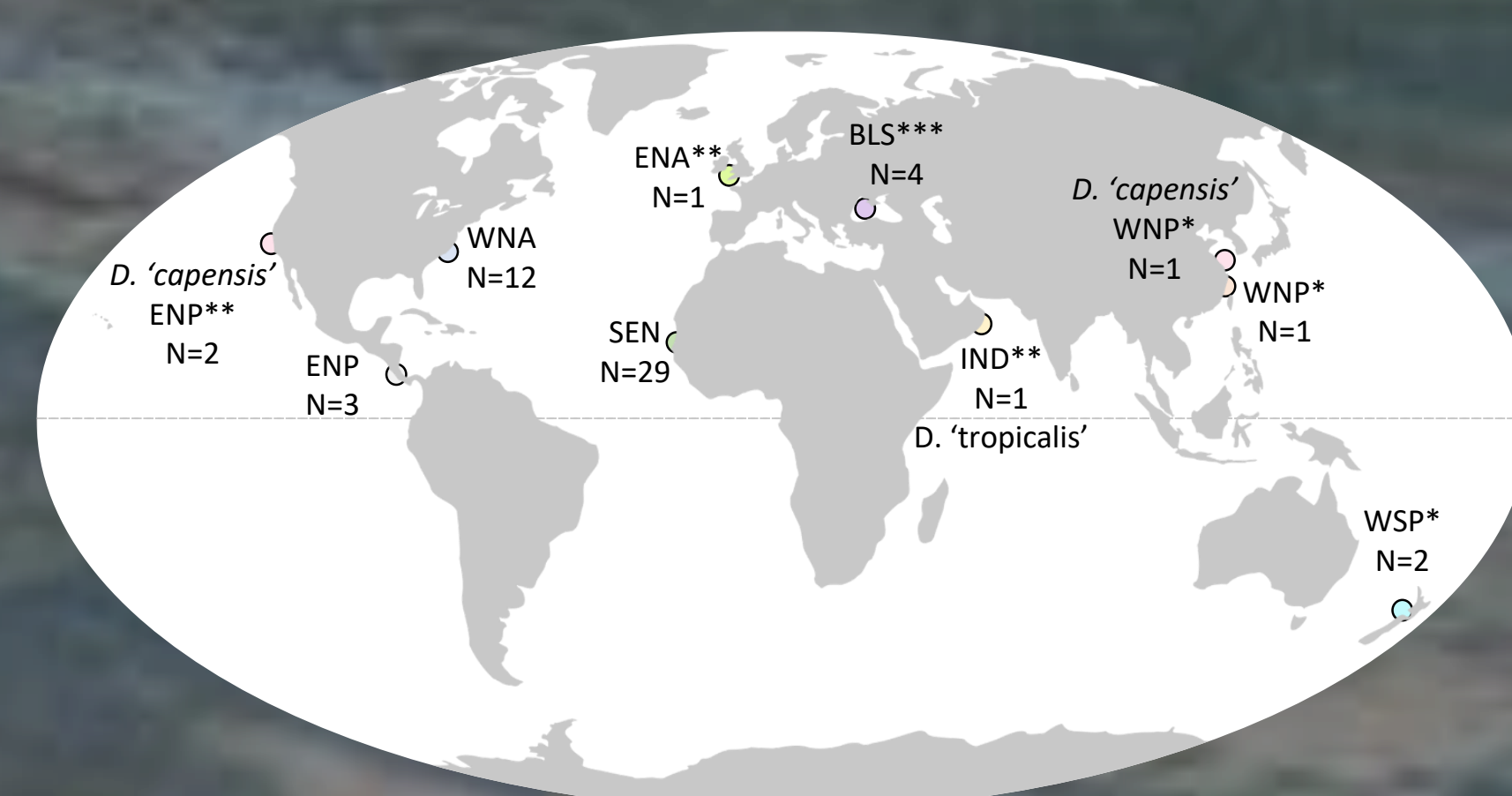
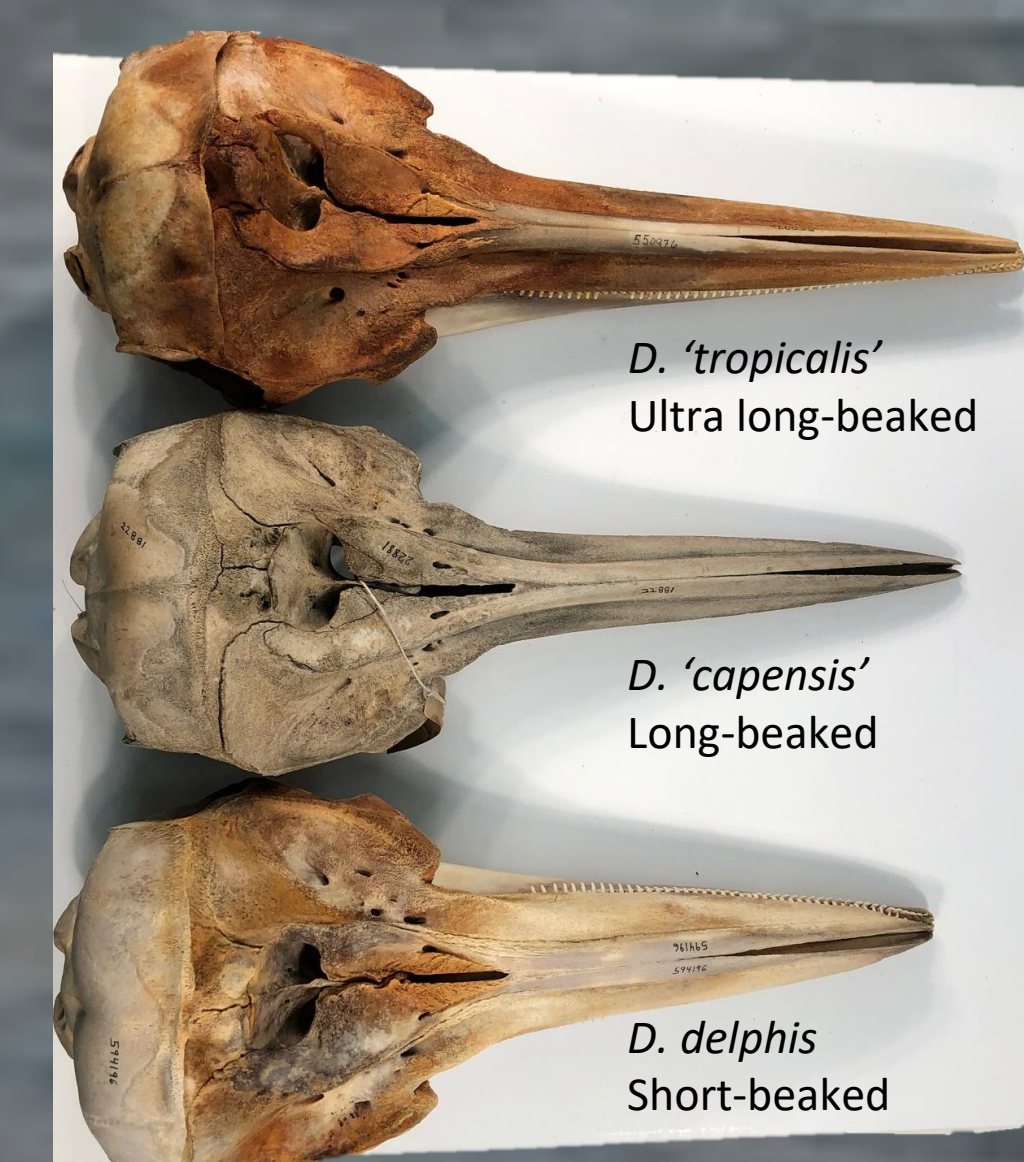
- Resolve subfamily Delphininae with whole mitogenomes
- Determine how morphotypes and geography impact *Delphinus* relationships

## Introduction

While the advent of next-generation sequencing has revolutionized our understanding of mammalian taxa, cetacean evolutionary history and relationships have remained challenging to understand. Slow evolutionary rates and global ranges are characteristic of this infraorder, which when paired with incomplete lineage sorting, introgression, and potential hybridization, make phylogenetic reconstruction difficult. In this study, we investigated the dolphin subfamily Delphininae, with particular emphasis on the genus *Delphinus*.

*Delphinus delphis* was described by Linnaeus in 1758 and is generally known as the short-beaked common dolphin. However, the long-beaked morphotype of the common dolphin has a more complicated taxonomic history. First described as *Delphinus bairdii* (Dall 1873) as an eastern north Pacific species, the morphotype was re-classified as part of a cosmopolitan sympatric species *Delphinus capensis* by Heyning and Perrin in 1994. *D. capensis* would later also include an ultra long-beaked morphotype, '*tropicalis*'. While the *D. capensis* designation was consistent with the skull morphology of short and long-beaked morphotypes in California (Figure 1a), genetic and more global assays have called the validity of this species into question (Natoli et al. 2006, Cunha et al. 2015, Fariás-Curtidor et al. 2017).

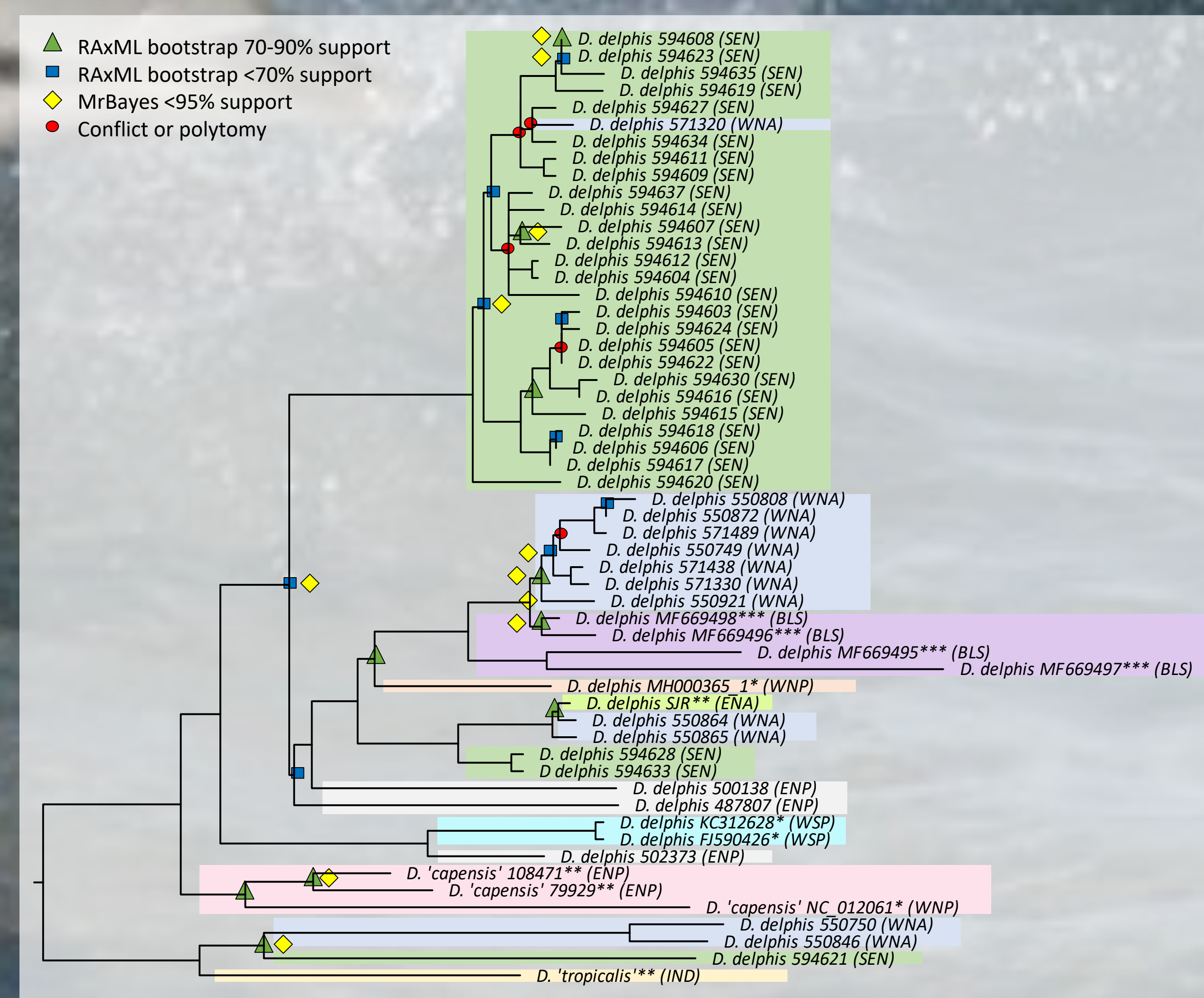
Using a global dataset of whole mitochondrial sequences, this study seeks to resolve some of the taxonomic issues of Delphininae with a concentration on resolving phylogeographic relationships within *Delphinus*. Here we have compiled the most genetic data for *Delphinus* populations to date.



**Figure 1a (Left).** Skulls of three *Delphinus* morphotypes: short-beaked (USNM 594196, North Carolina, USA), long-beaked (USNM 22881, South Korea), and ultra long-beaked (USNM 550976, Somalia). It is disputed whether all of these are simply one species *D. delphis*, two species (in which *D. capensis* includes all long-beaked specimens), or whether they are three species in which *D. tropicalis* and *D. capensis* comprise a long-beaked clade.  
**Figure 1b (Right).** Map of general sampling sites and origins of GenBank-derived sequences used in these analyses, including sample size from each region. \* = GenBank sequences, \*\* = unpublished sequences from McGowen et al. (in press), \*\*\* = GenBank sequences from ancient specimens (400-500 CE).

## Methods

- 87 samples collected opportunistically from recent strandings in the western north Atlantic, eastern north Pacific, and Senegal (Fig 1b)
- Representing species *Delphinus delphis*, *Feresa attenuata*, *Grampus griseus*, *Lagenodelphis hosei*, *Phocoena phocoena*, *Stenella attenuata*, *Stenella clymene*, *Stenella coeruleoalba*, *Stenella frontalis*, *Steno bredanensis*, *Tursiops truncatus*
- Extracted DNA using Qiagen Dneasy Blood & Tissue Kits in NMNH LAB
- Amplified mitogenome with primers from Morin et al. (2010) in 2-4 large segments using long-range PCR
- Samples pooled and sequenced as 150bp paired-end reads on Illumina MiSeq with the MiSeq Reagent Kit v2 Nano
- Quality analyzed with FastQC and MultiQC; reads trimmed with Trimmomatic
- Reads were mapped using the *Delphinus delphis* mitogenome reference sequence (MH000365.1) and assembled/circularized using default parameters with Geneious
- Added 3 individual mitogenomes from McGowen et al. (in press) and 371 from GenBank representing 30 delphinid species in total (Fig 3)
- All sequences aligned with MAFFT
- Conducted phylogenetic analyses with RAXML and MrBayes on CIPRES
  - Partitioned data by gene and codon (including separate partitions for each tRNA and control region) and used PartitionFinder to select the most appropriate partition scheme.



**Figure 2.** RAXML best tree of *Delphinus* sequences using PartitionFinder model, annotated with bootstrap support scores, MrBayes support scores, and any conflicts with MrBayes consensus tree. Nodes without annotation have over 90% RAXML bootstrap support and over 95% MrBayes support. Samples are colored by region and labeled with region codes. \* = GenBank sequences, \*\* = unpublished sequences from McGowen et al. (in press), \*\*\* = GenBank sequences from ancient specimens (400-500 CE). Samples from the same region group unreliably and many nodes have low support. Notably, *D. tropicalis* and *D. capensis* specimens do not form a monophyletic group as historically hypothesized. *D. delphis* samples from the Black Sea, often denoted as *D. delphis ponticus* (Amaha, 1994) are also paraphyletic.

## Discussion

- Not much support among basal relationships within Delphininae (Fig 3)
- Many conflicting and unsupported nodes within *Delphinus* (Fig 2)
- **Polyphyly among *Tursiops* and *Stenella*** within subfamily Delphininae
- **Unclear geographic groupings within *Delphinus***, with some groups showing geographic structure (Senegal, western north Atlantic)
  - Potentially explained by occasional migration between different ocean basins, or persistence of multiple mitochondrial lineages in populations through time due to large population sizes
- **Long-beaked dolphins do not form a monophyletic group**, even though '*capensis*' type from the north Pacific do
- **Black Sea dolphins (*D. delphis ponticus*) do not form a monophyletic group**
- Nuclear data (using ddRAD-seq or other method) needs to confirm these results within *Delphinus*
- More populations need to be included, especially more long-beaked representatives

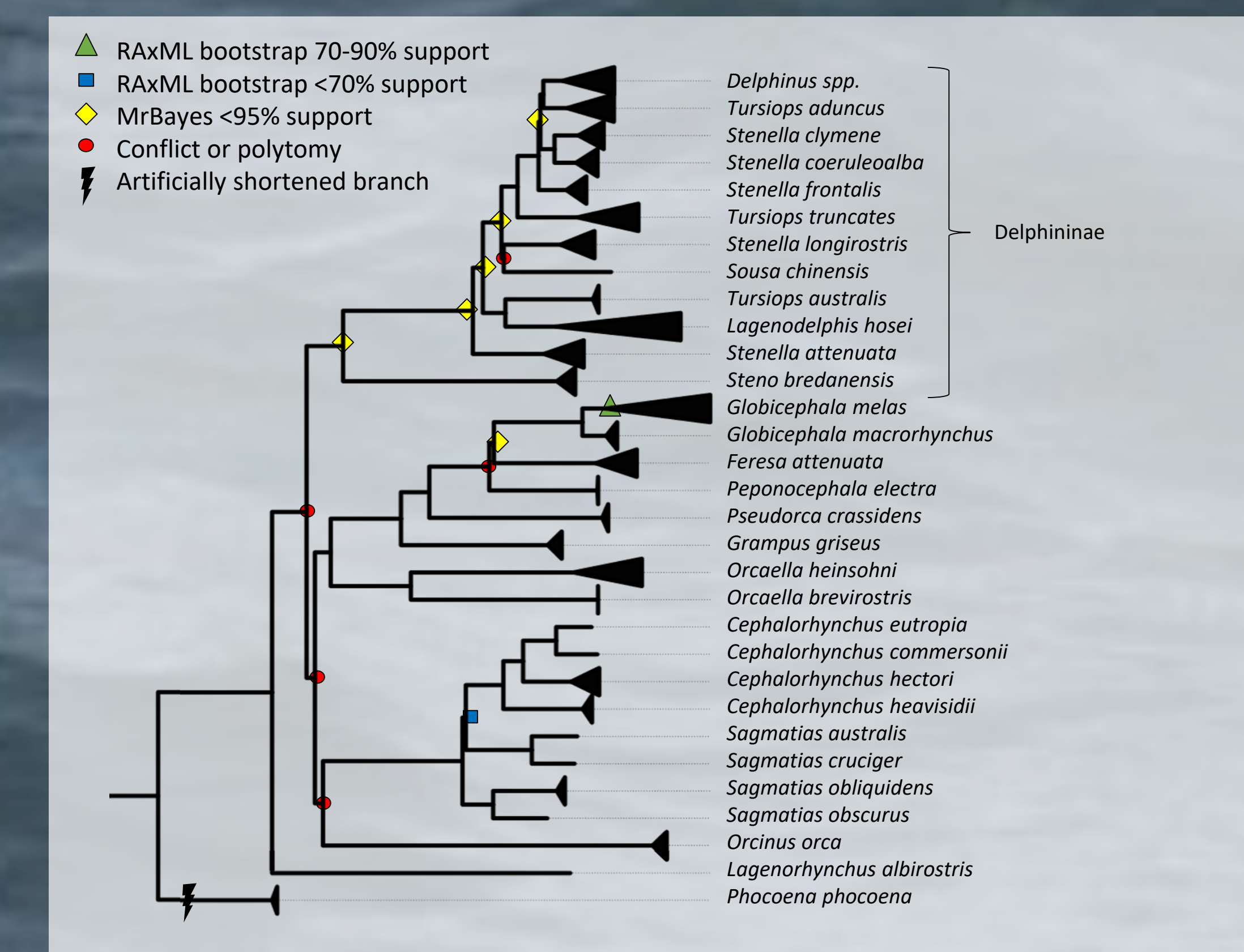
## Acknowledgments

We would like to thank Chris Huddleston, Daniel DiMichele, John Osofsky, Dietrich Gotzek, Tom Sargent (NMNH), Tomas Diagne (African Aquatic Conservation Fund) and Ellen Potter. Funding for this study was provided by NMNH start-up funds to MRM. Additional thanks to Gene Hunt and Virginia Power for coordinating the NHRE program and to the NSF for their NHRE sponsorship.



Cunha H.A., Lóizaga de Castro R., Secchi E.R., Crespo E.A., Lailson-Brito J., Azevedo A.F., Lazoski C., Solé-Cava A.M. (2015) Molecular and morphological differentiation of common dolphins (*Delphinus* sp.) in the southwestern Atlantic: testing the two species hypothesis in sympatry. *PLoS ONE* 10:e0140251.  
Fariás-Curtidor N., Barragán-Barrera DC, Chávez-Carreño PA, Jiménez-Pinedo C, Palacios DM, Calcedo D, et al. (2017) Range extension for the common dolphin (*Delphinus* sp.) to the Colombian Caribbean, with taxonomic implications from genetic barcoding and phylogenetic analyses. *PLoS ONE* 12(2): e0171000.  
Heyning J.E., Perrin W.F. (1994) Evidence for two species of common dolphins (genus *Delphinus*) from the Eastern North Pacific. *Contrib Sci Nat Hist Museum LA City*. 442:1-35.  
McGowen M.R., Tsakogea G., Alvarez-Carretero S., dos Reis M., Struëbig M., Deaville R., Jepson P.D., Jarman S., Polanowski A., Morin P.A., Rossiter S.J. (in press) Phylogenomic resolution of the cetacean tree of life using target sequence capture. *Syst Biol*.  
Morin P.A., Archer F.I., Fonto A.D., Vistral J., Allen E.E., Wardo P., et al. (2010) Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research*, 20(7), 908-916.  
Natoli A., Canadas A., Peddemors V.M., Aguilar A., Vaquerro C., Fernandez-Piqueras P., Hoelzel A.R. (2006) Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.). *J. Geol. Biol.* 19:943-954.  
Redfern, Jessica (2006) *Delphinus delphis* with calf [JPEG]. Retrieved from Protected Resources Division, Southwest Fisheries Science Center, La Jolla, California. swfsc.nmfs.noaa.gov/PRD/  
"San Jose" (2006) World Map Blank GMT [SVG]. Modified from commons.wikimedia.org

REU Site, OCE-1560088



**Figure 3.** RAXML best tree of all sequences using PartitionFinder model, collapsed to species level and annotated with bootstrap support scores, MrBayes support scores, and any conflicts with MrBayes consensus tree. Nodes without annotation have over 90% RAXML bootstrap support and over 95% MrBayes support. Delphininae resolves as a monophyletic clade, but with low Bayesian support at basal nodes. Polyphyly of genera is especially apparent within Delphininae.