

FINAL REPORT – SMALL AIDS IN AID OF RESEARCH (Society for Marine Mammalogy)

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Molecular investigation and characterization of hemotropic mycoplasmas in marine cetaceans and Amazonian and marine sirenians, Brazil.

Summary: Hemotropic mycoplasmas (hemoplasmas) are uncultivable, pleomorphic, small bacteria that infects mammals, including humans. In spite of descriptions in several wild species, there is no information regarding hemoplasma DNA presence in cetaceans and sirenians, and overall, little is known about its pathogenicity and transmission routes. We have recently detected hemoplasma DNA in blood of riverine dolphins (*Inia* spp.) and identified at least one novel hemoplasma species. The aim of this study is to further detect and characterize hemoplasmas in wild marine cetaceans, and in sirenians (*Trichechus manatus* and *Trichechus inguinus*) in Brazil.

Hypothesis: We hypothesize that, due to the diversity of arthropod vectors present in the Amazon biome, Amazonian manatees and riverine dolphins will present a higher occurrence of hemotropic mycoplasma when compared to marine manatees and cetaceans. We also hypothesize that hemoplasma species will present host specificity, and therefore will differ between marine and riverine species.

Results: We investigated the occurrence of *Mycoplasma* spp. in blood samples of marine cetaceans (n=108) that stranded alive or dead or were bycaught between 2011 and 2022 in the northeastern (48/108) or southeastern (60/108) Brazilian coast. Blood samples belonged to animals of the families Delphinidae (n=52), Pontoporiidae (n=39), Kogiidae (n=14), Balaenopteridae (n=2) and Physeteridae (n=1), comprising eighteen different species. Additionally, we analyzed 50 blood samples of 23 *Trichechus manatus manatus*

and 26 *Trichechus inunguis* under human care (rehabilitation or permanent residents). Mycoplasma DNA was detected in 16 out of 108 (14.8%) cetaceans' samples by a real time-PCR (384pb) or conventional nested-PCR targeting 1100 pb fragment of 16S rRNA gene and confirmed by direct sequencing. All tested manatees were hemoplasma-PCR negative. The occurrence rate observed in marine cetaceans was apparently lower when compared to the one previously found in river dolphins (64%) (*Inia geoffrensis* and *Inia boliviensis*).

We found significantly higher prevalence in adults and mixed habitat range marine cetacean species in comparison with juveniles and calves, and coastal species, respectively. However, the remaining variables did not show statistically significant differences.

Our findings indicate that hemoplasmas are circulating in both riverine and marine cetaceans, however not in sirenians. Additionally, our data suggest a hemoplasmas transmission route of different from vectors for aquatic mammals, probably associated with food intake, endoparasitism or direct contact. This possibility is also reinforced by the higher detection rate of hemoplasmas in cetaceans with mixed habitat range than in coastal species. Preliminary phylogenetic analyses have not shown host specificity for this pathogen as usually suggested, and the same sequence types have been detected in hosts from different species, which suggests interspecific transmission. Ongoing studies are investigating this hypothesis.

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