

Protocol for collection, preservation and shipment of endo and ecto-parasites of aquatic mammals

Antonio A. Mignucci-Giannoni, PhD

Carla I. Rivera-Pérez, BS

Inter-American University of Puerto Rico



Supplies and equipment needed

- | | |
|--|---|
| ☐ Disposable latex gloves | ☐ Cardboard or heavy stock paper |
| ☐ Forceps without teeth, razor or knife | ☐ Pencil |
| ☐ Plastic or glass vial | ☐ Black permanent ink marker |
| ☐ 70% ethanol or molecular grade ethanol (95-98%) | ☐ Whirl-pack plastic bag |

Scope and objectives

Endo and ecto-parasites can serve as indicators for aquatic mammal zoogeography and can help distinguish between closely related species or populations of the same species. Documentation of aquatic mammal parasites can also help diagnose health problems that an individual may be facing. This information can also be used forensically to determine disease processes or cause of death.

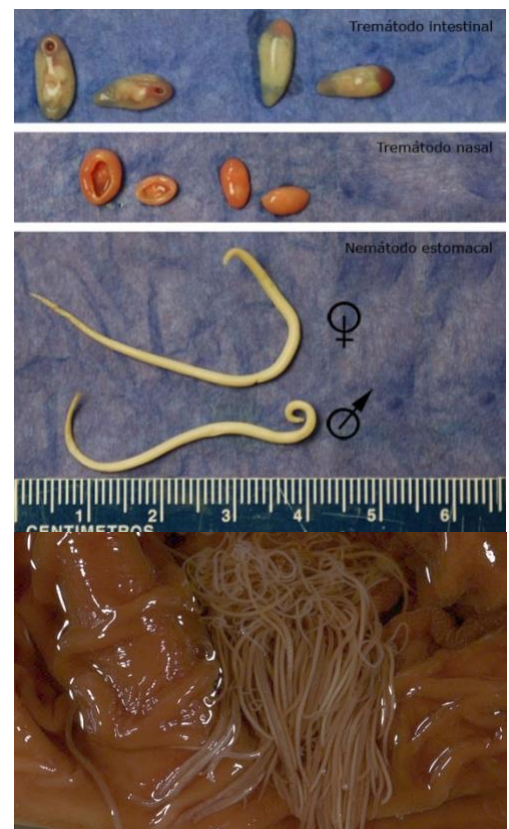
This study focuses on parasitological documentation of endo and ecto-parasites of whales, dolphins, and manatees in the Caribbean, Central and South America, with particular interest in flukes, worms, crustaceans and barnacles (epibionts) of manatees (*Trichechus* spp.). Also of interest is the documentation of the dolphin stomach worms, especially in the Amazon River dolphin (*Inia geoffrensis*). This study will use morphological and molecular methods to document the species found.

Basic parasitology of aquatic mammals

Aquatic mammals should be examined externally and internally for parasites and epibionts. In particular, we should examine the skin and fat, mouth and teeth, inner ear sinuses, nostrils or blowhole, lungs, heart, liver, pancreas, stomach(s), intestines, kidneys and urinary bladder.

Manatees have associated with their skin a tiny crustacean and barnacles. In the nostrils there is a fluke in the form of a small cup, while in the intestine they have a different fluke in the form of a large grain of rice with a red dot. In the stomach, manatees have a roundworm of cream color.

In general, dolphins have stomach worms which are of particular interest. These are typically of the genus *Anisakis*, which are identified only with molecular techniques. The Amazon River dolphin has a species of *Anisakis* parasite in the stomach, which is poorly understood by science and is in need for future study.



Collection

The collection of endo and ecto-parasites is quite simple. Using forceps without teeth, or sometimes just with gloved fingers, the individual worms can be collected and transferred to a storage container. It is important not to pinch too hard, as this will distort the morphology and may damage the anatomy of the worm. Nasal flukes are easily collected from manatees by introducing the pinky finger in each nostril as far as the length of the finger allows. Stomach or intestinal worms (acanthocephalans) that are not easily peeled off from the mucosa should be cut around the tissue (mucous) around the mouth of the worm, without harming their mouth parts, which is important for identification. Crustaceans and barnacles are collected with the edge of a razor or knife between the ecto-parasite and the animal's skin. Sometimes this can be done the same way with your fingernail.



Please collect at least 24 individual specimens representing each type of parasite. Note the approximate total number of parasites present in the carcass if possible. It is important to describe the collection location (lung, nares, stomach, small intestine, etc.) It is a good practice to collect a duplicate sample of each parasite at each location. One sample is left with the collector and the other is used to send to the laboratory for forensic identification. That way, there will always be a set of samples available should one get lost or destroyed in transit.

Storage

In previous years, parasites were fixed and stored in 10% formalin jars, but today this practice is not used as it does not allow for molecular studies. Today, all endo and ecto-parasites are preserved in either 70% ethanol, ethanol 95-98% or in molecular grade ethanol. Do not use isopropyl or rubbing alcohol because it damages the DNA in the sample.

The ratio of preservation liquid to sample is 9 to 1, meaning that each vial should be 1 part sample of parasites to 9 parts of ethanol. This ensures that the sample will be adequately preserved.

On a small piece of heavy stock paper or cardboard write in pencil the following basic information regarding the sample as follows:

Field number, date
Type of parasite
Stored in which liquid?
Location in host
Host
Geographic location, country
Collector

CCM191221Tm01 21 DEC 2019
Nematode
in Ethanol 70%
Stomach
Trichechus manatus
Guayama, Puerto Rico
José Pérez-Zayas



This card is included in the vial along with the parasites. A copy is written with permanent ink and attached to the outside of the vial. Vials should be kept in dark, cool, dry spaces, and checked periodically to ensure that the alcohol has not evaporated.

Packaging and shipping

No refrigeration is required for the ethanol vials with parasites. Prior to shipping ensure the cap is tight, over the seam with heavy tape, and place the vial into a Whirl-Pak or Ziploc bag; this will help if the vials or the ethanol leaks. It is good practice to place a paper towel or other absorbent material inside the bag should the sample leak. A separate plastic bag per vial also helps keep the sample together and expedites laboratory processing. Make sure copies of notes, necropsy records, and proper collection and shipping permits are included with the sample in its own Ziploc bags.

The vials with parasites can be sent to either of these addresses:

Carla I. Rivera-Pérez
Centro de Conservación de Manatíes de Puerto Rico
Universidad Interamericana de Puerto Rico
PO Box 361715
San Juan, Puerto Rico 00936 USA

Carla I. Rivera-Pérez
Centro de Conservación de Manatíes de Puerto Rico
Universidad Interamericana de Puerto Rico
500 Carr. John Will Harris
Bayamon, Puerto Rico 00956 USA

Thank You

Any questions or concerns please do not hesitate to contact us. Additionally, please notify us when samples of parasites will be sent. Our contact information is: carly@manatipr.org (phone number 787-923-6350) or mignucci@manatipr.org. Thank you for your interest in this research and participation in this project.