General recommendations for the Phylum Annelida

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Polychaeta is a class of annelids that includes approximately 12000 species of aquatic worms. The vast majority of these species are typical of the marine environment, but some forms occupy brackish water or freshwater environments. They can be errants (crawling or pelagic) or sedentary (diggers, tube-dwellers or borers). However, for beginners it might be difficult to distinguish the worms habit from fresh materials, out of its habitat.

Clitellata comprises approximately 9000 representative species. It is divided into 2 large groups: Oligochaeta (with few chaetae or bristles) and Hirudinomorpha (no bristles). They have reduced cephalic sensory structures; body externally homogenous, except for the clitellum. All clitellates are simultaneous hermaphrodites. Most are represented by terrestrial or freshwater annelids, although there are also many marine species.

Sampling

After collection (by hand, grab/dredge/trawl, brushing), specimens are maintained in ambient seawater containers. At this step, specimens are identified with:

- sampling date
- station number
- name species / taxon
- “GENOME” label (to indicate that this specimen will follow the ATLASea cold chain)

After sampling (dredging, trawling and brushing), specimens can be in poor condition. They should be treated as soon as possible and maintained in cold seawater while waiting to be processed. Identification must be carried out only with a stereomicroscope due to the small size of the specimens, or even with a light microscope for certain microscopic morphological characters (diagnostic) used to reach the species level.Then, pay attention to not squash specimens when mounted on slides, by using modeling clay in the corners of the cover slip or excavated slide.

Photography

Ideally, pictures should be taken in the highest quality resolution (macro lens recommended) and where no voucher specimen parts are retained the pictures will serve as voucher and should include identifying features, although many require observations with a microscope to reach species identification.
Specimens should be photographed in a glass container with sea water and individually.
Water should be clean and changed between each specimen.

If possible, discuss with the taxonomist to find out the important morphological elements to see, and therefore to photograph.

Take dorsal picture of the whole specimen.

Take closer picture of the head, posterior part (if present), and appendices, unusual features or colored patterns.

With the specimen, one picture is taken with a **scale**, the **code identifier** (e.g. ATLASea QR code, specimen **MNHN-IA** barcode) and the station label.

Dissection for DNA barcoding and Genome Sequencing

1. Whenever feasible, organisms should be sampled and preserved while they are still **alive**.
2. For anesthesia use sparkling seawater, cooled specimen (at least 15 min in a fridge), or magnesium chloride in the container.
3. Rinse and brush specimen in filtered sea water (FSW) and dab on clean tissue paper to remove contaminants and any excess mucus.
4. For large specimens, dissect at least **10 pieces** (approx. **300-500 mg** each). Cut each piece into smaller fragments before putting them in separate tubes (with unique identification labels). Recommended tissues: **mid body, or lateral part of mid-body** without digestive tract contents, if possible (rinse tissues thoroughly with FSW). Cutting only one side of the mid-body allow to keep the information of the length and the number of segments.

a. Parapods containing setae must be removed. The worms are then cut in smaller pieces. If possible, the digestive tract should be removed and each piece rinsed.

b. For worms in tubes, cut the tube lengthwise with a scissors to extract the worm (which tends to hide at the bottom of the tube). If there is a crown of tentacles (used to capture food), remove it. When the worm is large enough, open it lengthwise from the oral end (protostomium) and remove the contents from the digestive tract. Rinse all tissues thoroughly with FSW to remove gut contaminants, and then cut the tissues into small pieces < 5mm.

5. For small individuals (<300mg), put one individual per tube.
6. Ensure that all tissues from the same individual are correctly identified in the log sheet.
7. Weigh the tubes and scan the barcode on the log sheet.
8. Tubes should be **flash-frozen** in liquid nitrogen, transferred to a dry shipper for transportation and stored in a -80°C freezer until used.

Backup/Biobanking:

1. Dissect at least 1 and up to 10 pieces in separate tubes (with unique identification labels).
2. 10 tubes by specimen.
3. Tubes should be flash-frozen in a liquid nitrogen.

Voucher & Taxonomic Assignation samples:

Voucher will be stored at MNHN.

1. Keep the leftover specimen, as many parts/tissues as possible (head, posterior part, parapodia,...) or another individual from the same population and checked by a taxonomist as belonging to the same species.
2. Place the barcode **MNHN-IA** identifier and the station label with the specimen in tube/container.
3. Put 75-80% ethanol in the tube/container. There must be at least 10 times the volume of specimen in alcohol.
4. Put the tube/container with the others specimens in the ATLASea barrels for shipment to the MNHN.