General recommendations for Mollusca Phylum

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Molluscs, the largest marine phylum, encompass approximately 23% of all known marine organisms. Distinguished by their diverse forms, molluscs exhibit a remarkable array of species, such as snails, slugs (Gastropoda), clams (Bivalvia), squids, octopuses (Cephalopoda), and other distinct subgroups that are often overlooked. While the majority of mollusc species inhabit oceanic environments, ranging from intertidal to the abyssal zone, a significant portion also contribute to freshwater fauna and terrestrial ecosystems.

Mollusk Class	Examples	Estimated Number of Living Species	Habitat
Gastropoda	Snails, slugs, abalone, conch, limpets, sea hares, and sea butterflies	70,000	land, sea, and freshwater
Bivalvia	Clams, oysters, scallops, and mussels	20,000	sea and freshwater
Polyplacophora	Chitons	1,000	sea
Cephalopoda	Squid, octopus, ammonites, nautiluses, and cuttlefish	900	sea
Scaphopoda	Tusk shells	500	sea
Aplacophora	Small deep sea marine mollusks that resemble worms	320	sea
Monoplacophora	Deep sea mollusks with a cap like shell	31	sea
Conchifera, Rostroconchia, and Helcionelloida	Extinct	Extinct	Extinct

Sampling

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

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

/!\ Several molluscs species (Bivalvia, Cephalopoda, Gasteropoda) are declared as CITES (Convention on International Trade in Endangered Species) and need special permits to be sampled.

Photography

Ideally, images 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.
Specimen can be photographed on the support or in a glass container sea water.
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.

Bivalvia
Take picture of both sides of the shell.
After opening for tissue dissection or after mission, take picture of the inside of the two valve.
Gasteropoda
Take picture of the opening side and from behind the shell. In a glass container with sea water, let the animal come out of the shell to get a photo of the body (pattern color,..)
Cephalopoda
Take picture of both side of the specimen
Polyplacophora/Chitons
Keep the specimen in the container.
Take picture of the specimen from above, plates visible (unfolded animal).

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

Dissection for DNA barcoding and Genome Sequencing

1. Rinse and brush specimen in filtered sea water (FSW).
2. Whenever feasible, organisms should be sampled and preserved while they are still **alive**.
3. Anesthezied specimen with sparkling seawater or 1% ethanol. The time necessary will depend on the size of the animal. For bivalves ethanol 1% is shown to be more efficient.
4. Isolate the individual:
    - For **Gasteropoda**, if the animal can not be removed from the shell after anesthesia, gently break the shell with a vice. For very small specimens break the shell by squashing with a spatula on a p
    - For **Bivalvia**, open the shell with a knife (or let it open the shell in FSW)
5. **Avoid gut**. Recommended tissues are listed below.

6. Dissect at least **10 pieces** (approx. **300 mg** each). Cut each piece into smaller before putting them in separate tubes (with unique identification labels).
7. Ensure that all tissues from the same individual are correctly identified on the log sheet.
8. Weight the tubes and scan the barcode on the log sheet.
9. Tubes should be flash-frozen in a liquid nitrogen charged dry shipper and stored in a -80°C freezer.

Recommended tissues to sample

Bivalvia

- Adductor muscle
- mantle
- muscle from foot

Gasteropoda

- muscle from foot
- muscle from siphon
- columellar muscle

Nudibranchs:

- foot
- mantle
- Avoid gonads and gut.

For **turbospiral groups**: Avoid upper whorls of the body, mostly composed of the tissues of the digestib\ve gland and gonad.

Polyplacophora/Chitons

- a section of the foot
- only flesh (when it can be extracted from the shell)

Cephalopoda

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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 storage at MNHN.

1. Keep every parts of the shell even if it's broken or another individual from the same population and checked by a taxonomist as belonging to the same species.
2. If there is leftover tissus, keep muscle form foot or mantle.
3. Place the barcode **MNHN-IM** identifier and the station label with the shell/tissus in tube/container.
4. If there is only shell left put 75-80% ethanol in the tube/container. Otherwise, put the shell and the tissus in 96% ethanol. There must be 10 times the volumes of specimen in alcohol.
5. Put the tube/container with the others specimens in the ATLASea barrels for shipment to the MNHN.