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9. Taxon Group: Gastropoda

In 2 collections

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Darwin Tree of Life

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Abstract

This is part of the **collection** "DToL Taxon-specific Standard Operating Procedures (SOPs) for Marine Metazoa", lead by the Other Metazoa Working Group. The SOP collection contains guidance on how to process the various marine Metazoa species within the scope of the Darwin Tree of Life project. The guidance specifically refers to the tissue samples needed for DNA barcoding (which takes place at the Natural History Museum (NHM) and at the Marine Biological Association (MBA)) and outlines the dissected tissues required for whole genome sequencing, which takes place at the Wellcome Sanger Institute. Every specimen is submitted for DNA barcoding first before potentially being sent to the Wellcome Sanger Institute.

Definition: The class Gastropoda contains a vast total of named species, second only to the insects in overall number. They comprise of snails, slugs and whelks from marine, freshwater, and terrestrial environments, and typically have a large muscular foot for movement as well as (in certain species) a single, asymmetrical, spiral shell.

Including: Specimens over 5mm; priority towards species with no current genome (as of SOP publication); Acmaiedae, Calliostomatidae, Lacunidae, Littorinidae. Hydrobiidae, Turritellidae, Aporrhaiidae, Capulidae, Calyptraeidae, Triviidae, Naticidae, Epitoniidae, Muricidae, Turridae. Opistobranchia, including Acteonidae, Philinidae, Tritoniidae, Dendronotidae, Dotoidae, Goniodorididae, Onchidorididae, Polyceridae, Archidorididae, Janolidae, Flabellinidae, Facelinidae, Aeolidiidae.

Excluding: Specimens under 5mm (eg. Rissoidae, Barleeidae). It is noted there are many gastropod species that measure under 5mm but they will not be appropriate for this SOP.

See the Guidelines for important details and checklists.

Acknowledgements

Thank you to **Jon Ablett** at the Natural History Museum for assisting in assembly of this SOP.

Guidelines

Field sampling:

1. Environment to be sampled: Marine/brackish.

2. Trap/method of sampling: Individual collection by hand, intertidally or by diving. Possible incidental capture by dredge/trawl/grab.

Where possible, it is recommended to keep specimens alive after collection in cool boxes/buckets containing 'habitat' seawater, and transferring to holding tanks of (running or aerated) natural seawater on return to the laboratory. Note that subtidally collected animals will be more sensitive than intertidal animals, so should be sampled for tissues first.

This SOP can also be used for freshwater/terrestrial gastropod species if appropriate.

Note

Each specimen, regardless of species, must have its own relevant unique identifier (e.g. QR code) which will be attached to any subsequent tubes, genome or barcoding results.

For genome sequencing:

3. Specimens can be sampled live and held in holding tanks back at the laboratory.

Note

Advice on anaesthesia

Animals can be reversibly anaesthetised using isotonic magnesium chloride (75g $MgCl_2L^{-1}$) in freshwater (preferably distilled). The animal is placed into a container with the solution (enough to cover it) and left in a quiet place where it will not be disturbed as it needs to come out of its shell and crawl around for the anaesthetic to work. It takes longer for large specimens (may take hours), less time for small specimens (may take minutes) and does not work at all for some taxa. It tends to work best on active animals, not slower ones (unless they lack an operculum). The anaesthetic should ideally be collected for reuse, though it will become diluted and thus less effective over time. Note the anaesthetic can be used to slow down fast organisms, or to relax specimens prior to fixation.

Photography:

4. Photograph dorsal and ventral side of whole organism

Depending on shape of shell (high-spired to low-spired to no-spired), take two or more of the following aspects: apertural (spire in profile, with head-on view of aperture); lateral (spire in profile, with shell rotated 90 degrees so plane of aperture is parallel to axis of view, outer lip of aperture central); apical (apex of shell viewed head-on, with axis of shell-spiral parallel to axis of view); basal (opposite to apical view, looking back along axis of shell from aperture end, with umbilicus head-on if present).

Photographs to be taken in situ before collection or in the laboratory after collection but prior to dissection, as for many species, shells need to be cracked to extract soft tissue.

Photographs of the soft tissue such as tentacles, mantle and siphon can be helpful in ID and should be taken wherever possible. It may be easiest to collect these images before relaxing the animal, although if this is proving difficult the animal can be relaxing in phenoxytol or MgCl2.

5. The image should be taken in the highest quality resolution -a macro lens is recommended. The photos should be of high enough resolution to be diagnostic, when possible.

Photograph to include a unique identifier (e.g. QR code, specimen barcode) where possible; where no voucher specimen parts are retained the photograph will serve as voucher and should include identifying features.

Note

Further tips

Light reflection of the shell can be an issue when photographing outside of the water. Be aware of the reflection showing up in the photo and how this may impact on the ability to see diagnostic features.

Nudibranch Photography

Photograph nudibranchs in a shallow dish or a narrow tank. A flash light is best, but must be aimed from the side or at 45 degrees to the front glass or water surface. A black background is best for most species; this can be rendered out of focus and unlit by placing it well away from the back of the tank or bottom of the dish. Two small flashguns give the best results. Use a small aperture (f16 or f22) and move the flash closer or further away to alter the exposure. Trial and error may be needed to get the right exposure; increasing the magnification will mean increasing the light by moving the flash(es) closer. Experiment with different distances and magnifications.

Dissection for DNA barcoding:

6. Pieces of non-digestive tissue are best used for DNA barcoding. A section of foot is usually easiest to obtain, or a tentacle if not retracted.

For nudibranchs, remove a small piece of the tail.

Once the tissue for barcoding is removed, put the tissue in 100% ethanol. The rest of the frozen/live organism can then be dissected.

Dissection for whole genome sequencing:

7. Use lentil-sized subsample of soma/ body (relatively undifferentiated). If possible, avoid the surface tissue to decrease likelihood of including contaminants. Avoid any digestive tissue. For spiralled individuals, the digestive tissues and gonadal tissue tends to be contained in the apex of the spiral, and can usually be easily dissected out.

Dissect up to ten, lentil-sized pieces in separate tubes if possible.

Tissue should be frozen at at least -80°, for example in dry ice, a liquid nitrogen charged dry shipper or in a -80° freezer.

Storage of frozen tissue:

8. If barcoded tissue passes the DNA barcoding stage, subsequent frozen tissue of specimen to be sent to Wellcome Sanger Institute.

Note

Please refer to **DNA barcoding SOP v2.1**.

9. Leftover tissue from specimens must be sent to NHM for vouchering and long term storage.

Storage of voucher:

10. Vouchers to be sent to/kept at NHM. Include shell (if applicable) and any remaining tissue. Ensure to not break the shell if possible; where the shell needs to be broken try to do this as cleanly as possible, and/or include an un-dissected individual of the same species for reference alongside the broken shell and remaining tissue.

11. Vouchered tissue to be eventually preserved in 70-90% ethanol.

Note

Tips on dissection without breaking the shell

A hooked needle is usually preferable for extracting animal from the shell. This can be difficult, especially with an individual that has not been relaxed. It is also possible to drill a hole through the shell a short distance around the whorl from the edge and poke a needle through the hole to push tissue out the operculum.

Specimens can also be microwaved and then the tissue extracted very easily, leaving the shell intact. However, the efficacy of the subsequent DNA sequencing is uncertain.

Photo guide below:

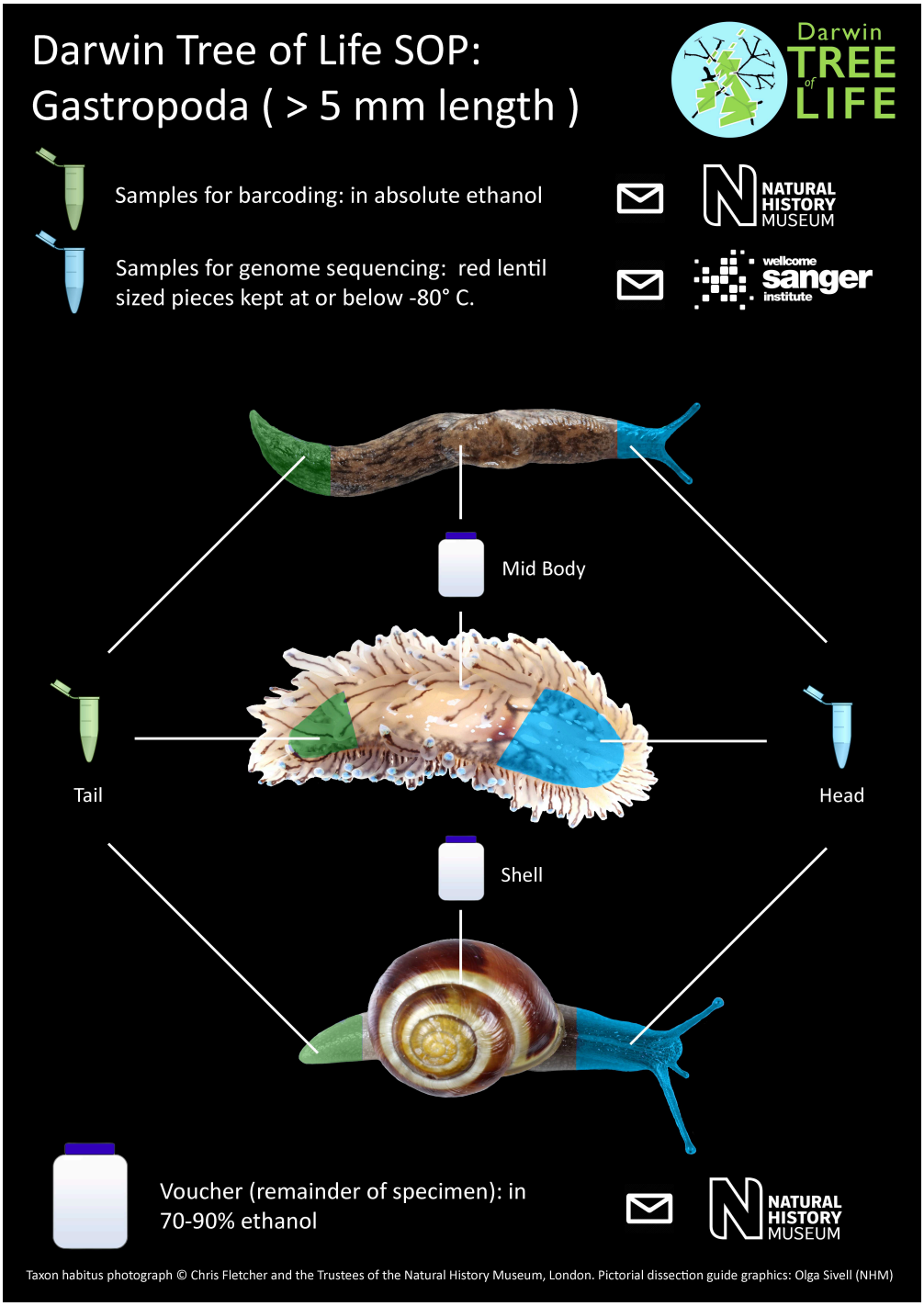

Photo guide assembly: Chris Fletcher

Troubleshooting

Protocol references

Further advice on nudibranch photography

http://www.seaslug.org.uk/nudibranchs/collect.html