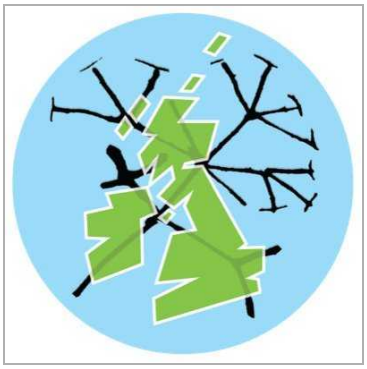

MAY 26, 2023

15. Taxon Group: Unitary Ascidiacea

In 2 collections

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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: Ascidiacea, commonly known as the ascidians or sea squirts, can be described as sac-like marine invertebrate filter feeders.They are characterized by a tough outer layer called a "tunic", in fact made of a polysaccharide. This SOP covers unitary or solitary ascidians, which form clumped communities attached at their bases.

Including: Families Cionidae, Ascidiidae, Corellidae, Pyuridae, Molgulidae, unitary Styelidae.

Excluding: Colonial Ascidiacea; see separate SOP in the complete SOP collection.

See the Guidelines for important details and checklists.

GUIDELINES

Field sampling:

1. Environment to be sampled: Marine and brackish.

2. Trap/method of sampling: Individual collection by hand, intertidally or by diving. Incidental capture by remote gear (dredge/trawl etc.) deployed for general collection

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Protocol status: Working
This is a working protocol that may be subject to changes in the future.

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across taxa.

For this phylum it is recommended they are photographed in situ before collection: see section on "Photography", point 4.

Only collect as many specimens as strictly necessary.

Specimens may suffer internal damage during removal from the substrate, which can severely limit their subsequent survival – extreme care needed. If feasible, consider collecting complete with substrate (e.g. shell) without damaging the habitat.

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 and frozen whilst still alive.

After collection, specimens should be kept alive in aerated and/or stirred/running seawater, and are likely to require processing within a day or so to avoid deterioration, particularly if damaged. A few hours delay may be beneficial in allowing guts to clear. Sublittoral specimens collected by diving are likely to deteriorate faster, thus may require prompt attention when returning to surface.

Photography:

4. Photo whole colony first in situ and, ideally, undisturbed before collection, plus close-ups. Note the muscular contraction when disturbed obscures features.

Before preservation also photograph additional surface detail of submerged specimen. Internal features (e.g. of branchial basket) are valuable and can be photographed during dissection.

For post-collection photography, specimens can be narcotized using 0.1 % v/v Propylene Phenoxetol in sea water; for many species, individuals will become more expanded as a result, with siphons remaining open during handling, allowing more detail to be documented and facilitating dissection.

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; when no voucher specimen parts are retained the photograph will serve as voucher and should include identifying features.

Dissection for DNA barcoding:

6. Prior narcotisation with 0.1% Propylene phenoxetol in seawater is a humane precaution and aids dissection by preventing muscular contraction. Specimen then to be removed from tunic and opened up by cutting down from inhalant siphon along ventral midline (see photo guide; bottom of this SOP). Cerebral ganglion (between siphons, generally a white blob) to be obliterated as humane precaution. Avoid damaging gut (likely to be silt-filled). Depending on species and condition, tissue sampled for barcoding and can be branchial basket (discard mucous feeding net if present), mantle, or siphon. Avoid collection of gut, digestive gland, tunic.

In large-bodied species with storage gonoducts (e.g. Ascidiidae, Ciona) it may be possible to collect substantial quantities of sperm from the sperm duct (white, alongside rectum); if intending to use sperm from duct, maintain specimens in constant light to prevent spawning.

Once the tissue for barcoding is removed, that tissue should be placed in 100% ethanol. The rest of the frozen/live organism can then be dissected. Less tissue is required for barcoding in comparison to whole genome.

Dissection for whole genome sequencing:

7. The same tissues recommended for DNA barcoding can be used.

Dissect tissue up to ten, lentil-sized (5mm) 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.

Note individual size varies very widely, depending on species/specimen.

Note

Most unitary ascidians spawn eggs and sperm, but some (e.g. *Corella eumyota, Dendrodoa grossularia, Asterocarpa humilis* and a few other styelids) brood their sexual (generally outcrossed) progeny in the atrial cavity. Avoid these progeny when gathering tissue for sequencing.

Parasitic copepods are frequent, particularly in the branchial basket.

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 and kept at NHM.

11. Vouchered tissue to be preserved in 70-90% ethanol. 80% ethanol concentration is recommended for soft tissued organisms.

Note

Ethanol versus formalin as a preservative

If facilities allow and there is sufficient material, initial fixation of a proportion of the voucher material in buffered formalin followed by thorough rinsing (tap water) and transfer to ethanol long-term is ideal to optimise anatomical detail. However, DNA analysis will be compromised by formalin fixation. It is recommended to undertake both options if possible.

Some species have calcareous spicules that may dissolve if preservative acidifies (e.g.formalin) or in RNAlater.

Photo guide below:

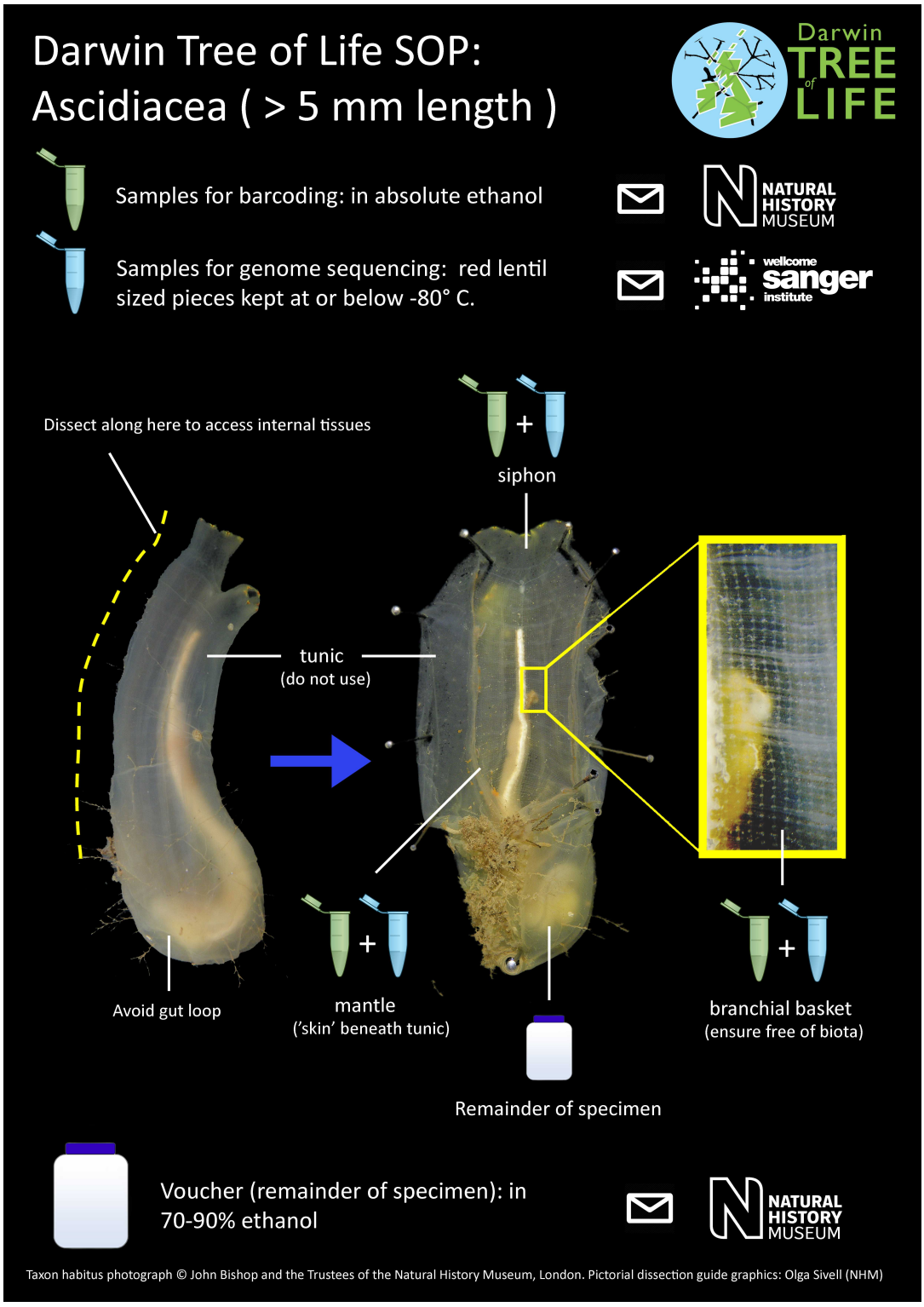

Photo guide assembly: Chris Fletcher