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6.Taxon Group: Colonial Ascidiacea

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: Colonial ascidians are when multiple individual ascidians live and interact closely with each other. Each individual in the colony has its own intake siphon for feeding, but several individuals may combine to share one exit siphon.

Including: Families Didemnidae, Polyclinidae, Polycitoridae, Holozoidae,Clavelinidae, Perophoridae, Diazonidae, colonial Styelidae

Excluding: Unitary (= 'solitary, non-colonial) Ascidiacea; for this, see separate SOP in the **collection**.

See the Guidelines for important details and checklist.

Guidelines

Field sampling:

1. Environment to be sampled: Marine and brackish

2. Trap/method of sampling: Collection of individual colonies by hand, intertidally or by diving; incidental capture by
remote gear (dredge/trawl etc.) deployed for general collection across taxa.

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. 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 (less time if damaged). A few hours' delay may be beneficial in allowing guts to clear. Sublittoral specimens collected by diving are likely to deteriorate faster, and may require prompt attention on surface.

Note

Definition of "colony" in this instance

Ideally a colony is a group of physically connected individuals (= zooids) derived by budding from a single founding individual, and thus sharing a single genotype. Replicate samples for whole genome sequencing can thus be taken from the same colony. If colonies are too small to yield sufficient replicates, more than one colony can be sampled but must be treated as different specimens (i.e. different genetic individuals). Only collect the whole colony if necessary; otherwise leave remainder undamaged. Note any presence of visible brooded embryos and potential contaminating species, and avoid if possible.

Photography:

4. Photograph whole colony in situ and, ideally, undisturbed before collection, including any numbered containers; plus close-up. Any muscular contraction when disturbed obscures features. Before preservation, also photograph additional surface details of submerged specimen, plus zooidal details if possible

Note

For post-collection photography, e.g. during processing (and as a humane precaution), colonies can be narcotized using 0.1 % v/v Propylene Phenoxetol in sea water. For many species, zooids will be less contracted as a result, with more open branchial baskets allowing more detail to be documented.

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. The sample for barcoding (smaller than required for whole genome sequencing) is removed and put in 100% ethanol. For small-zooid species (e.g. Didemnidae, Pycnoclavella spp.) part of a colony containing a few of the modular individuals (=zooids) may be necessary; the number of zooids required will depend on zooid size. For larger-zooid species (e.g. Clavelina, Stolonica, Diazona) a single or partial zooid should suffice, avoiding the tunic and gut.

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

Dissection for Whole Genome Sequencing:

7. If colonies are too small to yield sufficient replicates, more than one colony can be sampled, but different colonies must be treated as separate specimens (i.e. different genetic individuals).

The number of zooids required will depend on zooid size. For larger-zooid species (e.g. Clavelina, Stolonica, Diazona) a single whole or partial zooid should suffice, avoiding tunic, brooded progeny, and (if possible) gut.

Each sample must be frozen with minimum delay at -80°C or colder.

Taking ten samples per colony (specimen) is feasible for many species. Colony size varies very widely, depending on species/example.

Note

Additional information regarding colonial Ascidiacea whole genome sequencing

Most colonial ascidians brood sexual (outcrossed) progeny scattered throughout the colony, commonly associated with the zooids but found in the colonial tunic in some species. Samples lacking these embryos are required for whole genome sequencing.

In species in which zooids arise from runner- like stolons (e.g. Perophora spp.), different colonies may intermingle, so each sampled specimen should ideally comprise one physically continuous piece.

Colonies may be chimaeric following fusion with conspecific neighbours, sometimes discernible by the presence patches of different colouration (e.g.in Botryllus and Botrylloides).

Relatively isolated colonies with uniform colour are thus preferable.

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. 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 subsequent transfer to ethanol long-term is ideal to preserve the anatomical detail (although DNA analysis will be compromised by formalin fixation).

Otherwise, vouchered tissue to be directly preserved in 70 - 80% ethanol.

Note

Some species have calcareous spicules which may dissolve if preservative acidifies (e.g. formalin) or in RNA later.

Troubleshooting