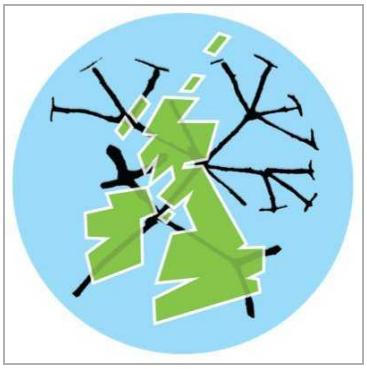

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Protocol status: Working
This is a working protocol that may be subject to changes in the future.

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3. Taxon Group: Bryozoa

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Darwin Tree of Life

Inez Januszczak

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: Bryozoa are a phylum of simple, aquatic inverterbrate animals, nearly all living in sedentary colonies.

Including: Cyclostomatida, Ctenostomatida, Cheilostomatida

Excluding: Phylactolaemata

See the Guidelines for important details and checklists.

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GUIDELINES

Field sampling:

1. Environment to be sampled: Marine and brackish.

2. Trap/method of sampling: Collection of individual colonies by hand, intertidally or

Keywords: Bryozoa, whole genome sequencing, DNA barcoding, Darwin Tree of Life Project, Wellcome Sanger Institute, Natural History Museum, Marine Biological Association, Cyclostomatida, Ctenostomatida, Cheilostomatida, SOP, Standard Operating Procedure

by diving; incidental capture by remote gear (dredge/trawl etc.) deployed for general collection across taxa.

Where possible, photograph in situ before collection.

Keep specimens alive after collection in cool boxes/buckets containing water from the habitat and transfer to holding tanks of running or aerated (sea)water on return to the laboratory.

Note that (ideally) a bryozoan colony is a group of physically connected individuals (= zooids) derived by budding from a single founding individual, and thus all zooids share a single genotype (but see section on dissection for additional comments). The colony is thus the 'specimen' (= genetic individual).

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 frozen and transported alive depending on the holding facilities.

Note

Even when kept in holding tanks, specimens will deteriorate, so sample preservation should occur as soon as possible (at most a few days).

Photography:

4. Photographs should be taken of the general colony form (lower magnification) plus higher magnification images of: frontal surface - encrusting colonies; frontal and abfrontal ('back') surface - erect colonies, ideally including shots at sufficient magnification for some characteristics of individual zooids to be resolved.

Images should be taken when specimens are live and prior to tissue dissection for sequencing. Colonies should be submerged. Relaxation/narcotization of bryozoan colonies is not recommended as the lophophores (tentacle rings) are then likely to be everted and will obscure important details of the zooidal orifice and frontal

surface.

Additional images of calcified bryozoans (i.e. excluding ctenostomes) should be taken with a scanning electron microscope (SEM) to enable accurate identification. This requires taking an additional sample of the specimen, ideally including key features such as ovicells, avicularia etc. if present This sample can be preserved in 100% ethanol until ready for SEM preparation.

To prepare the sample for SEM, remove any epibionts and bleach to remove organic material (10% solution – domestic bleach is fine), rinse in distilled water, air dry and transfer onto a carbon tab fixed to a specimen stub. Bleaching requires frequent monitoring to ensure that the specimen remains intact (especially lightly calcified and erect, jointed taxa).

Note

Whilst preparing the specimen for SEM, some of material can be left aside to act as the specimen voucher - see points 8 and 9.

Dissection for DNA barcoding:

5. A sample for barcoding (smaller than required for whole genome sequencing) is removed and put in 100% ethanol. Part of the colony containing just a few zooids is likely to be sufficient.

Dissection for Whole Genome Sequencing:

6. A bryozoan colony is a group of physically connected individuals (= zooids) derived by budding from a single founding individual, and thus all sharing the same genotype (but see Section 2). Replicate samples of zooids 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 different colonies must be treated as separate specimens (i.e. different genetic individuals).

Individual zooid bodies (polypides) cannot generally be removed from the colony matrix, so samples are pieces of colony including polypides plus the surrounding ‘skeletal’ material (calcareous, membranous, gelatinous, or chitinous). DNA yields will depend on density and size of zooids, so the ideal size of tissue per sample is difficult to prescribe.

Note

As a guide, sections of encrusting colonies might be 5-10mm square, comprising several tens of zooids. Ten samples per colony (specimen) is feasible for most species.

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

Surface contamination (including remains of substrate) and attached epibiotic organisms must be removed from the sample surface using fine forceps and brushes.

Live zooids must predominate within the colony sections sampled; moribund or senescent ‘empty’ zooids may be frequent in colonies, and often have just a dark speck – the ‘brown body’, a product of zooid regression – in the polypide space.

It is recommended to dissect tissue from the distal (youngest) parts of a colony, that is towards colony margins (if encrusting) or towards branch tips (if erect); these regions are the youngest and most active, are generally not yet brooding, and may have a lower level of external colonization by other species.

Note

Additional information regarding Bryozoan whole genome sequencing (life cycle related)

Ensuring tissue samples from a colony contain a single genotype may be difficult.

Most bryozoans brood sexual progeny (thus of genotype different from the parent colony) in zooids scattered throughout the colony. Samples *lacking* these embryos are required for whole genome sequencing. This necessitates avoiding occupied ovicells or other brooding structures (including the large gonozooids of cyclostomes), or conceivably removing the embryos mechanically. If complete removal is not possible (e.g in species with internally brooded larvae or those with immersed ovicells), the probable presence of embryos or larvae should be flagged on the species list and the manifest.

Particularly in species in which zooids arise from runner-like stolons, different colonies may intermingle, so each sampled specimen should ideally comprise one physically continuous piece.

Colonies may be chimaeric following fusion of conspecific neighbours, the coalescence often being recorded in the convergent pattern of zooidal budding, particularly in calcified forms.

Colony size varies very widely, depending on species/example.

Storage of frozen tissue:

7. 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.

The use RNAlater (or any reagent containing EDTA) is not recommended with bryozoans. Calcareous skeletons are dissolved, destroying taxonomic characters and making any subsequent handling very difficult.

Storage of voucher:

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

9. Vouchered tissue preserved in 100% ethanol.

Note

It can also be advised that voucher material is prepared during the processing for DNA barcoding - if possible, preserve a piece into bleach and mount it on a carbon tab for downstream SEMing.

Bryozoan SEM stubs (dry) can also acts as a voucher.

Unlike the case for samples destined for whole genome sequencing, voucher material should include brooding structures if possible, because of their taxonomic value.