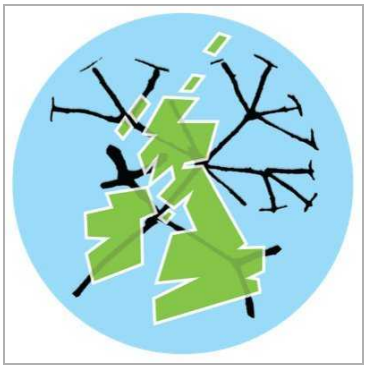

MAY 11, 2023

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

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1. Taxon Group: Anthozoa

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ABSTRACT

This is part of the collection "DToL Taxon-specific Standard Operating Procedure (SOP) 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: Anthozoa are a class of marine coelenterates comprising the corals, sea anemones, and related forms all of which lack medusa generation. They are distinguished by polyps with radial partitions or mesenteries projecting from the body wall into the gastrovascular cavity.

Including: Specimens larger than 5mm.

Excluding: Specimens smaller than 5mm.

See the Guidelines for important details and checklist.

GUIDELINES

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: Both bulk capture / single specimen targeted

Both methods of sampling may be used. Where possible, it is recommended to keep specimens alive after collection in cool boxes/buckets containing ‘habitat’ seawater and transfer to holding tanks of (running or aerated) natural seawater on return to the laboratory.

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 for up to 10 hours.

Note

If kept alive in holding tanks, specimens can be sampled live. However, specimens will deteriorate even in these conditions so sampling should occur as soon as possible. In situations where such facilities are not available (e.g. when at sea), there may be considerable time interval between collection and sampling whilst bulk haul is sorted.

Photography:

4. Oral and lateral views (higher magnification); additionally for colonials, image of gross colony morphology (lower magnification).

The image should be taken in the highest quality resolution - macro lens recommended.

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

Oral and lateral views (higher magnification); additionally for colonials, image of gross colony morphology (lower magnification).

it is recommended to photograph live prior to dissection, in situ and ex situ where possible. Relaxation may be useful for some taxa (menthol or MgCl2). For some Alcyonacea (e.g. Alcyonium) and Scleractinia (e.g. Caryophylla) with hard skeletons, examination of sclerites/corallum are/is needed for ID.

Dissection for barcoding:

5. Recommended tissues for DNA barcoding are outlined in the Notes below.

Note

Actiniaria (solitary): individual tentacle or piece of column tissue.
Alcyonacea (colonial): section of polyp tissue ('small amount') – dependent on polyp size. Blot to remove excess seawater and mucus.
Corallimorpharia (Corynactis; solitary but clonal): tentacle(s) or piece of column tissue, size dependent.
Ceriantharia (solitary): individual tentacle or piece of column tissue.
Pennatulacea (colonial): section of polyp or polyp leaf.
Scleractinaria (solitary or colonial): polyp tissue (for barcoding), larger specimens – tissue from mesentery (section of gastrovascular cavity); small specimens – 'entire sector' including skeleton submerged in lysis buffer).
Zoantharia (colonial): polyp tissue (suggestion).

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

For colonial taxa, ensure sampling of an individual colony. Remove all visible contaminants and epibionts, including any substrate, though gut contents and symbionts will remain.

Sample preparation should take place over dry ice, with sterile tools. A fresh scalpel blade should be used per specimen.

Dissection for Whole Genome Sequencing:

6. Specimens must must be sampled and frozen while still alive. The tissue for whole genome sequencing is removed, and immediately frozen on dry ice (-80). Refer to above notes for more detail on tissues that can be used.

The organism should be dissected into 5mm chunks.

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.

Storage of voucher:

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

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