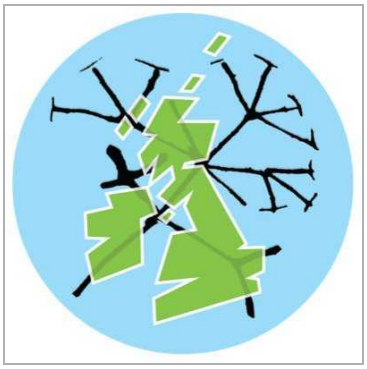

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Protocol status: Working
This is a working protocol that may be subject to changes in the future.

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12. Taxon Group: Porifera

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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 phylum Porifera, also known as sponges, are multicellular organisms that consist of bodies with pores and channels. These allow water to circulate through them; the water deposits nutrients and then leaves through a hole called the osculum, an excretory structure in the living sponge.

Including: Calcarea, Homoscleromorpha, marine Demospongiae.

Excluding: Freshwater species; Hexactinellida.

See the Guidelines for important details and checklists.

Acknowledgements
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GUIDELINES

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: Targeted collection of individual specimens by hand is

Keywords: porifera, DNA barcoding, whole genome sequencing, SOP, Standard Operating Procedure, Natural History Museum, Marine Biological Association, Wellcome Sanger Institute, sponges

ideal, intertidally or by diving. Also
capture by dredge/trawl deployed for general cross-taxon collection, although this carries risk of spicule cross-contamination and species' undisturbed appearance will not be recorded.

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

Use separate container per specimen to avoid spicule cross-contamination. Collect whole thickness, ensuring sample includes surface layer including the complete range of spicules represented. Note smell, consistency, production of mucus when damaged, presence of visible brooded embryos and potential contaminating species. For thin crusts, include substrate if possible to ensure basal layer of sponge collected. Only collect whole sponge if necessary; otherwise leave remainder undamaged.

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 in aerated and/or stirred/running seawater and are likely to require processing within a few hours to avoid deterioration, particularly if damaged.

Photography:

4. Photograph whole growth in situ before collection, plus close-ups. The sponge should not be dry; either photographed underwater in situ, fully immersed, or fully out of water but wet.

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. A piece of the soma/body (which is relatively undifferentiated) can be used for DNA barcoding.

Note that tissue for spicule preparation will also be required for specimen vouchering (see "Storage of voucher", points 10 and 11).

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.

Dissection for whole genome sequencing:

7. A piece of the soma/body (which is relatively undifferentiated) can also be used for whole genome sequencing.

Avoid inclusion of the sponge's external surface if possible, to reduce the likelihood of including contaminants.

Many sponges brood sexual (outcrossed) progeny scattered throughout the soma. Regions lacking these embryos are required for whole genome sequencing.

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

Collecting ten samples is feasible for many species. Thin crusts are likely to yield fewer samples.

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.

Reference material should ideally include a cleared section of the whole thickness and/or a spicule extraction slide-mounted or kept in ethanol (or this can be prepared from ethanol- preserved voucher material some time later).

Note

Spicules are structural elements found in most sponge species. Meshed spicules serve as the sponge's skeleton and provide structural support/ potential defense against predators.

If a spicule preparation is made and examined, inclusion of a photomicrograph of the spicules with the other images of the specimen is useful.

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