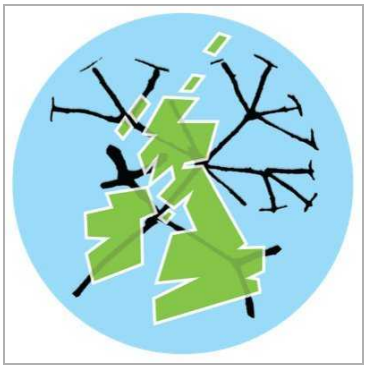

MAY 17, 2023

5. Taxon Group: Chitons

In 1 collection

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Darwin Tree of Life

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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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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: Chitons are marine molluscs of varying size in the class Polyplacophora, formerly known as Amphineura.

Including: Polyplacophora

Excluding: Specimens smaller than 5mm; non - UK listed species.

See the Guidelines for important details and checklists.

GUIDELINES

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: By hand, in the intertidal or subtidal zone of rocky or sandy shores, or as bycatch from sediment grabs etc. Although many species are intertidal, some exist more commonly in deeper waters. Most chitons prefer to attach to rocks, and so inspecting rocks is a recommended method of collection.

Keywords: Chiton, Chitons, SOP, Standard operating procedure, DToL, Darwin Tree of Life, Wellcome Sanger Institute, Natural History Museum, Marine Biological Association, Other Metazoa, whole genome sequencing, DNA barcoding, Amphineura

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 live and held in holding tanks, if available.

Photography:

4. Chitons can be easily damaged whilst removing them from rocks; ensure to photograph the ventral side first before removal.

After removal the chiton should be placed in phenoxytol. The chiton may curl up initially, but should relax into an open state and then the dorsal side can be photographed.

Note

Chiton gills are an important identification feature for some species, so ensure to get a clear photograph of the number of gills present. Features on the top of the shell should also be clearly visible for identification purposes. Additional close ups of these features can be taken if they are difficult to see in the initial ventral photo.

Setting up a small natural aquarium or environment can be useful for photography.

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

Dissection for barcoding:

5. A small section of the foot is recommended to use for barcoding.

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

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). If the animal is sufficiently large, dissect out any gut or gonad tissue before processing the remaining tissue.

The organism should be dissected into 5mm chunks.

Up to ten pieces in separate tubes.

Storage of frozen tissue:

7. If barcoded tissue passed 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.

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

Storage of voucher:

9. Vouchers to be sent to NHM.

10. Vouchered tissue preserved in 70-90% ethanol.

Note

Voucher specimen should have gills and girdle preserved - it is recommended to leave half the specimen intact.