May 19, 2023 Version 1

8. Taxon Group: Echinodermata V.1

DOI
dx.doi.org/10.17504/protocols.io.4r3l27d63g1y/v1

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Darwin Tree of Life

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Protocol Citation: Kesella Scott-Somme, Chris Fletcher, Inez Januszczak 2023. 8. Taxon Group: Echinodermata. **protocols.io https://dx.doi.org/10.17504/protocols.io.4r3l27d63g1y/v1**

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

Created: May 17, 2023

Last Modified: May 19, 2023

Protocol Integer ID: 82019

Keywords: Echinodermata, Echinoderms, Darwin Tree of Life Project, Wellcome Sanger Institute, Natural History Museum, Whole genome sequencing, DNA barcoding, Standard Operating Procedure, SOP, dissection, echinodermata, marine biological association, echinodermata adult, marine metazoa, various marine metazoa species within the scope, various marine metazoa species, dna barcoding, taxon group, echinoidea, dtol taxon, darwin tree of life project, aforementioned asteroidea, whole genome sequencing, species, tissue sample, darwin tree, dna, clypeasteroida, holothuroidea, sea cucumber, asteroidea, specimen, other metazoa working group, sop collection, marine, sea lily

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: Within Echinodermata adults are recognisable by their (usually five-point) radial symmetry and include starfish (Asteroidea), brittle stars (Ophiuroidea), sea urchins (Echinoidea), sand dollars (Clypeasteroida) and sea cucumbers (Holothuroidea), as well as the sea lilies or "stone lilies" (Crinoidea).

Including: Aforementioned Asteroidea, Ophiuroidea, Echinoidea, Clypeasteroida, Holothuroidea and Crinoidea,

Excluding: Species smaller than 5mm.

See the Guidelines for important details and checklists.

Guidelines

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: individual collection by hand, intertidally or by diving. Also capture by ship-towed gear (dredge/trawl etc.) possible.

Do not collect more than necessary.

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.

Narcotisation prior to dissection should be considered as a humane precaution, to dislodge associated organisms, and (ophiuroids) to prevent autotomy of arms. Propylene phenoxetol (0.1% in seawater), or Magnesium chloride (isotonic solution mixed 50:50 with seawater) or menthol crystals (allowed to dissolve in lidded specimen container) can be used.

Photography:

4. For UK species:

Oral and aboral views of whole specimen plus the Spicules if collected for ID.

Note

Regarding the different classes:

Asteriodea
Arrangement and structure of tube feet should be visible.
Close up of mouth plates.

Crinoidea
Close up of pinnule with gonad visible.

Echinoidea
Side view showing shape of test.
Close up of mouth.

Holothuroidea
Tube feet arrangement and form (if present).
Tentacles (if possible).
Ventral sole (if present).
Calcareous deposits.

Ophiuroidea
Close-ups of disc and arm bases.
Close ups of mouthparts with teeth and any papillae present.

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.

Note

Further photography tips

All echinoderms should ideally be relaxed for some time prior to photography, using magnesium chloride or similar. This is particularly the case for Holothuroidea (sea cucumbers), as without relaxing it may be difficult to get a photograph of the tentacles. If you are unable to get a photograph of the tentacles before processing, it is possible to dissect out the tentacles for photography, although they will still be in a retracted form. Faster moving Ophiuroides (brittle stars) may need to be relaxed for longer to ensure they are still enough to photograph, this also helps to open up the mouth parts for photography.

Spicules are a good identification feature for many echinoderms. These should be visible under a compound microscope if you dissect out a small amount of skin. You can soak the tissue in bleach for a short amount of time if needed.

Dissection for DNA barcoding:

6. The following tissues have been used as sources of DNA for DNA barcoding and whole genome if required.

Note

Asteroidea: Gonad or tube feet. Intact males may spawn substantial quantities of sperm; induction by injection with KCl if necessary.

Crinoidea: Cirrus or section of arm.

Echinoidea: Tube feet preferable, some species have little tissue so you may need to take a section of the test. Gonads could also be used.

Holothuroidea: Section of tentacle, tube feet, or section of body wall.

Ophiuroidea: Gonad; tube feet, generally as whole sections of arm—outer portion of arm sometimes specified.

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. Many echinoderms have less tissue due to the presence of spicules (sharp pointed structures), and so dissecting out 5mm sized sections may be challenging.

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

Note

Recommended tissues to sample

Asteroidea
Arm, or section of arm. Dissect slightly away from the body to ensure the arrangement of arms can still be recognised, and leave at least one arm attached to the main body if leaving a voucher.

Gonads can be present in the arm, so dissect out any gonadal tissue before processing (unless you can ensure it is unfertilized). Avoid the central disk as this is where the stomach contents will be, unless it is a large enough specimen to remove the stomach.

Generally large-bodied, c.10 samples per specimen probable.

Photo guide available for Asteroidea.

Crinoidea
Arms are easiest to dissect, particularly from still living specimens, for larger specimens you may be able to use a section of arm.

Usually less tissue can be removed, estimated a maximum of five samples for most specimens.

Echinoidea
The majority of tissue inside the test is stomach, intestine or gonad, all of which are best avoided for whole genome sequencing (unless you can be sure the reproductive tissues are unfertilized). Therefore, sections of the test itself (minus spines if possible) is best for processing.

Amount of tissue depends on overall body size; most species can get fairly large and so should be possible to have up to 10 samples per specimen.

Holothuroidea
Tentacle, retractor muscles, tube feet, or section of body wall to be used.

The body wall typically has thick sections of tissue, so if the specimen is of decent size, 10 samples should be possible.

Ophiuroidea
Gonad; tube feet, generally as whole sections of arm—outer portion of arm sometimes specified.

Usually less tissue can be removed, estimated a maximum of five samples for most specimens.

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.

Regarding tissues to retain for the voucher specimen; for relevant species species the mouth and arms are needed for identification. Keeping one arm attached to oral disk and taking other arms for sequencing is the easiest and quickest way to process. For Echinoidea the mouth with a section of the test should be sufficient.

Photo guide: Asteroidea

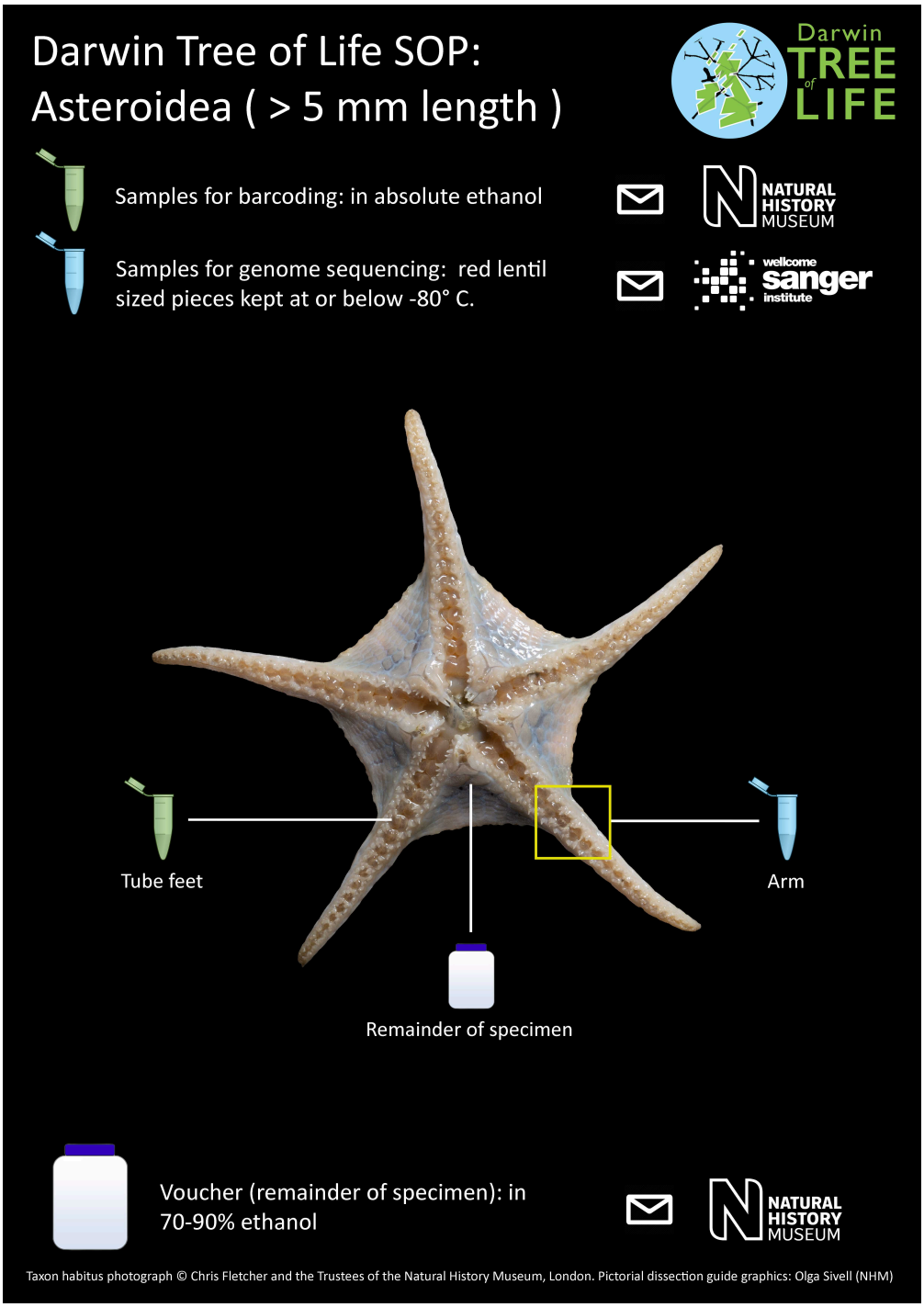

Photo guide assembly: Chris Fletcher

Troubleshooting