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14. Taxon Group: Sipuncula

In 2 collections

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Darwin Tree of Life

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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: Sipunculids, also known as peanut worms, are an unsegmented, marine, annelid worms. The body is divided into a bulbous trunk and a narrower, anterior section, called the "introvert", which can be retracted into the trunk. Although found in a range of habitats throughout marine environments, the majority of species live in shallow water habitats.

Including: Species/specimens larger than 5mm.

Excluding: Specimens 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: Live-caught specimens, through single target or bulk capture.

This may be done on shore by hand/net, sub tidally by divers/snorkelers by hand or net or from a vessel using appropriate capture methods e.g. dredge/trawl/grab.

Bulk habitat/substrate may also be collected where appropriate and then tray sorted.

Often, species can be identified live when anaesthetised (use appropriate relaxants for specific taxa). Live specimens can also be put temporarily in the fridge to help slow them down during identification.

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. For most taxa, specimens can be sampled and frozen live. Some species can only be identified to species level by dissection and may require euthanasia first.

Some taxa can be very difficult to dissect when frozen. Fast and accurate dissection on ice and flash freezing of tissue as soon as possible may be most appropriate for some species.

Photography:

4. Photograph the whole animal, ideally with its introvert fully extended. The animal may be relaxed or dissected to allow this to be seen.

Photograph close ups of the mouth and the introvert hooks (or lack of), as well as any other important external features specific to that taxa, i.e. the papillae on the surface of *Phascolosoma*.

For taxa where internal diagnostics are important dissect to reveal them and photograph in the same way, whole animal (showing all the internals) then close up of features, i.e. the contractile vessel of *Thysanocardia procera.*

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. For barcoding; use retractor muscles if possible, use the bodywall if not.

Rinse tissue in clean water to remove contaminants.

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

Note

This is a fairly difficult group to barcode. see paper below for some more details.

Schulze, A., Cutler, E.B. and Giribet, G., 2007. Phylogeny of sipunculan worms: A combined analysis of four gene regions and morphology. Molecular Phylogenetics and Evolution, 42(1), pp.171-192.

Dissection for whole genome sequencing:

7. For whole genome sequencing; use the bodywall, leaving internal features and introvert intact for vouchering purposes.

Rinse tissue in clean water to remove contaminants.

Dissect tissue up to ten, lentil-sized 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.

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.

11. Vouchered tissue to be preserved in 80% ethanol.

Note

The voucher should consist of the introvert and the internal features, plus any remaining tissue left after removal of tissue for barcoding and whole genome sequencing.

For some taxa specific external features should be kept if they are important for identification.

Note

Ensure the voucher container is kept with suitable label and information, mainly:

Specimen code
Species name
Collector
Identifier
Site
Date of collection

This should be on paper suitable for the long term storage of specimens e.g. resistall paper.