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11. Taxon Group: Polychaeta

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: Polychaetes are a paraphyletic class of generally marine annelid worms. Each body segment has a pair of protrusions called parapodia that bear many bristles made of chitin (called chaetae). More than 10,000 species are described in this class.

Including: All species/specimens larger than 5mm.

Excluding: Specimens smaller than 5mm.

See the Guidelines for important details and checklists.

Acknowledgements
Thank you to **Emma Sherlock** and Lenka Nealova at the Natural History Museum for reviewing this SOP.

Guidelines

Field sampling:

1. Environment to be sampled: Marine and brackish.

2. Trap/method of sampling: Specimens caught for genome sequencing will be live caught; single target or bulk capture (up to 10 specimens of the same species collected if possible).

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

Light traps may also be appropriate for some taxa.

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. Specimens can be sampled and frozen whilst still alive. 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 worm dorsally and ventrally. Close ups should include the head and other taxonomically important features for example the parapodia, elytra and operculum. Every species will have its own identifying features so these should be captured appropriately

For species in the subclass Sedentaria photograph the tube they are dwelling in if appropriate.

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. The mid body without the gut should be used for barcoding. Rinse in clean water and dab on clean tissue to remove gut contaminants and any excess mucus.

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. The mid body without the gut can also be used for whole genome sequencing. Rinse in clean water and dab on clean tissue to remove gut contaminants and any excess mucus.

Note

For large specimens with well developed parapodes - a few parapodes would be sufficient instead of a section of the mid body. For Sedentaria specimens; where there are a lot of tentacles present, those can also be used for whole genome instead of the whole body, which can be preserved more towards further morphological study (as a voucher or otherwise).

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

Further vouchering advice

The specimen head should be kept ensuring that all features (such as gills, tentacles, antennae, palps) are intact. Keep the posterior section intact.

Retained sections should have enough segments included to contain morphological representatives of gills, parapodia and chaetae.

Keep representative elytra for polynoids.

For Sedentaria keep the tube that the specimen was found in if possible.

If possible, keep an intact representative of the species collected from the same place at the same time to have alongside the dissected voucher specimen.

Photo guide below:

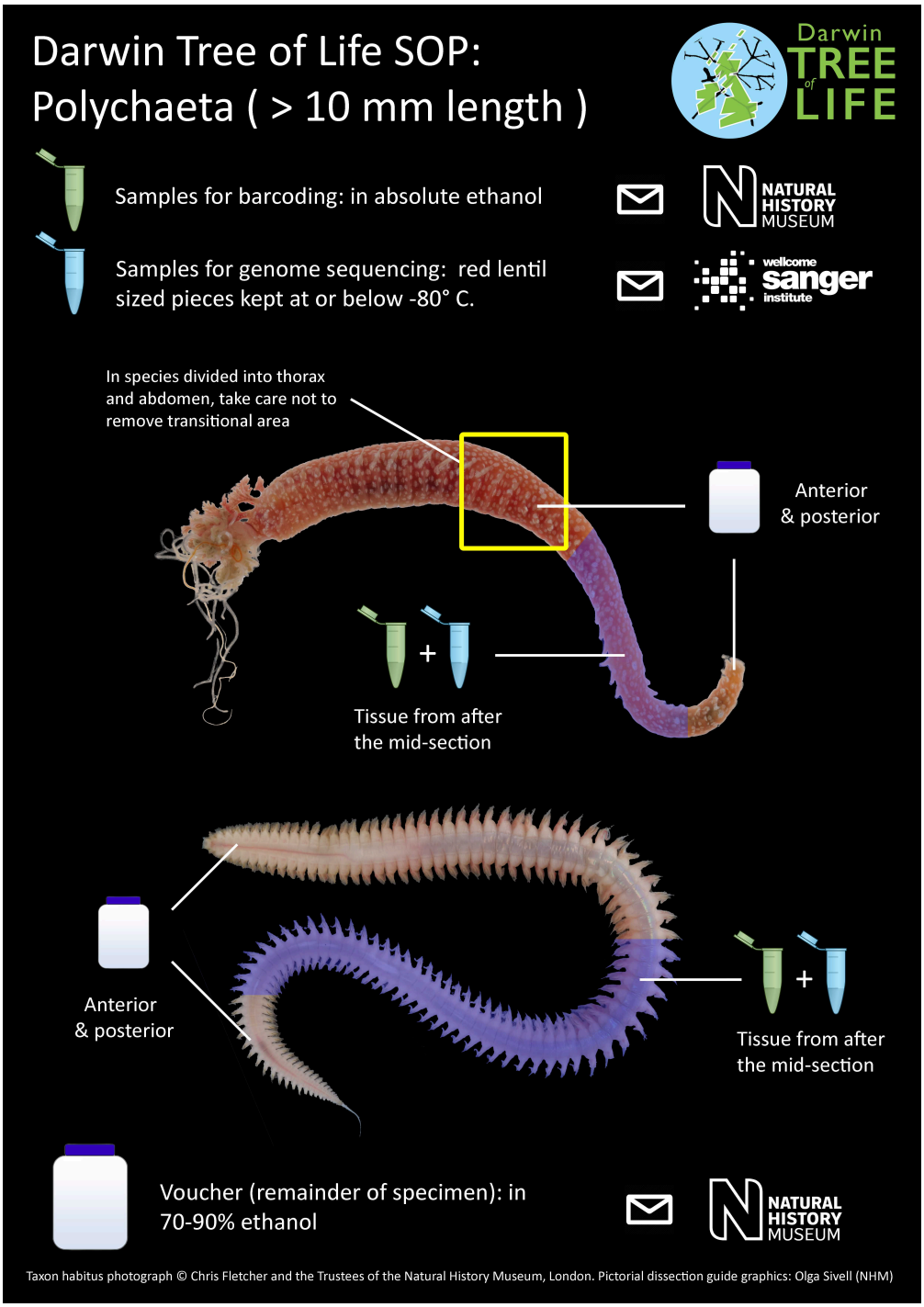

Photo guide assembly: Chris Fletcher

Troubleshooting