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7.3: Taxon Group: Crustacea - Cirripedia

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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.

In this instance, the subphylum Crustacea (SOP number 7) has been sub - divided into three particular groups; Decapoda (1), Peracarida (2) and Cirripedia (3). These other Crustacea SOPs can be found in the Marine Metazoa **[collection](#)** contents.

Definition: Cirripedia (barnacles) are a type of arthropod. They are exclusively marine, and tend to live in shallow and tidal waters, typically in erosive settings.

Including: Thoracica and Rhizocephala.

Excluding: Acrothoracica and species/specimens smaller than 5mm.

See the Guidelines for important details and checklists.



Guidelines

Field sampling:

Guidance regarding regulatory compliance: Prior to visiting a collection site, it must be determined whether the site is located within a Marine Protected Area and, if so, ensure that sampling permission has been obtained (on a site-by-site basis) from the relevant conservation body.

1. Environment to be sampled: Marine, brackish.

2. Trap/method of sampling: Individual collection by hand, intertidally or by diving. Possible incidental capture by dredge/trawl/grab.

Where possible, collect substrate/host barnacle is attached to – removing the barnacle will kill it. If it is necessary to remove the barnacle from the substrate it must be processed quickly at the point of collection.

After collection keep the specimens kept in an aerated, cooled (preferable) or ambient seawater. Seawater can be replaced every few hours in place of aeration.

Specimens may require processing within a few hours to avoid deterioration.

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. They must be anaesthetised by cooling on ice, in an ice and seawater slurry or in a domestic freezer.

Photography:

4. Photograph the dorsal (top) view and up to five lateral (side) views for larger or stalked specimens. Ensure all plates are imaged of any live specimens attached to a substrate/host, either prior to collection or after.

For unstalked species: do not remove from attachment site prior to photography as this will damage the plates.

The image should be taken in the highest quality possible - macro lens recommended for smaller subjects.

Photograph to include a scale and 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 DNA barcoding:

6. The recommended tissue types for DNA barcoding are listed below:

Note

Thoracica - Any part of organism excluding mouthparts and shell.

Rhizocephala - Any part of organism

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

Dissection for whole genome sequencing:

7. The recommended tissue types for whole genome sequencing are listed below:

Note

Thoracica - Any part of organism excluding mouthparts and shell.

Rhizocephala - Any part of organism

Dissect 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.

Mouthparts and shell to be retained as voucher, when possible.

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/kept at NHM.

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

