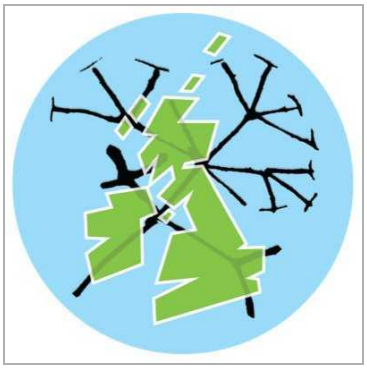

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Protocol status: Working
This is a working protocol that may be subject to changes in the future.

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2. Taxon Group: Bivalvia

In 1 collection

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Darwin Tree of Life

Inez Januszczak

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: Bivalvia are members of the phylum Mollusca, characterized by a shell that is divided from front to back into left and right valves. The valves are connected to one another at a hinge.

Including: Specimens larger than 5mm.

Excluding: Specimens smaller than 5mm.

See the Guidelines for important details and checklist.

GUIDELINES

Field sampling:

1. Environment to be sampled: Marine and brackish environments.

2. Trap/method of sampling: By hand; sorting through bulk sediment and substrate; gear thrown from an RV (e.g. dredge or grabs) or through the use of divers if sub

Keywords: Bivalvia, Bivalves, Mollusca, Mollusca, SOP, Standard, Operating, Procedure, Darwin Tree of Life Project, Wellcome Sanger Institute, Whole genome sequencing, DNA barcoding, Marine

tidal.

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 bivalve taxa, 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 (at -80) as soon as possible may be most appropriate for some species. Animals may die during dissection. Efficient dissection is recommended.

Photography

4. Profile of both the left and right valves on the exterior.

Once opened and dissection is complete, a detailed image of the interior of both valves with particular focus on diagnostic feature of the taxon, cardinal/taxodont teeth, internal scaring and so forth.

The image should be taken in the highest quality resolution - macro lens 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 (e.g. genitalia, wings or other) the photograph will serve as voucher and should include identifying features.

Dissection for barcoding:

5. Recommended tissue for barcoding: muscle from foot/adductor and mantle

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

Dissection for Whole Genome Sequencing:

6. Specimens must must be sampled and frozen while alive. The tissue for whole genome sequencing is removed, and immediately frozen on dry ice (-80). Recommended tissue for whole genome: muscle from foot/adductor and mantle.

The organism should be dissected into 5mm chunks.

Up to ten pieces in separate tubes.

> Note
>
> For small individuals where less than ten 5mm tissue samples can be collected, please dissect several individuals of the same species, treating each individual as a separate genotype. Avoid contamination and do not pool samples.
>
> Sample adult specimens, if possible.

Storage of frozen tissue:

7. 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.

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

Storage of voucher:

9. Vouchers to be sent to/kept at NHM.

Shell to be kept as intact as possible. Excess tissues to be carefully removed from shell and an intact individual to be included as well if possible (shell should be opened to allow preservative to reach all tissues).

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

Photo guide below:

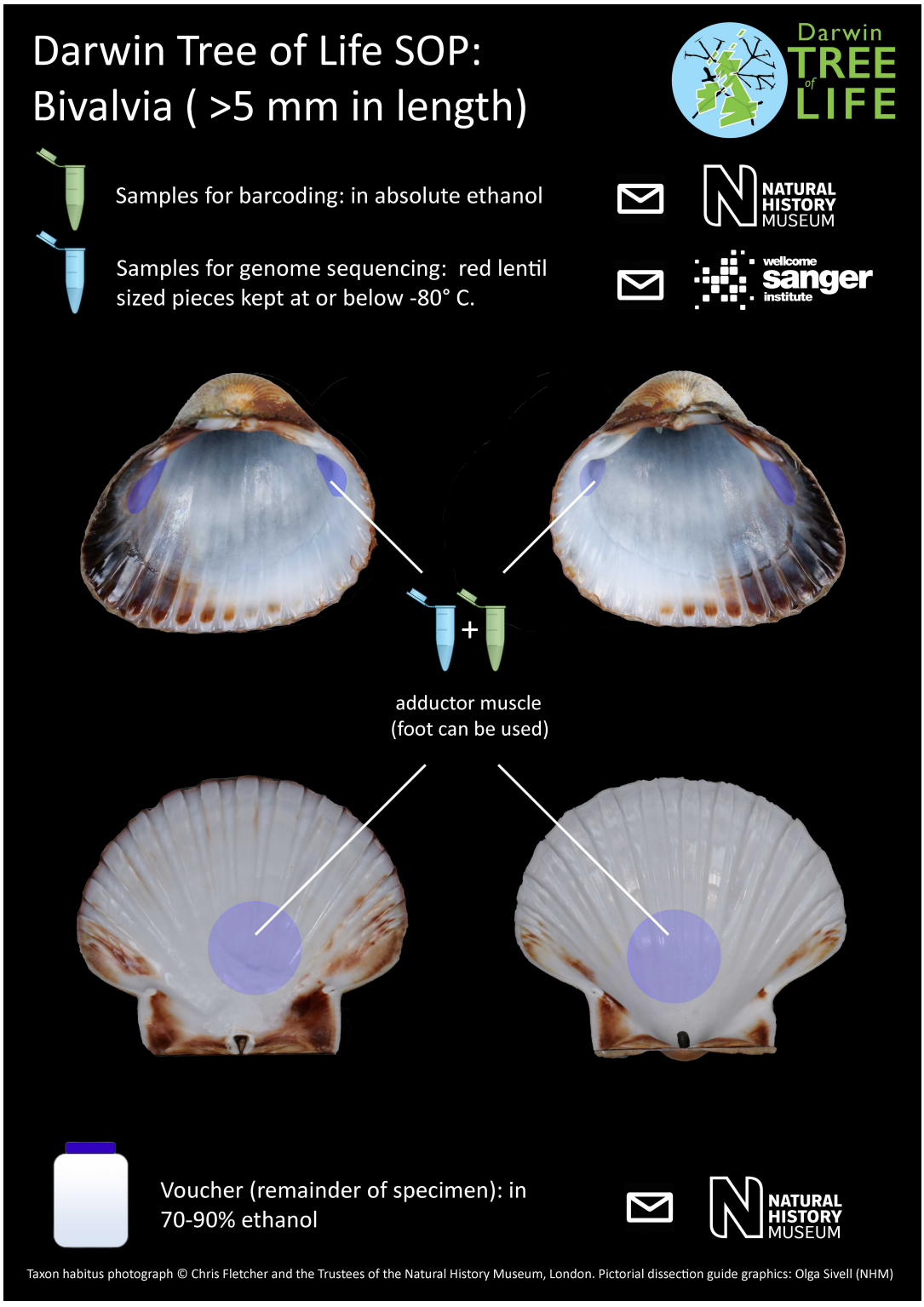

Photo guide assembly: Chris Fletcher