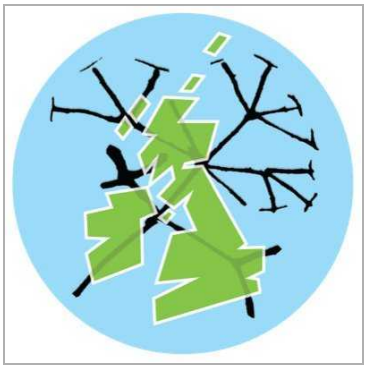

MAY 17, 2023

4. Taxon Group: Cephalopoda

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: A cephalopod is any member of the molluscan class Cephalopoda such as a squid, octopus, cuttlefish, or nautilus. These exclusively marine animals are characterized by bilateral body symmetry, a prominent head, and a set of arms or tentacles modified from the primitive molluscan foot.

Including: Octopodidae, Loliginidae, Sepiidae, Sepiolidae.

Excluding: Non-UK recorded species.

See the Guidelines for important details and checklists.

GUIDELINES

Field sampling:

1. Environment to be sampled: Marine.

2. Trap/method of sampling: Recommended collection via demersal or pelagic trawl, or caught in pots. In some instances they may be caught via hand net.

DOI:
dx.doi.org/10.17504/protocols.io.14egn2dozg5d/v1

Protocol Citation: Kesella Scott-Somme, Inez Januszczak 2023. 4. Taxon Group: Cephalopoda. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn2dozg5d/v1

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

Created: May 09, 2023

Last Modified: May 17, 2023

PROTOCOL integer ID: 81651

Keywords: cephalopoda, Octopodidae, Loliginidae, Sepiidae, Sepiolidae, DToL, Darwin Tree of Life Project, SOP, Standard Operating Procedure, whole genome sequencing, DNA barcoding, Marine, Cephalopoda, Marine Biological Association, Natural History Museum

Usually caught as bycatch- some species are solitary and can be more difficult to target. Success has been found via collection and rearing of the eggs.

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 (however, see Note under "Photography") but do not last long in captivity without a suitable enclosure and specialist care.

Photography

4. Photography of identification features can be difficult and/or stressful for live specimens. It is recommended to get at least one photograph of the specimen alive, but if it will cause unnecessary stress, wait until after euthanasia to photograph.

Note

Cephalopods are legally protected in the United Kingdom and must be euthanized correctly by a trained individual. After euthanasia, photographs of dorsal and ventral views should be captured, alongside close ups of the eyes, arms, tentacles and suckers. If visible, the shape and length of the fins should also be captured.

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; where no voucher specimen parts are retained the photograph will serve as voucher and should include identifying features.

Dissection for barcoding:

6. A small section of mantle, arm or tentacle could all be used for barcoding. It is recommended to rinse in filtered sea water and dab on tissue to remove mucus.

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. Animals are usually large enough for easy dissection. For Sepiolidae, Sepiidae and Loliginidae, the mantle can be cut down the centre to remove the contents of the body cavity, in suitably large individuals you may be able to remove small sections of the fin or mantle for genome sequencing. In smaller individuals you can use any tissue apart from the beak and gut contents (and cuttlebone for Sepiidae).

The organism should be dissected into 5mm chunks.

Up to ten pieces in separate tubes.

> Note
>
> Ideally for the voucher, as many of the arms/tentacles as possible should be preserved.

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, although can be initially stored frozen if required.