May 25, 2023

13. Taxon Group: Pycnogonida

In 2 collections

DOI
dx.doi.org/10.17504/protocols.io.x54v9d98mg3e/v1

Kesella Scott-Somme[1], Chris Fletcher[2], Inez Januszczak[2]
[1]Marine Biological Association; [2]Natural History Museum

Darwin Tree of Life

Inez Januszczak
Natural History Museum, London

DOI: https://dx.doi.org/10.17504/protocols.io.x54v9d98mg3e/v1

Protocol Citation: Kesella Scott-Somme, Chris Fletcher, Inez Januszczak 2023. 13. Taxon Group: Pycnogonida. **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9d98mg3e/v1**

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

Created: May 24, 2023

Last Modified: May 25, 2023

Protocol Integer ID: 82394

Keywords: Pycnogonida, Pycnogonid, Darwin Tree of Life, Wellcome Sanger Institute, Natural History Museum, DNA barcoding, Whole genome sequencing, SOP, Standard Operating Procedure, marine arthropod, marine arthropods of the order pantopoda, various marine metazoa species within the scope, marine metazoa, various marine metazoa species, sea spider, marine biological association, pycnogonid, pycnogonida, known species, specimen, identifiable specimen, species, dtol taxon, dna barcoding, taxon group, oceans around the world, darwin tree of life project, photo guide available for identifiable specimen, genome, order pantopoda, ocean, whole genome sequencing, other metazoa working group, darwin tree of life, darwin tree

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: Pycnogonids, also know as "sea spiders" are marine arthropods of the order Pantopoda. They are found in oceans around the world, with over 1,300 known species. Leg lengths range from 1 mm to over 70 cm. Most are toward the smaller end of this range in relatively shallow depths; however, they can grow to be quite large in Antarctic and deep waters.

Including: Specimens over 5mm.

Excluding: Specimens under 5mm.

Photo guide available for identifiable specimens under 5mm.

See the Guidelines for important details and checklists.

Acknowedgements
Thank you to **Jan Beccaloni** at the Natural History Museum for reviewing this SOP.

Guidelines

Field sampling:

1. Environment to be sampled: Marine

2. Trap/method of sampling: Individual collection by hand, intertidally or by diving. They are commonly found as bycatch living on algae, hydroids etc.

Pycnogonids can be well camouflaged and are often small, so may be difficult to spot in the field. One of the easiest ways to collect them is to collect their likely habitat and sit it in a bowl of seawater. As oxygen levels drop, pycnogonids will move around to find an escape. In the United Kingdom most pycnogonid species are associated with bryozoans, filamentous algae or hydroids, although they may also be under stones, on sublittoral soft sediments, or on other organisms (eg. anemones).

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.

Specimens can be held live in holding tanks back at the laboratory.

Photography:

4. Preferable to photograph either in-situ, or on its associated habitat in the lab. Some of the habitat should be taken as some species are associated with certain habitat types. Setting up a small natural aquarium or environment can be useful for photography.

A ventral and dorsal photograph of each specimen should be collected, alongside the notable identification features for that species.

Some specimens can be quite active, so it is best to relax using phenoxytol prior to photography.

Usual identification features to photograph are:

- Presence of chelifores/palps and ovigerous legs.
- Segmentation of legs, ovigerous legs and palps.
- Presence/absence of auxiliary claws.

- Proboscis size and position in relation to chelifores/palps.

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 gut and gonads can extend from the main body into the legs, so the distal part of a leg, a palp, or even a claw, chelifore or the proboscis (if sufficiently large) would be most suitable for barcoding.

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. Depending on the size of pycnogonid, the whole body may need to be frozen. For larger individuals legs are recommended, although if possible ensure no gut or gonadal tissue is present. Legs may need to be cleaned.

Dissect tissue up to ten, lentil-sized (5mm) 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 70-90% ethanol.

Note

If working with multiple specimens of the same species, it may be worth keeping an intact representative (from the same collection event/site) to have alongside the dissected voucher specimen.

Photo guide below:

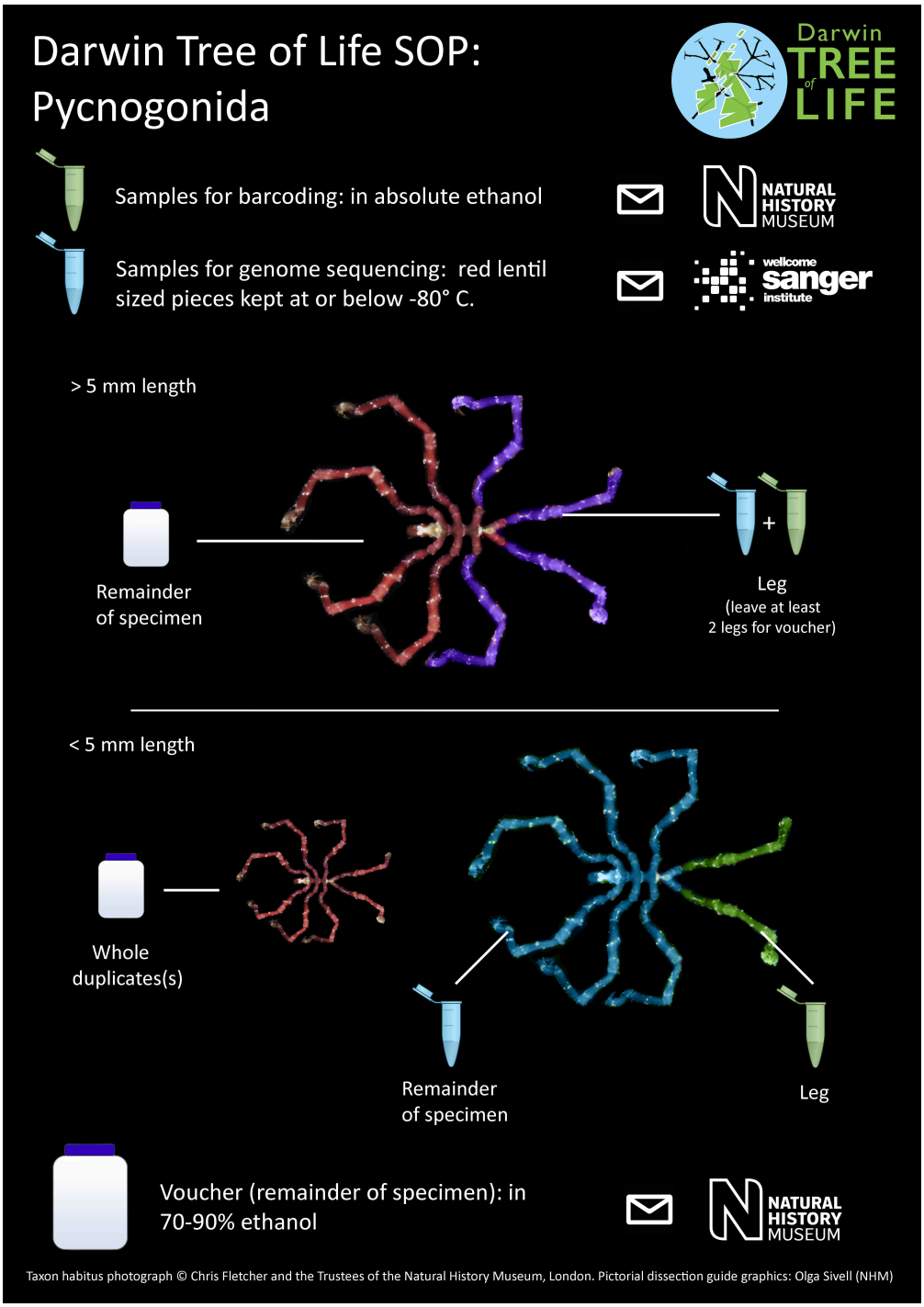

Photo guide assembly: Chris Fletcher

Troubleshooting