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Taxon group: Terrestrial Arthropods smaller than 5mm (TSS3)

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Darwin Tree of Life

Inez Januszczak

This is part of the collection "DToL Taxon-specific Standard Operating Procedure (SOP) for the Terrestrial and Freshwater Arthropods Working Group". The SOP collection contains guidance on how to process the various terrestrial and freshwater arthropod taxa 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 outlines the dissected tissues required for whole genome sequencing (WGS), 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.

TSS3 = Taxon specific SOP for Terrestrial Arthropoda smaller than 5mm

Note: For genome sequencing, anything smaller than a lentil (5mm), or estimated to be around 5mm, should be frozen whole (live) after photography. There are numerous species that fit under this category. Tissue or extract from Sanger will be used for DNA barcoding.

Including: Arachnidae (especially Acari, Pseudoscorpiones), Amphipoda (under 10mm), Collembola, Diplura, Protura, Symphyla, Pauropoda and terrestrial insects (incl. terrestrial and aquatic life stages of freshwater taxa) smaller than 5mm: Archaeognatha, Phthiraptera, Psocoptera, Siphonaptera, Strepsiptera, Thysanoptera, Zygentoma, also small species from remaining orders, e.g. Blattodea (‘Isoptera’), Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mecoptera (Boreidae), Hemiptera.

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Part of collection

DToL Taxon-specific Standard Operating Procedure for the Terrestrial and Freshwater Arthropods Working Group

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Field sampling:

1. Environment to be sampled: Terrestrial and freshwater.

2. Trap/method of sampling: Bulk capture and single specimen targeted (up to five specimens of the same species collected if possible).If aquatic, sorted in a tray of water with specimens still alive.

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:

Tissue preparation:

3. Specimens must be sampled and frozen while still alive.

Must be frozen at -80˚C or lower. Specimens to be identified (where possible up to genus as a minimum) and photographed prior to tissue preparation for genome sequencing.

Freshwater specimens should be imaged live, in water.

Photography:

4. Photography appropriate for taxon: Whole body, as appropriate.

The image should be taken in the highest quality resolution - macro lens recommended.

Photograph to include a unique identifier (e.g. QR code, NHMUK barcode) where possible.

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Dissection: Ensure all tube barcodes are linked with the original specimen

5. Specimen must not be larger than a lentil (~5 mm).

Whole specimen to be put in one tube and frozen.

Any corresponding tube IDs need to be linked with the unique specimen identifier.

Storage of frozen tissue:

6. All prepared tubes with frozen dissected tissue to be sent to Wellcome Sanger Institute.

The tissue or extract from genome sequencing at Sanger will also be used for DNA barcoding.

Storage of voucher:

7. After whole genome sequencing; the DNA extract should be sent to NHM, to act as a voucher. The photograph will also act as a voucher if the whole specimen was destructively sampled.

8. If any part of the specimen was preserved, store in a vial with 70- 90% ethanol.

9. Wet preparation technique to be used (vial with 70- 90% ethanol).

SOPs checked by experts.

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