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Taxon group: Non-larval Arthropods (TSS1)



Lyndall Pereira da Conceicao¹, Olga Sivell², Laura Sivess², Chris Fletcher², Gavin R. Broad², Liam Crowley³, Inez Januszcak²

¹UK BIOSCAN, Wellcome Sanger Institute, Hinxton, UK; ²Natural History Museum, London, UK;

³University of Oxford, Oxford, UK

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Darwin Tree of Life

Inez Januszcak

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.

TSS1 = Taxon Specific SOP for Non-larval Arthropods

Definition: Adult terrestrial and freshwater arthropods and immature life stages of hemimetabolous taxa; over 5 mm body length.

Including: Coleoptera, Hymenoptera, Diptera, Lepidoptera, other insect orders (Blattodea, Dermaptera, Ephemeroptera, Hemiptera, Mecoptera, Megaloptera, Neuroptera, Odonata, Orthoptera, Plecoptera, Raphidioptera, Trichoptera), Amphipoda, Isopoda, Diplopoda, Chilopoda, Araneae, Opiliones, Scorpiones.

Excluding: Specimens smaller than 5mm

Pictorial dissection guides available.

See the Guidelines for important details and checklist.

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

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.

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 as detailed here:

Taxon (Class or Order)	Dorsal	Ventral	Lateral
Amphipoda			✓
Araneae (spiders, harvestmen, mites, ticks)	✓	✓	
Blattodea (cockroaches)	✓	✓	
Chilopoda (centipedes)	✓	✓	
Coleoptera (beetles)	✓	✓	
Dermaptera (earwigs)	✓		✓
Diplopoda (millipedes)			✓
Diptera (true flies)	✓		✓
Ephemeroptera (mayflies)			✓
Isopoda (woodlice and waterlice)	✓	✓	
Hemiptera (true bugs)	✓	✓	
Hymenoptera (wasps, bees, sawflies and ants)	✓		✓
Lepidoptera (butterflies and moths)	✓	✓	
Mecoptera (scorpion flies)	✓		✓
Megaloptera (alderflies, dobsonflies and fishflies)			✓
Neuroptera (net-winged insects)			✓
Odonata (dragonflies and damselflies)	✓		
Orthoptera (grasshoppers and crickets)	✓		✓
Plecoptera (stoneflies)	✓		
Raphidioptera (snakeflies)			✓
Trichoptera (caddisflies)			✓
Scorpiones	✓	✓	

Photography appropriate for Non-larval Arthropods (TSS1)

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. Where no voucher specimen parts are retained (e.g. genitalia, wings or other) the photograph will serve as voucher and should include identifying features.

The photos should be of high enough resolution to be diagnostic, when possible.

Often, terrestrial species can be photographed live when anaesthetised (CO₂ or ethyl acetate are both effective) for diagnostic photography. When specimens are anaesthetised, you are also able to observe and photograph parts of the anatomy at high magnification under a microscope. These features may be less clear once the specimen is frozen.

Dissection:

5. Ensure all tube barcodes are linked with the original specimen.

5.1 Specimen must be larger than a lentil (~5 mm).

5.2 Leg(s) are to be dissected for DNA barcoding; the number will depend on size of the specimen, number of legs present and volume of tissue.

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

5.3 Dissection for whole genome:

One dissected section per tube is required with a unique barcode/ relevant unique identifier (e.g. QR code, NHMUK number) associated with the tube number.

Please use the following recommendations for taxon-specific dissection:

Insecta: In separate tubes: head, thorax, abdomen if they take up 5mm chunks; depending on body part size can be fragmented further. For specimens under 5mm see [section for TSS3](#).

Amphipoda: Remove pleopods for DNA barcoding and whole genome. The rest of the whole body would act as a voucher. This is recommended for specimens over 10mm. For specimens under 10mm, please refer to [section for TSS3](#).

Araneae: In separate tubes: cephalothorax, abdomen, legs (if any left after taking samples for barcoding); depending on size to be further fragmented.

Pedipalps of male specimens and abdomen of female specimens should be preserved in ethanol and retained as vouchers.

Opiliones: In separate tubes: cephalothorax, abdomen, legs (if any left after taking samples for barcoding).

Scorpiones: In separate tubes: prosoma (cephalothorax), mesosoma, metasoma, pedipalps (pincers); can be fragmented further if needed.

Diplopoda: Mid section of the body (trunk) should be dissected into approximately 5 mm chunks.

Head and seven anterior body segments, also last leg-bearing segment and terminal segments including telson should be preserved in ethanol and retained as vouchers.

Chilopoda: Mid body should be dissected into 5mm chunks.

Head and two anterior leg-bearing segments, also two to three last leg-bearing segments including last pair of legs should be preserved in ethanol and retained as vouchers.

Isopoda: Body dissected into 5 mm chunks (anterior & posterior).

Pleon and hind legs should be preserved in ethanol and retained as vouchers.

Immature life stages of hemimetabolous taxa: To be dissected as their adult stage. Pictorial dissection guides TSS1 available below for more specific dissection information.

5.4 Up to 10 sections per specimen to be dissected for genome sequencing (into separate FluidX tubes); could be more depending on arthropod size. Should be

dissected into 5 mm chunks.

Parts taken for vouchers should be stored in 70-90% ethanol, except wings which should be retained dry in an envelope. See section 8 and 9 for more details on taxon specific vouchers methods.

Ensure all tube barcodes are linked with the original relevant unique identifier (e.g. QR code number).

Storage of frozen tissue:

6. If prepared tube with frozen dissected tissue passes the DNA barcoding stage, it is to be sent to Wellcome Sanger Institute.

Please refer to [DNA barcoding SOP v2.1](#).

7. Leftover tissue from large specimens must be sent to NHM for vouchers and long term storage.

The samples for genome sequencing will be sent to Sanger.

Any sample extracts will be sent by Sanger to NHM for long-term storage.

Storage of voucher:

8. Vouchers to be sent to/kept at NHM, to act as a voucher.

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

Pictorial dissection guides TSS1 available below for more taxon specific vouchers information.

Voucher methods

For certain families genitalia may need to be dissected out (Diplopoda, Chilopoda, Araneae, Calyptrate flies) or dissected/slide mounted (various insect groups) to allow for positive identification.

The dissected genitalia or slides should be retained as a voucher, in a vial with 70-90% ethanol. Genitalia are removed by cutting the end of the abdomen off the freezing

specimen (ideally before it is completely frozen and then liable to ping off).

Wings in cellophane envelope: Lepidoptera, Mecoptera, Odonata, Raphidioptera

For some species of specialist interest, eg. in Lepidoptera, the abdomen can also be stored as a voucher for diagnostic purposes, alongside the wings.

SOPs checked by experts.

TSS1: Pictorial dissection guides

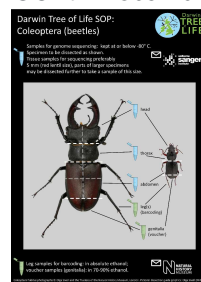


Figure 1: Coleoptera

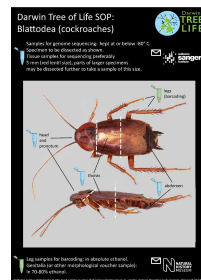


Figure 2: Blattodea

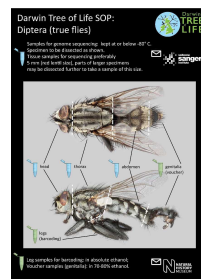


Figure 3: Diptera

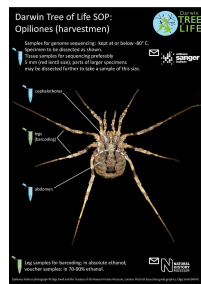


Figure 8: Opliones

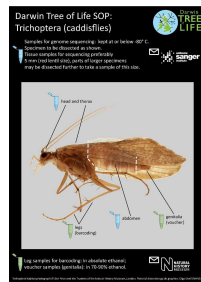


Figure 9: Trichoptera

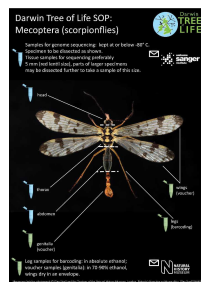


Figure 10: Mecoptera

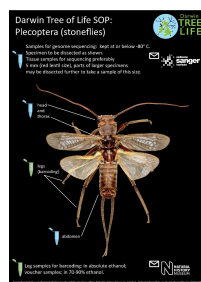


Figure 11: Plecoptera

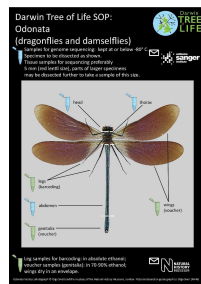


Figure 12: Odonata

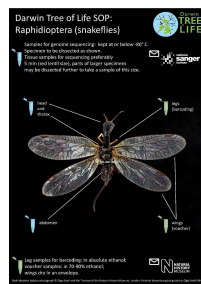


Figure 13: Raphidioptera

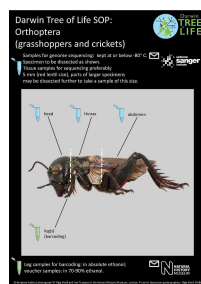


Figure 14: Orthoptera

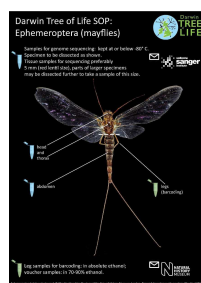


Figure 15: Ephemeroptera

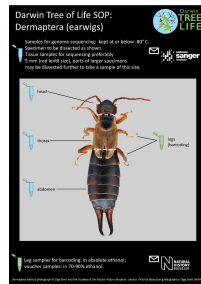


Figure 16: Dermaptera

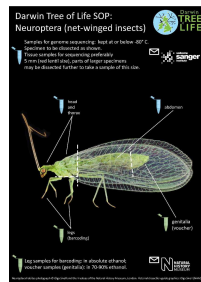


Figure 17: Neuroptera

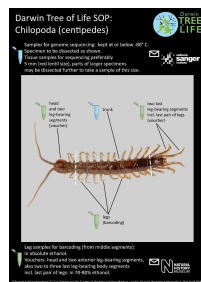


Figure 18: Chilopoda

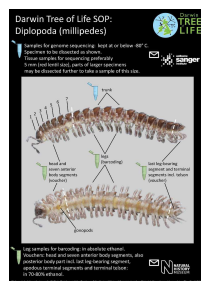


Figure 19: Diplopoda

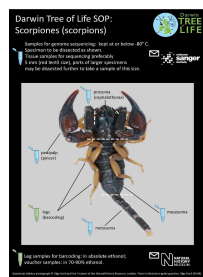


Figure 20: Scorpiones

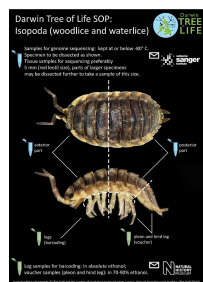


Figure 21: Isopoda

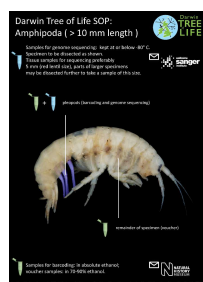


Figure 22: Amphipoda

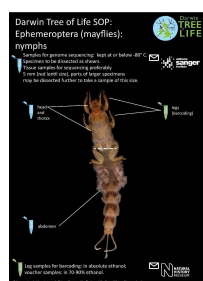


Figure 23: Hemimetabolous nymphs – example taxon, Ephemeroptera