May 04, 2022

Taxon group: Larval stage insects (TSS2)

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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.

TSS2: Taxon group: Larval stage insects

Definition: Holometabolous insect larvae and pupae

Including: Coleoptera, Diptera, symphytan Hymenoptera, Lepidoptera, Mecoptera, Megaloptera,Neuroptera, Raphidioptera, Trichoptera.

Excluding: Specimens smaller than 5mm.

See the Guidelines for important details and checklist.

DToL SOP Terrestrial and Freshwater Arthropod Taxa v4_IJ_with images.docx

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

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

Definition: Holometabolous insect larvae and pupae.

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Excluding: Specimens smaller than 5mm.

Field sampling:

1. Environment to be sampled: Terrestrial and freshwater.

2. Trap/method of sampling: Specimens will be live caught, single target or bulk capture (up to five specimens of the same species collected if possible).

If aquatic: collected with a kicknet; live specimens sorted in a tray of water.

Each specimen, regardless of species, must have its own unique ID (eg. QR code number) which will be attached to any 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. If the larvae originate from a gall it may also be retained or photographed to aid the identification process. Larvae reared from an egg cluster may also be sampled and used for ID verification of specimens from the same batch used for genome sequencing.

Freshwater specimens should be imaged live, in water.

Case-bearing larvae to be removed from the case prior to freezing and samples being taken.

Pupa to be removed from the pupal case/cocoon prior to freezing and samples being taken.

Photography:

4. Photography appropriate for taxon: Dorsal and lateral, additional views as required.

Plecoptera: ventral view.

Hymenoptera: Symphyta: both lateral views.

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

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

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

5.2. Part of specimen dissected for DNA barcoding**:** Leg(s), if present. Number of legs

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removed will depend on the size of the specimen, number of legs and volume of tissue.

If no legs; fragment of mid-section.

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: Frozen specimen to be dissected into separate tubes: head, mid section, abdomen if they take up 5mm chunks. If specimen chunks are larger than 5mm, further fragmentation is necessary. For specimens under 5mm see section TSS3.

For more detailed dissection guides per certain taxa, see section TTS2: Pictorial dissection guides.

5.4. Estimated number of sections per specimen dissected for genome sequencing (into separate FluidX tubes): 5.

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

Storage of frozen tissue:

6. If passing the DNA barcoding stage (refer to DNA barcoding SOP v2.1), prepared tubes with frozen dissected tissues to be send to Wellcome Sanger Institute.

7. Leftover tissue from large specimens to be sent to NHM for vouchering 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. Tissue to be sent to/kept at NHM as voucher (see pictorial dissection guides).

9. The voucher is to be curated in spirit (vial with 70-90% ethanol).

10. For voucher preparation technique, all larvae and voucher samples to be stored in 70-90% ethanol.

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Head capsules or whole larvae may be slide-mounted later for ID verification.

The recommended vouchers to be retained are: for Coleoptera (Figure 25), head/thorax, tip of abdomen with urogomphi is present, head capsule for legless larvae; for Diptera (Figure 27), the head capsule and tail end; for Hymenoptera, the head capsule; for Trichopetra (Figure 26), post-removal from case, the head capsule and tail end.

Coleoptera - head and thorax, also tip of abdomen with urogomphi if present; for legless larvae: head capsule.

Diptera - the head capsule and tail end

Hymenoptera - head capsule

Trichoptera - post removal from pupae, head capsule and tail end.

SOPs checked by experts.

TSS2: Pictorial dissection guides

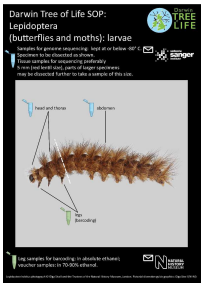

Figure 24: Lepidoptera larvae

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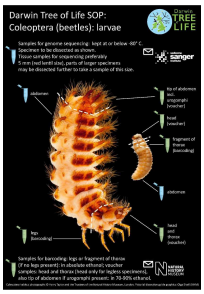

Figure 25: Coleoptera larvae

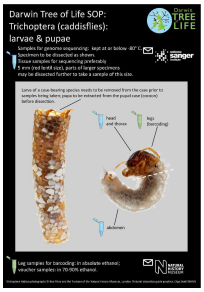

Figure 26: Trichoptera larvae

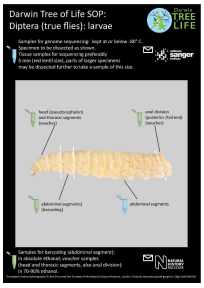

Figure 27: Diptera larvae

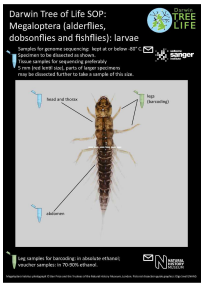

Figure 28: Megaloptera larvae

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