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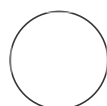
**Protocol status:** In development  
 We are still developing and optimizing this protocol.

## DToL Specimen Vouchering Standard Operating Procedure: Lepidoptera (V.1)

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Darwin Tree of Life



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### ABSTRACT

This Standard Operating Procedure (SOP) contains guidance on how to voucher Lepidoptera specimens submitted to the Darwin Tree of Life (DToL) project. The guidance specifically refers to the assembly of Lepidoptera vouchers at the Natural History Museum (NHM) in London.

For guidance on sampling and preparation for whole genome sequencing, please refer to the [DToL Taxon Specific Standard Operating Procedure for the Terrestrial and Freshwater Arthropoda Working Group](#).

**Definition:** A voucher is a permanently preserved specimen that is maintained in an accessible collection. In genomics, vouchers serve as the physical evidence for the taxonomic identification of genome assemblies.

This is version one of this SOP, and may be subject to change in the future.

### GUIDELINES

This Standard Operating Procedure (SOP) outlines in more detail the voucher preparation of specimens within the insect order Lepidoptera.

Collection and preparation of Lepidoptera specimens for barcoding and full genome sequencing should refer to [DToL Taxon Specific Standard Operating Procedure for the Terrestrial and Freshwater Arthropoda Working Group](#).

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#### Note

Please note this SOP is specifically referring to the handling of Lepidoptera specimens that have been flash frozen at -80C.

Though the vouchers themselves, once removed, do not need to be kept at -80C, the rest of the specimen must remain within the cold chain as per the [DTOL Taxon Specific Standard Operating Procedure for the Terrestrial and Freshwater Arthropoda Working Group](#).

This SOP focuses mainly on using wings as the primary voucher for Lepidoptera, although other vouchering methods are mentioned.

#### Note

Please ensure each specimen is accompanied with all its relevant metadata (Date of collection, Collector, Identifier, Collection location, Species, Specimen ID, associated tubes).

The Natural History Museum (London) will only accept and voucher specimens that have this information.

### **Before vouchering the specimen**

Take a high-quality photograph of the specimen from both ventral and dorsal positions (lateral if possible) ensuring that any key ID features are visible before taking the voucher. This is to aid any identification verification as the specimen will later be dissected. Ensure when storing the image it is labelled with the unique identifier (e.g. QR code, specimen barcode), and if possible, for the physical barcode to be included in the image.

### **Vouchering the specimen**

Note that the whole specimen must be on dry ice whilst the vouchers are being removed.

A high quality of care must be taken when removing the wings from Lepidoptera specimens. The specimens are delicate, and the wings (re. the voucher) are liable to break or become damaged if handled incorrectly.

### Note

If genitalia dissection is required for identification confirmation of the specimen, remove the tip of the abdomen and place in a glass vial containing 80% ethanol, with a corresponding specimen ID label. As the specimen will be frozen any careful dissection will have to occur after the voucher has defrosted.

- 1) Remove each wing (both sets) from the Lepidoptera specimen. These must be gently detached from the base of the wing, to cause minimal damage. Using fine forceps is recommended.
- 2) For smaller Lepidoptera (such as micromoths) it can be easier to remove the wings after sampling the head/thorax/abdomen of the specimen.
- 3) Place the wing vouchers within a glassine envelope, ideally 75x55mm (see Figure 1 for guidance). The vouchers should be placed in the left-hand corner of the envelope. Place the physical barcode in the envelope if storing temporarily.
- 4) Record the specimen barcode number along with the corresponding Lepidoptera species checklist number in an excel spreadsheet (see note section below).



**Fig. 1:** Wing Voucher placement in glassine envelope

### **Labels to be included with the voucher**

Below is the description of the labels to be included with each set of Lepidoptera

wing vouchers, all of which should be printed in Arial Font.

#### Note

**Genus:** Genus labels should ideally be in Arial font sized 10 (if the names do not fit, reduce font size) with the Genus name italicized and capitalized in bold, beneath that the name of the author and year of description (See Figure 6). Note if the specimen has been moved from its original Genus, both author and year should be placed in brackets.

All Genus labels should be placed in 1.00cm x 3.66cm (Excel) cells within an 18.28cm wide grid, measuring 5 x 27 cells.

**Species:** As Genus labels but species name should be in lower case (See Figure 6).

**Species checklist number:** The Checklist number for each species must be taken from the [Agassiz Lepidoptera checklist](#) for each specimen. These must measure 0.51 cm x 1.59cm and be in Arial size 7 font (See Figure 6).

**Barcode:** The given specimen barcode.

**Data label:** This label lists the key information about the specimen. This should include Country, District/County/Shire, Precise Locality, Decimal Latitude and Longitude, Collection method, Collector name(s), Date and Acquisition number.

Layout example:

Scotland  
Wester Ross  
Beinn Eighe NNR  
51.6637, -0.9445  
Light Trap  
10/09/2021  
Dr John Smith  
AQ-ENT-2021-111\*

\*Any museum related acquisition information, if relevant

**Determinations label:** Showing species name at identification (including author) and name of identifier.

#### Preparing the voucher for inclusion into the NHM Entomological collections

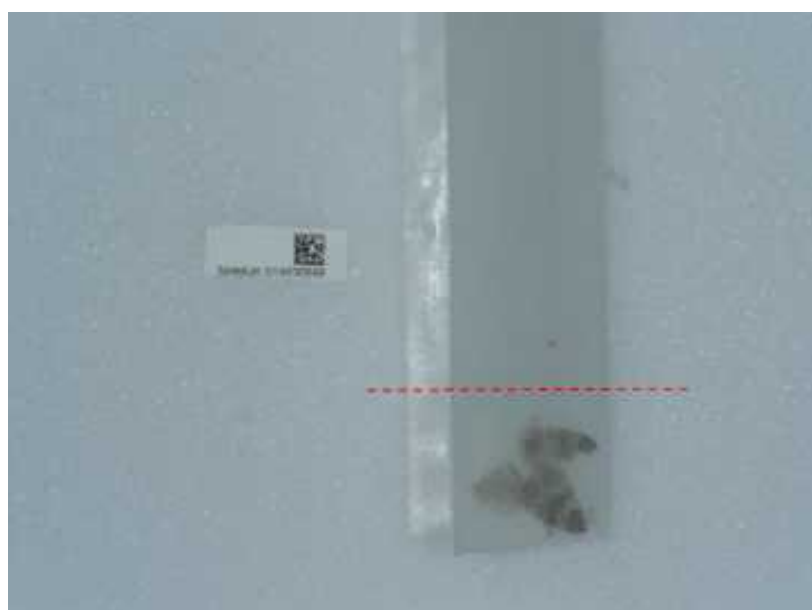
All vouchers of the same species should be stored together. (Please see Figures 5, 6 and 7 for a visual representation of the box layout comparing individual and multiple voucher boxes).

For storing vouchers, white cardboard unit trays lined with plastazote should be used. The dimensions will vary depending on the number of vouchers placed in each box. The below refers to typical unit tray sizes used in a museum context.

Individual voucher: size A5 (dimensions 56mm x 42mm)

Multiple vouchers: size A4 (dimensions 112mm x 42mm)

- 1) Remove the barcode from the glassine envelope containing the voucher.
- 2) Ensure the wing vouchers are placed together in one corner of the glassine envelope. Fold the envelope in half, vertically. Cut the envelope horizontally just above the fold line (Figure 2). If the wing vouchers are too large for a vertical fold at the halfway point - adapt the folding points accordingly.



**Fig. 2:** Vertical folding of envelope showing cutting point.

- 3) Using a number 5 entomological pin, pin the left-hand edge of the envelope containing the voucher on a plastazote sheet. Ensure the pin does not pass through the voucher. Push the pin with voucher through to stage 1 of a pinning block (the deepest stage) (see Figure 3 and Figure 4).



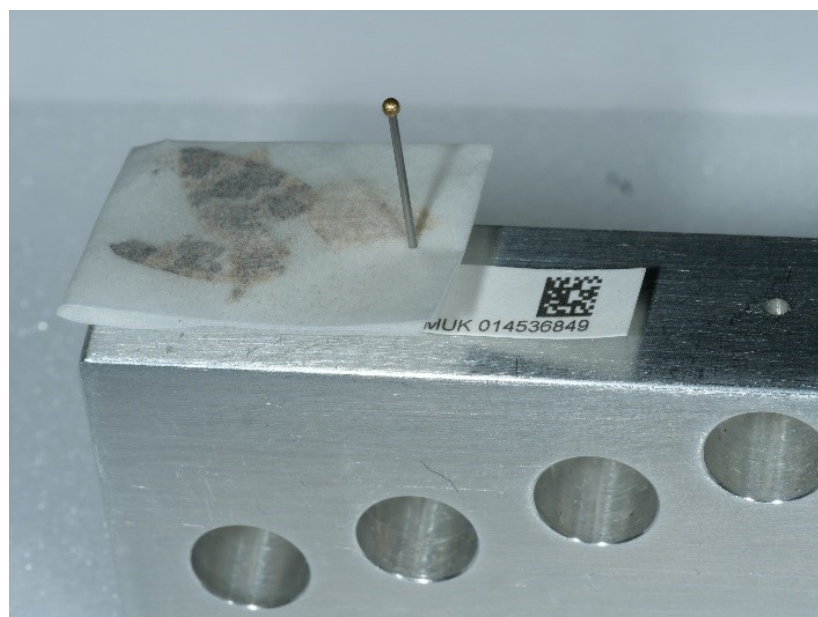
**Fig. 3:** Pinning placement on cut envelope.

4) Using the pin holding the voucher, pin the printed, pre-cut information labels. Order of pinning and pinning block stages for labels are as follows (See Figure 4 and 5):

**Barcode:** Stage 2. Ensure barcode is visible from above.

**Data label:** Stage 3

**Determinations label:** Stage 4



**Fig. 4:** Voucher and label positioning against pinning block.



**Fig. 5:** Specimen with all data labels pinned.

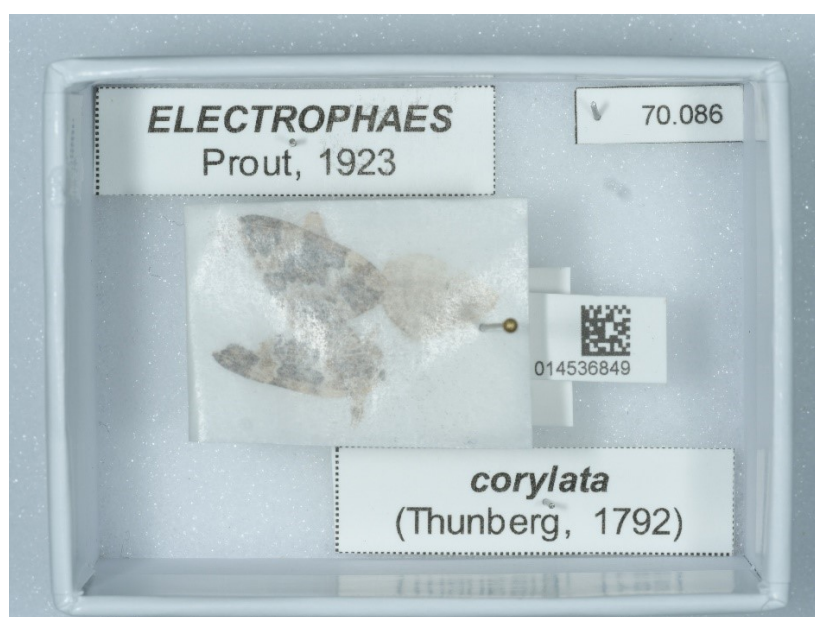
5) With individual vouchers; pin the voucher and information labels into the center of the plastazote lined unit tray.

Using No.3 cabinet point pins, pin in the species, genus and checklist numbers into the box (see Figure 6):

**Genus:** Top left

**Species:** Bottom right

**Checklist number:** Top right





**Fig. 6:** Label placement in the unit tray.

#### Note

When you have multiple vouchers of the same species, place them all in the A4 longer unit tray.



**Fig. 7:** Example layout.

**Photo credit:** Dominic Phillips