Collecting Live Invertebrate Specimens for Darwin Tree of Life

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Important Note from DToL on the Environmental and Biological Impact of Sample Collection

Samples and specimens will be acquired with the minimum possible impact on biodiversity, the local habitat and wider environment, and the species itself, and should be balanced with achieving the scientific goals of DToL.

Where a species is abundant in one location, but rare in another, collection of specimens of that species from the locale in which it is more abundant is preferred, unless there are good scientific reasons why an alternative location should be selected.

Where there is no applicable law, permit, licence requirement or regulation relating to a species (for example, relating to rarity or endangered status), or to a particular habitat (such as Site of Special Scientific Interest (SSSI) or Marine Conservation Zone (MCZ) status), steps should be taken to ensure that collection occurs with no or minimal impact on the species or habitat (which may include consideration of effects on other species), and advice sought on conservation status in cases of doubt.

The Project will not condone the acquisition of samples or specimens where individuals or organisations have conspired to acquire these for DToL illegally.

Please also see the online publication for the Amateur Entomologists' Society code of conduct for collecting insects and other invertebrates for best practice while field collecting: https://www.amentsoc.org/publications/online/collecting-code.html

Your contribution to the Darwin Tree of Life

We greatly appreciate you volunteering your time and effort in helping us achieve our targets in the ambitious **Darwin Tree of Life** project. Where we will be sequencing the genomes of all 66,000+ animals, plants, fungi and protists in the British Isles. A project on this scale has never been done before. Genome results from this project will be released immediately and openly shared in the hopes to transform the way we do biology, conservation and biotechnology.

What the Darwin Tree of Life offers you

Collectors and identifiers will automatically be given **authorship*** on the published Genome Note if their specimen is used in generating the genome. Please see an example of a Genome Note **here**. If your sample is not used for genome sequencing, it will contribute to the DNA Barcode Reference Library for UK species and its DNA barcode will be uploaded to the Barcode Of Life Database (BOLD). **Your specimen will be visible in our collection to the wider research community and the public**.

*We note that storage of names with affiliations in a database brings the DToL system under the aegis of the GDPR regulations, and we must ask collaborators to agree to their data being stored in relevant databases at the NHM and Sanger and to those data being propagated to secondary databases (including ENA and the final collections of record).

The DToL partners:

Meet the NHM Sample Team

Olga Sivell
Research Assistant

Laura Sivess
Research Assistant

Chris Fletcher
Research Assistant

Lyndall Pereira
Sampling Coordinator

What are we looking for?

In this call we are looking for **live terrestrial, freshwater and marine invertebrates** identified to species level.

While we will sequence the genomes of every species in the British Isles, we have certain targets that we aim to meet in the first phase of the project. Having a **wide taxonomic coverage** is crucial for future research and understanding the vast differences in the genomes of different species. This means **common species are also important** to our goal.

- We aim to sequence **1 - 2 species per family**, from different genera (where possible) by end-2022.
- We also aim to "**bank**" a further **3 - 4 species per family** (where possible) for the second phase of the project (2022 - 2025).

Bank it or sequence it?

- Other priorities we have for choosing species to be sequenced in the first phase include species where there is expressed **research interest**, they are **rare/endemic/iconic** species or have **agricultural/conservation/economic** importance. We will endeavor to **sequence** these in the first phase.
- Species that are smaller than 5mm will be used in Research & Development projects to improve DNA yield for genome sequencing. These will also be **banked** for the second phase.

Live Lists of Species Collections and Target Progress

Please see our **LIVE SPECIES LISTS** that update weekly with species* collected and stored in the NHM freezers and those submitted into the genome sequencing pipeline. Specific priority species** are also listed in the tables:

For a list of **Insect** species and target progress:

https://tinyurl.com/DToLinsects

For a list of **Non-Insect Invertebrate** species and target progress:

https://tinyurl.com/DToLinverts

*The taxonomy in these lists are continually being reviewed and updated, please do let us know if/when you see any issues.

** Priority species listed are from surveys, regulatory bodies and other suggestions and are not exhaustive. It does not show all taxonomic coverage priorities (these are dependent on what is available/feasible to collect), we welcome taxon requests.

Nominate your favourite species for DNA sequencing

Suggest a species to be sequenced by the Darwin Tree of Life Project

 https://tinyurl.com/dtol-suggest

Thank you for participating.

Recording the biological data

Phone App vs Spreadsheet

It is very important to record meaningful and accurate biological information for each specimen collected.

We highly recommend using the "***NHM DNA Vouchering***" project on the **EpiCollect5** phone app for this, you **do not need to sign in** to enter the biological information for your specimen. We will be able to access the information as soon as you have uploaded it from your device.

Detailed instructions on using the EpiCollect5 app can be seen at the end of this document or at this address: https://tinyurl.com/NHMDNA

If you prefer not to use a phone app, then an excel spreadsheet with instructions will be provided by one of our team via email (darwintreeoflife@nhm.ac.uk).

NHM DNA Vouchering

How to Collect and Send samples to the NHM

Due to continuing restrictions with travel and social distancing, we are initiating a huge drive to meet our species target collections in three main ways:

A. By **post** (terrestrial species)
B. By **accessing captive bred populations** (for freshwater, marine and terrestrial species), and
C. By **"roving" bioblitzes** (for freshwater, marine and terrestrial species)

If you are interested in collecting live specimens for the Darwin Tree of Life, please contact our NHM DToL team by emailing darwintreeoflife@nhm.ac.uk and we will provide you with collection kits and advice on collecting permissions.

We are also happy to receive specimens preserved in ethanol, identified to species level. These will be used to contribute to the UK DNA Barcode Reference Library that is used to confirm species throughout the sequencing process in the DToL project.

B. By Post (NHM will supply materials and cover all postage)

1. **Contact the NHM and request a collection kit**
   Please email darwintreeoflife@nhm.ac.uk to let us know which taxon groups you would like to target. Some species do better in the post than others. We will also make sure you are placed onto the NHM system so that you can be reimbursed for postage. Collection kits consist of 50ml tubes, barcoded NHM labels, paper towelling, tubes with 70% ethanol in a rack and a set of instructions.

2. **Ensure you have permission to collect**
   You do not need any permissions when collecting on your own land/ in your garden. If you would like to collect on privately owned land, then you will need landowner permission. Designated land (Trusts, SSSIs etc) may need special permission to collect (see interactive maps here: https://magic.defra.gov.uk/). We are happy to advise on possible permissions when you email us.

3. **Collect and identify the sample (one specimen per tube)**
   If using the EpiCollect app, all site information can be easily recorded while at the collection site.

4. **Please record the data for the specimen via App or spreadsheet**
   Using either the EpiCollect App: *NHM DNA Vouchering* or the excel spreadsheet provided. It is vital that you record the correct **NHMUK Barcode Label** with the specimens so that we can associate the data when it arrives.
   If using the excel sheet, then please email the completed sheet to darwintreeoflife@nhm.ac.uk before posting the specimens.

5. **Put damp (not wet) paper-toweling into the tube**
   It is important to make sure that the paper towel is not soaked with water. For molluscs, you can include a small piece of lettuce.

6. **Please place the provided NHMUK Barcode Label into the tube lid with an extra piece of paper towel in the tube entrance**
   This ensures the label does not get wet. All collection information should be on the epicollect app or in the spreadsheet.

7. **Keep specimen cool in the fridge until post day**
   Most specimens can be kept cool in the fridge for up to a week or more before being posted. Although it is best to post specimens as soon as possible.

8. **Post overnight guaranteed on Mondays, Tuesdays or Wednesdays ONLY**
   Please place tubes in a padded envelope or in a box with additional padding for protection, and address as follows:

   > **Live Specimens for Darwin Tree of Life**
   > **Molecular Collections Facility**
   > **The Natural History Museum**
   > **Cromwell Road, London, SW7 5BD**
   >
   > **Email darwintreeoflife@nhm.ac.uk**

B. By Live Laboratory/ Captive-bred populations

Populations of invertebrates are usually captive bred for study (e.g. species that are agricultural/ conservation/ economic importance or have other research interest), and so their genomes can provide immediately useful information.

Please let us know if you have access to any live cultures/populations of British or Irish invertebrate species that you think would be suitable for the Darwin Tree of Life project by emailing darwintreeoflife@nhm.ac.uk.

C. By Roving Bioblitz

Our team will be travelling around the country making pre-arranged live specimen pick-ups from collectors. This is particularly useful for aquatic species and species that do not travel well in the post, and for fulfilling species targets and gaps (please see the links to the Live Lists for priority species on page 4).

If you are interested in being involved, please let us know your taxonomic area of expertise using this survey link: https://tinyurl.com/InvertExperts or by emailing us directly at darwintreeoflife@nhm.ac.uk.

Thank you for your participation and contribution to the success of the Darwin Tree of Life project
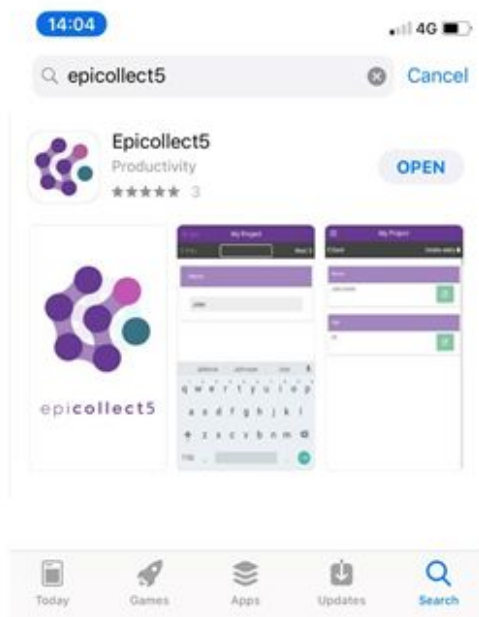
NHM DNA Vouchering using Epicollect

Version: 1.0
Published Date: July 2020
Author: Heather Allen

Data Capture in Field: NHM DNA Vouchering

Fig 1: Download EpiCollect5 app in your app store (in this case for iOS)

BACKGROUND – EPICOLLECT5 APP:

Prior to collecting in field please download the app through your apps service on your phone (simply search EpiCollect5) (Fig 1). EpiCollect5 is free to download and is developed and maintained by Imperial College London. A 'project form', has been designed by the NHM.

Please use ONLY this app for data capture in field. If there are issues with the app/isn't working – please contact Heather h.allen@nhm.ac.uk. Alternatively, you can use the shared Sanger manifest for your Data Capture.

EpiCollect5 uses data networks (2G/3G/4G or wireless) for data exchange. However, a data connection IS NOT required for data gathering and is only needed when loading a project onto a mobile device and for synchronising data. This allows data gathering to proceed in areas without data coverage (i.e. remote areas).

The app works on the basis that there can be many specimens collected from one site. Many prep records can then be created for each specimen. Note that there is a separate project and guidance document for prep records.
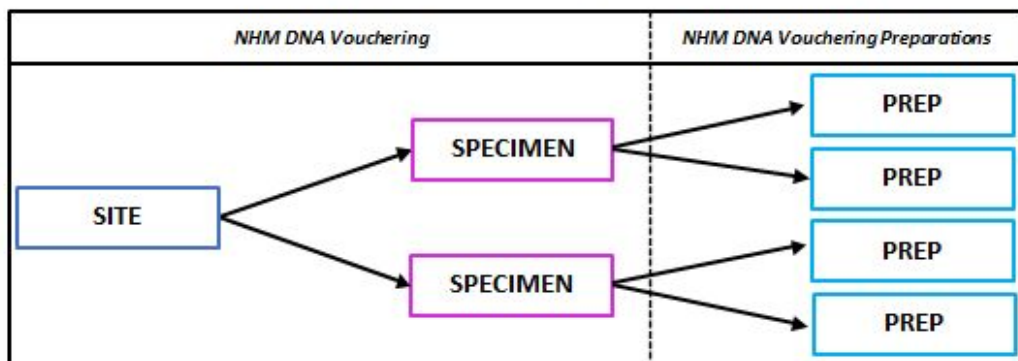
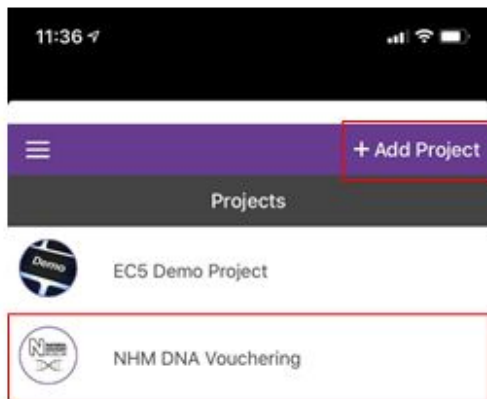*Fig 2: Data structure for collecting in EpiCollect*

Fig 3: Adding the "NHM DNA Vouchering" project

PRIOR TO DATA CAPTURE IN FIELD:

1. Download the EpiCollect5 app from the app store (Fig 3).

2. Open the app. Add the project by clicking on the "+ Add Project" button at the top right of the screen and searching for "NHM DNA Vouchering". When it appears, click on it (Fig 3).

3. This will now appear on your Projects Homepage. Click on it.

CAPTURING DATA IN FIELD:

4. You will be brought to "Site entries" screen. Click "+ Add entry" at the top right of the screen (Fig 4).
   If you have not yet created any entries this screen will read "No entries found" (Fig 4). If you have, your entry groups will appear here (Fig 5).
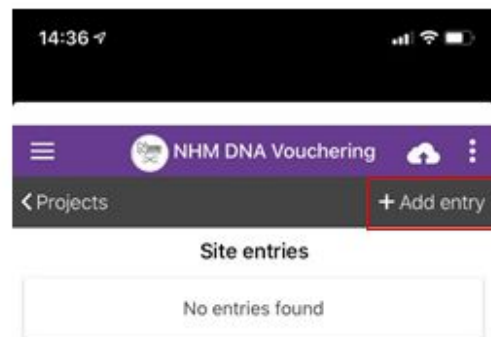

Fig 4: "Site entries" screen

5. You will now be on the "Collection Details" screen. Here you can enter:
   a. "Data Entered By" – this is a required free text field.
      Note that the "Data Entered By" field is for the person physically using the app – there is an option further down to enter the names of collectors.
   b. "Collection Date" – this is a required field that will automatically default to the current date. You can however change the date if required.
   c. "Collector Affiliation (optional)" – this is an optional free text field.
   d. "Collector 1" – this is a required free text field. In addition, if you scroll down further you will see "Collector 2 (optional)" and "Collector 3 (optional)" which can be filled out in the same way should there be more than one collector.
   Click "Next".

6. You are now on the "Site Details (required)" screen. Here you can enter:
   a. The "Site Name (for reference)" – this is a required free text field that is purely for reference purposes; your completed site entry will use this as its name.
   b. Your location using your phone's GPS by pressing the "Update location" button.
   c. If you feel for any reason that your phone's GPS is not accurate, you can manually enter "Latitude", "Longitude" and "Grid Reference" (scroll down on the app to find these options). If you are happy with the coordinates given by your phone, ignore these fields.
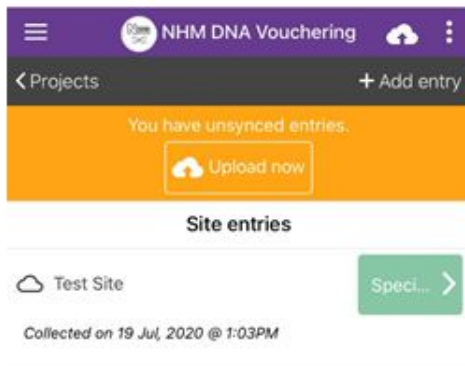
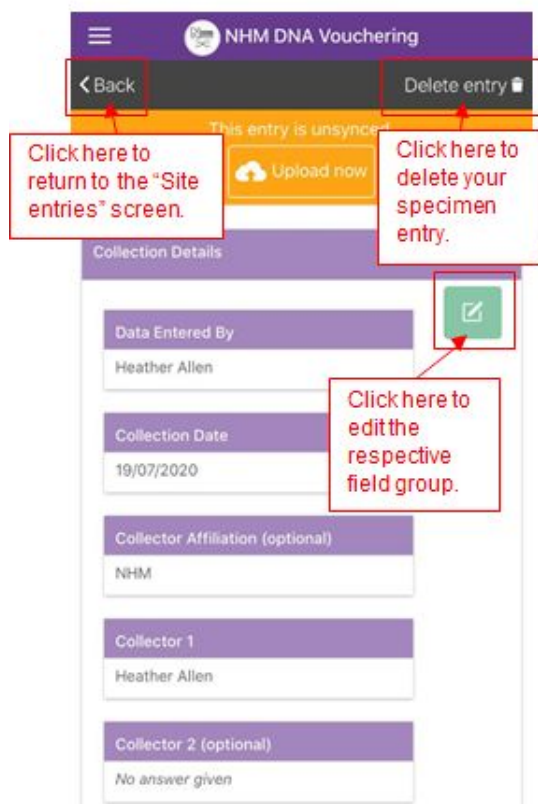Fig 5: "Site entries" screen with a site record on it
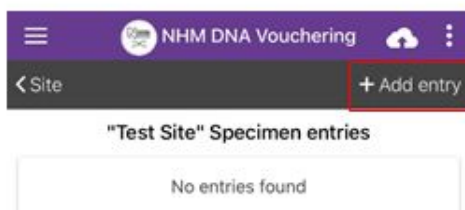

Fig 6: Viewing your site record

Fig 7: "Specimen entries" screen

d. "Habitat Description (optional) - this is an optional free text field where you can add in as much (or as little) description of the habitat as you want i.e. damp mossy ground in moderate shade. Click "Next".

7. You must now click "Save entry". This will bring you back to the "Site entries" screen. The site record that you have just created will be displayed on this screen (Fig 5). From here you can:
a. Create specimen records within your site record by proceeding to step 8.
b. Add another site entry by following on from step 4 again.
c. View the site record you have just created by clicking on it.
From this screen (Fig 6) you can:
i. Press the edit icon next to any field to edit it. Once you have made your changes, click "Quit" and then "Save" to return to your site record. Click "Back" to return to the "Site entries" screen.
ii. You can also delete your record from this screen by clicking "Delete entry". After you click this you are automatically taken back to the "Site entries" screen.
d. Upload your entries by proceeding directly to step 16.

8. Add in a specimen record by first clicking the green "Specimen" button (Fig 5). You will be brought to the "Specimen entries" screen specific to the site record you are creating the specimen record within (Fig 7).
Note that this screen will hold any specimens you have already input.

9. You should then click "+ Add entry" to start your specimen record. You will be brought the "Sample ID" screen. Here you can add:
a. "Tube Barcode" – click on the "Scan" button and then hold up your tube barcode in front of your camera to scan your tube. In the event that your tube will not scan, you can enter the barcode manually using the "IF UNABLE TO SCAN Type Tube Barcode" field.
If a Collector is making up their own specimen code, the latter field should also be used. This code should follow the convention of the Collector's initials, followed by their own given number sequence.
Note that one of these fields **must** be filled out.

b. "Specimen Image (optional)" – this is an optional field (you may have to scroll down on your phone to see) where you can attach a picture of your specimen if you wish. You can either take a photo or choose an existing one from your photo library.

c. "Specimen Collection Method" – this is a required field where you must pick an option from the dropdown list.

d. "Preservative Used" – this is a required field where you must pick an option from the dropdown list.

Click "Next".

10. You must now choose whether or not you are going to identify your specimen at the site:

a. If you answer "Yes" to "Identify Specimen Now?", you want to identify your specimen now. Click "Next" and proceed to step 11.

b. If you answer "No" to "Identify Specimen Now?", you want to identify your specimen at a later stage. Clicking "Next" after "No" will take you directly to the "Notes" section so you can skip steps 11 - 12 and go directly to step 13.

11. If you have chosen "Yes" for "Identify Specimen Now?" you will now be on the "Identification" screen. Here you should input:

a. "Scientific Name" – this is a required free text field.

b. "Higher Taxonomy (Order or Higher)" – this is a required free text field.

c. "Common Name (optional)" – this is an optional free text field.

d. "Sex" – an optional field where you can choose from the dropdown list.

e. "Lifestage" – an optional field where you can choose from the dropdown list.

Click "Next".

12. You should then enter the name of the identifier in the "Identified By" free text field. This is a required field.

Click "Next".

13. You are now on the "Notes" screen. This is an optional free text field where you can anything you feel is important. You do not have to write anything in this field. Click "Next".

14. You will then be prompted to choose whether or not your records are complete. Choosing "Yes" indicates that your records are ready to be uploaded to the NHM database. Click "Next".
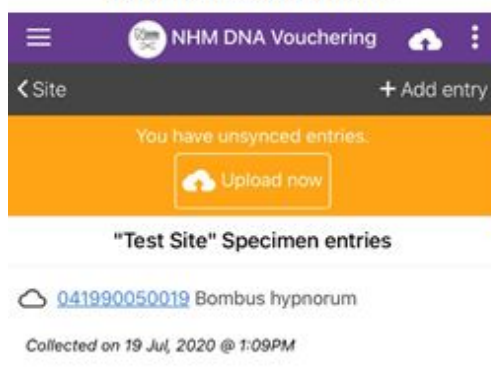

Fig 8: "Specimen entries" screen with a record on it

15. Click on "Save entry". You will then be taken back to the "Specimen entries" screen (Fig 8). From this screen you can:

a. Choose to enter another specimen record for your site by clicking "+ Add entry" (i.e. repeat steps 9 - 15).

b. View the specimen record you have just created (its title will be its barcode and scientific name) by clicking on it.

i. Press the edit icon next to any group of fields to edit them. Once you have made your changes, click "Quit" in the extreme top left hand corner. A dialog

box will then pop up; click "Save" to return to your specimen record. Click "Back" to return to the main "Specimen entries" screen.

ii. You can also delete your record from this screen by clicking "Delete entry". After you click this you are automatically taken back to the "Specimen entries" screen.

c. Go back to the "Site entries" screen by clicking "Site".
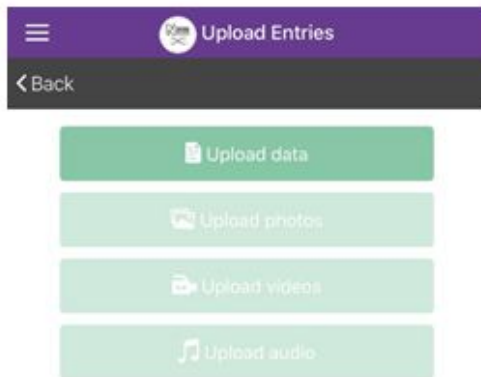d. Upload your entries by proceeding to step 16.

Fig 9: Uploading your data

UPLOADING YOUR ENTRIES:

16. Once you have captured all your data you can upload your records. Select "Upload now" (Fig 8). The "Upload now" button will appear on both the "Site entries" and "Specimen entries" screen when you have unsynced entries. Both your site and specimen entries will be uploaded at once, regardless of which screen you're on when you click this button.

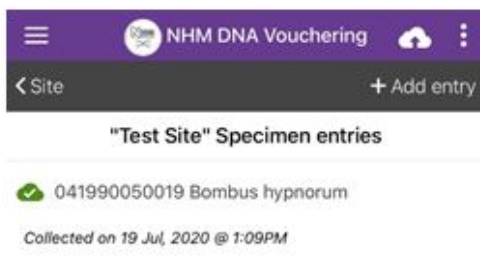*Note that you will need internet access for this.*

17. Then select "Upload data" (Fig 9).

18. Then "Upload photos".

Note that the "Upload photos" button will not be active until the data has been uploaded. This button will only become active if you have photos to upload.

You can check that your entries have been uploaded as a green cloud with a "tick" in the centre of it will appear next to all uploaded entries (Fig 10). Entries that have not yet been uploaded have a white cloud next to them.

Fig 10: The green cloud with a "tick" will appear next to all uploaded entries

You can amend your data at any point but please remember to synchronise your entries by repeating steps 16 - 18.