# BIODIVERSITY GENOMICS EUROPE WP4

## High Mountain Systems - Arthropod sampling with Malaise traps

## SOP

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# Introduction

The Biodiversity Genomics Europe (BGE) Consortium has the overriding aim of accelerating the use of genomic science to enhance understanding of biodiversity, monitor biodiversity change, and guide interventions to address its decline. The objective is to establish functioning biodiversity genomics networks, data generation and pipelines to characterize biodiversity, and to improve management intervention and biomonitoring programs by practical application of genomic tools.

Arthropod biodiversity is mostly unknown and highly understudied, despite its importance for ecosystem functionality (Outhwaite et al., 2022; Srivathsan et al., 2023). Studies have identified that insect biodiversity changes are mostly driven by intensive human land-use and climate change (Outhwaite et al., 2022), but how these factors interact under different systems is unclear. Identifying the drivers of arthropod biodiversity loss requires allocating resources for species discovery, and understanding how community composition is shaped worldwide (Srivathsan et al., 2023). The use of DNA barcoding (Herbert et al., 2003) and metabarcoding (Taberlet et al., 2012) techniques, represent an effective way to identify diversity by analyzing bulk samples of specimens (Young and Herbert, 2022).

Within the BGE scope, one of the objectives is to assess pan-European patterns of species diversity and community composition in key systems, establishing baseline sampling in mountain ranges across Europe to track biodiversity shifts associated with climate change. The “High Mountain Systems - Arthropods” case study is designed to evaluate how arthropod and pollinator communities change (species diversity and composition) along altitudinal gradients in selected mountain systems in Europe, showcasing the use of DNA-based tools.

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# Sampling Design

For this Case Study, sampling will be carried out in mountain systems of different European countries representing different biogeographic settings. Malaise traps (Figure 1) will be placed in each mountain for sampling arthropods at different altitudinal gradients. A Malaise trap is a tent-like trap that will canalize arthropods to go to the top of the trap, for them to fall into a collection tube attached to the top central section of the trap, containing 96% ethanol. For the BGE, we are following the procedures stated in the Global Malaise Trap program, where you can find further information about the program.

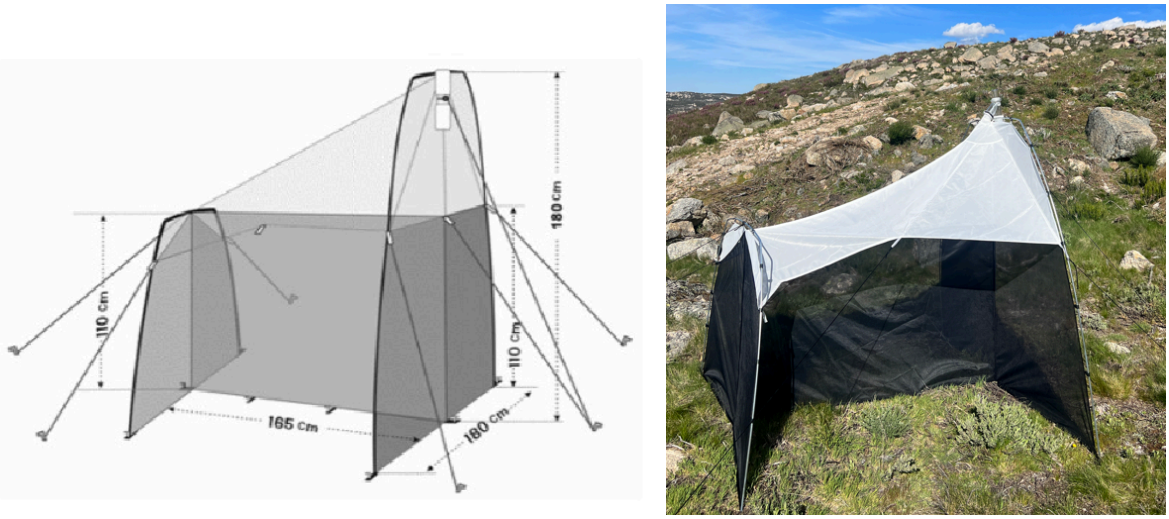


**Figure 1. Scheme of a Townes Style Malaise trap used in the present study and its approximate dimensions (left); and example of a Malaise trap set in Serra da Estrela, Portugal (right).**

For each Mountain range, an altitudinal gradient will be sampled, setting Malaise traps at five elevation sites (Figure 2). The selection of these sites should be done according to each mountain system's characteristics, i.e. including representative vegetation of each gradient. Sampling should start at the highest point accessible (or feasible to access every week), and then go down at regular intervals. A pair of Malaise traps will be placed per site/elevation, at least 50 m from each other. Malaise traps will operate for a total of 20 weeks, with samples collected from each trap at weekly intervals. From each pair of traps, one sample will be used for characterising the arthropod community using DNA metabarcoding; and the other sample will be used as a backup to the former, in case of damage or other operational issue, or as a source of specimens for morphological studies and to be sequenced as fresh material aiming to complete the DNA barcoding reference database. Detailed information about how to set the Malaise traps is described in page 8.

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Sample tubes must be collected every week during 20 weeks, trying to be as consistent as possible to collect the samples on the same day of the week during all the sampling time. This will give a total of 200 bulk samples per mountain range that will be sent for DNA metabarcoding analyses. Please note that once removed from the Malaise trap, **sample tubes should not be reopened for any reason to avoid contamination**. Store the samples safely at room temperature and avoid light exposure.

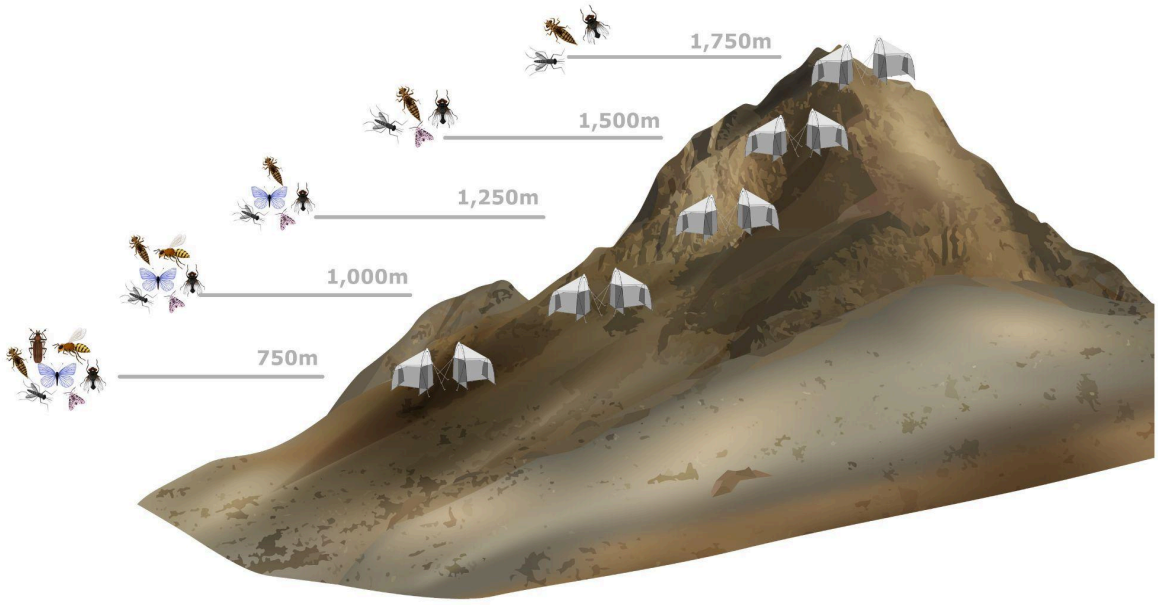


**Figure 2. Example of the altitudinal gradient sampling scheme.**

The *PlutoF* platform (Kessy et al. 2010) will be used as a workbench for processing the metadata. It includes a mobile app, *PlutoF Go*, that will be used for data entry during fieldwork. A set of 250 stickers with unique QR codes will be supplied to each partner institution (200 + 50 extra) for adding them to each collection tube, prior to sampling. More information regarding *PlutoF* usage, labels and procedures will be provided further in the document.

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# Materials

*List of materials needed for Malaise trap setting and sample collection*

A. Malaise trap (includes trap, plastic and metal stacks, cords and provisional collection tube)
B. Extra cords (IMPORTANT: the cords supplied with the traps are faulty, more to read in the section "Before sampling")
C. 500mL sterile collection tubes (see Wide-Mouth LDPE Bottles with Closure link)
D. 96% Ethanol
E. Sticky labels with predefined QR codes (provided by BIOPOLIS-CIBIO)
F. Transparent tape (to provide extra fixation for the sticky labels on the tube)
G. Cable ties
H. Hammer
I. Gray duct tape
J. Extra stacks

# Permits and documentation

It is highly important to make sure all the permits and necessary documentation are ready before sampling. To prevent any delays, check regulation and start processing your permits as soon as possible.

Permission from local authorities, property owners, rangers, or protected areas stakeholders can be another factor of potential delay or cancellation. Make sure you formalise the authorisation to sample on your selected area on time, and if possible, obtain a written confirmation.

Have copies of any legal documentation ready in case they are needed.

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# Before starting

Make sure you have all sampling materials organized, to have sorted fieldwork logistics (e.g., vehicle, budget, personnel, a fixed day of the week for collection, etc.), to consider habitat characteristics, sampling location, storing equipment, obtaining all necessary permissions/authorization for collecting specimens (see previous section).

It is advisable to have more than one site chosen for each altitudinal gradient, when possible, as a backup site. This is particularly relevant if the sites selected for this project are new for the team, as is important to consider anthropogenic factors that may disrupt the traps, like hiking visitors, private landowners, cattle, vandalism, etc.

Malaise traps ordered for the BGE project contain a plastic grid (located in the collection mechanism in the top of the trap) that comes within the trap. For the High Mountain System sampling it needs to be removed prior to setting the trap. Otherwise arthropods larger than the size of the grid will not be sampled. In Figure 3 is shown an example of how to remove the plastic grid and to secure the mesh back using cable ties.

**Figure 3. Example of how to remove the plastic grid included in the Malaise trap.**

NOTE: The cords provided with the Malaise traps are showing to be too weak and will probably give up after normal usage. Please make sure you buy stronger material cords and place them instead. The extra cords can be useful as spares or to reinforce the structure when necessary.

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To optimize time of sampling and storing, the QR codes stickers should be placed on the sterile tubes and reinforced with tape prior to sampling. Partner's coordinators must be registered in the *PlutoF* platform ("Become a user": Register → fill in details) in order to have access to the corresponding project (High Mountain Systems - "name of partner institution") when using the *PlutoF Go* app, to appear as a collector. Alternatively, project coordinators can add "persons" into the platform (Menu "Persons" → Add → fill in details) if the collector will be a person that will be only involved in fieldwork. Make sure you have downloaded the app and enter your personal data correctly. Further information on *PlutoF Go* is given on page 13. Support will be provided whenever needed before, during and after the fieldwork (see contact details at the end of this document).

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# Setting Malaise traps and sample collection

The design of the trap relies on insects being attracted to the highest and brightest part of the trap. When setting up the trap, ensure that the part that collects the insects (the trap head) is facing uphill. Ideally, position the trap perpendicular to the path of insect flight, in areas with minimal undergrowth, such as forest edges, clearings, or elevated locations. Take into account potential disruptions by wildlife or humans, as well as the direction of the prevailing winds.

Once you have chosen a location, follow the Malaise trap instruction sheet to securely assemble the trap. A video of how to set up a Malaise trap can be found in this link. When possible, fasten the front and/or back ropes to nearby trees to provide extra support, and use metal pegs to attach the bottom rings of the traps to the ground (Figure 4, left), placed in opposite directions to the trap. Particularly if your sites are located in areas of strong winds, it is advisable to attach the trap poles to a 1 m - 1.5m stake or post at the highest points to prevent the trap from toppling over. Use gray tape to reinforce the joints of the trap's metal frame and increase its stability and resilience (Figure 4, right).

**Figure 4. Metal stack/peg placed at one of the extremes of the Malaise trap (left). Gray tape placed in the joints of the trap metal structure for reinforcement (right).**

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When assembling the trap, make sure to put tension in all the extremes, and that the head structure in the front (i.e. the tent-like metal structure) is parallel to the tail structure support. To ensure the entrance is fully open, imagine you are an insect and fly into the trap towards the trap head (Figure 5), making sure that there is a clear path/entry to the collection tube at the top of the trap). Check to not over-stretch the mesh, as this will most likely block the path too.

**Figure 5. Arthropod view to “the light” path.**

Once the trap is set (Figure 6, left), carefully attach the *BGE.HMS* labeled collection tube tightly to the trap head, with 96% ethanol (around ¾ would work), and secure it with the white straps on the trap (Figure 6, right). Take pictures of the trap set and its location. Begin collecting on a day of the week when you can reliably return for the duration of the sampling period.

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**Figure 6. Malaise trap set in Serra de Estrela (left). Example of a collection tube correctly placed and secured in the trap using the white straps around it (right).**

Every week, two tubes will be collected per altitudinal site, making a total of 10 tubes per week, during 20 weeks (200 tubes). Take some extra tubes with you to the field in case something happens when swapping tubes. Each collection tube should be handled carefully to prevent contamination. **On the collection day, the tube must be topped up with 96% ethanol**. REMEMBER: When collecting the tubes from the traps each week, tubes should be closed and not opened again.

There could always be problems or eventualities with the Malaise traps (e.g. vandalized, cattle playing ground, extreme weather). Placing the two Malaise traps away from each other and in places that cannot be easily seen might help. If something happens to the trap, you can set one of the replacements nearby, and take a note if the sample was retrieved or lost. Institutional signs may also help to protect the traps.

After sampling, make sure that all the tubes are well closed and store them at room temperature. In the *PlutoF* platform, complete any missing information.

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# Shipment

For the BGE *HMS Arthropods* and the *Pollinator Communities* projects, samples will be shipped to Dr. Brent Emerson, based in the Institute of Natural Products and Agrobiology, (Instituto de Productos Naturales y Agrobiología, IPNA-CSIC), in Canary Islands:

*Brent Emerson*
*Island Ecology and Evolution Research Group*
*Instituto de Productos Naturales y Agrobiología (IPNA-CSIC)*
*C/Astrofísico Francisco Sánchez 3*
*La Laguna, Tenerife, Canary Islands, 38206, Spain*

Before shipping the samples, make sure that the IPNA-CSI lab has confirmed the availability to receive the samples (partners will ship samples in different rounds). Contact details will be provided by emailing the main contact of this SOP (at the end of the page).


For shipping, It is needed to remove as much ethanol as possible from each tube, leaving only enough to keep the arthropods “moist”. To do this, please make sure you are working in a sterile environment, i.e. laboratory, and open each tube using gloves to decant the supernatant ethanol. Be careful to not cross-contaminate any tube.

**Full instructions** of how to ship Malaise traps tubes are described in the “Malaise traps - Bulk samples Shipping instructions” document, please refer to the information when needed.

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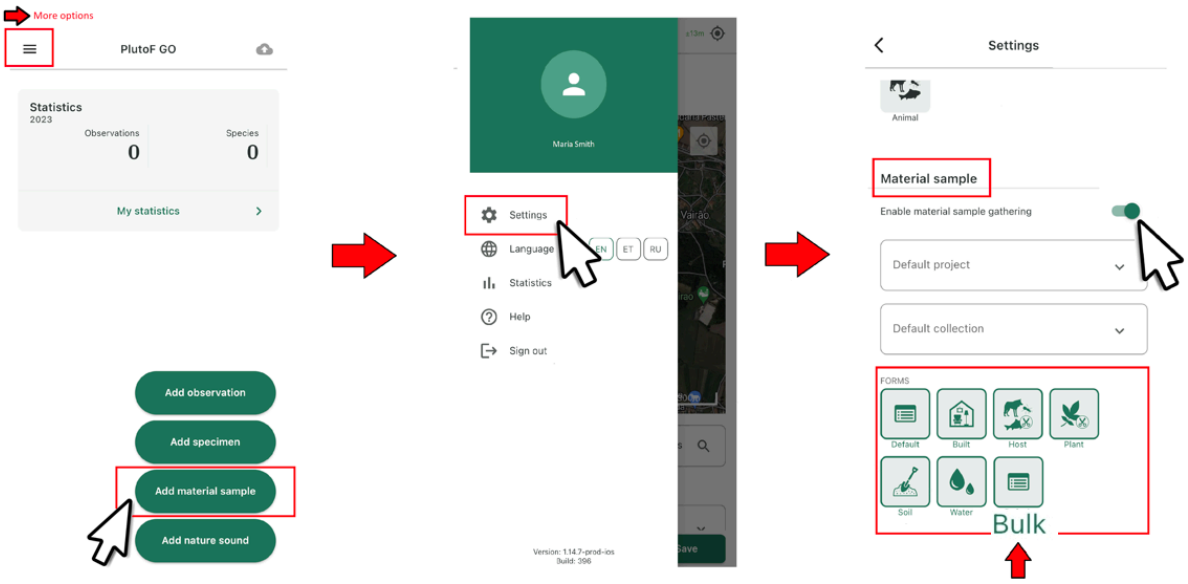
# Registering samples in *PlutoF Go* app

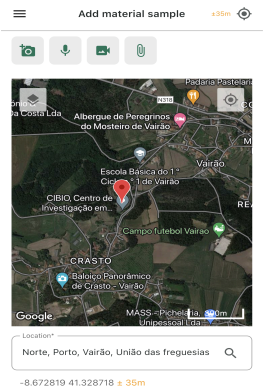
*PlutoF* is an online data management and computing service provider for biological data. *PlutoF Go* is the app that will be used to record samples directly in the field. Before using the app, the data collector should be registered on the *PlutoF* website. This can be done by the user in the option "Become a user", or to be added by the BGE project manager directly on her/his workbench[1]. You can fill all the available information within the Bulk sample option, but it is required at least to have the data detailed below:

1. Open the ***PlutoF Go*** app

2. Go to **Add material sample** box (If this option is not visible in the main page, go to Settings, scroll down to Material sample, activate Enable material sample gathering and make sure the Bulk form is highlighted in green as well).




3. The **Location** button will show your position in the map. You should record all the necessary information at the moment of the sample collection, therefore it should capture the coordinates detected by your device's GPS. If there is no internet signal you can still get the coordinates by pressing the compass icon (top right inside the map box).




[1] The *PlutoF* project manager will be the only one authorized to add any person to the working project. Any team member will be automatically notified by email when added to any project.

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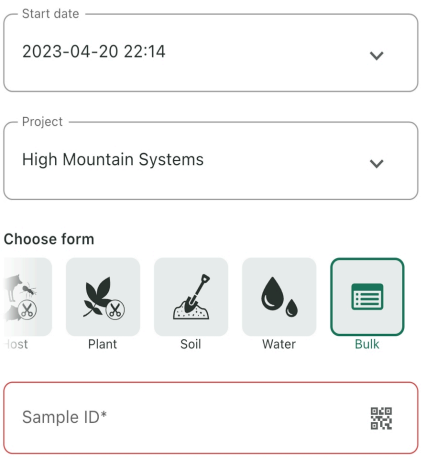



4. **Start date:** insert the collection date (when the tube is removed from the trap).

5. **Project:** choose the "*High Mountain Systems - Partner*" option. *Partner*: Choose the project according to your institution (compulsory field).

6. **Choose form:** select "Bulk".

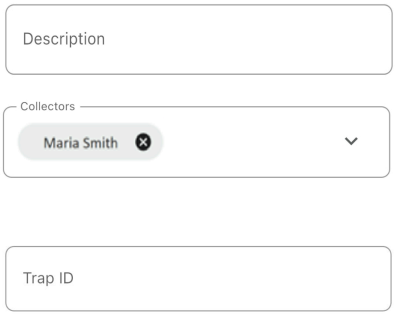
7. **Sample ID:** click on the code icon and point your device camera to the corresponding QR code provided for the sampling. QR codes are unique and cannot be added multiple times (compulsory field).

8. **Subcode:** Add Partner ID, location of gradients/stages + trap in a consistent way for all the sampling, for example:


**Portugal (*PT*), Stage 1 (*S1*) (~1000m altitude), Malaise trap a (*a*) and Malaise trap b (*b*) = *PT.S1a, PT.S1b***

***PT*, Stage *2* (~1300m altitude), Malaise *2a* and Malaise *2b* (PT.2a, PT.2b).** And so on until the last altitudinal stage (***PT.S5a* and *PT.S5b***)

These subcodes will correspond to the same traps during all the sampling period; each *BGE.HMS00XX* entry should have these subcodes

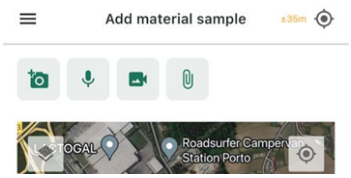


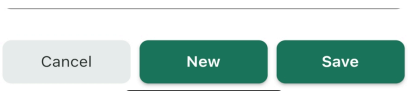

9. **Description**: Write "Placement date of trap or collection tube in Malaise trap [date]" (i.e. the day when the Malaise trap was set)

10. Add **Collectors:** Any person has to be previously registered/added in the *PlutoF* platform

11. Add a **Trap ID**: an identifier for each trap (optional).

12. Add at least one photo of the Malaise trap and its environment using the panel that is in the top of the Material sample menu. Use the **camera + icon** (top left) to generate a picture (see Figure 5 for an example). Additionally (optional), you can add any extra information/metadata you think convenient




13. Review all the information submitted is accurate and click "**Save**" at the bottom of the screen

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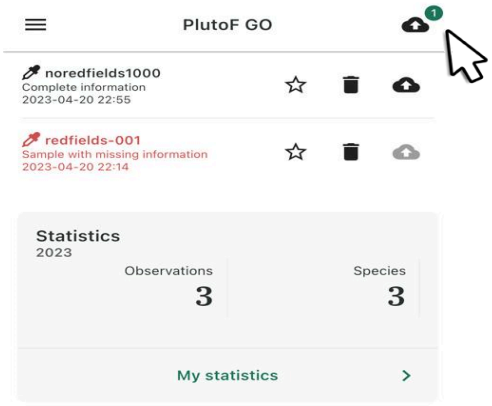


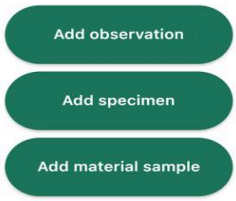


14. In the main screen, your entries will be waiting in the queue to be synchronized. Click on the **cloud icon** on the top right to do it.

In the image, there is one good sample entry (dark letters, intense color) that can be synchronized, and another entry with missing information (red letters, faded color) that will not be able to sync until the missing information is filled. This can be done by clicking on the entry and revising the info submitted.

If you cannot sync a sample, click back to that entry and check your institutional project is selected. Save, and try to sync again.

Make sure of having an internet connection to sync your entries, and try to sync often to ensure the data is saved.

15. *You are ready for the next site sampling collection*!

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# Useful contact

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