BIODIVERSITY GENOMICS EUROPE WP4

Pollinator Communities - Malaise trap sampling

SOP

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Laura Nájera-Cortazar [BIOPOLIS-CIBIO]

Sónia Ferreira [BIOPOLIS-CIBIO]

Pedro Beja [BIOPOLIS-CIBIO]

Associação Biopolis - CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos [BIOPOLIS - CIBIO]

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Introduction

The Biodiversity Genomics Europe (BGE) Consortium has the aim of accelerating the use of genomic science to strengthen understanding of biodiversity, monitor its status, and develop informed strategies to address its decline. One of the main objectives is establishing international biodiversity genomics networks, data generation and pipelines to characterize biodiversity, improving management of interventions and biomonitoring programs by the application of genomic tools. The present standard operating procedure (SOP) is adapted from the High Mountain Systems - Arthropod sampling with Malaise traps SOP (Najera-Cortazar et al. 2024), developed within the BGE.

Pollinators are crucial for terrestrial ecosystems. Both ecologically and agriculturally, they perform important functions that involve many classes of interactions, and support the majority of the global plant diversity (Ollerton 2017). Insects are one of the most important groups of pollinators (Ollerton 2017). It has been evaluated that insect biodiversity changes are mostly driven by climate change and intensive human land-use, including habitat loss by land transformation and agricultural intensification (Goulson et al., 2015; Ollerton 2017; Outhwaite et al., 2022). Given the intensification and homogenisation of agricultural land uses in the countryside, the use of gardens as refugia for biodiversity in urban areas might become of particular importance. In fact, given the increasing intensity of farming across Europe, involving for instance increases in field size, loss of non-agricultural habitats (e.g., hedges, woodlots), and increasing use of agro-chemicals, among other changes, we expect that urban habitats will be increasingly important for a range of species that are now rare or absent on farmland.

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The Pollinator Communities case study will analyze pollinator diversity using DNA barcoding (Herbert et al., 2003) and metabarcoding (Taberlet et al., 2012) techniques, using bulk samples of specimens collected during fieldwork sampling (Young and Herbert, 2022). Specifically, the study will contribute to: i) Enhance the inventory of pollinators (and other arthropods) using European urban and agricultural habitats, including exotic species; ii) set a baseline for future monitoring efforts on pollinators (and other arthropod) temporal trends in European urban and agricultural habitats; iii) to quantify differences in pollinator community attributes between urban and agricultural habitats (e.g. Alpha and beta diversities); iv) to identify pollinator species traits associated with urban living; and v) to engage citizen scientists and the wider community in pollinator sampling activities.

Figure 1. Examples of Malaise traps set in an Urban garden (left) and in an Agricultural field (right) in Seia, Portugal.

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Sampling Design

The *Pollinator Communities* case study will involve flying arthropod pollinators sampling with Malaise traps (Figure 2) set in pairs of sites across Europe. A Malaise trap (Figure 2) is a tent-like trap that will direct 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 or higher concentration (Najera-Cortazar et al., 2024). For the BGE, we are following the procedures stated in the Global Malaise Trap program where you can find further information about the program. The design for Malaise trap samping was first described in the High Mountains Systems - Arthropods SOP (Najera-Cortazar et al., 2024). Detailed information about how to set the Malaise traps is described in page 9.

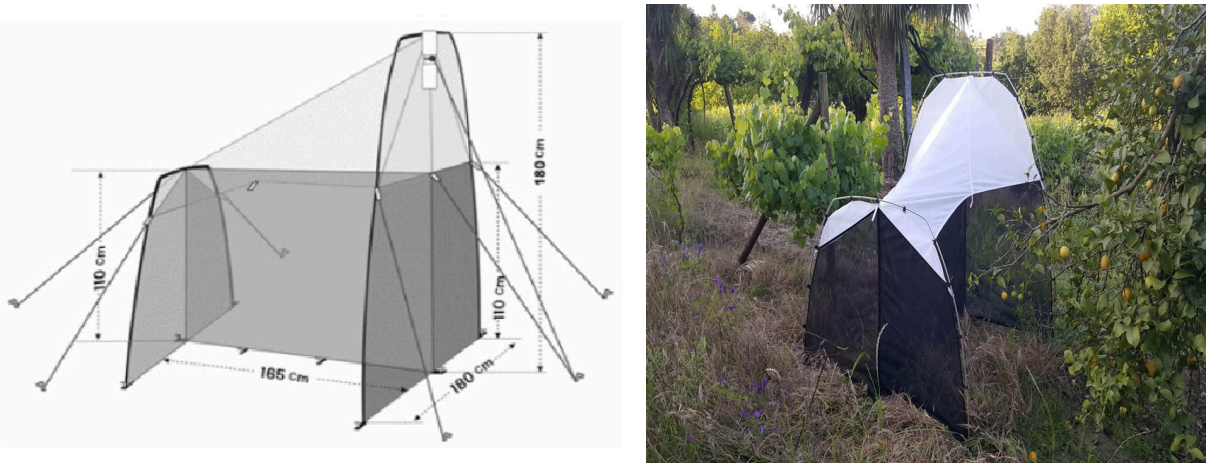

Figure 2. Scheme of a Townes Style Malaise trap and its dimensions (left); and example of a Malaise trap set in an agricultural field in Portugal (right).

Each collaborator is expected to select and sample at least three different pairs of sites for five weeks each. Each pair of traps should correspond to one site in an **Urban Garden** and another one in an **Agricultural Field** (Figure 1 and 3), which should be located at no more than 20 km from each other. If there is only one pair of Malaise traps available, sampling should be done in a rotating manner: One site pair sampling for five weeks, then moved to a different site for five weeks, and then to another one for another five weeks. In total, it would be 15 weeks of sampling. Each trap will collect flying arthropods through a 500 mL collection tube filled (around 300 mL) with at least 96% ethanol (i.e. 96% or more), that will be used for characterizing the pollinator community using DNA metabarcoding. Ethanol volume can be adjusted in the following week of sampling if it was too much or too little for that site's trap.

Samples will be collected each week, by swapping the "filled" tube of each Malaise trap for a new one. Sample tubes must be collected during **five weeks** on the same day of the week during all the sampling time (e.g. tube collection from one or all pairs will be each Monday). Each **pair** set will generate **two tubes/samples per week**, giving a total of **10 bulk samples**

per five weeks of sampling, that will be sent for DNA metabarcoding analyses. **Please note: once removed from the Malaise trap, tubes should not be reopened for any reason to avoid contamination.** Make sure that the arthropod mass is submerged in ≥96% ethanol and store the samples safely at room temperature in a fresh place, away from light exposure.

The definition of **Urban Garden** and **Agricultural Field** sites are dependent on the types of environments available in each particular area sampled. The following conditions should apply for site selection, except if otherwise agreed with the project's coordinators, Prof. Pedro Beja (pbeja@cibio.up.pt) and Dr. Laura Najera (la.najera@cibio.up.pt):

- **Agricultural Field** sites should be located in areas where land cover is predominantly agricultural (e.g., annual crops, permanent crops) within about 2 km of the site, and to be in or at the edge of an agricultural field where productive agricultural areas (i.e., excluding farmhouses, hedges, woodlots, ponds, and other non-productive habitats) are dominant within about 100 m of the site. Any type of agricultural site can be considered (e.g., cereals, vegetables, fruit orchards, among others), but excluding areas dominated by greenhouses, livestock pastures, and urban vegetable gardens.

- **Urban Garden** sites should be located in areas where land cover is predominantly urban (e.g., housing, roads, urban green areas) within about 2 km of the site, and to be in a garden where green areas are dominant within about 100 m of the site. Gardens can be botanic gardens, public leisure gardens, or private backyard gardens, among others. Ideally, the garden should have a mixture of habitats, including woodlots or at least isolated trees, flower beds and meadows.

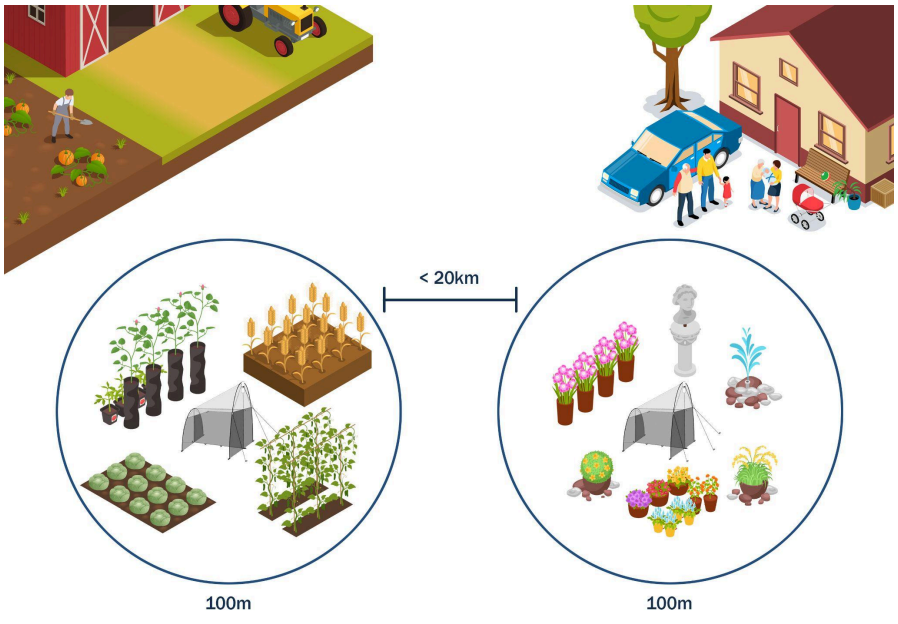

Figure 3. Sampling design, showing an example of an Agricultural field (left) and an Urban garden (right), separated from up to 20 km. The blue circle denotes land cover dominance.

Materials

List of materials needed for Malaise trap setting and sample collection

A. A pair of Malaise traps (ez-Malaise Trap II mode, see BugDorm store)
B. Extra cords, metal stacks, etc. for mounting (more to read in the section “Before sampling”)
C. 500 mL sterile collection tubes (see Wide-Mouth LDPE Bottles with Closure link)
D. ≥ 96% Ethanol
E. Sticky labels with predefined QR codes to be read by the *PlutoF Go* app[1] (.pdf will be 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. Aluminum foil (Optional, to cover the tubes during sampling)

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. QR codes will be supplied by email to each partner institution 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.

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 formalize the authorization 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.

[1] The *PlutoF Go* app can be found for Android, and for iOS systems. Instructions and other information can be found in the link provided. A quick guide for PlutoF Go is provided in this document.

Before starting

The following instructions are taken from Najera-Cortazar et al. (2024). 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 several options of pair sites chosen when possible, as backups. 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. It is important to ensure that each pair site is accessible during its five weeks of sampling, and that tube collectors will be available during that period.

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 Pollinator communities sampling it needs to be removed prior to setting the trap. Otherwise arthropods pollinators larger than the size of the grid will not be sampled. In Figure 4 is shown an example of how to remove the plastic grid and to secure the mesh back using cable ties.

Figure 4. Example of how to remove the plastic grid included in the Malaise trap.

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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 (Pollinator Communities - "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 pollinators (the trap head) is facing uphill. Ideally, position the trap perpendicular to the path of arthropod's flight, in areas with minimal undergrowth, such as forest edges, clearings, or elevated locations. Take into account potential disruptions by wildlife, 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 5, 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 5, right).

Figure 5. 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 6), 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 6. Arthropod view to "the light" path.

Once the trap is set, carefully attach the BGE.PC labeled collection tube tightly to the trap head, filled with ≥96% ethanol, and secure it with the white straps on the trap (Figure 7). Take pictures of the trap set and its location. Remember to begin collecting on a day of the week when you can reliably return for the duration of the sampling period (five weeks). Additionally, you can cover the tube with aluminum foil, to keep a more stable temperature.

Ethanol recommendations: as previously mentioned, the tubes should be filed approximately with 350 mL of ethanol. After the first collection, you would be able to monitor if you needed more ethanol or less, so you can adjust the quantity of ethanol per tube, and per site. During hot weeks, ethanol could evaporate faster, or you might have more specimens than expected, so you can add more or less depending on how your collections are.

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Figure 7. Example of a collection tube correctly placed and secured in the trap using the white straps around it.

Every week, two tubes will be collected per urban/agricultural pair site, making a total of two tubes per week, during five weeks (10 tubes per pair). 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. Remember: when collecting the tubes from the traps each week, **tubes should be filled with ethanol, closed and not opened again**.

Each pair of sites should be selected under previous agreement with landowners/stakeholders and having any necessary permit/documentation. Despite this, there could always be problems or eventualities with the Malaise traps (e.g. vandalized, cattle playing ground, extreme weather). 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, have enough ethanol to cover the specimens (top it up if needed), and store them at room temperature, away from light exposure. 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
e-mail: bemerson@ipna.csic.es

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). Please confirm this with project coordinator Laura Najera (la.najera@cibio.up.pt) and Brent Emerson.

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 only under these conditions and at a unique time, open each tube using gloves to decant the supernatant ethanol.

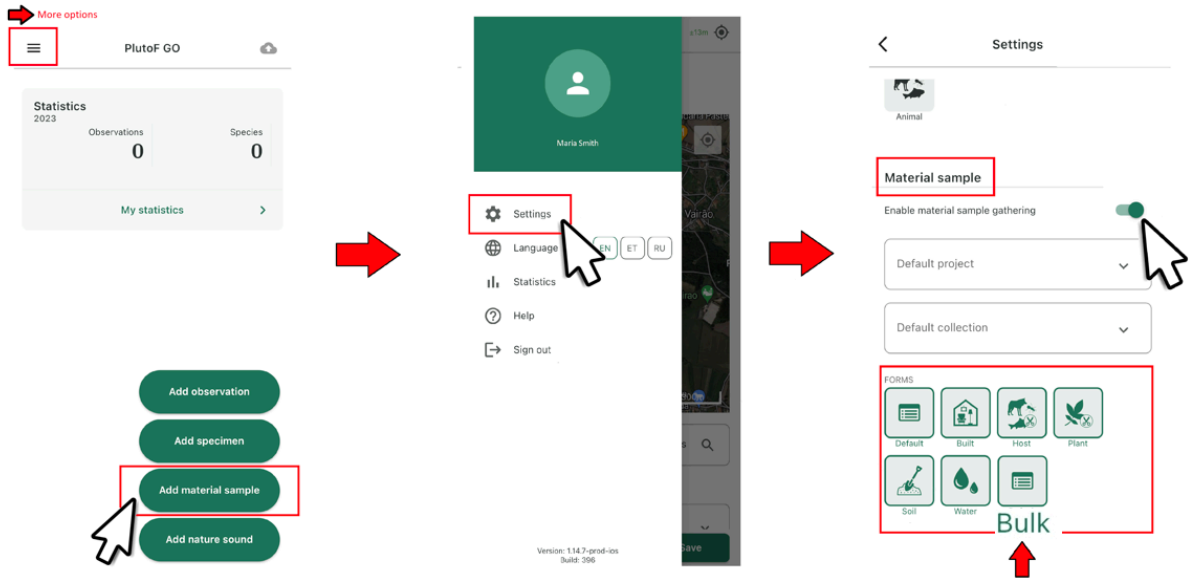
Full instructions of how to ship Malaise traps tubes are described in the “Malaise traps - Bulk samples Shipping instructions” living document, please refer to the information when needed.

Registering samples in *PlutoF Go* app

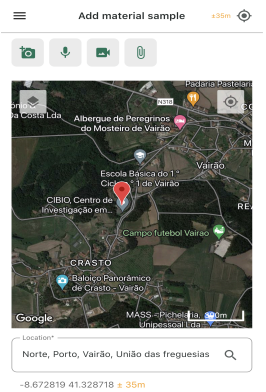
PlutoF is an online data management and computing service provider for biological data. *PlutoF Go* is the app that will be used to record samples directly in the field. Before using the app, the data collector should be registered on the *PlutoF* website. This can be done by the user in the option "Become a user", or to be added by the BGE project manager directly on her/his workbench[2]. 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

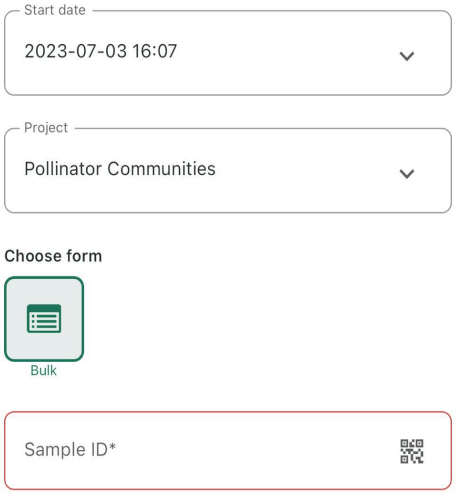
2. Go to **Add material sample** box (If this option is not visible in the main page, go to Settings, scroll down to Material sample, activate Enable material sample gathering and make sure the Bulk form is highlighted in green as well).



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).



[2] 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.



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

5. **Project:** choose the "*Pollinator Communities - Institution*" option. Choose the project according to your institution acronym (compulsory field).

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

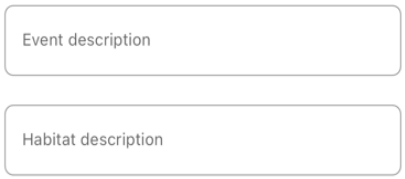
7. **Sample ID:** click on the code icon and point your device camera to the corresponding QR code provided for the sampling. QR codes are unique and cannot be added multiple times (compulsory field).

8. **Subcode:** Add the country prefix of your study, point, Institution prefix, Agricultural/Garden + trap in a consistent way for all the sampling, for example for Portugal **PT.CIBIO.A1, PT.CIBIO.G1**:

- **Pair 1: Agricultural Malaise 1 (A1) and Garden Malaise 1 (G1) =** these subcodes will correspond to each *BGE.PC00XX* sample collected from the same traps, every time you visit them
- **Pair 2: Agricultural Malaise 2 (A2), and Garden Malaise 2 (G2).** And so on until **Pair 3**… If you sample three pairs of sites, you will have A1,G1; A2, G2; and A3, G3. Please record location and all the required details per each trap set

9. **Description**: Write "Placement date of Malaise trap [date] or "Placement date of the collection tube [date]". This to know how long was the tube sampling in the trap

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



11. **Event description**: Describe any relevant events during the sampling week (e.g. strong wind/rain, external disturbance by human/animal; etc.)

12. **Habitat description:** Describe any information about the site you have sampled. For example: type of garden, % of flower coverage, taxonomic description of plants (if known), type of agricultural field, types of crops, % of crop coverage, position of trap respective to the crop land, etc. Pictures of the trap's surroundings (point 14) will help to support this

13. **Trap ID**: an identifier for each trap, if needed

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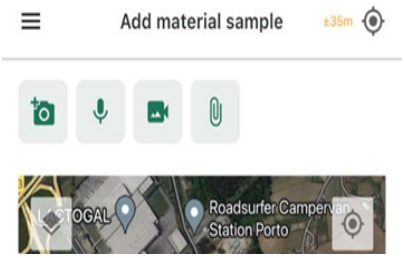
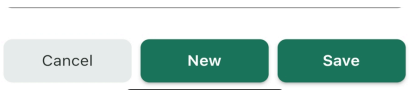
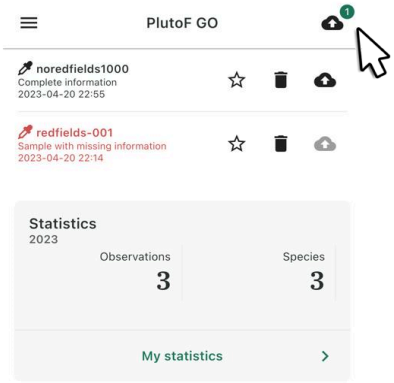
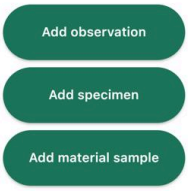
14. Add at least one photo of the Malaise trap and its environment using the panel that is in the top of the Material sample menu. Use the **camera + icon** (top left) to generate a picture (For example, see Figure 1 and 7). You can add any extra information or metadata you think convenient using the media bar (more photos, videos, etc.), anything you think will be useful for further assessment of the site, vegetation, etc.



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







16. 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. Keep in mind that entries with media files (pictures, videos, etc.) will take longer to sync, and more internet to load.

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

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Useful contact

Laura Nájera Cortazar | Associação Biopolis - CIBIO – Centro de Investigação em Biodiversidade e Recursos Genéticos [BIOPOLIS - CIBIO] la.najera@cibio.up.pt