PROTOCOL FOR THE COLLECTION OF LEAF SAMPLES FROM PHOENIX SPECIMENS FOR DNA EXTRACTION FOR GENETIC ANALYSIS

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1.- Preparing for the field trip. Field sheet. Tools.

To carry out the genetic study of *Phoenix* samples, the first thing to do, obviously, is to know which specimens are going to be studied.

To do this, you have to choose the populations (regions, areas... whatever you choose) on the map and make a file for each population with the specimens to be studied in that population.

Assign names and codes to the populations and specimens. Attached is the sheet that we use when we go to the field.

Population / UTM: Sample / UTM: Height: nº Photo: ♀□ ♂□ ?□ Observations:	Population / UTM: Sample / UTM: Height: nº Photo: ♀□ ♂□ ?□ Observations:

Figure 1. Example of a field sheet with data for each specimen

Tools: Needed at least: pruning shears, knife or razor, cloth or paper for cleaning, alcohol to disinfect tools between samples, paper envelopes, permanent markers, pencils, silica gel, tape measure, plastic boxes for storing samples; aluminium trays, cookers.

2.- Selection of the specimen

Data to be entered on the record sheets for each specimen

- ✓ Location of the population by GPS (UTM). Include surface or working area if necessary.
- ✓ Location of the specimen by GPS (UTM).
- ✓ Assign a code to the individual. *We usually put a code according to the name of the population and list sequentially; e.g., Gomera-Tazo (GTZ001).*
- ✓ Sex of specimen (male, female, undetermined).
- ✓ Height of the individual. *Approximate or exact. Depends on the specimen.*
- ✓ Remarks. Describe any characteristics. Presence of fruits, state of the fruits, state of the specimen, presence of very dry leaves, presence of pests... whatever you consider.
- ✓ Take photographs of the specimen. We usually take 4-5 photos of the specimen with details of some parts (leaves, trunk, etc.) and 2-3 general photos where the whole specimen can be seen. In the case of *Phoenix canariensis* it is important to have evidence of the morphological differences between *P. canariensis*, *P. dactylifera* and its hybrids. We also take several photographs of the area and its surroundings.

- ✓ Write down the camera numbers (codes) of each specimen on the cards. *We take a photo in the air or put our hand in front of it or take any other unusual photo that serves to separate the set of photos of one specimen from the set of photos of the next specimen.*

Photo 1. Collecting the position of the specimen using GPS

3.- Cutting of the leaves

Only 4-5 leaflets are needed to store and dry the samples.

A piece of a leaf is cut off. We usually cut it towards the apical end so as not to waste plant material. It does not matter from which area the samples are collected, but it is very important to collect the parts of the leaves that have green leaflets.

The youngest (greenest) leaves are more difficult to obtain and collect because they are at the apex and bud of the specimen and are usually further away. Any leaf can be picked, if it can be seen to be **green and healthy**.

Photo 2. Cutting a piece of green leaf. The 4-5 leaflets to be stored are then cut from this piece.

Photo 3. Cutting a piece of green leaf. The 4-5 leaflets to be stored are then cut from this piece. ***It is not necessary that the cut leaf is so large. This leaf was cut for other purposes.***

We often use poles with scissors to reach the leaves of very tall specimens. It is advisable to buy a similar tool (Photo 4-5).

Photo 4. Picking up leaves. On the shoulders of a partner or via a pole.

Once we have the leaf, we cut 5-7 leaflets of between 10-12 cm. Clean them well with a piece of paper or a damp cloth with water. Leave to dry. In this way we eliminate dirt and the presence of phytophagous, insects and even some fungus or other organisms from the leaves. And keep them in the envelope with their corresponding code (Photos 5, 6, 7).

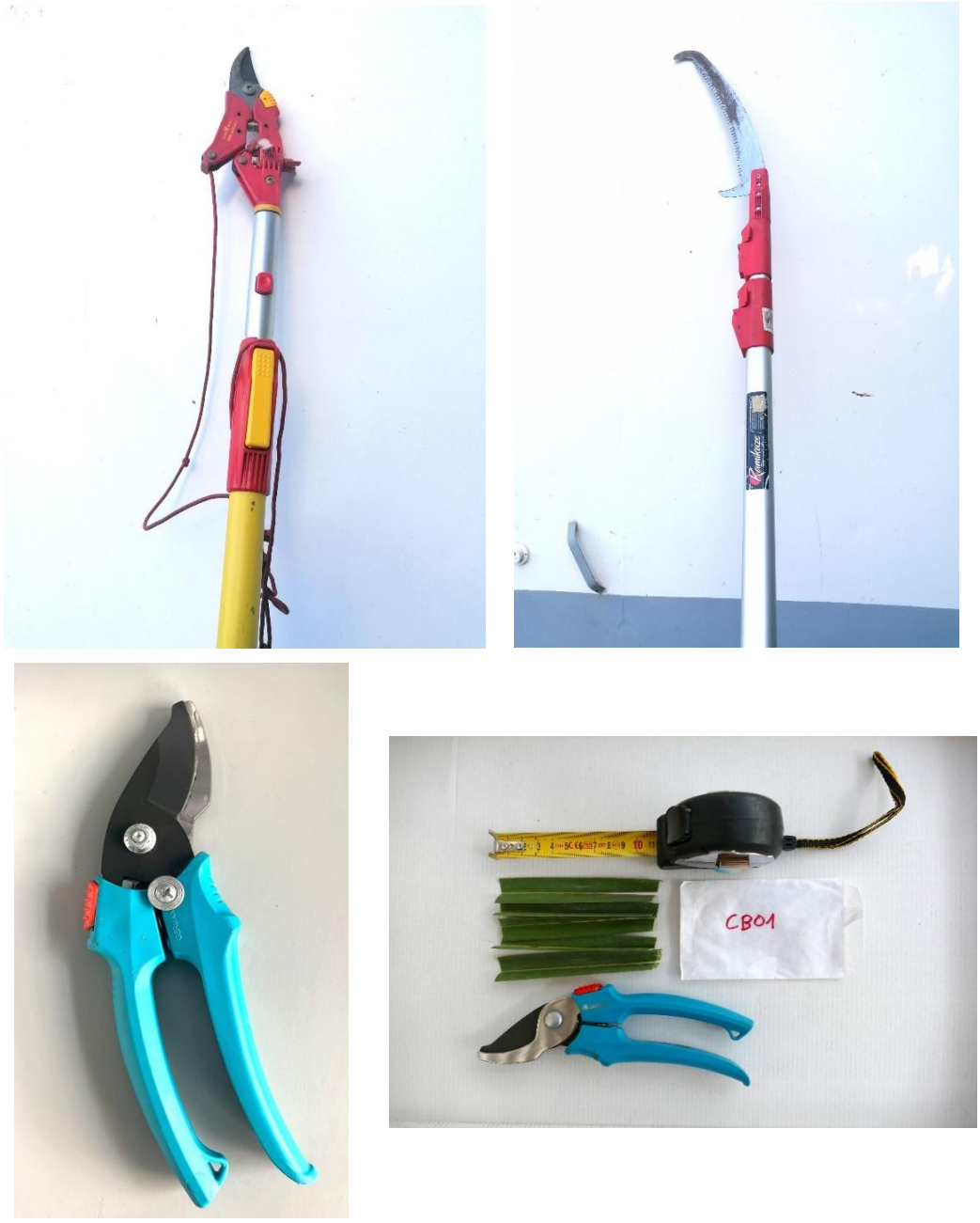

Photo 5. Tools for cutting leaves and leaflets. Pole shears. Saw and pruning shears. Size of leaflets to put in a paper envelope.

Photo 6. Cutting the leaflets to put in the envelope.

Photo 7. Leaflets with organic debris or insects that need to be cleaned and removed before storing the leaflets.

Cut the leaflets to a size that allows them to be placed in a paper envelope (10-12 cm). Place the leaflets in the marked envelope, write the code of the specimen on the envelope and optionally the codes of the photographs, and seal the envelope (photo 8).

Photo 8. Paper envelope with leaflets and copy code

It is not necessary to close the envelope tightly (sealing it) because it will be used later. Just fold the end of the envelope several times, so that the sample does not slip out of the envelope. A paper clip or some adhesive tape can be used for extra security (Photo 9).

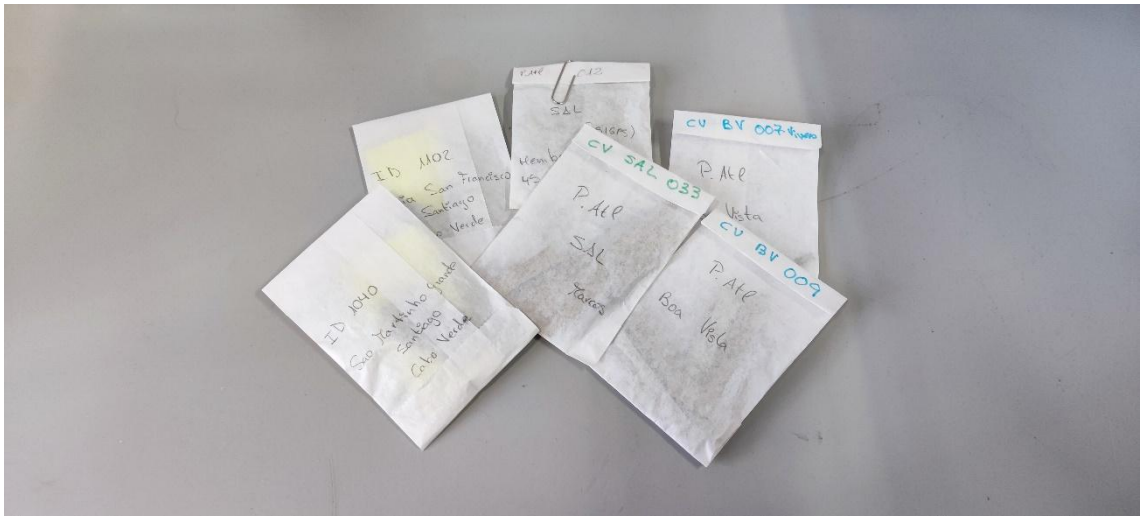

Photo 9. Paper envelope with the leaflets and copy code. See the position of the clip that helps to prevent it from slipping off, so that it can be opened and closed as many times as necessary. Or invisible tape that is easy to peel off.

4. Drying and storage of leaves

The drying process is very important for proper storage. The samples (leaves) should always be well dried. *Phoenix* leaves are dry leathery leaves, but during the first phase of the process it is important that the envelopes are well soaked in silica gel from the moment they are collected.

The envelopes with the samples are placed in a zip-lock bag or a box with a lid and plenty of silica gel. Usually by the next day the silica gel has absorbed the moisture and changes colour to dark green. It is at this point that it needs to be replaced and renewed with new gel.

This process of renewal of the silica gel is done in plastic boxes and will last approximately 3 to 4 weeks until the gel no longer changes colour. Several dozen envelopes with the samples can be stored inside the boxes. At this point, the samples are ready for DNA extraction and can be stored for months (Photos 10-11).

Photo 10. Sealed sample envelopes in plastic boxes with silica gel (orange balls inside the box).

Photo 11. Detail of sample envelopes in storage boxes.

The boxes are stored in a dry place. They must be hermetically sealed. They can last for a long time (years) (Photo 12).

Photo 12. Storage of samples in boxes. It is important to renew the silica gel every six months or as soon as it is observed that the gel has turned.

IMPORTANT: During storage of the samples, the condition of the silica gel must be checked, and the gel must be renewed from time to time**.** The gel is not discarded, it can be reused several times. For this purpose, it is placed in trays and placed in an oven

at 135ºC for 5 hours (photo 13). We put it in aluminium trays inside the oven and once the gel is reactivated (it changes colour), we turn off the oven and let it cool inside. When it has cooled, we store it in a hermetically sealed container away from light.

Photo 13. Gel in the oven for reactivation

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