Illustrated protocol for palm leaves sampling, drying and sample preserving for subsequent DNA extraction, as implemented by CRA-FSO in Sanremo (Italy)

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This protocol follows Jean-Christophe PINTAUD's (IRD Montpellier, FRANCE) recommendations

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- **Phase 1. Marking palm individuals to be sampled**

Palm trees to be sampled should be individually marked, allowing us to recognize each tree in the future and repeat the sampling procedure if needed. In Italy, we used a code consisting in a double information: a first number indicating the sampling zone (for example: zone 93) and a following number indicating the individual palm tree (for example: tree ID number 001). So, an individual mark can be written as the following: 93.001, i.e. palm ID number 001 in the zone 93 (**Fig.1 and 2**).

Fig.1. Palm tree 93.001

Fig.2. Sampling zone N. 90 (Sanremo, Italy)

- **Phase 2. Sampling**

Recently expanded leaves are preferred for sampling, as they give the best yield and quality of DNA. Unexpended leaves are more difficult to access and may be rich in unwanted compounds that can affect DNA purification. Old leaves are heavily sclerified, which complicate grinding, reduce the percentage of DNA-containing tissues, and may be affected by necrosis, parasitism and epiphylls that bring contaminant DNA. Sampling can be performed by means of a cutter (**Fig.3**).

Fig.3. Cutting a young leaf

Fig.4. Telescopic carbon fiber pole

In case of tall trees, a carbon firber pole with a blade screwed at its extremity (**Fig.4 and 5**), or other devices (**Fig.6**), allow to reach young leaves.

Fig.5. zirconium-steal blade used with the carbon fiber pole

Fig.6. Elevator

Leaflets are cut in pieces and the central vein is removed, and the fragments are put into plastic bags (**Fig.7, 8 and 9**).
Each bag should report the following information:

- palm species (for example: *Phoenix rupicola*);
- palm ID number (for example: 90.004);
- sampling area (for example: Sanremo, ITALY);
- date of sampling (for example: 10th May 2010)

Fig.7.

Fig.8.

Fig.9

- **Phase 3. Drying and sample storage**

The best way to store leaf samples is to dry them. Once dried, both metabolic and decomposition processes are stopped and samples can be kept at room temperature for an indefinite time. While waiting for the drying process, each sample envelope can be stored in a freezer (-85°C); such a low temperature stops metabolic and decomposition processes, too.

Drying can be performed following two possible procedures:

a) Silica gel drying, inside airtight plastic bags or airtight boxes (suitable in case of small samples, **Fig.10**)

b) Freeze-drying, using a freeze-dryer (suitable for larger samples; in case of palm tree leaves, it takes 4-5 days; **Fig.11**)

Fig.10. Airtight plastic bag containing a leaf sample and some silica gel

Fig.11. Freeze-dryer

Degree of drying can be checked visually (**Fig. 12 and 13**).

CAUTION: an incomplete lyophilisation may result in failure of sample preserving!

Fig.12. Leaf sample after incomplete lyophilisation:
TO BE AVOIDED

Fig.13. Leaf sample after complete lyophilisation:
GOOD FOR PRESERVING

Once dried, samples can be preserved in airtight boxes (**Fig.14 and 15**) or airtight plastic bags (**Fig.10**). Some silica gel must be put inside the boxes or the plastic bags containing the samples, in order to adsorb residual humidity.

Fig.14 and 15. Airtight box containing dried leaf samples and, on the bottom, some silica gel

A silica gel containing an indicator that changes its colour when passing from a dry to humid status should be preferred (**Fig.16 and 17**), allowing us to understand when the gel is exhausted and must be replaced.

Fig.16. Dry silica gel

Fig.17. Humid silica gel

- **Phase 4. Preparing sub-samples for DNA extraction**

To perform DNA extraction from stored samples, smaller sub-samples must be prepared. According to IRD Montpellier's protocol, **45** mg of dried tissue for each sample must be weighted (**Fig.18 and 19**), fragmented in tiny pieces and put into small (airtight) tubes (**Fig. 20 and 21**). Of course, each tube must have an ID number.

Fig.18.

Fig.19.

Fig.20.

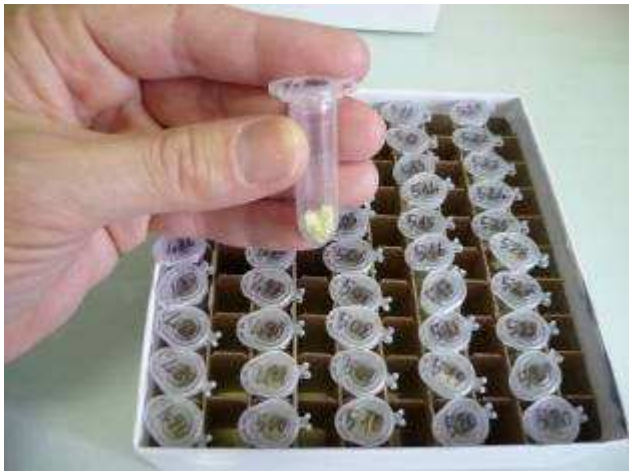

Fig.21.

What remains of the original dried samples should be used to create an archive for future analyses.
Samples can be put into paper envelopes and kept into airtight boxes containing silica gel, for an indefinite period of time (**Fig. 22**).

Fig.22. Samples archive