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## Short Communication

# Forensic Palynology in Argentina. An alternative treatment for tape method is proposed

### Abstract

Forensic Palynology contributes to the evidence of the crime site and sometimes the moment of the fact by the study of pollen and spores and other palynomorphs present in the scene. Pollen morphology is sometimes exclusive for a plant species and some of them grow in specific areas besides plants produce pollen at certain times of the year. Some of the methods used to obtain the samples are here described, and they depend on the material to be analyzed. An alternative treatment for the tape method is proposed. Little is known about palynomorphs in forensic scope in Argentina, although some cases have been satisfactorily addressed. This paper describes three cases in which pollen was important evidence in this country.

## Introduction

Palynology is the study of pollen and spores and some structures related to them like acritarchs, dinoflagellates cysts and chitinozoans. The results obtained may contribute to different scientific disciplines like biostratigraphy, taxonomy and evolutionary studies in plants, allergy studies, melissopalynology and archaeological palynology, a description was made by Bryan [1]. Forensic palynology is the study of pollen and other palynomorphs for evidence at a crime scene [2-4], linking the accused to the victim and the place. The questions "Where?" and "When?" can be answered because the pollen morphology is sometimes exclusive for a plant species and some of them grow in specific areas besides plants produce pollen at certain times of the year. Several papers describe the importance of Palynology as a forensic discipline [3-5]. Forensic palynology was introduced in criminal cases in Argentina in July 1984, in a non-resolved crime. At that moment limitations to trace evidence analysis and the technological capabilities was limited. Actually new technologies have been applied to resolve some problems in the trace evidence, and are currently trying to prove the effectiveness of the methods to achieve introducing this discipline in the legal field [6-8]. However, little is known about palynomorphs in forensic scope in Argentina, although some cases have been satisfactorily addressed. This paper describes three cases in which pollen was important evidence. It proposes an alternative treatment for tape method, too.

## Results and Discussion

### Palynology importance

Some plants produce spores or pollen grains for reproduction, pollen grains are responsible to transfer the male gametes from the anther to the stigma of the female floral organ for fertilization takes place. A pollen grain is between 10 and 200  $\mu\text{m}$  (most common in 30-60  $\mu\text{m}$ ) and fungal, mosses and ferns spores size have similar values. The importance is that the pollen grains and spores have an outer wall of sporopollenin, very resistant even with treatment with acids,

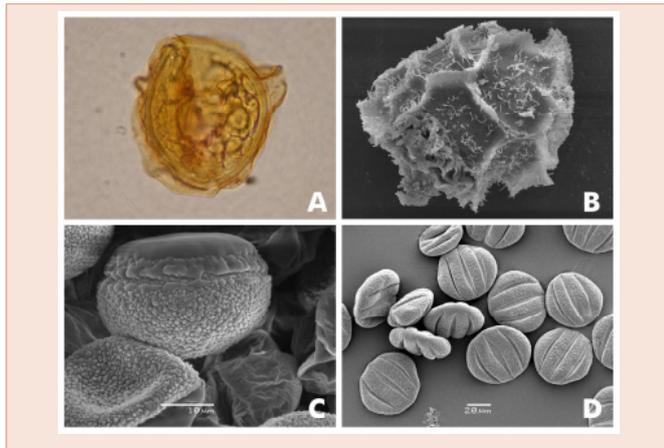
so fossil elements can be thousands or millions of years in sediments. They are also relevant in this case because pollen grains and spores from different species can be very distinctive and, on or in the surface of soil, reveal the vegetation on the site. If it is on clothes, mud, or other elements and the sample contains pollen from plants with limited distributions it will be a good clue in determining the scene of the crime.

### Palynomorphs features

Pollen may be released as a single grain, or *en masse*, as a compound. They can vary quite a lot in size (from about 10 to nearly 100 micrometers) and in shape: (Ellipsoidal, spherical, or with sacs) symmetry, presence and number of apertures, shape, size and disposition of the sculpturing on the outer wall surface in agreement with the plant that originated it. However, in some cases like Pinaceae or Poaceae (grass family) you cannot say with accuracy because the grain morphology is common to several genus and species. It is therefore very valuable uncommon presence of pollen types, for example, Lamiaceae, (*Salvia guaranitica* A. St.Hil ex Benth, Figure 1D) species belonging to this family had similar grains but they grow in limited spaces. Pollen and spores morphology is very accurate and may determinate exactly the plant which originated it (Figure 1).

### Dispersion mode

Ferns, Gymnosperms or Angiosperms produce large amounts of spores or pollen. The dispersion can be carried out by abiotic vectors (wind, water) or biotic vectors (birds, insects or rarely mammals), it is also influenced by climatic factors like wind. Some aquatic plants release pollen into the water (Ruppiaceae [9]), therefore, produce large quantities because the possibility of reaching the female gametes is low. Other plants release pollen and the wind carries it, also in these plants the production is very high (*Pinus* sp). The animal-pollinated plants have reduced production of pollen grains according to the specificity of the vector [10]. Plants also bloom at certain times of the year. For example, if plum pollen is obtained we know that in the



**Figure 1:** Spores and pollen grains with distinctive features. A- *Blechnum auratum subespecie auratum* (Fée) R. M. Tryon & Stolze (Blechnaceae) fern spore. B- *Blechnum cordatum* (Desv) Hieron (Blechnaceae) fern spore. C- *Nymphaea sp* (Nymphaeaceae) pollen grain. D- *Salvia guaranitica* (Lamiaceae) pollen grain. A- Photograph with ML and B-C-D with SEM.

Southern Hemisphere trees are in bloom in springtime (August to September).

### Which materials and methods are used in this discipline? [4]

Forensic palynology samples should be taken by a competent palynologist who knows the forensic aspect. Such specialists will know how to collect samples not contaminated and what precautions should be taken to remain free of contamination during storage time prior to conducting the study, during the treatment and extraction in the laboratory and in the process analysis. He must know the sample amount, necessary for the treatment [4].

The material to be treated is very diverse and the professional must select the best alternatives to test the scene. Samples of mud, clothes, hair, nails, wrappers, rugs and parts of a car may be used. For chemical processing sample, like soil samples, the standard KOH (deflocculation), acetylation (cellulose and organic matter removal) and hydrofluoric acid (silicate removal) method is used [5]. Where pollen substance adheres to cloth it can be obtained by sticky tape [11]. The tape will be cut into smaller portions and then treated with the acetolysis method [12]. This method has advantages such as its low cost, easy transportation, rapid processing [8]. Small samples may be put directly in a test tube, add glacial acetic acid to dehydrate the sample, centrifuge and decant, and then processed with acetolysis. If the material amount is scarce the acetolysis is not recommended here because much material is lost during processing by this technique, only dehydration and centrifugation is suggested for good results. If the pollen sample is stored in 70% ethanol we can put a drop of liquid glycerin gelatin and mix. Then, with a clean dissecting needle touch the mix in the tube and then, place the jelly with the pollen on it onto a slide, warm it gently over a flame until the jelly melts (don't allow the glycerin jelly to boil) and lower the coverslip carefully over the molten jelly. For permanent slides you can seal the sample with parafin wax. [13] Observe with LM.

When the material to be analyzed is scarce or difficult to recognize it is proposed in this paper an alternative treatment: a) dry material like small pieces of rugs, nails, hair, etc. may be mounted without treatment on metal stubs with glue or double-sided adhesive tape. b) If the material was centrifuged the residue will be placed on the stub and allowed to air dry. b) Coat the pollen with 300 Å of gold palladium (60/40) using a sputter-coater. c) Observe the material with SEM (Figure 1, B-D). This technique is usually applied in pollination studies where it is necessary to investigate whether or not insects transport pollen on the body [14].

### Cases in which pollen provided evidence

**Case 1: Lucy- Alex** (Names have been changed): Lucy's corpse was found on February 26 in a lagoon in the southern Neuquén city, Argentina, associated with the Limay River and do not know how it got there. The main suspect, Alex, said he had never been in that area, but shoes and carpets Alex's car had traces of pollinium of *Ludwigia uruguayensis* (Michx.) Greuter & Burdet (Onagraceae) a plant that grows on the banks of Limay River and not in other areas of that city.

It was also noted in the victim's shoes the presence of "aromo pollen" (*Acacia caven* Molina), this tree is not common in Neuquén and two trees were found in the front door of Alex's house, indicating that the victim has been there. Alex was convicted for this and other evidence, and sentenced to 21 years in prison.

**Case 2: Natalia:** Natalia was found dead in the tree nursery of Miramar city, Buenos Aires province, Argentina, an area of forest, over 500 hectares planted with varieties of pine and eucalyptus. She was raped and murdered. One of the dilemmas was whether if she had been killed there or if the body had been moved. The foreign pollen found in the socks and the scarcity of pollen from the forest, determined that she was moved after death. Pine and eucalyptus trees produce a very high amount of pollen, sometimes it is known as "pollen rain".

**Case 3: Juan:** A photojournalist was murdered in General Juan Madariaga, Buenos Aires, Argentina, in 1997. A group of divers found the camera near Route 11. This was the first concrete evidence of the crime committed and involved one of the suspects. Researchers suspect that may have been drawn by the murderers in their flight to La Plata after the crime, but the presence of plankton, different diatom species not belonging to the lagoon where the victim chamber was found throw new doubts.

### Discussion and Conclusion

Forensic palynology is a discipline little known in Argentina, even in scientific sites. Cases presented here are the only recognized for Argentina. It has been rarely applied in the United States (Bryan et al. 1990) and actually it is adopted in countries like England and especially New Zealand, where usually the pollen evidence is used in court. Its aid is invaluable as an evaluation on the site or the time when the incident occurred. Still a long way off is necessary before these data were accepted as evidence in Argentina, but in recent years much has been achieved. It also depends on the Argentine palynologists to extend science to court. The scanning electron microscope is



proposed here as an alternative tool for greater definition, biologists and of course palynologists have access to this technology.

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