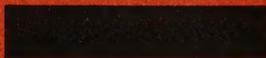


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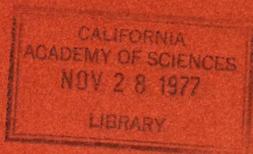
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No. 13

David M. Jarzen

THE POLLEN AND SPORE REFERENCE COLLECTION
AT THE NATIONAL MUSEUMS OF CANADA



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OF CANADA

David M. Jarzen
National Museum of Natural Sciences
Ottawa

Syllogeus No. 13

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Les Musées nationaux du Canada

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Ottawa, May 1977

THE POLLEN AND SPORE REFERENCE COLLECTION AT THE NATIONAL MUSEUMS OF CANADA

David M. Jarzen

INTRODUCTION

The collections held by a Natural Science Museum are as varied as are the ways in which they are stored and in the ways in which they are used. Fish in bottles, dinosaur bones in plaster casts, bird and mammals stuffed and lined neatly in drawers, sea shells cleaned and placed in individual boxes, and dried plants pressed and affixed to herbarium sheets are but a few of the collections maintained by the National Museum of Natural Sciences in Ottawa.

One collection which is unique in both its nature and methods of collection and preservation is the Pollen and Spore Reference Collection of the Palynology Section of the Paleobiology Division. What makes the collection unique is the smallness of the objects being collected. The pollen and spores of plants are not visible to the naked eye, inasmuch as they measure only 20 to 100 microns, or approximately 2/100 to 1/10 of a millimeter. To locate and examine pollen and spores, the researcher must use a compound research microscope (fig. 1). To store the collection, the pollen and spore grains are mounted onto glass microscope slides and kept in boxes of 100 slides per box. The space required to store an adequate collection is thus not of the magnitude required to store fish, sea shells or dinosaur bones.

This paper is an attempt to explain why the Museum maintains a pollen and spore collection, and the methods involved in collecting and preparing such a collection.

The pollen and spore collection was established (1974) in order to obtain reference material of living plants with which comparisons to fossil pollen and spores could be made. These comparisons are necessary if a researcher hopes to be able to identify the kinds of plants which were growing during the past eras of our Earth's history.

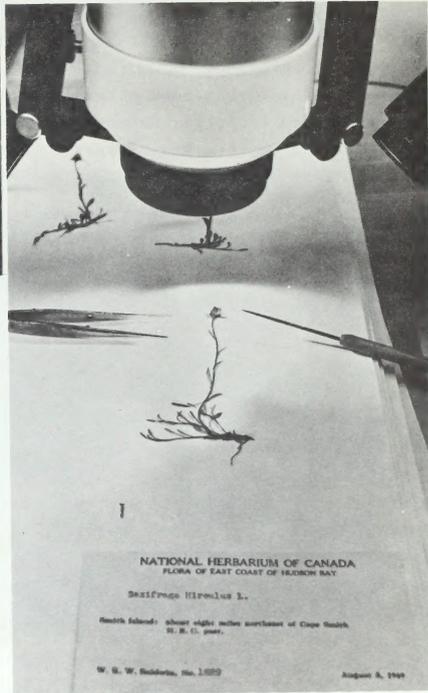
It is not often realized that the pollen and spores of plants are preserved as fossils in the rock layers of our Earth. It is in fact true that often when the rock layers have not preserved the leaves and fruits or seeds of plants the pollen and spores of these plants are well preserved, and preserved in great abundance. Because of this abundance of well preserved "microfossil" material the scientist can frequently identify the parent plant which produced the pollen or spore grains. It is indeed fortunate for the investigator that pollen or spores often have unique architectural and surface features which make their identification possible (see Plate I).

The Pollen and Spore Reference Collection of the National Museum of Natural Sciences is stored at the Palynology Laboratory, Ottawa, Canada.



FIGURE 1. A compound binocular research microscope similar to this instrument is a necessary piece of equipment for the study and photography of pollen and spores.

FIGURE 2. A few anthers of *Saxifraga hirculus* L. are carefully removed from the herbarium sheet. The label provides information relative to location, collector, date collected etc...



COLLECTING AND PROCESSING TECHNIQUES

Proper collection and processing techniques are prerequisite to a valuable and useable collection. Pollen and spores can of course be collected from living plants growing in the fields and forests around us. This method however, is not often used, inasmuch as the collector, unless he is a trained botanist, may not know the exact species which he is collecting. A more common practice is to obtain pollen and spores from labelled herbarium sheets, directly at a herbarium, where each preserved plant has been carefully examined and properly named by a professional botanist.

The herbaria of the world maintain collections of plants which have been identified by experts in the art of taxonomy and nomenclature. Thus each plant has its own set of documented information such as the latin generic and specific names, the exact locality where it was collected, the name and collection or field number of the collector, and often other information such as habit and habitat data. Additionally each sheet has its own unique herbarium number. With such well documented or vouchered specimens, the pollen and spore collector can be assured that what is added to his collection is properly identified.

Once permission has been obtained from the curator of a herbarium, the pollen and spore collector may begin his task of carefully removing a few stamens (the pollen-bearing organs) or a few sporangia (the spore-bearing organs of ferns etc...) by using a tweezer or needle (see fig. 2) and placing them into small sequentially numbered envelopes. Onto a pad of paper on which the same sequence of numbers has been entered, the collector records all information presented on the herbarium sheet label. On the average, about 100 specimens of pollen or spores can be collected during a day's work.

It is very important that the collector uses sound judgement while making his collections. Type specimens, that is the specimen on which the original name of the species was made, should never be used for pollen or spore material. Type specimens are extremely valuable and obviously, once destroyed, can never be replaced. Specimens with only a few flowers, or specimens which would lose some major portion of their bulk should likewise not be collected. The pollen and spore collector must be properly trained in the professional use and respect of herbarium specimens and equipment.

After the pollen or spore material has been collected and the data dutifully recorded it is ready for laboratory processing (figs. 3 and 4, illustrate some equipment and methods employed in the processing of polliniferous material).

A series of chemical treatments are necessary in order to remove the surface oils common on pollen and spores, so that the intricate details of the surface sculpture are clearly visible. The extraneous plant material, e.g. petals, sepals, stamens, and anther walls, must be removed to provide a

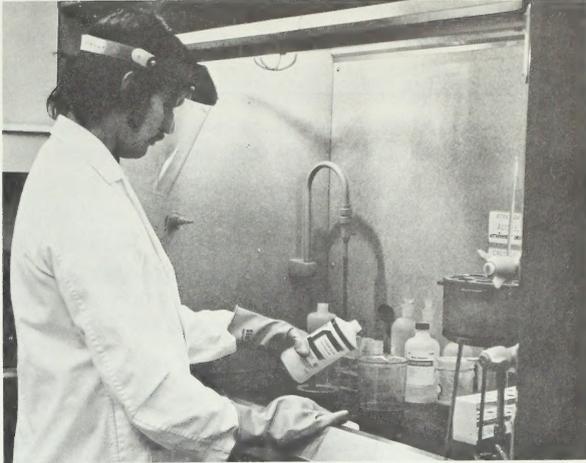


FIGURE 3. *Some laboratory techniques may involve the use of dangerous chemicals. The use of a fume hood and safety equipment help protect the laboratory technician.*

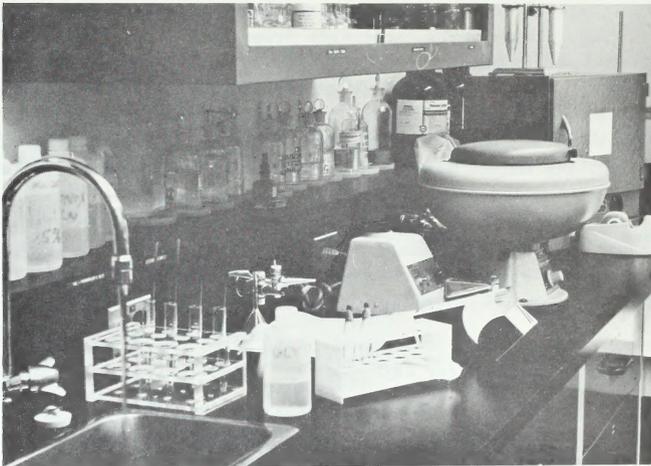


FIGURE 4. *A view of the pollen laboratory bench showing some of the equipment and materials used in the preparation of pollen and spore samples.*

clean slide preparation.

The steps involved in processing a sample are enumerated below:

Preparation Technique for Modern Reference Pollen and Spores

1. Place pollen-bearing material into a series of numbered 15 cc centrifuge tubes and fill about one-half ($\frac{1}{2}$) full with 5-10% KOH.

In order to identify which species of plants are represented in each of the tubes, it is necessary that they be numbered sequentially and a list kept of the tube contents. Enter this information on the processing record sheet. Each tube should contain an equal amount of KOH. This is necessary to insure that the centrifuge is balanced.

2. Place the tubes in the water bath at about 90° C for 2 or 3 minutes, remove and allow to cool slightly.
3. Pour (carefully) the contents of the first set of numbered tubes through a fine mesh screen into a second set of numbered tubes. Pour slowly so as to allow for a break-up of the surface tension of the KOH.

It is obvious that the contents of tube number one of the first set must be poured into tube number one of the second set, etc. It may be desirable to remove large fragments of plant material (e.g. flower parts, sporangia fragments, etc.) with a glass rod or spatula before straining. It is advisable to use separate rods or spatulas for each sample, to lessen contamination.

4. Centrifuge at 1500 rpm for 2 or 3 minutes, decant the coloured supernatant and wash twice with distilled water. Centrifuge and decant after each wash.
5. Wash once with glacial acetic acid.

This step is necessary because water and the acetolysis mixture to be used in the following step, can react violently.

6. Prepare the acetolysis mixture. Fill each tube about one-half ($\frac{1}{2}$) full, and place in the water bath (ca. 90° C) for about 3 to 4 minutes.

The acetolysis mixture is prepared by pouring one (1) part of concentrated sulfuric acid (H_2SO_4) into nine (9) parts of acetic anhydride. If eight samples are being prepared, a suitable method is to pour 5 cc of acid into 45 cc of the acetic anhydride. NOTE: This should be done under a fume hood and the mixture treated with care. It can cause severe burns, particularly when warm.

7. Remove the tubes from the water bath and allow to cool slightly, centrifuge, and decant.

NOTE: It is not possible to discard the acetolysis mixture directly into the sink. It would react violently with the limited amount of water present. Fill a large beaker (2,000 ml) to over-flowing with water (keep a supply of water running into the beaker at all times) and slowly pour the mixture, one tube at a time into the large reservoir of water.

8. Wash once with glacial acetic acid, centrifuge and decant.
9. Wash twice with distilled water.
10. Wash once with a 1:1 (50%) mixture of glycerine and distilled water, decant and allow to drain for at least 30 minutes.

The 30 minute period is a minimum time; overnight drainage is not excessive. To drain, turn the tubes upside down in test tube rack. Cover with a paper towel. If a number of samples are being prepared it is possible to begin a second set while the first is draining. By staggering the samples in this way, it is possible to prepare as many as 32 samples per day (using an eight capacity centrifuge).

After the samples have been drained they are ready for slide preparation. Several mounting media are used to prepare slides and include: Canada balsam, silicone oil, Permount (Fisher Scientific), glycerine jelly, Lucite and others. The reference pollen and spores of the National Museums of Canada are mounted in glycerine jelly because of the ease of use of this medium and its fine refractive index. The cover glass of the slide is ringed with Glyptal varnish (General Electric) in order to provide an air tight seal, thus minimizing drying out of the glycerine jelly. Once each slide is properly labelled, catalogued alphabetically by genus and species, onto 3 x 5" index cards and entered into a systematically arranged master catalogue binder they are ready for use by the investigator.

Before the slides are placed into the collection, the palynomorphs are examined and described as to their morphological features. The description and measurements of each species are recorded onto 5 x 8" data cards (E-Z Sort System) which are then filed according to a system based on morphologic features. This system is particularly useful when a fossil pollen or spore can not be routinely identified. The E-Z sort cards are punched-out along their margins with the morphological information relative to that particular species. Through a series of elimination (piercing appropriate holes with a long needle) several cards can be removed from a larger stack; the cards removed

all represent species which bear the set of morphologic features selected by the investigator. Eventually one or more species will be chosen which have features similar to the unknown fossil species. Fig. 5 is an example of such a card properly punched and described.

THE SCOPE OF THE COLLECTION

When examining the prepared microscope slides of material from a fossil locality, the researcher is soon aware that many different kinds of microfossils may be present in his samples. Among the terrestrial vegetation the spores of ferns, fungi, mosses and the like are mixed with the pollen of conifers and other gymnospermous plants and the pollen of the flowering plants. The number of flowering plant species growing on the Earth today probably ranges between 250,000 to 350,000! Certainly, all these plants will not be found in one fossil assemblage, but the more familiar the researcher becomes with the pollen and spore types of a large percentage of land plants the more secure will be his identification.

In building a reference pollen collection for the National Museums of Canada emphasis is placed on acquiring a good cross section of many plant groups, families and lower taxa, especially those plants of tropical and subtropical floristic regions. The emphasis on tropical plants is based on the preponderance of these forms in the ancient sediments of Cretaceous age (63-75 million years before present) from western Canada. The vegetation of much of western Canada during this time was probably very similar to the present day vegetation of the Indomalaysian region.

To date the pollen and spore collection numbers about 4,000 species. A comprehensive listing of all taxa with data relevant to location, collector, and herbarium where collected is now in preparation and should be completed by early 1978.

A listing of the major plant groups by family is presented here to provide a general understanding of the scope of the present collection:

NON-FLOWERING PLANTS:

MYXOMYCOPHYTA (slime molds)
HEPATOPHYTA (liverworts and horned liverworts)
BRYOPHYTA (peat mosses and true mosses)
PSILOPHYTA (wisk ferns)
MICROPHYLLLOPHYTA (lycopods)
ARTHROPHYTA (*Equisetum*)
PTEROPHYTA (ferns)

Aspidiaceae	Dicksoniaceae	Loxomaceae
Blechnaceae	Gleicheniaceae	Marattiaceae
Cyatheaceae	Gymnogrammeaceae	Ophioglossaceae

Osmundaceae
Parkeriaceae

Polypodiaceae
Schizaceae

Sinopteridaceae

GYMNOSPERMOUS PLANTS

CYCADOPHYTA (the cycads)
Cycadaceae

GINKGOPHYTA (*Ginkgo*)
Ginkgoaceae

CONIFEROPHYTA (cone-bearing plants)
Cupressaceae Taxaceae
Pinaceae Taxodiaceae

GNETOPHYTA
Ephedraceae Welwitschiaceae
Gnetaceae

THE FLOWERING PLANTS

MONOCOTYLEDONEAE

Abolbodaceae	Haemodoraceae	Pontederiaceae
Alismataceae	Heliconiaceae	Potamogetonaceae
Araceae	Hydrocharitaceae	Restionaceae
Bromeliaceae	Iridaceae	Sparganiaceae
Burmaniaceae	Juncaceae	Strelitziaceae
Butomaceae	Lemnaceae	Taccaceae
Centrolepidaceae	Liliaceae	Trillaceae
Commelinaceae	Limnocharitaceae	Triuridaceae
Cyclanthaceae	Mayacaceae	Typhaceae
Cyperaceae	Musaceae	Velloziaceae
Dioscoreaceae	Orchidaceae	Xyridaceae
Eriocaulaceae	Palmae	Zosteraceae
Flagellariaceae	Pandanaceae	
Gramineae	Philydraceae	

DICOTYLEDONEAE

Acanthaceae	Annonaceae	Berberidaceae
Aceraceae	Apocynaceae	Betulaceae
Achatocarpaceae	Aquifoliaceae	Bignoniaceae
Actinidaceae	Araliaceae	Bombacaceae
Adoxaceae	Aristolochiaceae	Boraginaceae
Aizoaceae	Asclepiadaceae	Bruniaceae
Altingiaceae	Balanophoraceae	Burseraceae
Amaranthaceae	Balsaminaceae	Buxaceae
Anacardiaceae	Begoniaceae	Cabombaceae

Cactaceae	Guttiferae	Piperaceae
Campanulaceae	Haloragidaceae	Pittosporaceae
Canellaceae	Hamamelidaceae	Plantaginaceae
Capparidaceae	Hernandiaceae	Plantanaceae
Caprifoliaceae	Hippocastanaceae	Plumbaginaceae
Caricaceae	Hydrangeaceae	Polemoniaceae
Caryophyllaceae	Juglandaceae	Polygalaceae
Cephalotaceae	Labiatae	Polygonaceae
Celastraceae	Lauraceae	Portulacaceae
Chenopodiaceae	Lecythidaceae	Primulaceae
Cistaceae	Leguminosae	Proteaceae
Cneoraceae	Lentibulariaceae	Psiloxylaceae
Cochlospermaceae	Linaceae	Pyrolaceae
Combretaceae	Loranthaceae	Ranunculaceae
Compositae	Lythraceae	Resedaceae
Connaraceae	Magnoliaceae	Rhamnaceae
Convolvulaceae	Malpighiaceae	Rhizophoraceae
Coriariaceae	Malvaceae	Rosaceae
Cornaceae	Marcgraviaceae	Rubiaceae
Corylaceae	Melastomataceae	Rutaceae
Crassulaceae	Meliaceae	Salicaceae
Cruciferae	Menispermaceae	Salvadoraceae
Cucurbitaceae	Monimiaceae	Santalaceae
Cunoniaceae	Moraceae	Sapotaceae
Diapensiaceae	Moringaceae	Sapindaceae
Dichapetalaceae	Myricaceae	Saururaceae
Dilliniaceae	Myristicaceae	Saxifragaceae
Dipentodonaceae	Myrsinaceae	Scrophulariaceae
Droseraceae	Myrtaceae	Simaroubaceae
Ebenaceae	Myzodendraceae	Solanaceae
Ehretiaceae	Nelumbonaceae	Sterculiaceae
Elaeagnaceae	Nyctaginaceae	Theaceae
Ericaceae	Nymphaeaceae	Thymelaeaceae
Eucommiaceae	Olacaceae	Tiliaceae
Euphorbiaceae	Oleaceae	Tovariaceae
Fagaceae	Onagraceae	Trigoniaceae
Flacourtiaceae	Opiliaceae	Turneraceae
Fouquieriaceae	Oxalidaceae	Ulmaceae
Fumariaceae	Papaveraceae	Umbelliferae
Gentianaceae	Parnassiaceae	Urticaceae
Geraniaceae	Peperomiaceae	Vahliaceae
Gesneriaceae	Phellineaceae	Verbenaceae
Gomortegaceae	Philadelphaceae	Violaceae

Vitidaceae
Vochysiaceae

Winteraceae
Zygophyllaceae

STORAGE AND MAINTENANCE OF THE COLLECTION

The collection of prepared slides are stored in plastic slide boxes of 100 capacity, on shelves alphabetically arranged by family. In this way those pollen grains with similar morphological features will be grouped together for easier examination. The room in which the slides are stored is air-conditioned inasmuch as extreme heat could cause a melting of the glycerine jelly mounting media, and thus total loss of the slide contents.

Much of the routine maintenance involves keeping the laboratory as free as possible from outside pollen or spore contamination. Pollen and spores (always present) from outside sources could enter a preparation and if more abundant than the pollen or spore material being processed, could be misleading as to the true identity of the final prepared material.

Additionally, the cataloguing system involves several cross-referenced sources so that the researcher can draw upon the collection in quite different ways. At the present time, a particular pollen or spore type can be retrieved in the following ways: (1) based solely on its morphology; (2) by its unique number or (3) taxonomically by knowing its family generic and/or species names. With only 4,000 entries to catalogue and retrieve the manual card/file index system works well and quickly. Once, however, the collection reaches 15,000 or 20,000 entries (an estimated 5 to 8 year period of time) a computerized system will be required similar to that described by McAllister et al. (1972), as utilized in the fish collections of the National Museum of Natural Sciences.

USE OF COLLECTION

The major use of the pollen and spore collection is in the identification of fossil pollen and spore types. As mentioned earlier, the surface features and general morphology of pollen and spores is often sufficient to allow identification to family, genus or sometimes species. By becoming familiar with the general gross features peculiar to certain plant groups, the researcher can narrow down his search for a living pollen type which approximates the fossil forms he has recovered.

Even if the fossil type cannot be assigned to a comparable living plant family, genus, etc... the comparisons to certain "groups" of living plants may still provide valuable information. The fact that a fossil flora was dominated by gymnosperms and ferns which no longer exist, can be useful information in describing the nature and direction of plant evolution during the Earth's history. Often enough however, especially with more recently deposited rock types, the contained fossil pollen and spores are very similar if not indeed identical to present day pollen and spores (see for example;

Wijmstra, 1968; Germeraad, Hopping and Muller, 1968).

With the proper identification of a fossil flora, considerable information as to the climate, plant migrations and evolutionary trends of the past can be elucidated.

Another use of the pollen and spore collection is as a taxonomic tool. Botanists, when describing or revising the status of a plant taxon (e.g. family, genus or even species) could well benefit through the use of the pollen and spore collection. Some recent taxonomic studies of plant taxa in which pollen or spores were used as a criteria in delineating genetic relationships are those of Breckon and Falk (1974); Gastony and Tryon, (1976); Graham and Graham, (1971) and Wagner (1974).

When preparing a pollen or spore sample it is to the advantage of the researcher to prepare several (3-6) slides from the same residue. One of these, usually the finest preparation, is kept for the permanent reference collection, the remainder are stored separately and are used for exchange purposes. Other institutions which maintain pollen and spore reference collections also prepare exchange material and are willing to "swap", on a one-to-one basis, prepared slides. In this way the preparation of one sample of which six slides are prepared can in return provide for the acquisition of five additional different species of prepared material. One single institution need not collect and prepare all the material needed for research purposes, an open and free system of exchange facilitates the growth of many fine research collections. For a listing of the institutions with which the National Museum of Natural Sciences has made exchanges see Jarzen (1976).

A listing of all material for which exchange slides are available is prepared and updated every two years and sent to researchers who are known to maintain their own pollen and spore collections.

SUMMARY

The foregoing is but a brief survey of the nature and scope of collecting and maintaining a pollen and spore reference collection as a tool for scientific research. It is hoped that eventually the collection maintained at the National Museum of Natural Sciences will be a fine source of material from which other institutions throughout Canada and perhaps elsewhere may benefit. As a tool for modern taxonomic studies, or for comparison purposes to identify fossil pollen and spore material the collection becomes an invaluable resource.

Pollen and spores can do more for man than cause hay fever and other allergies, for their uniqueness and ubiquitous occurrence can provide clues to the proper identification of the living and the past vegetation of our Earth. Perhaps pollen and spores could be classified among nature's most intricate architectural wonders.

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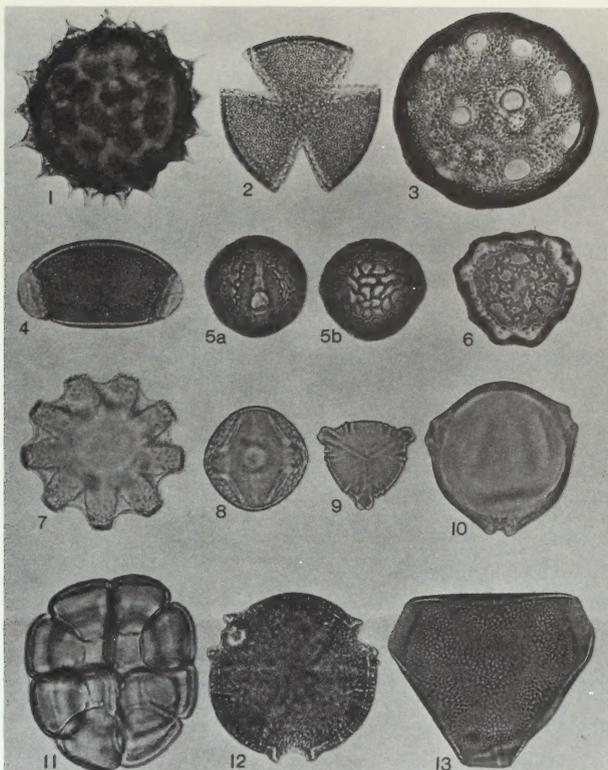


PLATE I. Representative examples of the diversity in size, morphology and surface architecture of some pollen grains of flowering plants:

1. *Abutilon crispum* Malvaceae, 71 μ . 2. *Cephalocereus brooksianus* Cactaceae, 108 μ . 3. *Cyphomeris gypsophiloides* Nyctaginaceae, 139 μ . 4. *Dryandra rippistiana* Proteaceae 41 μ . 5a. *Eriosema glaziovii* Leguminosae 22 μ . 5b. *Eriosema glaziovii* Leguminosae 22 μ . (same grain as in 5a, but at different focal level to illustrate the surface pattern. 6. *Eriosema crinitum* Leguminosae 27 μ . 7. *Dinemagonum gayanum* Malpighiaceae 55 μ . 8. *Centrosema pubescens* Leguminosae 68 μ . 9. *Cuphea wrightii* Lythraceae 29 μ . 10. *Betula lenta* Betulaceae 45 μ . 11. *Acacia mellifera* Leguminosae 50 μ . 12. *Stahlia monosperma* Leguminosae 59 μ . 13. *Adenanthos meisneri* Proteaceae 34 μ .

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