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THE PALYNOTHECA COLLECTION AND THREE-DIMENSIONAL MODELS OF POLLEN GRAINS AND SPORES AS TEACHING RESOURCES FOR A MEANINGFUL LEARNING OF BOTANY

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Abstract: This article aims to explain the use of the biological collection of microscope slides containing pollen from Angiosperms and Gymnosperms and spores of bryophytes, ferns and lycophytes, or Palynotheca, and the respective three-dimensional models, as a strategy for didactic representation of components of plant biodiversity that are not visible to the naked eye, which can help to reduce "botanical blindness". This phenomenon occurs due to the difficulty of human beings in establishing a direct relationship with plants. By relating theory to practice, in a continuous discovery approach, students are interested. The use of technological equipment such as optical microscopes and stereomicroscope and the existence of an organized collection with microscope slides containing pollen grains and spores characteristic of several botanical families facilitates morphological and taxonomic learning in the classroom and in Environmental Education events. In addition, the use of three-dimensional anatomical and morphological models as a tangible didactic resource and facilitator in the perception and understanding of microscopic plant shapes and structures can help when microscope slides and microscopes are not available. Despite being incipient, the use of both resources has shown relevant results in the teaching of Biology as a concrete and contextualized strategy for the inclusion of plants in the school routine and in the dissemination to the general public, including the visually impaired, providing a playful and significant information on the contents of Botany. In addition, Palynotheca is fundamental for the development of research in various branches of scientific investigation.

Keywords: Botanical blindness; Biological Collection; Scientific divulgation; Teaching botany; Palynology.

INTRODUCTION

The learning of Botany is often neglected due to approaches based on the memorization of terminologies that add to the precarious perception of plants and insufficient biological knowledge, resulting in little interest of students and distancing from the natural world, which is reflected in the " botanical blindness" of modern societies. "Botanical blindness" is defined as the "inability to perceive the relevance of plants in the environment", which makes it difficult to recognize the importance of plant biodiversity, which can result in negative actions of environmental impact (Wandersee & Schussler, 2001; Salatino & Buckeridge, 2016). Hershey (1996) already said that literature for teaching Botany is underused and difficult to access. Botany teaching is often seen as arid, boring and out of modern context, reflecting a high load of prejudice (Salatino & Buckeridge, 2016). Therefore, it is desired that the teacher, when planning his Botany class, keep in mind that his theoretical-methodological approach makes the entire teaching-learning process effective, preferably with an active exchange of information, where everyone is subject to learning by observing the natural environment, adapting theory to the practices necessary for the deepening of knowledge.

Meaningful learning can be defined as the interaction of new knowledge with an existing one, in which both are modified, adding more information to the central theme, and helping the student to build scientifically accepted meanings (Moreira, 2005). According to Stanski et al. (2016) for the effective teaching of Botany, which favors meaningful learning, it is important that the topics covered in the classes are worked in a more concrete and attractive way, avoiding the lack of students' interest in the contents. Teaching through a routine and mechanical class that uses only lists of scientific names and the textbook, with words totally isolated from the student's daily life, and without interaction with plants, causes distance from the theme, resulting in little learning.

Themes focusing on pollen grains and spores and their environmental functions do not have great representation in schools and universities, as it requires the teacher to develop strategies for the visualization of these microscopic structures, requiring, in addition to the theoretical contextualization of the theme and sub-themes, the practice of field collection of Angiosperm flowers, Gymnosperm cones, sporangia from the fern frond and bryophyte capsules, with subsequent preparation of microscope slides in the laboratory.

A recent bibliographic survey from 2017 to 2020 carried out by Costa et al. (2021) in the Scielo and Google Scholar databases revealed that among the 128 articles and final course assignment, master's and doctoral works on the teaching of Botany in Brazil, some showed improvements, with new forms of approach, such as explanations about facilitating practices and the use of games, seeking dynamic forms of content presentation. However, no significant contributions were noted on the themes of pollination, pollen grains or spores, demonstrating still little creativity in contextualizing such subjects.

An exception is the work of Stanski et al. (2016) who used multi-mode representation of pollen grains and pollination (conceptual maps, blackboard and chalk, questionnaires, photographs, microscope slides containing pollen, floral parts, optical microscopes, stereomicroscopes), associated with oral explanations during the activities, to make meaningful learning effective, bringing advances in knowledge on the subject to 35 students enrolled in the seventh grade (eighth year) of Elementary School at a school in Paraná.

Also in this regard, tutoring was carried out in 2020 with students of the undergraduate course in Biological Sciences of the Federal Institute of Education, Science and Technology of the South of Minas (IFSULDEMINAS), Campus Poços de Caldas, in order to encourage learning about the central themes "Biological collections" and "Palynotheca". The tutoring included the practice of collecting the botanical material in the field and preparing the microscope slides containing pollen in the laboratory, with subsequent observation under an optical microscope. It was found, after applying questionnaires to the students, that the insertion of the Palynotheca as a pedagogical practice in the classroom was able to instigate critical thinking, as the use allowed the morphology of pollen grains to be correlated with the species of source plants. Consequently, the importance of scientific collections for expanding knowledge about plant biodiversity and its potential use in the development of environmental studies was revealed to students (Souza et al., 2021).

Furthermore, with this same approach, Rodrigues et al. (2022) presented the results of a training course for Biological Sciences teachers in the region of Bico do Papagaio, Tocantins, which included the collection of plants and the making of a Palynotheca. In the end, the course participants showed motivation to present the same pedagogical resources learned in their classrooms, which can enrich the students' learning.

Both in basic education and in higher education, the lack of interest in theoretical and unattractive classes is a challenge for teachers, especially in subjects focused on natural sciences that involve abstract, complex and challenging themes (Biagolini & Piacitelli, 2016; Souza et al., 2021). In the case of observing microscopic structures contained in microscope slides, it is necessary to use technological tools, such as microscopes and stereomicroscopes, which generates a lot of interest in students and can facilitate learning (Stanski et al., 2016). However, these equipments are not always available in Brazilian classrooms. Thus, a viable alternative is the construction of three-dimensional models, which can facilitate the thematic approach in Environmental Education events, including for the visually impaired (Martarello et al., 2022).

Playful activities, such as the use of didactic games, practical classes in laboratories and in the field, using computers and other multimedia resources, are instruments that facilitate the teaching and learning process, and can be interesting pedagogical resources, if used properly and with due planning (Queiroz et al., 2021).

In this sense, the making of three-dimensional didactic models presents itself as a tangible and visual pedagogical tool for students that arouse curiosity, imagination, interest and interaction among students (Corte et al., 2018; Souza et al., 2021). For example, three-dimensional models present valuable contributions in the representation of plant structures and histological sections (Souza et al., 2021). The visualization of two-dimensional images as three-dimensional structures requires the ability of abstraction by students, making the use of models essential (Ceccantini, 2006).

Three-dimensional models stimulate visual and sensory memory and enable meaningful learning (Corte et al., 2018), helping to perceive microscopic structures for both sighted and visually impaired students in inclusive education, developing creativity and promoting autonomy and participation for students with disabilities (Faria et al., 2013; Freitas & Castro, 2019).

These models can be used to overcome the lack or limitation of didactic resources and the unavailability of expensive equipment, such as microscopes, in addition to their potential use complementing Palynotheca (Ceccantini, 2006; Souza et al., 2021). They can be applied at all levels of education, both in theoretical and practical classes, making the theme of the class more interactive, also serving in exhibitions and other educational events (Souza et al., 2021).

The commercialization of didactic models usually presents a high value, being the manufacture of these models a versatile and more economical option. The materials that can be used are the most diverse, including cardboard, acetate, styrofoam, modeling clay, putty, cold porcelain (biscuit) or even plaster and recyclable materials (Ceccantini, 2006) . Therefore, the development and use of didactic models must be encouraged in universities and research institutes (Amâncio et al., 2013) . Another relevant aspect to be considered for the use of the models is the development of activities and other extension programs carried out by universities and research institutions, which aim to bring the community and the elementary and high schools closer together, in order to share scientific knowledge (Borges et al., 2019).

Fontes et al. (2019) sought to develop didactic models based on the native Brazilian flora, promoting greater proximity between the student and the content, and contributing to the appreciation of local/regional biodiversity. The model made was based on the fruit of *Cereus fernambucesis* Lem. (Cactaceae) prepared for the subject "Plant morphology" of the Biological Sciences Degree course at the Federal University of Espírito Santo, Campus São Mateus. Low-cost, everyday materials were used for its preparation, such as balloons, newspaper sheets, bond paper, crepe paper, cotton, dehydrated papaya seeds, white glue, brush, scissors and stiletto.

In practical classes of Paleontology, to understand the theme "phytofossils", Biagolini and Piacitelli (2016) developed models applying the plant impression of leaves in clay. The activity was developed with students from the 7th year of Elementary School who collected the leaves to make the models. The authors highlighted that the entire process of preparing the materials was presented as a playful activity stimulating student learning.

Ribeiro and Carvalho (2017) applied pre- and post-tests on several areas related to Botany in order to assess difficulties and prior knowledge of students in the 2nd and 3rd year of high school. The most difficult subjects were developed with the making of three-dimensional models produced by the students, such as: plant cell, chloroplast and their structures, leaf with macro and microscopic structures and other models that represented the reproductive organs of angiosperms, gymnosperms, ferns and bryophytes. The materials used were: modeling clay, biscuit, styrofoam, fabric paint, brush, scissors, ruler, glucose, cardboard, EVA, hot glue gun and refill, white glue, styrofoam glue, white sheet, pen and mechanical pencil (Ribeiro & Carvalho, 2017). The experience of making three-dimensional didactic models was interesting and helped students learn about macro and microscopic structures in places where optical equipment was lacking (Ribeiro & Carvalho, 2017) .

Silva et al. (2016) used three-dimensional didactic models in the teaching of Cellular Anatomy (animal and plant) in the 7th year of Elementary School. The authors carried out a pre-test, accompanied by a theoretical exposition on the subject and later making models, which were produced by the students themselves from low-cost materials, such as Styrofoam board, paints, EVA and self-adhesive labels. At the end, a post-test was carried out to evaluate the use of didactic models for the students' assimilation of complex and abstract concepts, whose results were relevant. The evaluation indicated an increase in the success rates, showing that the ludic and motivating activity was effective for the students' learning. The materials produced were exposed to the other groups at the school, and this way, the students were able to learn about and discuss the anatomical structures, which brought interest and curiosity (Silva et al., 2016) .

Seeking a more consistent, pleasurable and dynamic teaching of Botany Borges et al. (2019) developed practical activities for students in the 6th year of Elementary School with the production of didactic material focused on the themes of cell biology, anatomy and plant physiology. The models were made by the students themselves and used in elementary schools, as well as served as a model for making other materials in higher courses of Bachelor's Degree in Biotechnology, Degree and Bachelor's Degree in Biological Sciences and Bachelor's Degree in Forest Engineering, in the disciplines of "Physiology Vegetal" and "Plant Anatomy and Morphology" from the Federal University of Pampa – Campus São Gabriel, Rio Grande do Sul. According to the authors, the didactic models have durability with the possibility of application in more classes, facilitating and helping teachers in their practices.

Ceccantini (2006) developed three-dimensional models for teaching plant anatomy in the discipline of "Structural Botany" at the Federal University of Paraná. According to the author, didactic models can be used in the evaluation process and in the construction of skills by students.

Faria et al. (2013) made three-dimensional models from anatomical slides of plants in light and scanning electron microscopies. The materials used were acrylic dough, clay and plaster, with a silicone mold, and the final material was produced in polyester resin and painted manually. The activities were developed in the project "From Macro to Micro: a journey through the plant world" developed by the Museum of Natural History and Botanical Garden of UFMV (MHNJB), Minas Gerais, with an audience of 30 students, aged 25 to 60, Subsequent recordings were made to register the students' perception, evidencing satisfaction on the part of the participants.

Amâncio et al. (2013) aimed to make models of plant ovaries in three dimensions. The activity was carried out with students from the discipline of "Morphology, Systematics and Phytogeography of Angiosperms" from the Agronomy Course at the Federal University of Cariri, Ceará. The proposed models were built with paper of different colors, scissors and glue.

Freitas and Castro (2019) used three-dimensional tactile models as a pedagogical resource for visually impaired students in the 3rd year of high school in a public school in Fortaleza, Ceará. The themes covered biomolecules and cytology, subjects that students had the greatest difficulties in assimilating. The materials were built by school monitors from modeling clay, paraffin, cardboard, pressed wood, wire, glue, biscuit, paints, brushes and a pen. According to the authors, the students were able to handle the models identifying the structures, as well as understanding their complex drawings and schemes. Inclusive education is challenging, especially in science teaching, and according to the authors, it must encompass a school where everyone has the opportunity to access knowledge construction, respecting their capacities and limitations.

Michelotti and Loreto (2019) used three-dimensional didactic models of cell biology, made of biscuit, for classes of the 8th and 9th grade of Elementary School, covering visually impaired and sighted students. The models were made by the researchers themselves representing the cells with their respective characteristics, shapes and sizes. According to the authors, the use of didactic models enabled both visual and tactile observation, promoting the assimilation of the studied content.

Perini and Rossini (2018) used didactic models to understand floral biology content (floral parts, the sexual systems and pollination), for two classes of the 3rd year of high school, with the application of a previous questionnaire to identify the students' knowledge. The didactic models were elaborated by the authors themselves from styrofoam balls, EVA, wire, colored pens, hot glue, ink and beans, low-cost resources, easy to maintain and apply. According to the authors, they were of great importance as a teaching tool.

Corte et al. (2018) carried out a collaborative project between the university and a high school in Vitória, Espírito Santo, covering Botany contents that students had greater difficulties. The activities involved 1st and 2nd year classes with subsequent application of the material produced in 3rd year classes. A model of the life cycle of ferns, another of the reproductive structures of angiosperms and another representing floral fertilization, were made by the students themselves with biscuit dough. The process was an opportunity for students to exchange knowledge, develop autonomy, and collaborate in collective construction. The materials used were of low cost, easy handling and high durability.

Regarding published works on three-dimensional models of pollen and spores, these are scarcer, with only the one by Sousa & Araújo (2020) and Martarello et al. (2022).

Sousa & Araújo (2020), in the search for tools to facilitate the teaching and learning of Palynology in a teacher training course held at the University of the State of Bahia, Paulo Afonso Campus, used the papier-

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mâché technique and scraps as a support for the making of three-dimensional models of the microscopic structures of pollen grains in the course "Introduction to Palynology". The flowers were collected by future teachers and after theoretical explanations and bibliographic consultations on the species collected, microscope slides containing the pollen grains were prepared in the laboratory. The subsequent observation of the structures took place under light microscopy, and the subsequent design for the making of three-dimensional models of pollen grains in papier-mâché from the observed species was carried out with the artistic guidance of the professors in the university's art room. The experience proved to be quite favorable, where the course participants manipulated, experimented, in addition to having fun and socialized, and the three- dimensional models of pollen grains formed a didactic collection that will serve, among other applications, to popularize the theme in Fundamental schools of the region.

Martarello et al. (2022), aiming to present alternative teaching materials at public scientific events, developed models of pollen grains and spores made with cold porcelain dough (biscuit). The confection of palynological structures was based on scanning electron microscopy and on specific scientific literature according to their morphological characteristics. The presentations took place at the "I and II Scientific Circuits of the IBt Environment Week (Institute of Botany)", "VIII Ecological Restoration Symposium" and the "26th Annual Meeting of the Institute of Botany (RAIBt)", carried out by the Environmental Research Institute (formerly Institute of Botany) in the city of São Paulo. The three-dimensional models were related to the reproductive aspects of plants and their respective floral visitors and pollinators and the public was very interested and attracted by this playful pedagogical resource. Thus, the authors mentioned that the use of three-dimensional models that show the shape of pollen, together with explanations about its biological and ecological function, was effective in disseminating to the lay public the importance of Palynology for the conservation of biodiversity.

POLLEN GRAINS AND SPORES

Our flora is very rich and varied, however, little studied from a palynological point of view. Palynology, a branch of Botany that studies the pollen grains of Angiosperms and Gymnosperms, as well as the spores of bryophytes, ferns and lycophytes, has contributed a lot to Science. The term Palynology was created in 1944 by Harold A. Hyde and David A. Williams and is derived from the Greek verb palunein (παλυνειν), which means to scatter, to sprinkle, and is similar to the Latin *pollen,* which means fine flour. The definition of Palynology is, in a narrower sense, the science that studies the external morphology of pollen grains and spores. In a more modern approach, this science encompasses analyzes of the structure and formation of pollen, spores and other palynomorphs (various organisms and structures with acid-resistant organic walls), in addition to studies on their mechanisms of dispersion, deposition and preservation in environments.

The pollen grain is located inside the anthers (Figure 1) of the flowers and corresponds to the male gametophyte, a multicellular structure originated from the haploid (n) phase of Angiosperms and Gymnosperms and containing the male reproductive nucleus. Pollination is the transport of the pollen grain from the anther to the stigma.

The underside of the blade of ferns and lycophytes have sori, which are groups of sporangia. Spores are produced by meiosis

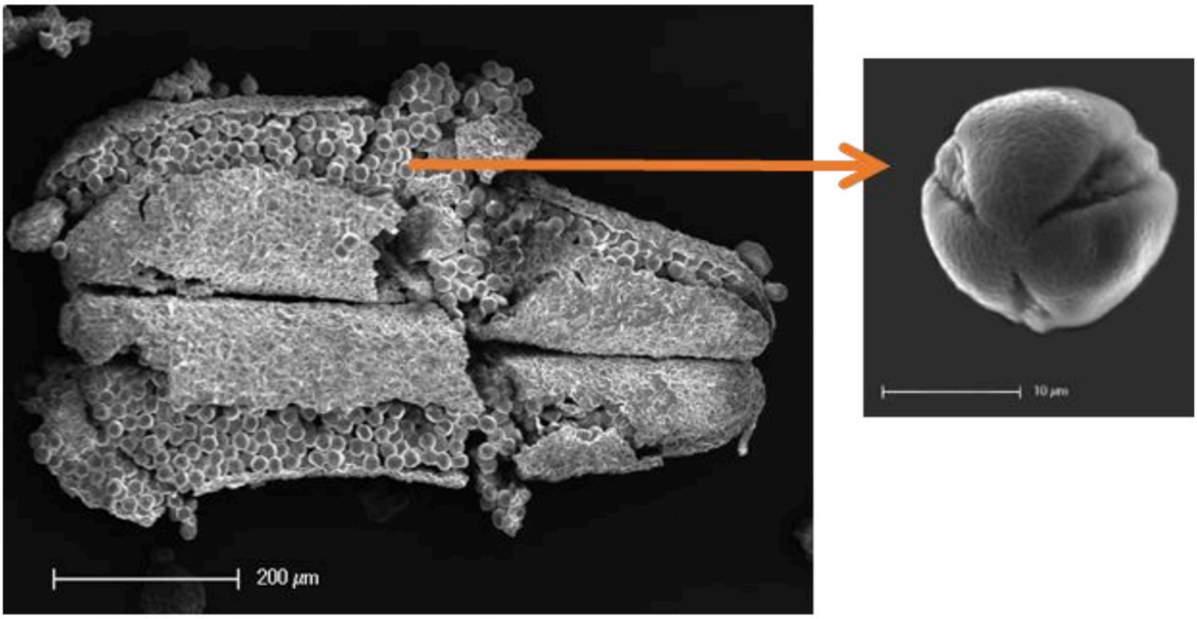

Figure 1. Anther with two thecae full of pollen grains (left) and an isolated pollen grain (right) from *Zornia diphylla* (Fabaceae). Scanning electron microscopy images. Source: Cynthia F.P. da Luz. cales: 200 µm (anther with pollen on the left), 10 µm (pollen on the right).

within the sori. When released by the sori, and under suitable conditions, the spores germinate, giving rise to a gametophyte.

In bryophytes, the sporophyte is composed of a seta and a capsule in which the spore mother cells are present, which, by meiosis, give rise to spores. When the capsule dries, the spores are released and, when they fall into a suitable environment, they germinate and transform into a new gametophyte.

The pollen grains and spores have sporopollenin on their outer wall (exine), which is a substance chemically resistant to high temperatures and organic matter decomposition agents, preventing their dehydration and protecting them, helping to maintain spore-pollen viability for a while, depending on the plant species. Thus, they remain in the environment, air, water, animal nests, etc., and can even fossilize and accumulate in soils and sediments.

Palynology applications include knowledge of past vegetation and past climate through fossilized palynomorphs, plant species sought by bees to collect nectar and pollen, allergenic plants that release pollen into the air causing pollinosis, aid in solving of cases of homicide and other crimes by pollen and spores contained in the clothes, hair and shoes of suspects, among others. Even Palynology is essential in surveys of bee flora, which is closely linked to the survival of bees.

For an effective inclusion of pollen grains and spores in the school routine, the challenge is to find tools that bring theory closer to practice and facilitate the understanding of specific contents. Therefore, teaching resources must be accessible according to the reality of each teaching and research institution. In this sense, the construction of palynotheca and three-dimensional models of pollen grains and spores can facilitate meaningful learning on the subject and the dissemination to the general public of the importance of pollination.

This work aims to contribute with didactic-methodological strategies that use Palynology and three-dimensional models of pollen grains and spores, seeking alternatives that facilitate the teaching of specific contents related to Palynology.

MATERIAL AND METHODS

MAKING THE PALYNOTHECA

For the recognition of pollen grains and spores, it is necessary to carry out a detailed study of these plant structures contained in microscope slides deposited in a biological collection called Palynotheca, which allows a comparison with the grains observed in samples of honey, collectors of pollen from the air, and recovered from sediments taken from the bottom of lakes, peat bogs, rivers and oceans, which have been deposited over time.

The Palynotheca is a collection of microscope slides containing pollen grains and spores taken from plants of different botanical families, whose slides are usually placed horizontally in special cabinets (Figure 2).

The pollen or spore is extracted from exsiccates deposited in institutional herbaria or from fresh material, whose species determination was carried out by specialists. This biological collection aims to generate, organize, perpetuate and disseminate palynological knowledge and can be used for didactic purposes from elementary to higher education and for the general public (Luz et al., 2014).

For pollen grains and herborized spores, the usual method of preparation is that of Erdtman (1960) where they undergo physical-chemical laboratory preparation called acetolysis. The pollen or acetolysed spore is placed on microscope slides containing glycerin gelatin and sealed with paraffin, making it permanent. Preparation by acetolysis needs great care as it requires the use of highly corrosive chemicals, acetic acid, acetic anhydride and sulfuric acid. This technique is used to obtain a better definition of all the details and patterns of the wall (exine) of pollen grains and spores.

As a standard in spore-pollen morphology studies, at least 3 flower buds near the anthesis are removed directly from the fresh plant or from herbarium exsiccates. These are stored in paper envelopes (same as those used in herbaria) inside a box containing silica gel to prevent hydration (Figure 3).

You can also store fresh flower buds taken directly from plants in nature in flasks with acetic acid or 70 ° GL alcohol, after which the liquid is centrifuged.

Figure 2. Asteraceae pollen grain (on the left), microscope slides labeled with the species data and highlighting the tip of the glass-writing diamond pen for labelling the microscope slides (in the center) that are stored in the drawers of the Palynotheca (on the right) of the Instituto de Pesquisas Ambientais, São Paulo. Image of the pollen grain in light microscopy. Source: Cynthia F.P. da Luz. Pollen image scale: 10 μm.

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Figure 3. Blue silica gel (left), paper envelopes and flowers being dissected under a stereomicroscope (center), and detail of the Asteraceae flower bud in the Petri dish (right). Source: Cynthia F.P. da Luz.

Formaldehyde, a fixative widely used in Botany, corrodes the exines and must never be used in Palynology. Botanical material stored in any formalin-containing fixative is no longer suitable for palynological studies. Exines are also soluble in 40% chromic acid and in the sulfo-chromic mixture, and are not preserved.

The Classical Acetolysis method (Erdtman, 1960) eliminates the protoplasmic content of the pollen and the microscope slides have great durability. The methodology is as follows (Figure 4): (a) remove pollen material from the anthers of well-developed flower buds near to anthesis in a Petri dish and place it in a centrifuge tube with 5 mL acetic acid (each species in a separate tube to avoid contamination between samples), remaining for at least 24 hours before acetolysis (the same can be done for spores); (b) centrifuge (preferably 1500 to 1800 revolutions per minute/rpm) for 5-10 min and decant the supernatant; (c) in the centrifuge tube, place 4.5 mL of acetic anhydride and 0.5 mL of sulfuric acid (9:1). Care must be taken, as the reaction is explosive when in contact with water! Immediately dip the centrifuge tubes with the acetolysis mixture into a beaker containing hot water in a laboratory water bath. Start the water bath with the temperature of the water inside the Becker close to boiling (from 80 to 100 °C); (d) place a glass rod in each tube and gently mix the contents at regular intervals; (e) keep the water bath boiling slowly for 1 to 2 min (for some species it is necessary to reduce the boiling time to a minute and a half, or even half a minute, otherwise the grains will knead a lot). The bath must be placed in a fume hood to avoid nasal aspiration of vapors, which are very irritating and toxic; (f) when the required time is reached, close the centrifuge tubes with the respective caps and place them still hot in the centrifuge. Centrifuge for five minutes at 1500 to 1800 rpm; (g) after centrifugation, discard the supernatant in a specially reserved container (never in the sink drain, to avoid corrosion of the pipes); (h) add distilled water to the spore-pollen residue to make up the volume to 10 ml. Place a clean glass rod in each tube. Shake each tube, add one by one the two drops of ethyl alcohol or acetone, shake again (this prevents foaming). Centrifuge and decant; (i) add to the spore-pollen residue a mixture of 5 ml of water with glycerin in equal parts. Let it remain in this solution for half an hour or until the next day; (j) centrifuge and, when decanting, keep the centrifuge tube upside down in a centrifuge tube rack with absorbent paper; (k) start assembling the slides by positioning a cube of glycerin gelatin (about 1mm^3) that was previously inserted into the bottom of the centrifuge tube to collect the spore-pollen residue. Place the gelatin cube in the center of the microscope slide; (l) Melt gelatin carefully under heat, avoiding boiling.

Figure 4. Erdtman's acetolysis steps (1960). Source: Cynthia F.P. da Luz.

Place the coverslip in the center of the slide and seal it with paraffin.

The pollen material is also stored in pure glycerin for years after it has been chemically prepared.

The Direct method (Wodehouse, 1935) is used for polliniferous material taken from fresh flowers, which does not eliminate the protoplasm from the pollen, but the microscope slides are highly durable. The methodology is as follows (Figure 5): (a) place one or several anthers of well-developed flower buds near to anthesis in a drop of alcohol 70 º GL on the center of the microscope slide (the same can be done for the spores); (b) dissect the anthers (or sori from ferns and lycophytes and capsules from bryophytes), removing pollen grains and spores, cleaning the residues with cotton wool; (c) slightly heat the slide until dry; (d) with the help of a stylet previously flamed red (to sterilize it), place a cube of glycerin gelatin (about 1mm ^{3}) in the center of the microscope slide. If the pollen material is oily, transfer the grains to another slide and clean the oil halo formed well; (e) carefully heat the gelatin until it melts, without boiling, mixing it with the stylet; (f) place a coverslip on top; (g) seal the sides of the entire coverslip with paraffin scraps, heating it carefully, or with liquid paraffin, without boiling the gelatin so as not to create bubbles between the pollen grains and spores. The amount of gelatin previously placed must not have been excessive so as not to reach the edges of the coverslip and not allow a complete seal; (h) let it cool and clean the slide successively with: scraping the excess paraffin with a razor blade, and after that, gently so as not to tear the coverslip, with cotton wool soaked in alcohol and a tissue.

For staining, in case of very clear material, glycerin gelatin is used, to which, before consolidating, a few drops of dilute aqueous 1% basic fuchsin solution have been added.

Microscope slides are deposited in the institutional Palynotheca only after pollen and spores have been measured, morphologically described, photographed, and presented in scientific publications. This way they become testimony material for future consultations.

Figure 5. Stages of microscope slide containing pollen and spores preparation by the Wodehouse method (1935). Source: Cynthia F.P. da Luz.

Figure 6. Binocular light microscope (left), micrometer drum (center), micrometer drum coupled to a light microscope (right) of the Environmental Research Institute, São Paulo. Source: Cynthia F.P. da Luz.

Measurements are performed using a micrometre drum coupled to the eyepiece of a light binocular microscope (Figure 6).

The morphological characters of pollen grains are genetically determined and are presented through descriptions, using standardized pollen terminology through the use of palynological glossaries (Figure 7), accompanied by photographs and drawings, often allowing the identification, at the level of taxonomic hierarchies of the plant that originated them.

The morphological analysis comprises the following descriptive sequence: regarding the Pollen Unit (number of grains); as for Polarity; as for Symmetry; as to Size; as to Shape; as to the Ambitus (contour in polar view); as for the Apertures; as for the Surface Zones; as to the appearance of the surface or Ornamentation and as to the layering of the wall or Texture.

It must be avoided that the representation of images is done incorrectly. By international convention, the pollen of eudicotyledonous Angiosperms is always represented with one of the openings facing downwards (Figure 8). In addition to light and scanning electron microscopy images, pollen grains can be represented through India ink drawings (Figure 9).

CONFECTION OF THREE-DIMENSIONAL MODELS OF POLLEN GRAINS AND SPORES

Among the topics addressed in Botany, Palynology, with pollen grains and spores, encompasses the study of these microscopic structures that present a wide variety of morphological characters used in their identifications. Therefore, the creation of three-dimensional didactic models of these structures can be used as pedagogical resources that complement theoretical and practical classes (Sousa & Araújo, 2020; Martarello et al., 2022) .

The three-dimensional models of pollen grains and spores can be made from cold porcelain dough (biscuit), using needles and/or pins of different thicknesses and shapes and other objects to mold the biscuit dough (Figure 10). Cold porcelain has an affordable price and durability for the material produced.

The representation in three-dimensional models of palynological structures must be based on light microscopy (LM), scanning electron microscopy (SEM) and on the specific scientific literature that describes them, respecting the following descriptive order: pollen unit (number of grains), polarity, symmetry, grain shape, ambitus, number, position and character of apertures, surface appearance or exine ornamentation and exine structure.

The three-dimensional models made of biscuit have low cost, are durable and can be used as a playful and attractive complementary didactic resource in public events or in the classroom, being that they contribute significantly to the teaching and learning process of students and interested parties.

Figures 11 and 12 show several examples of pollen grains and pollinaria observed in light and scanning electron microscopies and their respective three-dimensional models.

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Figure 7. Palynological catalogs used to describe pollen grains. Barth & Melhem (1988) (left); Punt et al. (2007) (center); Hesse et al. (2009) (right).

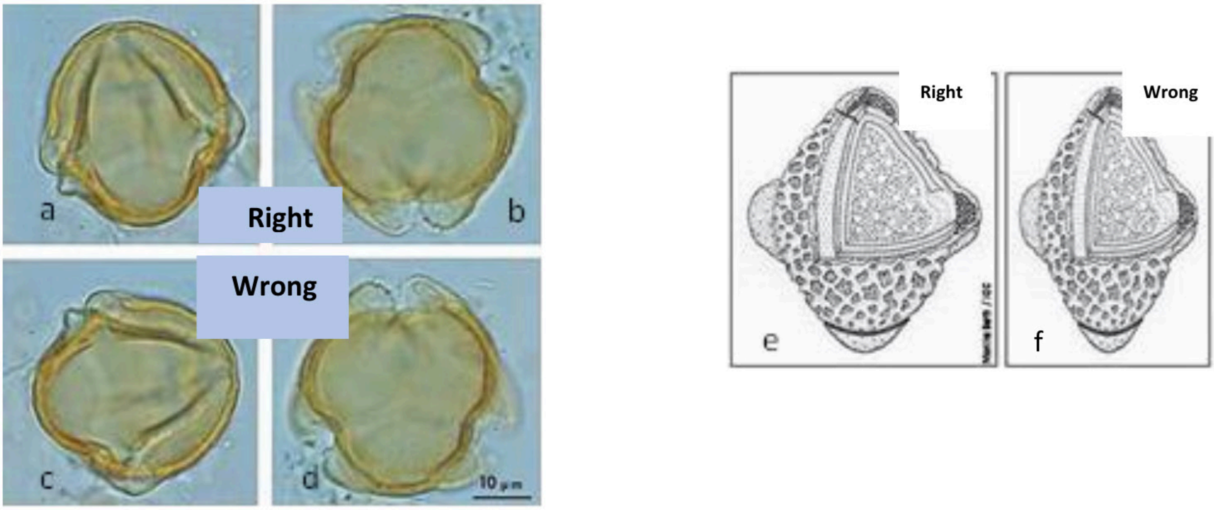

Figure 8. Example of right and wrong representation of pollen grain placement according to international convention. **a, b, c, d**: Pollen of *Aureliana paniculata* (Solanaceae) seen under the light microscope; in equatorial view top left (a), correct position; in polar view top right (b), correct position; below (c, d), incorrect positions. **e, f**: Indian ink scheme of a pollen grain characteristic of the genus *Caryocar* (Caryocaraceae, genus of pequi *C. microcarpum*). The left image (e) is correct, without lateral compression; the image on the right (f) was laterally compressed, which is usually done to fit within an available space, which must not be done. Source: Barth (2013). Scale: 10 µm (a, b, c, d).

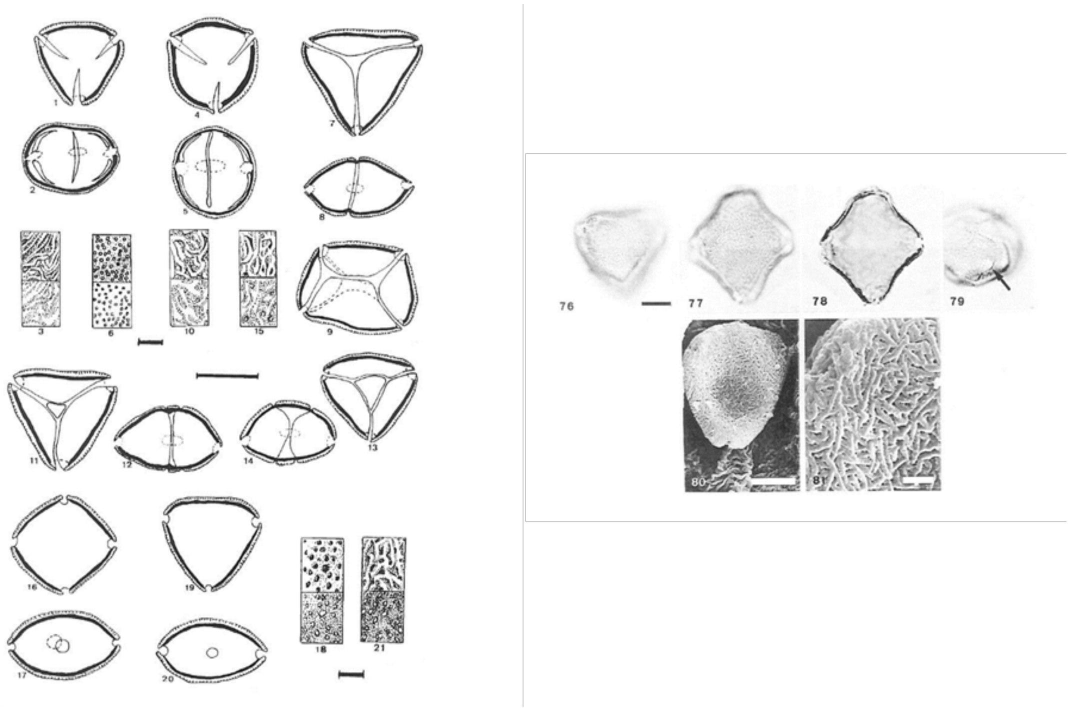

Figure 9. Example of India ink sketches (left) and images in light and scanning electron microscopies (right) of pollen grains of several tree species of Sapindaceae from Santa Catarina, Brazil. Source: Luz & Barth (1999).

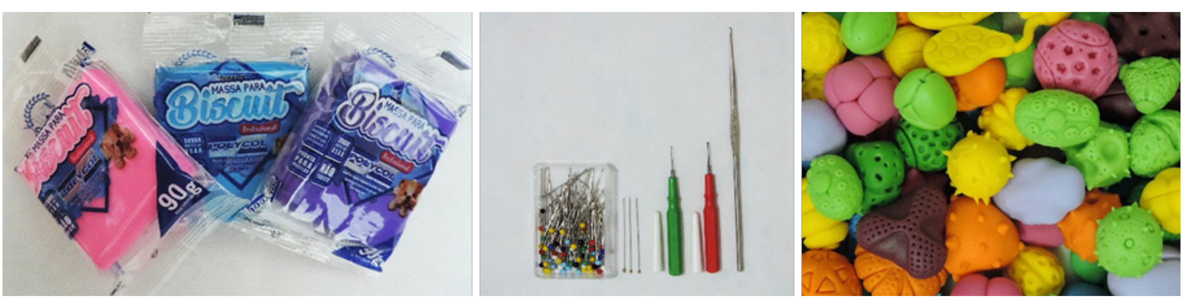

Figure 10. Cold porcelain dough (biscuit) (left), needles of different types (center) and three-dimensional models of pollen grains (right). Source: Natalia S. Martarello.

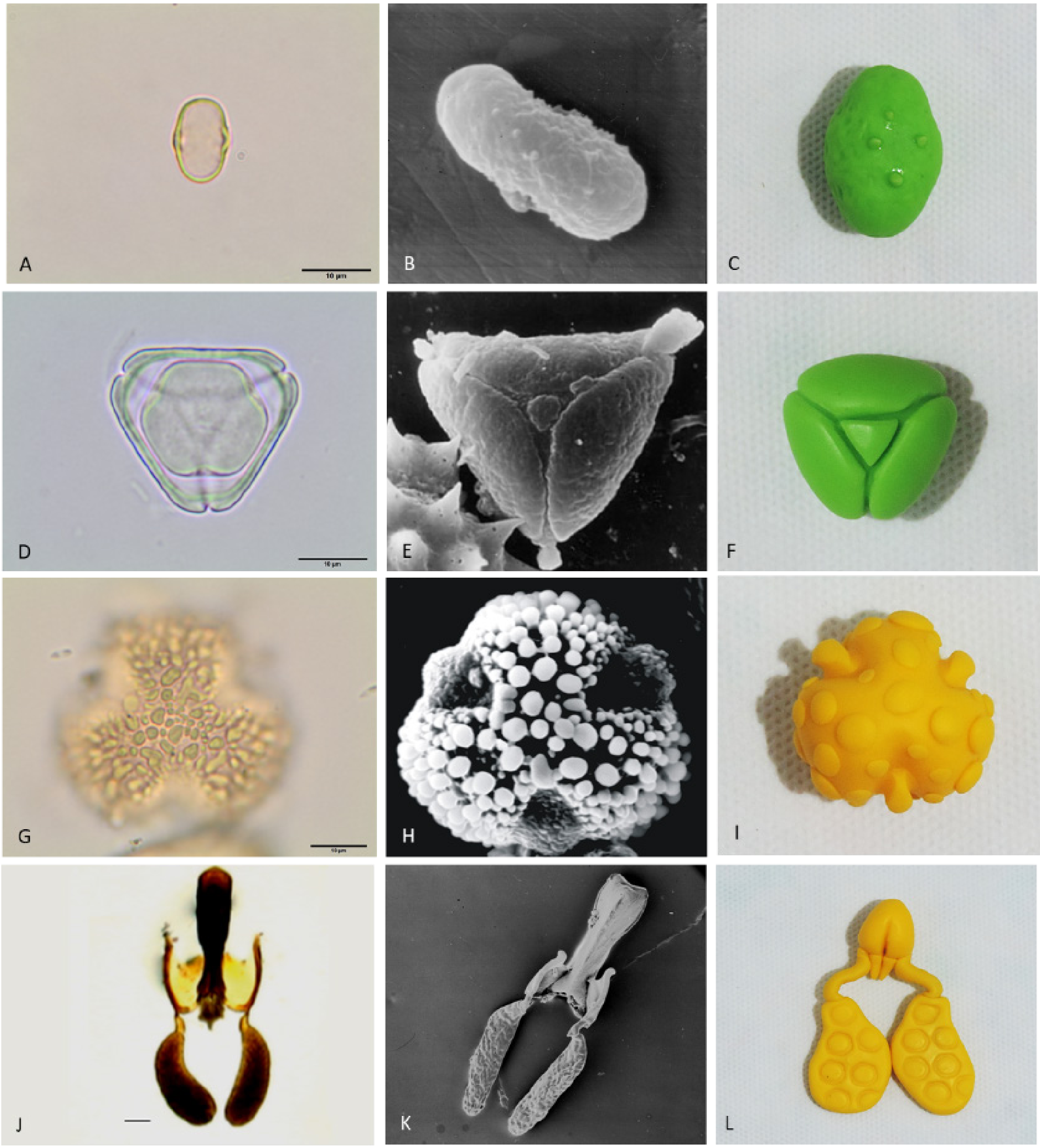

Figure 11. Pollinarium and pollen grains under light microscopy (A, D, G, J); Pollinarium and pollen grains in scanning electron microscopy (B, E, H, K); Pollinarium and pollen grain models made of biscuit (C, F, I, L); *Cecropia* pollen in frontal equatorial view (A-C); *Eucalyptus* pollen in polar view (D- F); *Ilex* pollen in polar (G-I) view; pollinarium of *Oxypetalum* (J-L). Source: Maria Amélia V. da Cruz- Barros et al. (2006) (H); Cynthia F.P. da Luz (A, B, D, E, G, J, K); Natalia S. Martarello (C, F, I, L). Scales: 10 µm (A, D, G), 100 µm (J).

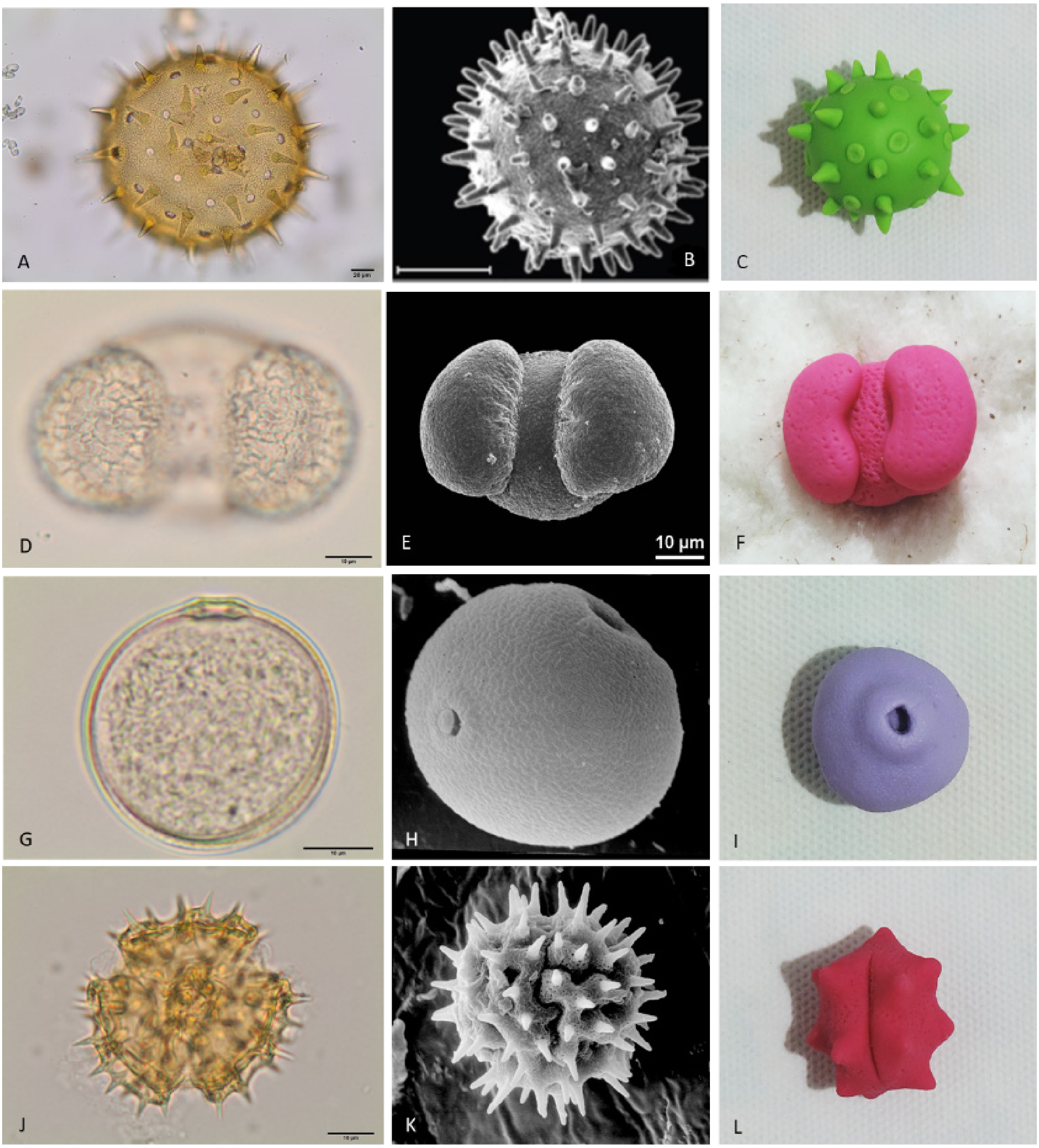

Figure 12. Pollen grains under light microscopy (A, D, G, J); Pollen grains in scanning electron microscopy (B, E, H, K); Pollen grain models made of biscuit (C, F, I, L); pantoporate apolar *Pavonia* pollen grains (A-C); *Pinus* pollen grains seen from the distal face (D-F); Poaceae pollen grains in equatorial view (G-H) and polar view (I); *Vernonia* pollen grains in polar view (J-K) and equatorial view (L). Source: Angela Maria da S. Corrêa et al. (2012) (A-B); Halbritter, H. (https://www.paldat.org/search/genus/Pinus) (E); Cynthia F.P. da Luz (D, G, H, J, K); Natalia S. Martarello (C, F, I, L). Scales: 20 μm (A), 50 μm (B), 10 μm (D, E, G, J).

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