2012

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Dario Piombino-Mascali
Institute for Mummies and the Iceman

Albert R. Zink
Institute for Mummies and the Iceman

Karl J. Reinhard
University of Nebraska at Lincoln, kreinhard1@mac.com

Melissa Lein
University of Nebraska-Lincoln

Stephanie Panzer
Department of Radiology, Trauma Center Murnau

See next page for additional authors

Published in *Journal of Archaeological Science* 40:4 (April 2013), pp. 1935–1945; doi: 10.1016/j.jas.2012.11.002

Submitted January 24, 2012; revised November 2, 2012; accepted November 4, 2012; published online November 24, 2012.

Dietary analysis of Piraino 1, Sicily, Italy: The role of archaeopalynology in forensic science

Dario Piombino-Mascali,[1] Albert R. Zink,[1] Karl J. Reinhard,[2] Melissa Lein,[2] Stephanie Panzer,[3] Arthur C. Aufderheide,[4] Rachel Rachid,[5] Wanderley De Souza,[5] Adauto Araújo,[6] Sérgio A.M. Chaves,[6] Sara LeRoy-Toren,[2] Isabel Teixeira-Santos,[6], and Juliana M.F. Dutra[6]

1. Institute for Mummies and the Iceman, EURAC, Bolzano, Italy
2. Forensic Science Degree Program, University of Nebraska–Lincoln, Lincoln, USA
3. Department of Radiology, Trauma Center Murnau, Murnau, Germany
4. Department of Pathology, University of Minnesota-Duluth, Duluth, MN, USA
5. Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
6. Escola Nacional de Saúde Pública Sérgio Arouca, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil

Corresponding author — K. Reinhard, tel 402-474-4238, email kreinhard1@mac.com

Abstract
Pollen from intestinal contents of mummies, backed by macrofloral analysis, provides important clues to diet, medicines, and season of death. Intestinal contents were recovered from the Piraino 1 mummy from the "Sepulcher of the Priests," Piraino Mother Church, in the province of Messina, Sicily. Using standard palynological methods and pollen concentration technique, we quantified the numbers of pollen grains per gram of coprolite. The pollen spectrum was dominated by Polygalaceae, the Milkwort Family. Polygalaceae pollen is rarely found in archaeological sites. Based on comparison to published keys, we determined that the pollen came from a species of *Polygala*. *Polygala* contains species with medicinal value. We found nine other pollen types. Traces of *Potamogeton* (pondweed) was observed in preliminary scans. Multiple grains of Brassicaceae (mustard family), cereal (cultivated grains), *Typha* (cattail) and Cheno-am were found. Single grains of *Castanea* (chestnut), Fabaceae (bean family), *Salix* (willow), and Solanaceae (tomato family) were found. The preservation of the pollen was poor except for *Polygala* and the cereal pollen. Brassicaceae and Cheno-am have been part of the natural pollen spectrum in Sicily since ancient times. Cereal grains were consumed with prepared food. Importantly, background arboreal pollen was nearly absent. This indicates that Piraino 1 died during months of low pollination. The absence of olive pollen is important since this plant in Sicily reaches its maximum pollination in May and June and tapers off rapidly by mid-June. Therefore, absence of the key warm season airborne pollen type suggests a post-June death. Macrofossils, especially residue from grape pulp, indicates a death in September to November. His cause of death was likely a result of multiple myeloma. The methodological differences between archaeopalynology and forensic palynology are summarized. We suggest that the palynological methods presented here should be adopted for human remains analysis in forensic palynology.

Keywords: palynology, Sicily, mummy, Piraino, *Polygala,* radiology, multiple myeloma

1. Introduction

Archaeopalynology is a quantitative field with long-established methods. Forensic palynology is a new and emerging field of developing pollen techniques and interpretation. Like other fields of forensic science, palynology must adjust to the recommendations of the National Academy of Sciences National Research Council related to standardization of methods (NRC, 2009). Reviewing papers published in a 2006 *Forensic Science International* volume dedicated to forensic palynology, the condition of forensic palynology is deficient with regard to methods in comparison to archaeopalynology and geopalynology. Indeed, the collection of articles presented in the volume threatens to put the field behind since it is presented in a peer-reviewed literature subject to Daubert criteria. As the fledgling field of forensic palynology addresses NRC guidelines, we feel that the long established standards of archaeology can be applied to forensic science (Bryant and Holloway, 1983; Bryant and Williams-Dean, 1975; Dean, 2006; Hevly, 1981; Kelso and Solomon, 2006). We are presenting the pollen analysis of a mummy from Sicily, not

only for the intrinsic value of pollen data, but also as a beginning point to introduce present archaeopalynological standards to be adopted by forensic palynologists. This is one step toward correcting the methodological vagaries present in the forensic palynological literature.

Our case example is an Italian mummy. Italian mummies reflect a rich heritage from various time periods, socioeconomic backgrounds, and cultural nuances. Especially after the 14th century, hundreds of spontaneous and anthropogenic mummies have been documented (Fornaciari, 2006). For the most part, these are naturally mummified individuals. Desiccation may be promoted in crypts and tombs environments that suspend decomposition. However, artificial mummification was achieved for famous public figures and also for families of the upper social classes (Ascenzi et al., 1998).

In some areas of the south of Italy, including the island of Sicily, mummification resulted from a local funeral custom. This involved draining cadavers and subsequent exposure to drying conditions. In fact, a large number of mummies can be found in this region (Farella, 1982; Piombino-Mascali et al., 2011). We are currently studying a series of individuals preserved by spontaneous-enhanced mummification in the Piraino Mother Church, in the province of Messina, northeast Sicily. There, twenty-six bodies of religious dignitaries are preserved in a crypt. The bodies tentatively date to the late 18th to mid-19th century AD (Piraino Parish Archives, unpublished data). One of these, Piraino 1, an unidentified adult male is concerned in this paper. Examination of this mummy revealed pleural adhesions, dental enamel hypoplasia and calculus. X-ray investigation revealed slight/moderate spinal arthritis and lytic lesions located on the skull, ribs, humeri and pelvis.

Piraino 1 contains coprolites in the lower digestive tract. One sample was submitted for dietary and parasitological analysis. Previous analysis (Kumm et al., 2010) focused on the parasitological analysis of Piraino 1. That analysis revealed the highest recorded archaeological infection with whipworm, *Trichuris trichiura*. Over 34,000 eggs per gram of coprolite were discovered which represents an infection with an excess of 100 worms.

The pollen from intestinal contents of mummies provides important clues to foods, medicines, and season of death. We analyzed the pollen and macrofossils from Piraino 1 to determine what he intentionally ate and drank before death, and what season of the year he died. We are presenting our data in context of radiographic information that reveals Piraino 1's disease condition at the time of death. This analysis relates directed to the successful attempt of Italian forensic palynologists in using pollen to gauge the season of death by examination of pollen on modern corpses (Montali et al., 2006)

2. Materials and methods

Within the framework of the "Sicily Mummy Project", the 26 Piraino bodies were inspected and sampled. Twenty-three of them were also examined by a mobile digital radiography system (Dragon DR, CXDI-50G, Canon/Sedecal) which was constructed inside the crypt (Piombino-Mascali et al., 2011) (Figure 1). The individual concerned in this paper was labeled as Piraino 1, and comprises an unidentified spontaneous-enhanced adult male mummy (Aufderheide, 2003). Visceral samples, coprolites, were obtained via a minimal opening of the abdomen of Piraino 1. Two coprolite samples were put in a sealed sterile centrifuge tube in Sicily at the site of the autopsy and subsequently sent to the Palynology Lab at the University of Nebraska–Lincoln, School of Natural Resources (UNL SNR). Therefore, the samples were protected from contamination in transit from Sicily to Nebraska. The UNL SNR lab is a filtered air, positive pressure, environmentally controlled facility which minimizes contamination. Gloves and lab coats were worn to prevent pollen from analysts' hands or clothing. A subsample was sent to the Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (ENSP/FIOCRUZ). Thus, analysis was carried out blindly in separate labs.

In Nebraska, a subsample of the coprolite was removed from the centrifuge tube and was weighed. The subsample was 2.54 g. The coprolite was rehydrated in 0.5% trisodium phosphate for 48 h. A dissolved tablet of *Lycopodium* (batch 124,961,

Figure 1. View of the "Sepulcher of the Priests", Piraino Mother Church, in the province of Messina, Sicily. Piraino 1 is under examination in this image.

containing 12,542 *Lycopodium* spores), was added to the rehydrated coprolite. Thus, we added approximately 4,938 *Lycopodium* spores per gram of coprolite. Then the sample was disaggregated in distilled water in a 600 ml beaker using a stir bar and stir plate. The disaggregated remains in fluid were screened through a 250 µm mesh with a distilled water jet. The water and microscopic residues that passed through the screen were collected in a beaker and concentrated by centrifugation. Samples of the concentrated microscopic remains were scanned for dietary residues and parasite eggs. The macroscopic remains were transferred to filter paper and dried for separate analysis which is on-going. Preliminary images of macroscopic remains were made with a Syncroscopy Auto-Montage digital microscope system at the University of Nebraska State Museum Biodiversity Synthesis Lab. This system eliminates depth of field limitation problems by automatically capturing the in-focus regions from a range of focal planes and combining them into a single fully-focused, high resolution image.

After the parasite analysis was complete (Kumm et al., 2010), the main chemical processing necessary for pollen analysis was acetolysis. This process dissolves cellulose, chitin and other materials, there-by concentrating the pollen. Acetolysis also darkens the pollen which makes pollen morphology apparent. The microscopic remains were in a 50 ml, conical bottom centrifuge tube. Preliminarily, we washed the sample in distilled water. "Wash" means that the sample was mixed with a vortex stirrer in 15 ml s of fluid. Then 30 more milliliters of fluid were added to the tube. The tube was centrifuged and the supernatant was poured off, completing the wash. After the distilled water wash, the sample was washed with glacial acetic acid. This must be done to remove the water since acetolysis solution reacts violently to water. We mixed the acetolysis solution of 8 parts acetic anhydride and 1 part sulfuric acid. Typically, acetolysis solution is mixed at a 9:1 ratio. However, the large amount of cellulose in the samples warranted the use of a more reactive solution of 8:1. The microscopic remains were mixed with the solution with a vortex stirrer and the tube was placed in a water bath at 99 °C for 10 min. We centrifuged the tube and poured the supernatant into a hazardous waste container. We wash the residue with glacial acetic acid. Then we washed the sample multiple times in distilled water until the supernatant was clear. The sediment was transferred to a 2 dram glass vial in glyerine for archival storage.

We mounted drops of sediment on microscope slides using applicator sticks. Cover slips were then placed on the preparations and sealed with commercial nail polish. Following standard palynological procedures, 200 pollen grains were counted. Then two additional slides were scanned for rare taxa. Preliminary lab assessment of microscope slides was done with a micromaster compound microscope at 400×. The final count was done with a Jenaval compound microscope using 400 and 1,000×. A Zeiss Axioscope compound microscope was used to do the final microimaging.

At ENSP/FIOCRUZ, the analysis was carried out in the same general manner, with additional analysis of phytoliths and Scanning electron microscopy (SEM) imaging of pollen. For bright field analysis a Nikon eclipse E200 microscope was used. Images were captured with Infinity 1 camera (Lumenera Corporation) using Image Pro 6.3 software (Media Cybernetics).

After pollen concentration, as described previously, SEM analysis was conducted and acetolyzed pollen grains were suspended in a drop of saline buffer, and then transferred to glass coverslip, allowed to dry at room temperature (Krachai et al., 2009). Small broken pieces of this glass coverslip were mounted in stubs using a double side carbon tape (TED PELLA Inc.). Images were taken under low vacuum mode at QUANTA 250 (FEI Company).

3. Results

3.1. Paleopathological findings

We found multiple small osteolytic changes of the skull. The chest radiograph revealed distinct osteolytic lesions with fracture of the second and fifth rib, an expansile osteolytic lesion of the eighth rib and small osteolytic lesions of the ninth and tenth rib in the left hemithorax (Figures 2 & 3). The third rib was partially missing, due to resection for further biomedical investigation. The fourth rib showed a fracture without the presence of osteolytic changes. The right hemithorax doubtfully showed small osteolytic changes of the fifth and sixth rib. The seventh rib was fractured without any visible osteolytic changes. There was a questionable osteolytic lesion in the distal part of the right scapula. We found small osteolytic lesions of the distal humerus on the right side and of the proximal humerus on the left side. On the pelvic radiograph an osteolytic lesion of the right pubic bone was detectable. Both proximal femora showed a very inhomogeneous bone structure with doubtful small osteolytic changes. None of the described lesions showed new bone formation.

Furthermore, radiographs revealed degenerative changes of the cervical, thoracic and lumbar spine and a moderate scoliosis of the thoracic spine. Post-mortem dislocation between two vertebrae was found in the upper thoracic spine. Piraino 1 wears a necklace, clearly visible on the x-rays.

The described osteolytic changes are compatible with the diagnosis of multiple myeloma or skeletal metastasis. Multiple myeloma, also called plasma cell myeloma, is a malignant neoplastic proliferation of the plasma cells in the bone marrow. Multiple myeloma is the most common primary malignant bone tumor. Skeletal metastases are the most common secondary bone tumor. They can be classified as purely osteolytic, purely osteosclerotic, and mixed osteolytic-osteosclerotic. A differential diagnosis between multiple myeloma and the lytic form of metastatic carcinoma is difficult. Both processes affect the same age group (older age group, particularly after the age of fifty years) and involve essentially the same areas of the skeleton which are the vertebral column, the ribs, the pelvis, the skull and the proximal femur and humerus (Steinbock, 1976; Resnick, 2002: 2188–2197, 4274–4301; Chhem and Brothwell, 2008). Furthermore, in paleoradiology the image quality

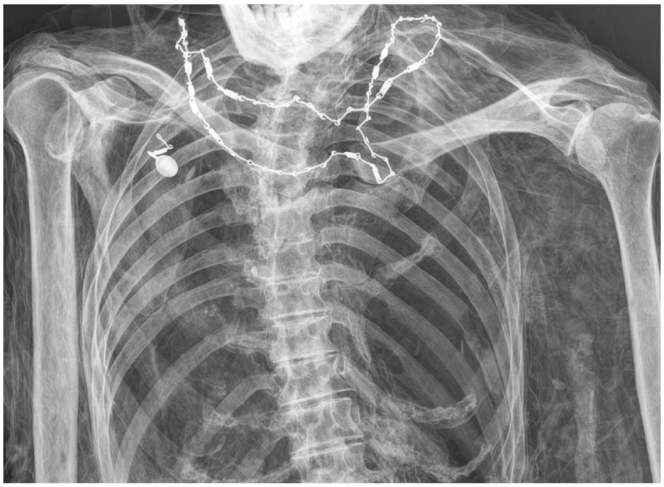

Figure 2. Antero-posterior radiograph of the chest showing a necklace and another foreign material possibly related to the necklace with projection to the upper right hemithorax. Multiple osteolytic changes of the ribs, as well as small osteolytic changes of the left proximal humerus and the right distal humerus.

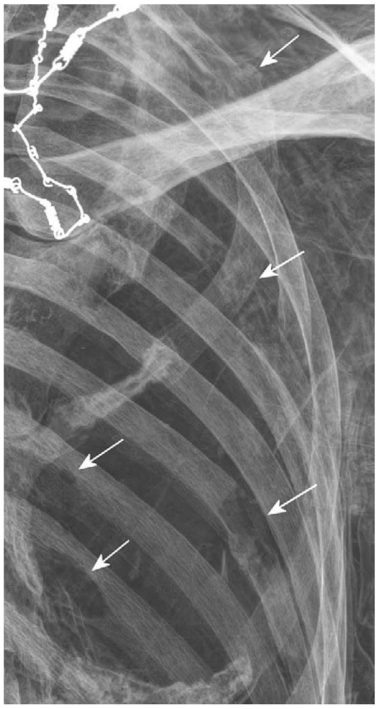

Figure 3. Detail out of left hemithorax illustrating distinct osteolytic lesions with fracture of the second and fifth rib, an osteolytic lesion of the eighth rib, and small osteolytic lesions of the ninth and tenth rib.

is often reduced due to superimposition by clothing and other foreign materials and radiographs cannot be performed in the standardized projections because of the stiffness and fragility of the mummies. In Piraino 1 we definitely found osteolytic changes in the skull, the ribs, the humeri and the pelvis. The second and fifth rib on the left side showed accompanying fractures which seemed to occur during life-time because of a dullness of the fracture edges. An adequate evaluation of the spine was not possible which could have provided important findings for a possible discrimination between multiple myeloma and skeletal metastases. Based especially on the lack of new bone formation and the punched-out character of the lesions, we favor the diagnosis of multiple myeloma in this case. However, a purely osteolytic form of skeletal metastases would also be possible.

3.2. Macroscopic coprolite findings

The macroscopic remains were dominated by grape (*Vitis* sp.) stems, pips, and fruit skins (Figure 4). Grape stem fragments were very common in Piraino 1 and were the most abundant remains. However, it is strange to find grape stems in digesta from any part of the world. Usually seeds and fruit coats are found. This aspect of Piraino 1 consumption is therefore unique and begs explanation.

Besides grape residue, we also found remains from a succulent fruit, with a yellowish pulp and peel of a Rosaceae (Figure 5). Analyzing the peel at the microscopic level and comparing with modern fruit, we concluded that the fruit was from the *Prunus* sp., which includes peaches, plums, cherries and apricots.

Less common were fragments of pine nuts (Figure 6) and small fragments of wheat chaff. Even though wheat pollen and chaff were consistently found, extensive examination of microscopic remains showed no wheat starch. This suggests that the wheat was prepared in a way that emulsified the starch and rendered starch unidentifiable after digestion. We hypothesize that he consumed wheat-derived foods such as pasta or bread.

3.3. Palynological findings

There were nine pollen types found in the 200 grain count. The pollen counts and calculated concentration values are presented in Table 1. In addition to the 200 grain count, one grain of *Potamogeton* (pondweed) was observed in preliminary scans. Polygalaceae pollen, consistent with the genus *Polygala* dominated the pollen spectrum (Figure 7). Pollen grains of the Polygalaceae are rarely found in archaeological sites. However, the morphology and taxonomy of the family have been thoroughly studied (Banks et al., 2008; Eriksen and Persson, 2007; Furness and Stafford, 1995). Multiple grains of Brassicaceae (mustard family), cereal (cultivated grains), *Typha* (cattail) and Cheno-am were found. Cheno-am refers to a similar pollen morphology produced by over 1000 species in the goosefoot family Chenopodiaceae and the pigweed genus *Amaranthus* (Reinhard et al., 2006). Single grains of *Castanea* (chestnut), Fabaceae (bean family), *Salix* (willow), and Solanaceae (tomato family) were found. The preservation of the pollen was poor except for *Polygala* and the cereal pollen. Most *Polygala* pollen grains were intact but flattened. Brassicaceae and Cheno-am have been part of the natural pollen spectrum in Sicily since ancient times (Yll et al., 2006).

4. Discussion

4.1. Archaeological interpretations

Piraino 1 had multiple disease conditions. The enamel hypoplasia may signal a disease episode in early life. The pleural adhesions resulted from healed lung conditions such as pneumonia. He had degenerative disease of the spine which is typical of adults as represented by Italian mummies (Ventura et al., 2006). Piraino 1 was also suffering a severe whipworm infection at the time of his death. Finally, the radiographic data presented here shows that he suffered from probable multiple myeloma. Clearly, he could have experienced symptoms from the parasitism and cancer, and residual symptoms from lung infection.

The dried plum fruit known as prunes, is an effective laxative and is also stomachic (Grieve, 1971). The bark can be used as a febrifuge (Chiej, 1984). Members of the genus contain amygdalin and prunasin, substances that break down in water to form hydrocyanic acid (cyanide or prussic acid). In small amounts this compound stimulates respiration, improves digestion and gives a sense of well-being (Brown, 1995).

The grape residues of seeds, fruit skin and pedicles are difficult to interpret. It appears that these were crushed and are byproducts of juice production. The general antioxidant properties of skin and seeds have been well defined (Vitseva et al., 2005). Flavonoids found in fruit skin and seeds are useful in treating some cancers and cardiovascular disease (Yilmaz and Toledo, 2004).

Pollen analysis of coprolites from burials, mummies, and refuse has successfully revealed medicinal treatments (Chaves and Reinhard, 2006; Reinhard et al., 1991; Shafer et al., 1989) as well as diet (Reinhard and Hevly, 1991). The analysis of Piraino 1 was designed to address diet and medicine for this ailing individual.

The most common pollen type was *Polygala*. *Polygala* flowers produce distinctive pollen grains (Krachai et al., 2009). Each pollen grain has 10–13 grooves (colpi), each groove contains a pore, and the pores are elongate. In palynological terms, the shape of the pollen grains are prolate and the aperture type is stephanocolporate. Because of the pore morphology and orientation, each grain can appear to have a ring of translucency around its equator (Figure 7). The Piraino 1 pollen is distinct enough to identify it to a cluster of nine species in a single genus. The fact that there

Figure 4. **Grape (*Vitis* sp.) remains. Analysis of macroscopic remains is on-going.** However, over half of the identifiable remains are from grapes. It appears that clusters of grape berries, commonly called bunches were crushed and the resulting pulp was eaten. The upper image shows an intact seeds. Most seeds were fragmented. The middle images show a stem pedicel from the side and end. The lower image shows the berry skin. No *Vitis* pollen was found.

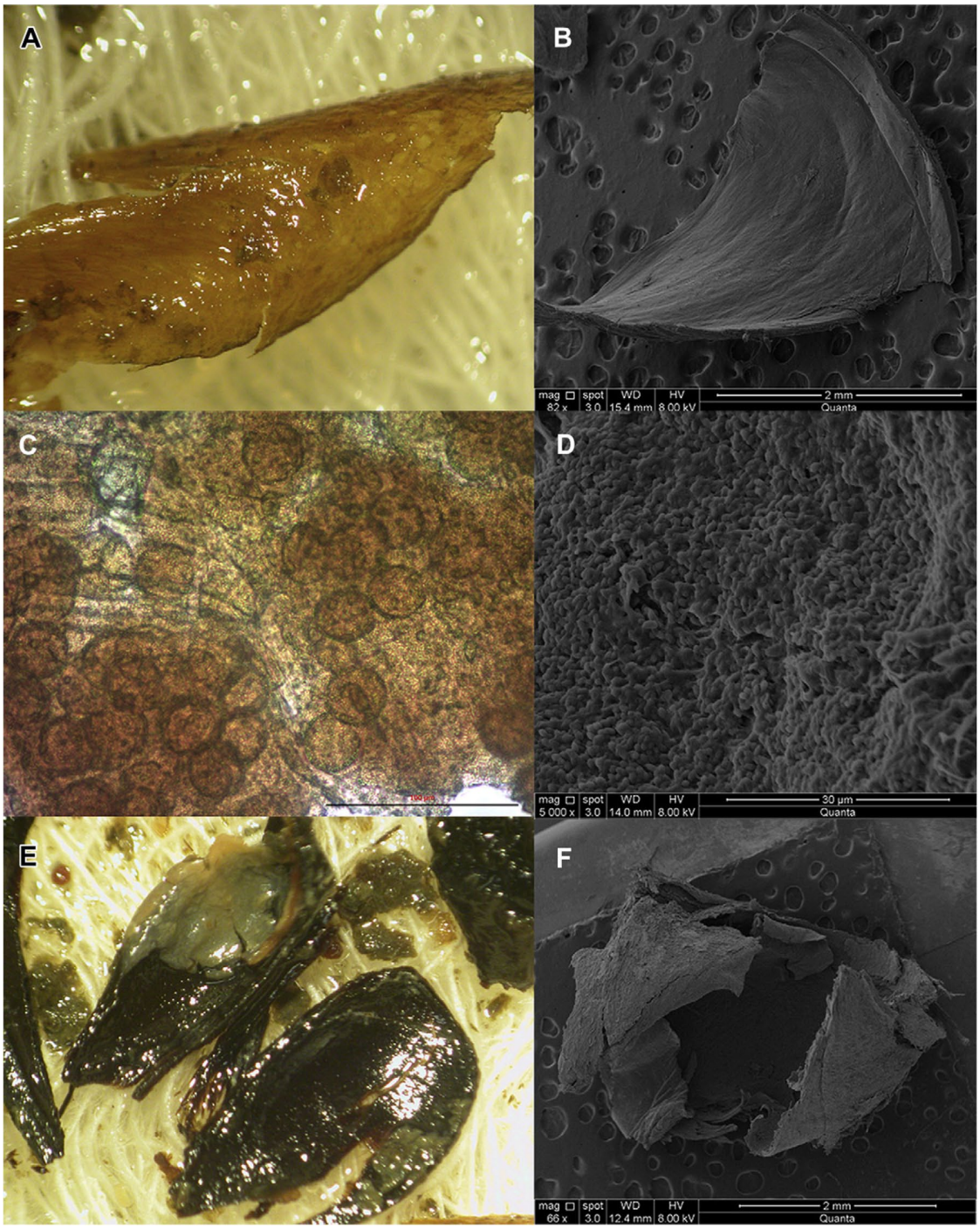

Figure 5. (*Prunus* sp.) fragments under light microscopy (A,C,E) and scanning electron microscopy (B,D,F). The upper images (A&B) show the skin (exocarp) consistent with *Prunus* drupe fruits. The middle images (C&D) compare favorably with pulp (mesocarp) of *Prunus* drupe fruits. The lower images (E&F) are of seed fragments that might be associated with the other remains. However, we are unsure of the taxonomic origin of the seed fragments.

are fewer than 14 colpi on each grain indicates that the pollen comes from a group of species classified as the *Polygala vulgaris* type. This type includes the species *Polygala alpestris*, *Polygala alpina*, *Polygala amara*, *Polygala amarella*, *Polygala calcarea*, *Polygala comosa*, *Polygala serpyllifolia*, *Polygala nicaeensis*, and *P. vulgaris*. *Polygala* pollen dominated the pollen spectrum of Piraino 1 and had a high concentration value of 10,826 pollen grains per gram. It is an insect-pollinated type (entomophilous). These facts combined show that Piraino 1 intentionally consumed a substance derived from *P. vulgaris* type flowers or foliage with flowers. Importantly, botanist Cupani (1713) records the presence of plants of the species *Polygala* in Sicily.

The Italian name for *P. vulgaris* is Erba Bozzolina. The presence of a type of *Poygala* in Sicily in the 1600s indicates that this species was present and known (Cupani, 1713). Obviously we assume that between Cupani's work and the time of Piraino 1's life, this plant did not disappear from the island. Therefore it is common sense to believe that Bozzolina would be used also in the 1700s and 1800s. According to botanist Gastaldo (1987: 190–193), *P. vulgaris* is more rare in the center and the south of Italy, but other

Figure 6. Nut fragments. We believe that the traces of nut shell in Piraino 1 are consistent with pine nuts based on the smooth surface of the exterior (upper image) and the membrane covered interior. Also, the wall of the nut is composed of parallel columns more consistent with pine than hardwood nuts.

Table 1. Pollen count and calculated concentrations for the pollen types observed.

	Direct count	Percentage	Pollen concentration value
Lycopodium	78		
Polygala	171	85	10,826
Brassicaceae	6	3	380
Cereal grains	5	2	317
Typha	5	2	317
Cheno am	3	1	190
Castanea	1	Trace	63
Fabaceae	1	Trace	63
Salix	1	Trace	63
Solanaceae	1	Trace	63
Unidentifiable	9	4	570

Polygala species are indeed present, such as *P. vulgaris* var. preslii, also known as *Polygala preslii* Sprengel (Bozzolina di Sicilia).

What was this *Polygala* substance? The absence of plant parts attributable to *Polygala* species indicates that the pollen was not eaten with food. It is more likely that the pollen was consumed with a drink. Following the logic of Reinhard et al. (1991) and Chaves and Reinhard (2006) pollen evidence such as this is suggestive of the consumption of a "tea", in other words foliage or flowers that were dispersed in water which was strained of large particles and drank. Of the species in the *P. vulgaris* type, *P. vulgaris* itself was used as a medicinal tea in Turkey for several purposes including as an expectorant and also to treat lung problems (Johnson, 1999: 650).

Recently, archaeopalynologists developed a progression of logic that can be applied to determine whether potential medicinal pollen in mummies or coprolites was intentionally ingested or accidentally ingested (Chaves and Reinhard, 2006;

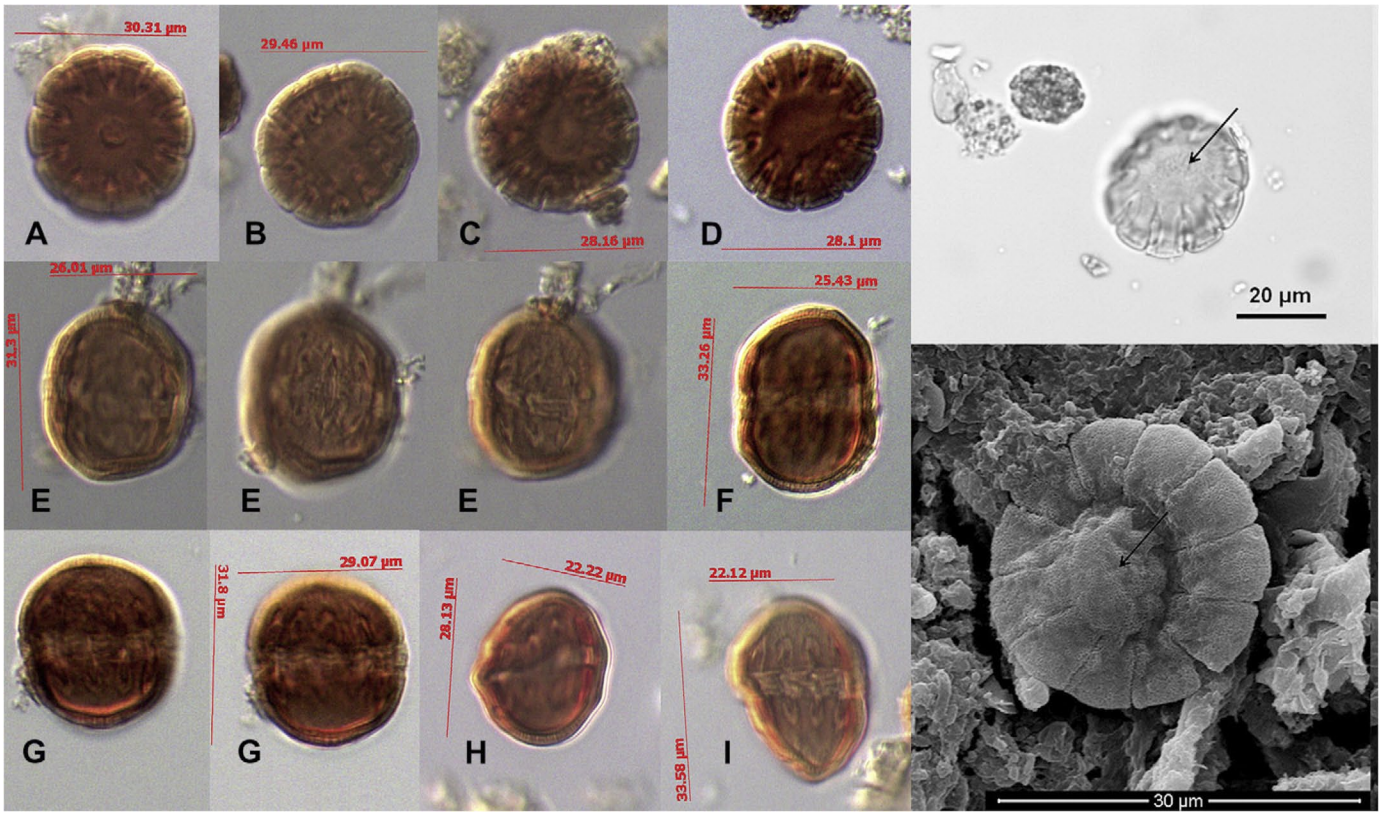

Figure 7. *Polygala* pollen light microscopy and scanning electron microscopy. A–D are polar views of four different grains. Equatorial views of five different grains are shown in E (three views), F, G (two views), H and I. I shows a deformed grain. The right hand images show a fine reticulum on the surface as indicated by arrows.

Reinhard et al., 2007). Chaves and Reinhard (2006) analyzed pollen types in ancient Brazilian coprolites from possible medicinal plants. They found pollen from ten different plant genera that had medicinal implications. The coprolites ranged in age from 8450 ± 80 BP and 7230 ± 80 BP and were excavated from Piauí, Brazil. They asked several key questions of the pollen evidence of medicinal plants. Is there evidence of prehistoric pathology that would have required treatment? Does the therapeutic property of the plant match paleopathological medicinal needs? Is it likely that pollen from the therapeutic plant will be carried by the part used for medicine? Is it likely that pollen will persist in the prepared medicine? Does the pollination strategy of the plant in question (insect vs. airborne dispersal) prevent its representation in the normal pollen rain? Can pollen be used to make a precise identification of a medicinal genus or species? Is the amount of pollen present in the coprolite consistent with a medicinal use?

The evidence from Piraino 1 addresses positively all of the criteria established by Chaves and Reinhard (2006; Reinhard et al., 2007). Insect-pollinated *Polygala* is not a part of the pollen spectrum that would have been inhaled. *Polygala* pollen is distinct enough to make a precise identification of a specific cluster of species. The amount of pollen signals intentional use. Medicinal use is documented for one species in the cluster, *P. vulgaris* (Dall'Acqua et al., 2002). The active compounds isolated from *P. vulgaris* include antitumor agents for cancer treatment. Dall'Acqua and his colleagues recovered medicinal compounds from foliage and roots. Therefore medicinal preparation could have included pollen that was consumed by Piraino 1 specifically for his cancer symptoms.

It is noteworthy that another Asian *Polygala* species produces polygalasaponins that have antiamnesic (Xu et al., 2011), anxiolytic, and sedative–hypnotic activities (Yao et al., 2010). Extracts from this species, *Polygala tenuifolia*, have been used for centuries (Chung et al., 2002) to treat behavioral and psychological symptoms in Korea and China. This species is not evident in Piraino 1. *P. tenuifolia* has a different pollen morphology than that found in Piraino 1 and is not endemic to Sicily. Also, medicinal extracts are derived from the roots, not foliage or flowers. Therefore, pollen would not likely be present in medicinal preparations from *P. tenuifolia*. Thus, following the method of Chaves and Reinhard (2006), *P. tenuifolia* must be ruled out as a potential plant used by Piraino 1.

Other pollen types are dietary or possibly dietary. The cereal grain pollen is consistent with wheat, *Triticum aestivum*. Five grains were found and measured. The pollen concentration value for cereal grains is 317 grains per gram. Cereal grains, like wild grass pollen grains, are spherical structures with one pore. The pore is surrounded by a thickened area called an annulus. Unlike wild grasses, cultivated cereals have relatively large pollen grains. Wheat pollen ranges in diameter between 40 and 70 µm. The annulus ranges between 11 and 16 µm. The surface of wheat pollen is covered by small bumps called verrucae. Specifically, verrucae are more than 1 µm wide, broader than they are high and are not constricted at the base. We observed these characters on the cereal grain pollen which leads us to conclude that *T. aestivum* is represented in Piraino 1's intestinal contents. Therefore, it is very likely that the *T. aestivum* was part of Piraino 1's diet.

Brassicaceae pollen was present in the intestinal contents. Two main dietary sources of Brassicaceae pollen are cauliflower and broccoli. However, the pollen was poorly preserved and it is impossible to determine whether the pollen originated with edible species or another source. The Brassicaceae pollen was not abundant enough to make for strong evidence of diet (Reinhard et al., 2007).

Seasonality of death, as explored by Montali et al. (2006) for northern Italy, can be determined from pollen on corpses. They

found that the pollen associated with corpses corresponds to the amount and types of pollen in the air at time of death. When the dietary types are excluded from the total pollen concentration value, the amount of pollen of airborne types is very low. Only 633 pollen grains per gram from environmental types were found. Of these, *Typha* pollen grains were probably drank with water. The pollen concentration values of arboreal environmental types is very low, just 316 grains per gram. The pollen preservation of the environmental types was poor. This suggests that the pollen recovered from Piraino 1 was released from source flowers some time before they were ingested.

Reinhard et al. (2006) showed that human coprolites from arid regions contain tens of thousands of pollen grains per gram from coprolites produced in months of pollination. From Sicily, ancient hyena coprolites have been studied (Yll et al., 2006). Since hyenas are carnivores, they serve as a good proxy gauge of the quantity and diversity of pollen that is ingested from ambiental sources. The pollen concentration values from the hyena coprolites ranged from 1476 to 12,142 pollen grains per gram from the natural pollen rain with an average of 5777 pollen grains per gram. Montali and her colleagues show that the amount of pollen in the air for northern Italy crashes in October and rebounds with the beginning of pollination in February. The low pollen concentration for Piraino 1, and the poor preservation of the pollen, indicates that Piraino 1 died during a season of low or no pollen production. This indicates that he died in late autumn, winter, or early spring. Importantly, the key summer pollination type, olive, is absent in Piraino 1. The absence of olive pollen is important since this plant in Sicily reaches its maximum pollination in May and June and tapers off rapidly by mid-June. Therefore, absence of the key warm season airborne pollen type suggests a post-June death (Orlandi et al., 2005, 2006). The season of death can be tightened down by addressing the macroscopic remains. The most abundant remains, grape seeds, skins and pedicles, represent the use of fresh grapes. The grape harvest can occur from September to November in Sicily, but usually in October. Therefore, a death at harvest time is suggested by the remains, or shortly thereafter.

In conclusion, the pollen spectrum is limited in Piraino 1. It is dominated by types that are not found in the natural pollen rain. The presence of cultivated grain pollen and *P. vulgaris* type indicates intentional use of these plants. The find of *P. vulgaris* type is a good candidate for medicinal treatment of Piraino 1's ailments. The near absence of environmental pollen and abundance of grape pulp indicates a death in a seasonal range from late fall to early spring, probably in the autumn around October.

4.2. Transferring archaeopalynology methods to forensic palynology

Reinhard et al. (2007: 539) reviewed the application of palynology to archaeology. They state, "There are four essential parts of a pollen study of archaeological materials, and each must be done thoroughly to produce reliable interpretations. First, the archaeological samples and control samples must be collected carefully to ensure against potential pollen contamination. Second, laboratory pollen extraction must be done in a contamination-free facility and techniques that are used must not destroy or damage the pollen. Third, recovered fossil pollen should be compared with modern pollen reference samples to ensure correct identifications. Fourth, once the pollen analysis is complete, it is critical that the resulting data be interpreted as logically and correctly as possible making assumptions only about those pollen types which seem to fit logically into patterns of either background or economic categories" (Reinhard et al., 2007: 539). With regard to the fourth part of study, they identify several common errors. These include including failure to quantify, failure to have proper laboratory conditions and over-interpretation of trace pollen types. Archaeopalynologists avoid these errors by applying pollen concentration to a minimum of 200 grain counts, maintain positive pressure laboratories with filtered air, and applying good judgment to their pollen data including adequate use of control samples.

Some authors in forensic palynology apply these considerations to their work, and indeed their methods have conceivable application to archaeology. For example, Donaldson and Stephens (2010) developed a pollen method for sourcing tobacco. Riding and his colleagues define how pollen is trapped and replaced on footware. Zavada et al. (2007) demonstrated the importance of cloth as pollen traps. All of these forensic applications could be applied to archaeological materials.

Among other workers, forensic palynology is sometimes a search for a "key pollen grain" (Riding et al., 2007) to make a legal case. Often, this is done in the absence of quantification. One of the basic questions in forensic palynology is how many pollen grains must be counted per sample. Geopalynologists debated the question of how many pollen grains should be counted in the early part of the 20th century (Geisler, 1933). Barkley's (1934) work on the statistical reliability of pollen counts representing the source pollen defined 200 grains as the standard goal. In the 1998 review of palynological techniques, key leaders in archaeopalynology set 200 grains as a standard count (Dean, 1998; Smith, 1998; Gish, 1998). There are exceptions to this rule, but the exceptions require exceeding 200 grains. Reinhard and his colleagues find that coprolites with high pollen concentrations due to consumption of polliniferous foods will require higher pollen counts to identify environmental taxa (Reinhard et al., 2006, 2012). Studies focusing on recovery of specific types such as cultivated grains from ancient fields will also require larger counts. But, in archaeopalynology, 200 grain counts are the minimum standard.

Reviewing the forensic palynology literature, there is little consensus regarding standardization of pollen grain counts. Horrocks (2004) notes that most palynologists count at least 100 grains. Horrocks and Walsh (2008) suggest that 100–200 grain counts are sufficient. Mildenhall, (2006a & 2006b) counted to 200–400 grains per sample. Wiltshire and Black (2006) and Wiltshire (2006) state that in their work, "as many palynomorphs as possible are counted, even though the law of diminishing returns may be considered to operate in counts above 300." Wiltshire and Black (2006) counted as many as 2000 pollen grains per sample. Wiltshire (2006) does not disclose the number of grains she counted. Montali et al. (2006) worked with swabs from autopsies and therefore had variable recovery of pollen grains from diverse samples. Their counts ranged from a low of 34 to a high of 457. Some forensic palynologists are totally silent regarding the numbers of pollen grains they count to come to their conclusions (Brown, 2006).

Pollen concentration methods are also standard in archaeopalynology. Maher (2000: 1) defined methods for the International Union for Quaternary Research Sub-Commission on Data-Handling Methods. He stated "It is good practice to add a known quantity of exotic spores to a pollen sample. Even if the analyst does not intend to measure pollen concentration or influx, the presence of the exotic in an otherwise barren slide effectively proves the sample's indigenous pollen was not accidentally decanted during processing. Although the exotic can be introduced in a number of ways, I have found marker-grain tablets (Stockmarr, 1971 & 1973) to be the most convenient." Previously, in an article that formalized methods for archaeo-

palynologists, Bryant and Hall (1993) wrote that "One of the recent advancements in archaeological pollen processing has been the regular use of tracers to evaluate the concentration of fossil pollen per unit volume, or unit weight, of a sample." Today, pollen concentration is universally applied in archaeology.

Among archaeopalynologists that apply their experience to forensic cases, pollen concentration methods are used (Bryant and Jones, 2006). However, there is a surprising disregard of the demonstrated value of pollen concentration among other forensic palynologists (Maher, 1981). The resistance to pollen concentration quantification is exemplified by the following quotes from forensic science literature. "Exotic marker spores were not added due to pollen and spore concentration values having little worth in such situations" (Brown, 2006: 206). "No attempt is made to assess the concentration of palynomorphs by the standard method of counting in relation to known numbers of added exotics (Stockmarr [6]). This technique is inappropriate where counts cannot be related to absolute amounts of original sample" (Wiltshire and Black, 2006: 226). "No attempt is made to assess the concentration of palynomorphs by the standard method of counting in relation to known numbers of added exotics [6] as this technique is inappropriate for forensic investigation", (Wiltshire, 2006: 242).

In this analysis, pollen concentration was essential in demonstrating low environmental pollen representation and therefore a death during a period of low pollination. Referring to Table 1, the wind pollinated environmental types (*Typha*, Cheno-am, *Castanea*, and *Salix*) amounted to only 633 pollen grains per gram. This is remarkably lower than the data for carnivore coprolites from Sicily that averaged 5777 environmental pollen grains per gram (Yll et al., 2006). Therefore, pollen concentration is essential in demonstrating the low pollen count compared to other studied remains.

Another potential error in forensic science is over-reliance on trace pollen types. As noted above, forensic palynology sometimes involves a search for a "key" pollen grain to make a case. Reinhard et al. (2006) present over-interpretation of trace evidence as a common error in palynology. Trace pollen types could come from contamination in the field or lab. Therefore, greater weight should be placed on pollen types that are abundant enough to represent real trends in pollen data. To come to the wrong archaeological conclusion by over-interpreting trace pollen grains is regrettable. However, in a legal case, the result of error could involve depriving one of his/her liberty if a false conviction is reached or result in legal action against the palynologist if the error is recognized. Therefore, forensic palynologists should be as careful as archaeopalynologists in preventing the over interpretation of trace pollen grains.

We believe that the current state forensic palynology is inconsistent regarding quantification methods. For forensic science over-all, the NRC has noted deficiency in methods and establishing error rates. The first steps in forensic palynology toward accommodating the new reality of forensic science is to identify the minimum number of pollen grains to be counted and adopting pollen concentration methods. This is essential in the United States where the NRC recommendations will take effect. For this purpose, archaeopalynology can show the way for forensic palynologists.

Acknowledgments — We are most grateful to Father Calogero Musarra, Archpriest of Piraino, and to anthropologist Mario Sergio Todesco, for advocating the project; funding by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico); FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro); CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). We extend thanks to the University of Nebraska State Museum Biodiversity Synthesis Lab for the use of the Syncroscopy Auto-Montage digital microscope system for imaging pine nuts and grape seeds.

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