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Pollen evidence of medicine from an embalming jar associated with Vittoria della Rovere, Florence, Italy

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Published in *Journal of Archaeological Science: Reports* 21 (2018) 238–242

doi:10.1016/j.jasrep.2018.06.039

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Submitted 5 March 2018; revised 30 June 2018; accepted 30 June 2018; published 21 July 2018.

Abstract

Various samples of human viscera fragments, sponges, and cloth were collected from embalming jars belonging to members of the Medici family of Florence. One jar was labeled with the name Vittoria della Rovere, who died in March of 1694. This jar contained viscera fragments that were identified as a section of collapsed intestine. The intestine of the Vittoria della Rovere sample contained a large concentration of pollen belonging to the Myrtaceae family. The Myrtaceae pollen was sometimes observed in clusters during analysis, which is indicative of purposeful ingestion of flowers, buds, or a substance derived from floral structures. Thus, the high concentrations and clustering of Myrtaceae pollen grains recovered from this sample are reflective of dietary or medicinal practices. Scanning electron microscopy indicated that the pollen was from cloves, *Syzygium aromaticum*. It is most likely that Vittoria della Rovere consumed cloves for medicinal or culinary reasons shortly before death.

Keywords: Archaeopalynology, Embalming jars, Medicinal plants, Scanning electron microscopy, Myrtaceae, Italy, Medici

1. Introduction

The Medici family of Florence, Italy, first came to power in the fourteenth century. Commerce and banking led to their rise of power and kept them in power for nearly three centuries. The family backed the ascension of four popes, giving them influence in all of Christendom and securing their sway beyond Florence. The remains of family members buried within the San Lorenzo Basilica, the church where the Medici Chapels are located, have been studied as part of a research project (Fornaciari et al., 2007; Giuffra et al., 2009). Several studies involving the health of the family through time have been completed since the project's beginning (Fornaciari et al., 2009; Giuffra et al., 2010). Further studies examined the embalming and autopsy techniques used in Italy during this time period (Giuffra et al., 2016). The entomological and arachnological examinations of the jar contents have been published previously (Morrow et al., 2016). The present study focuses on the recovery of pollen from an embalming jar that was also entombed within the San Lorenzo architectural complex. Pollen grains, presented here, are the most intriguing discovery from the jar.

The sample was one of 10 collected from embalming jars exhumed in 2010 from the Old Sacristy of the church, built in the fifteenth century. Analysis was done at the University of Nebraska-Lincoln in the

Palynology Laboratory, School of Natural Resources and the Microscopy Core Research Facility, Center for Biotechnology. The jars had been used to collect materials used during the embalming process of members of the Medici family (Giuffra et al., 2016; Marinozzi and Fornaciari, 2005; Morrow et al., 2016). In 2011, samples from the jars were taken at the Department of Anatomy, Histology, and Forensic Medicine of the University of Florence, where the jars were temporarily stored prior to reinterment. During the 2011 sampling, labels were found on two jars, indicating specific family members associated with the jars' contents (Lippi, 2006). One contained the viscera of Anna Maria Luisa de' Medici, the last descendant of the Medici family, who died in February of 1743. The other contained the viscera of Vittoria della Rovere, the grandmother of Anna Maria Luisa de' Medici, who died in March of 1694. It is probable that some of the other jars also contained material associated with these two individuals. However, there were no labels on other jars to confirm their identities. This study focuses only on the analysis of the Vittoria della Rovere (VdR) jar.

2. Material and methods

The materials in each of the ten embalming jars were examined to determine the composition of the sample (Morrow et al., 2016). Some jars contained cloth and sponge remains used in the mummy preparation process, while others contained sections of intestine removed from corpses. The VdR sample consisted of intestinal tissue fragments. The analysis of this material is the focus of the present study.

The VdR sample was weighed and observations prior to rehydration were recorded. It weighed 2.91 g. It was then rehydrated using a 0.5% trisodium phosphate for approximately 48 h. Following rehydration, three *Lycopodium* tablets (Batch #124961; containing approximately 12,500 spores/tablet) were dissolved in HCl and then added to the rehydrated samples. The use of exotic spores to quantify pollen in ancient samples was developed by Stockmarr and is now standard with mummy studies (Piombino-Mascali et al., 2013; Reinhard et al., 2006, 2017; Stockmarr, 1971).

The 37,500 *Lycopodium* spores added to the 2.91 g sample from VdR equate to about 12,887 *Lycopodium* spores per gram of the sample. The sample was then disaggregated in a 600-ml beaker using a

magnetic stirrer before being screened through a 250 μ mmesh and rinsed with distilled water. The selection of a 250 μ mmesh optimizes the separation of pollen and parasite remains. Macroscopic remains, primarily insects, were collected from the superior surface of the mesh while microscopic remains were screened through the mesh and into a beaker. Macroscopic remains were placed onto filter paper and allowed to dry before further examination. Microscopic remains were concentrated via repeated centrifugation and analyzed for the presence of mites, mite eggs, parasite eggs, starch granules, and other microfossils (Morrow et al., 2016). Following these analyses, acetolysis was employed for palynological investigations.

Processing samples using acetolysis is a common practice for pollen analysis (Piombino-Mascalì et al., 2013; Reinhard et al., 2006, 2017). The process dissolves cellulose, chitin, and other materials, leaving primarily resilient microfossils, such as the sporopollenin of pollen grains. Acetolysis also darkens the pollen grains, which makes the pollen morphologically apparent. For this process, microscopic remains were transferred to a 50 mL centrifuge tube. They were washed with distilled water, and then centrifuged prior to decanting. This process was repeated with glacial acetic acid to prevent a reaction between the acetolysis solution and any residual water. The acetolysis solution was prepared as a 9:1 ratio of acetic anhydride to sulfuric acid. The solution was then added to the sample and once again vortexed to ensure that the residues were thoroughly mixed with the solution. Samples were then placed in a hot water bath of approximately 99 °C for ten minutes. After 10 min, the sample was centrifuged and the acetolysis solution was decanted into a hazardous waste container. The sample was then washed once with glacial acetic acid and subsequently washed multiple times with distilled water.

Following acetolysis, the material was transferred to a 2 dram archive vial using 95% ethanol and then glycerin was added for archival purposes. For analysis, drops of sediment from the sample were removed from the vial using an applicator stick. Drops were placed on a microscope slide, mixed with glycerin and then secured with cover slips. A compound microscope was used at 400 \times and 600 \times to perform a two hundred grain count over the span of three slides. *Lycopodium* spores were counted during this process so that an approximate count of pollen grains per gram of material could be determined.

This was achieved using the following formula: pollen concentration = $[(p/m) \times a]/w$, where p was the number of pollen grains counted, m was the number of marker grains (*Lycopodium* spores) counted, a was the number of *Lycopodium* spores added to the sample, and w was the total weight of the sample prior to rehydration (Piombino-Mascalì et al., 2013).

Steps were taken to avoid contamination and maintain lab safety. Importantly, Myrtaceae flowers had never been processed in the lab prior to this analysis. Therefore, there could be no contamination from floral sources. Only sterile centrifuge glassware was used and all other equipment was cleaned thoroughly to ensure that no contaminants were introduced. The Palynology Laboratory is a filtered air, positive pressure, environmentally controlled facility that minimizes contamination. The lab's two research compound microscopes are Jenaval and Nikon instruments. The Jenaval compound microscope has differential interference contrast setting (DIC) and polarized light capability. It has 10 \times , 25 \times , 40 \times , and 100 \times objectives. The Nikon Eclipse compound microscope is designed for palynology, starch analysis, and parasitology. It has polarized light capability with 10 \times , 40 \times , 60 \times , and 100 \times objectives. It has image capture and analysis capabilities for bright-field and polarized settings.

For scanning electron microscopy (SEM), ~30 μ l of the top layers of the prepared samples (in 100% ethanol) were pipetted and placed onto a 10mm \times 10mm polycarbonate membrane (200 nm hole size) on a paper filter. After air-drying for 2–3 min, each of the membranes was placed onto a double-sided adhesive conductive tape on a SEM sample mounting stub. The samples were further dried at 42 °C in a sample oven overnight before being sputter coated with a thin layer of chromium (~5 nm thick) using a Denton Vacuum Desk V sputter coater. Samples were examined on a Hitachi S-4700 Field Emission SEM and images were collected at different magnifications (1,000 \times –5,000 \times).

Morphometric analysis of pollen images from SEM was used to describe Myrtaceae species (Thornhill and Crisp, 2012; Thornhill et al., 2012a, 2012b). Specifically, one metric feature, the colpus length divided by overall length (C/L), was used for species determination. Images were analyzed by direct measurements on printed images and by digital measurement using Adobe Photoshop CC. Measurements were made only on images with pollen having an orthogonal orientation

relative to the plane of focus, to avoid errors from perspective. Additionally, independent measurements of the same images were made by three individuals and averaged for analysis, to minimize any potential subjective measurement errors.

3. Results

3.1. Light microscopy

Most mummy intestinal and coprolite analysis features quantification through light microscopy. With LM, the pollen of Myrtaceae have long been recognized as easily recognizable at the family level (Erdtman, 1952). The Myrtaceae pollen concentration for the sample from this individual equates to 20,574 pollen grains per gram of intestinal remains. Other pollen types recovered in traces were *Pinus*, Poaceae, *Populus*, and *Castanea*.

3.2. SEM analysis microscopy

The genera and species within the Myrtaceae are difficult to identify without advanced microscopy. Using scanning electron microscopy, we were able to visualize the ultrastructure of the pollen. SEM analysis showed that the pollen grains featured scabrate sculpturing and were parasyncolpate, in other words, the colpi do not meet at the pole. Instead they form a triangular shape in the middle of the polar region known as the apocolpial field. Although it appears that apocolpial islands are present on some pollen grains, this may be an artifact of pollen grain distortion that led to elevated interior portions of the apocolpial fields. It is worth noting here that both *Syzygium* and *Eucalyptus* are parasyncolpate. Of the three genera, *Myrtus* is distinct in having a brevicolpate morphology. The pollen from the mummy is parasyncolpate. Therefore, *Myrtus* was eliminated from consideration as being a source of the intestinal pollen.

SEM has been extensively used to characterize Myrtaceae pollen (Thornhill and Crisp, 2012; Thornhill et al., 2012a, 2012b). Drawing from the characteristics for *Eucalyptus* and *Syzygium* described in these articles, we assembled the morphological data from seven *Eucalyptus* species and 11 *Syzygium* species that were characterized using

SEM. Six of the *Syzygium* species exhibit islands of exine in the apocolpial triangle, usually closely fitting the edges of the field. This was consistent with the VdR pollen. Two of the *Eucalyptus* species exhibit apocolpial islands, but only one exhibits closely fitting islands. Therefore, the morphology of the apocolpial region of the VdR specimens is closest to *Syzygium*. One metric feature, the colpus length divided by overall length (C/L), can be used to distinguish the species. Cloves, *S. aromaticum*, have the lowest C/L value of any of the *Eucalyptus* and *Syzygium* species presented in the literature. This value, 29, is very close to the value we obtained from the VdR pollen of 29.8. Finally, we compared the morphology via SEM between the VdR specimen, pollen from commercial spice *S. aromaticum* buds and *Eucalyptus* pollen tablets (Fig. 1). The VdR pollen is closest in morphology to *S. aromaticum*. The VdR pollen grains were unlike published SEM images of *Eucalyptus*. The VdR pollen exhibited more pronounced sculptural elements compared to the relatively smooth sculpturing of *Eucalyptus*. The VdR pollen is most consistent with the size, metrics, and surface ultrastructure of *S. aromaticum*. Therefore, we conclude that the pollen in the intestinal segments of Vittoria della Rovere originated from cloves.

4. Discussion

Eucalyptus and cloves are in two distantly related tribes, the Eucalypteae and Syzygieae respectively (Wilson, 2011). *Eucalyptus* pollen has been extensively studied in comparison to genera in four other tribes through SEM. The pollen morphology can be used to contrast *Eucalyptus* with genera in these tribes (Thornhill et al., 2012a). *Syzygium* (cloves) has been compared to genera in four other tribes in the Myrtaceae (Thornhill et al., 2012b). Drawing from the characteristics for *Eucalyptus* and *Syzygium* described in these articles, we found that there is considerable overlap in metric and nonmetric traits. We also reviewed the pollen morphology of myrtle (*Myrtus*) because this genus had been found in mummy context, apparently used in the processing of corpses (Vermeeren and van Haaster, 2002). Interestingly, a few of the Myrtaceae grains were found in clusters of pairs, triads, and tetrads. The grains appeared in a variety of taphonomic states

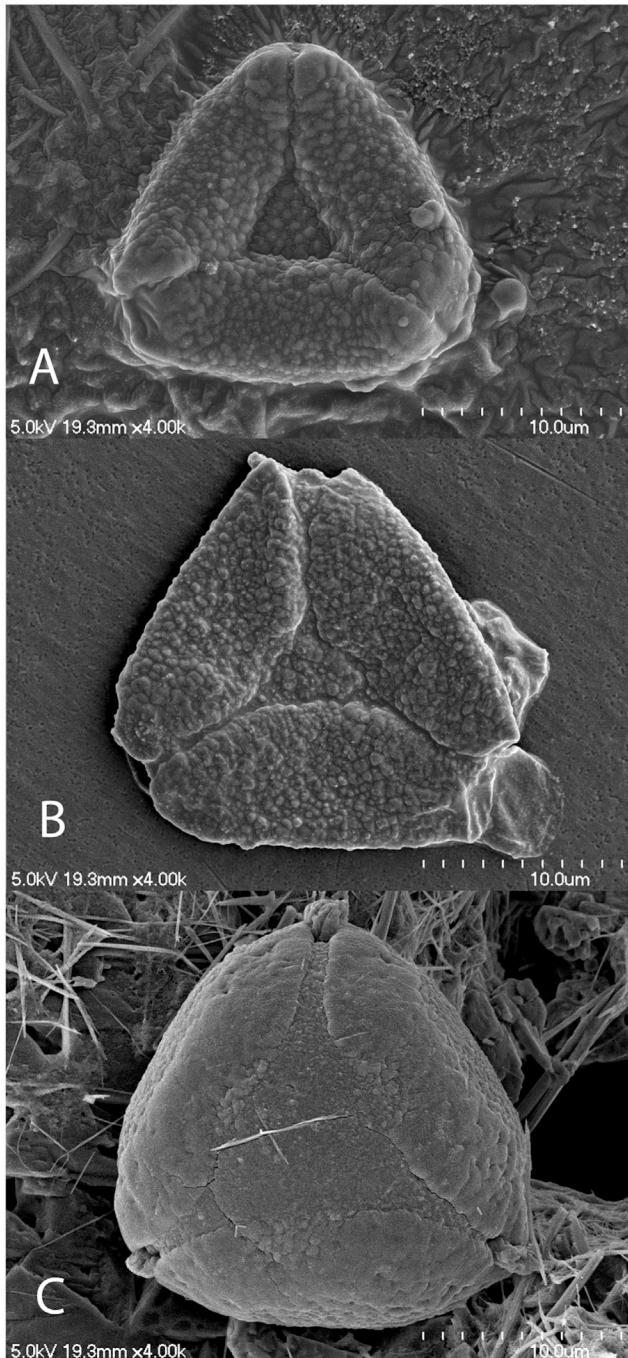


Fig. 1. The Vittoria della Rovere Myrtaceae pollen compared to modern controls. An example of the pollen from Vittoria della Rovere is shown in image A. Image B shows a pollen grain from a modern clove flower, *Syzygium aromaticum*. The flower was dissected in the Palynology Laboratory at the University of Nebraska-Lincoln School of Natural Resources. Image C represents *Eucalyptus* pollen. The *Eucalyptus* pollen grains were processed from pollen tablets used to spike samples for quantification. The tablet was from Stockmarr *Eucalyptus* batch 903772. All grains are parasyncolpate meaning that the three grooves, colpi, do not meet at the pole but form a triangular shape known as the apocolpial field. The *Eucalyptus* example exhibits shorter colpi and a larger triangular area. The exine ornamentation is more pronounced in the cloves and the Vittoria della Rovere examples. This comparison shows that the mummy sample is more consistent with cloves, *Syzygium aromaticum*.

ranging from pristine to degraded. Additionally, a pollen grain with four pores and colpi was found, representing a noteworthy variation of this pollen type.

The likelihood that *Eucalyptus* is represented is reduced by the fact that Australia was not 'discovered' by Europeans until 1770, which is

almost 100 years after Vittoria della Rovere died. This genus was introduced in the last two centuries to Italy and we cannot find references to the plant in Italy before the 19th century (La Mantia, 2013). Thus, it is not possible for the pollen grains to have been *Eucalyptus* as it was not yet known to Europe. *Myrtus* is native to Europe and it was possible that it could have been this Myrtaceae (Özkan and Güray, 2009). However, *Myrtus* pollen is brevicolpate and does not match what was found in the jar. Cloves are indigenous to the Moluccas (Cortés-Rojas et al., 2014). From the 8th century, cloves became increasingly popular in Europe and were a focus of Venetian trade. The Portuguese in 1514 and the Dutch in 1605 established spice trade including cloves (Rosengarten, 1969). Thus, cloves were a plant product available in Italy at the time of Vittoria's death.

We considered the fact that direct ingesting of flowers, or products made from flowers, is signaled by high concentration and the presence of pollen clumps (Chaves and Reinhard, 2006). The dried flower buds of cloves, *S. aromaticum*, have been used both as a culinary spice and for medicinal purposes. Medicinally, this plant has been used to treat gastrointestinal issues by improving peristalsis of the intestines and increasing the production of hydrochloric acid in the stomach (Balch and Balch, 2000). Cloves can also be used for treating toothaches by application to abscessed teeth (Alqareer et al., 2012). In order to understand the therapeutic use of cloves in the early modern era, an important source is represented by the commentaries of Pietro Andrea Mattioli, a doctor and naturalist from Tuscany, on the work by Pedanius Dioscorides, a Greek physician, pharmacologist, and botanist and author of *De materia medica* (On medical matters) who lived in the 1st century AD. The former includes cloves in the chapter dedicated to pepper, and mentions the fact that, if ingested, cloves are helpful for the stomach, liver, heart, and brain. In addition, he provides a recipe in the form of an infusion, prepared with ground cloves mixed with wine or water, in order to stop sickness and stimulate the appetite. Finally, he states that cloves can also be used to cure some diseases of the head such as epilepsy (Mattioli, 1744). These plants were widely traded during the times of the Medici. Because no macroscopic evidence of the dried buds from cloves was recovered during analysis, it is possible that Vittoria della Rovere drank cloves in a fluid preparation.

An alternative explanation for the presence of the Myrtaceae pollen is that it could have been introduced during the embalming or burial process of Vittoria della Rovere's body. Cloves have been used in concoctions for embalming cadavers (Marinozzi and Fornaciari, 2005). In a study of pollen from the embalming of the Dutch royal family in the late 1400s to early 1500s, two species of Myrtaceae were reported, one was identified as cloves and the other was identified as myrtle (Vermeeren and van Haaster, 2002). It was assumed that the pollen came from the embalming mixture or from the body lying directly atop a bed of herbs and spices to mask the scent of decay (Marinozzi and Fornaciari, 2005). It would be reasonable to consider that the VdR Myrtaceae pollen entered that jar in one of these two ways in the embalming processes of Italy near the same time period. Other studies of the embalming practices used in Italy around the time of the deaths of the later members of the Medici family have shown that a variety of aromatics, including oils infused with lavender, rose, and chamomile, were commonly used for anointing the body (Giuffra et al., 2011). In ancient Egyptian embalming practices, herbs and spices were employed in nearly every step of the process as post organ removal flushes, packing the body cavities, and as a wash following the desiccation of the body (Baumann, 1960). Using Myrtaceae species to produce aromatic effects from mixing them into an embalming concoction would also explain the clusters of Myrtaceae grains that were observed during the pollen analysis. Though the initial assumption surrounding clusters of pollen was that they were ingested, it would also be practical to propose that cloves were used in large amounts and applied directly to the body in a concentrated form. Because the samples used for the present study were originally preserved in jars, it would even be possible for cloves to have been purposely placed in the jars to mask the odors associated with the process of embalming.

Counter to these arguments is the fact that the pollen grains were only found in abundance within the intestine of Vittoria della Rovere and not in material from any of the other jars. Additionally, there was no macrobotanical evidence of the cloves recovered from the samples, which further contrasts the idea that they may have been used for the purpose of preventing offensive embalming odors. Whole cloves are quite durable and, had they been placed in the jars, woody bud fragments would likely have been recovered. The absence of macroscopic

remains suggests a dietary or medicinal origin of the pollen grains recovered during these analyses.

What we know about the life of Vittoria della Rovere suggests that she may have had a need for medicinal plants, such as cloves. She is described in historical sources as having been lethargic, having avoided muscular exertion, and having loved sleep since the early days of her youth. She also had a number of health conditions characterized by notable obesity. At age 60, she was confined to a sedentary life, and by age 62 she was half-immobilized by edema to the lower extremities. This latter detail suggests that – at least since 1685 – she suffered from a cardiac insufficiency related to a fatty infiltration of the heart and a subsequent alteration of the coronary arteries (Pieraccini, 1986). Beyond the severe heart pathology, the autopsy of her remains revealed renal failure, which likely contributed to her being confined to her bed near the end of her life. As a result, she had endured both edema and extreme sleepiness. She also exhibited catarrh, in which the nose and air passages become filled with mucus. She was enormously hydropic. The best explanation for the Myrtaceae pollen in Vittoria della Rovere is that it was ingested as a medicine to aid digestion for a woman who had fallen into ill health.

Authors' contributions – Karl Reinhard, Kelsey B. Lynch, Annie Larsen, Johnica J. Morrow, Braymond Adams, Marina Amaral, Julia Russ, and You Zhou conducted analysis and experiments. Karl Reinhard, Leon Higley, Donatella Lippi, Johnica J. Morrow, and Dario Piombino-Mascali wrote the paper. All authors reviewed the final manuscript.

Conflicts of interest – None.

Acknowledgments – We are grateful to Elsa Pacciani, of the Archaeological Superintendence of Florence, for advocating and supporting the project, as well as to Silvia Marinozzi, La Sapienza University of Rome, for her valued advice. We also thank Andrew Thornhill for his precious comments.

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