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# Paleogenomic and Ancient DNA

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## GEORGIOS PAPANICOLAOU AND THE HISTORY OF UTERINE CERVIX SCREENING

V. G. Vellone

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Since the end of the 19th century, exfoliated cancer cells had been described in all of the types of specimen in which we find them today. However, it was not until Drs. Papanicolaou and Traut published their account of the diagnosis of uterine cancer from exfoliated cells (1941 and 1943) that cytopathology acquired the strength to develop into the powerful presence that it has in human medicine today.

Born to a physician father on May 13, 1883, at Kyme Greece, he received MD from University of Athens in 1904 and served as an assistant surgeon in the military until 1906. After his military service, Dr. Papanicolaou returned to Kymi and reluctantly practiced medicine with his father. He was not interested in medicine, but yearned for a career of scientific research.

In the spring of 1907, Dr. Papanicolaou left for Jena, Germany, to begin his postgraduate studies under Professor Ernst Haeckel, and earned a PhD in zoology from University of Munich in 1910. Returning to Greece, he met and married Andromachque Mavroyeni, later known as Mrs Mary Pap, who became a pillar of support for him. Prospects of better opportunities brought the couple to US on Oct 19, 1913 where he became assistant in Pathology Department of New York Hospital and later in 1914 as assistant in Anatomy at Cornell Medical School.

It was at Cornell where Dr. Papanicolaou worked examining vaginal smears of guinea pigs to determine the existence of a menstrual cycle in them. In 1920, he started studies on human vaginal cells. He observed cancer cells in vaginal smears and realised great potential of this simple test in early diagnosis of cancer.

He introduced this low cost screening test for early detection of cancer at a medical conference in Michigan in 1928, which met skepticism and resistance from medical community.

Diagnostic potential of vaginal smear in early detection of cervical cancer was validated by scientific studies culminating in in publication of famous monograph titled "Diagnosis of Uterine Cancer by Vaginal Smears" in 1943 by Dr Papanicolaou and Dr Herbert Traut. This revolutionary diagnostic test was

named Pap smear which has saved lives of millions of women by early diagnosis of cancer of cervix.

Although Georgios Papanicolaou is generally credited for the invention of the cervical cancer screening test by cervical cytology the Romanian physician Aurel Babes was the true pioneer in the cytologic diagnosis of cervical cancer. Babes presented his findings to the Romanian Society of Gynaecology in Bucharest on 23 January 1927. His method of cancer diagnosis was published in a French medical journal, *Presse Médicale*, on 11 April 1928, but it is unlikely that Papanicolaou was aware of it. Furthermore Babes' method is radically different from Papanicolaou's method. Differences included the sampling method, the fixation and staining technique, and the interpretation of the results regarding cases of cervical cancer.

The cytological examination of the uterine cervix smears according to Papanicolaou was introduced in Italy in the early fifties by the efforts of Professor Mario Tortora in Naples and then in Ferrara

Dr Papanicolaou died on February 18, 1962 of heart failure and was buried in New Jersey. This great cytopathologist was honoured by USA in 1978 and by Greece in 1973 and 1978 by releasing commemorative stamps.

## PALEOGENOMIC AND ANCIENT DNA

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### Introduction

The DNA is a nucleic acid that contains the genetic information necessary for RNA and protein biosynthesis. DNA extracted from past samples (teeth, bones, faeces, etc.) is defined ancient DNA (aDNA) and needs, to preserve, low level of oxygen, fast decrease in water content and, above all, according to the Arrhenius equation, low temperature. From 1985 (first aDNA extracted from a mummy) with the introduction of the Polymerase Chain Reaction (PCR) several studies of paleogenomic were born, but it is essential to identify some possible errors such as fragmentations, contaminations and post-mortal mutations [1]. The applications of the paleogenomic are: evolutionary biology, population studies, studies of the pathogens and microorganism.

### Materials and methods

We report 3 cases studied by the Division of Paleopathology of Pisa:

a) Ferrante I, king of Naples (1431-1494). The natural mummy showed round white formations of the pelvis infiltrating the abdominal wall (fig. 1a-b).

b) Maria of Aragon (1503-1568). The well-preserved artificial mummy had a small pedunculate arborescence neof ormation in the right inguinal region (fig. 2a-b).

c) Andean female mummy (so-called 'Fi9') dated 10<sup>th</sup>-11<sup>th</sup> century A.D by radiocarbonium analyses. The natural young mummy presented a marked megavisceral syndrome characterized by megacolon, megaesophagus and cardiomegaly (fig. 3a-b).

It was possible to perform complete autopsies and collect tissue samples utilized for histological analyses and DNA extraction.

**Results**

a) Histology performed on the round formations confirmed the diagnosis of colorectal adenocarcinoma. Amplification of aDNA highlighted a point mutation of the codon 12 in K-Ras oncogene responsible for the cancer [2] (fig. 1c).

b) Macroscopic and histological aspects seemed peculiar of condyloma acuminatum, a papillomavirus-induced squamous lesion also called “venereal wart”. Molecular study revealed the presence of HPV 18, a virus with high oncogenic potential. Automated sequencing of several clones revealed 100% similarity sequences of both HPV 18 and JC9813 DNA, a putative novel HPV with low oncogenic potential [3] (fig. 2c)  
 c) Analysis of the gut microbiome (paleofeces, descending, transverse and ascending colon) underlined the massive presence of Clostridiceae. Sequences homologous to HPVs in the mummified gut (descending colon) was particularly surprising. It was detected also the *Tripanosoma cruzi*; by comparing a partial sequence homologous to the large ribosomal subunit alpha of the presumptive ancient *T. cruzi* with modern strains, we suggest that this pathogen may have a more remote origin than previously expected. We also found sequences associated with putative beta-lactamases, penicillin-binding proteins, resistance to fosfomycin, chloramphenicol, aminoglycosides, macrolides, sulfa, quinolones, tetracycline and vancomycin, and multi-drug transporters [4] (fig. 3c).

**Conclusion**

a) The alimentary “environment” of the Neapolitan court of the XV century, with its abundance of natural alimentary alkylating agents (red smoked meat), well explains the acquired mutation of K-Ras.

b) This represented the first molecular diagnosis of HPV in mummies. HPV is a very old virus that evolved together with man.

c) *Streptococcus*, *Staphylococcus*, *Bacillus* and *Pseudomonas* sequences were identified in the mummified gut, opening the opportunity to investigate possible mechanisms by which these bacteria are preserved. The detection of sequences homologous to those of pathogens such as *T. cruzi* and HPV indicate their presence in the Americas prior to European colonization. The presence of antibiotic-resistance genes in an 11th century pre-Columbian Andean mummy is intriguing as antibiotics were introduced recently. The presence of beta-lactam antibiotic resistance is certainly not unexpected in any culture, as would be in the case of resistance to any natural rather than a semi- or completely synthetic antibiotic as a result of exposure to natural antibiotic-producing microbiota originating from the environment (e.g. soil); however, vancomycin, particularly, was discovered more than 50 years ago, and vancomycin-resistance genes have been mainly implicated with the increased use of this antibiotic. The presence of antibiotic-resistance genes in the ancient human gut microbiome clearly indicates that these genes pre-date therapeutical use of these compounds and that they are not necessarily associated to a selective pressure of antibiotics use. Identification of pathogens and antibiotic-resistance genes in ancient human specimens will aid in the understanding of the evolution of pathogens as a way to treat and prevent diseases caused by bacteria, microbial eukaryotes and viruses.

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Figure 1a. The mummy of Ferrante I  
 B. White round formations  
 C. point mutation of the codon 12 in K-Ras oncogene

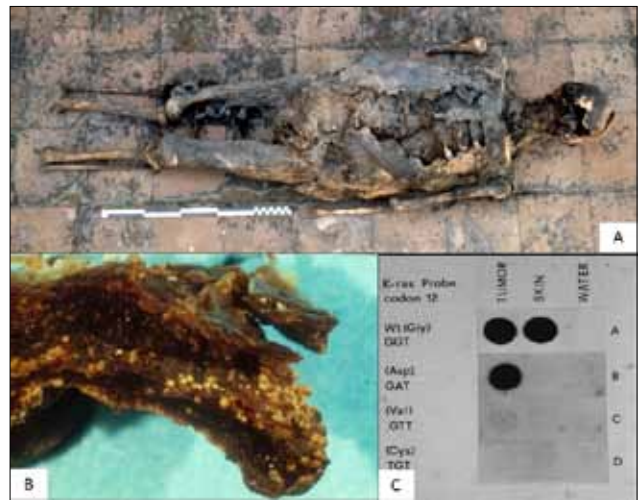


Figure 2 A. The mummy of Maria of Aragon  
 B. The pedunculate arborescence neoformation  
 C. Automated sequencing of several clones revealed 100% similarity sequences of HPV 18

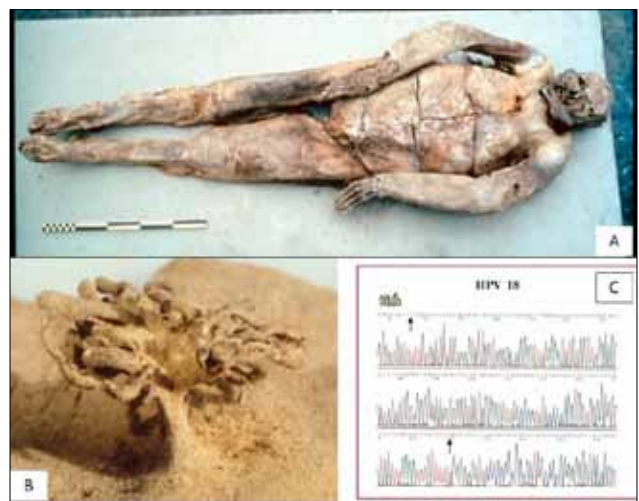
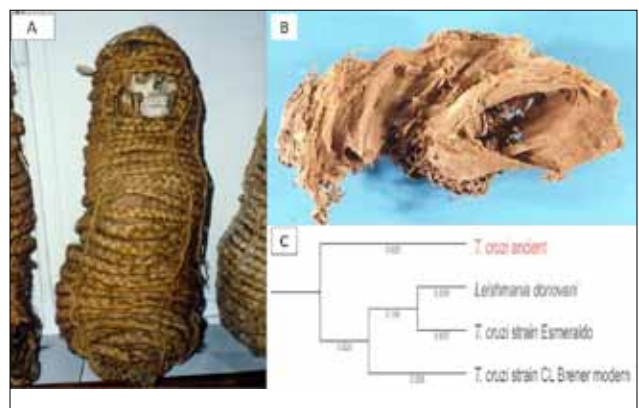


Figure 3 A. The mummy of Fi9  
 B. The megacolon  
 C. Phylogenetic reconstruction of large ribosomal subunit alpha from ancient T. cruzi and modern T. cruzi (CL Brener and Esmeraldo)





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## ADVANCED MORPHOLOGIC AND COMPOSITIONAL INVESTIGATIONS IN PALEOPATHOLOGY

L. Ventura

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### Introduction

Human remains can give us a huge amount of information about disease in past times, helping us to better understand contemporary pathological conditions. As a multidisciplinary science, paleopathology is widely based on modern investigation techniques and pathologists involved in the study of ancient diseases should be aware of the countless options available today. Beside the well-known morphology-based methods (such as anatomical dissection, histology, radiology and endoscopy) there is a growing number of analytical techniques that may be used to extract information from human remains (1). Among the different approaches employed, advanced morphologic and compositional methods play an important role in paleopathological investigation (2). The purpose of this speech is to illustrate the personal experience in the application of such methods through a series of practical examples (3-6), in order to outline a basic guideline for pathologists.

### Methods

Different ancient materials were studied using the following techniques: binocular stereomicroscopy (BSM), phase-contrast microscopy (PCM), scanning electron microscopy (SEM), also with energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD) analysis, and Fourier transform infrared (FT-IR) spectroscopy. The features of the samples and the information needed addressed the choice of the most suitable method from case to case.

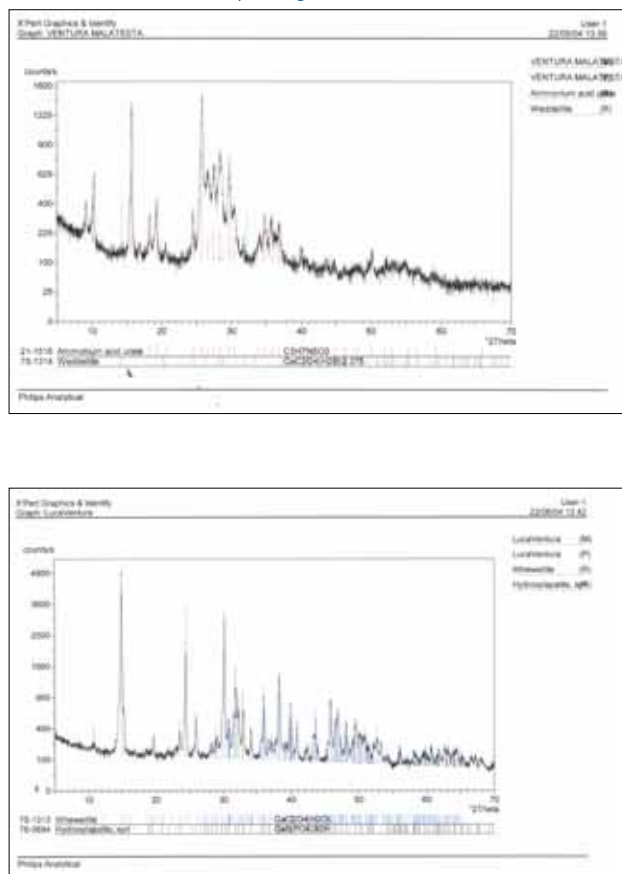
### Results

The renal stones found in the mummies of Pandolfo III Malatesta, Lord of Fano (1370-1427) and of an anonymous nobleman from Popoli (XVIII century) were investigated using BSM, SEM/EDX, and XRD (Fig. 1). Such methods enabled us to disclose the morphological details of the surface and the inner portions of the stones, along with their elemental and chemical compositions (ammonium acid urate and weddellite for Pandolfo; whewellite and hydroxylapatite for the nobleman from Popoli).

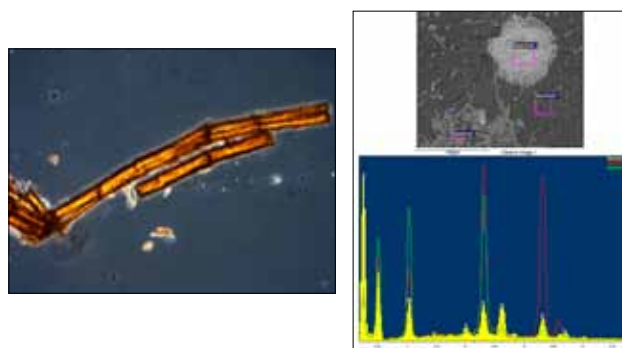
The content of four canopic jars from the Egyptian Museum of Florence, belonged to an anonymous individual of the New Kingdom (1550-1069 BC, XVIII-XX Dynasties), underwent investigation by BSM, PCM, and SEM/EDX (Fig. 2). Paraffin and methacrylate histology allowed to identify lungs with silico-anthraxis, and intestinal content with starch particles. One sample melted away after processing for methacrylate embedding leaving only entwined fibers related to the linen fabrics used to wrap the organs. Furthermore, chemical constituents of natron salts (sodium chloride, sulphate and carbonates) used during embalming were identified.

A XX century female mummy of 32-40 years from the church of San Michele Arcangelo in Sermoneta displayed all but one (the right fifth) clear, white fingernails. The fourth left nail

**Figure 1.** XRD analysis of the renal stones of Pandolfo (left) and the nobleman from Popoli (right).



**Figure 2.** Phase-contrast microscopy of linen fibres (left). Natron salts as demonstrated by SEM-EDX (right).



was carefully extracted from its bed and submitted to BSM, and SEM/EDX (Fig. 3), in order to establish chemical composition of the white substance. BSM allowed to appreciate differences between dorsal (polished) and ventral (unstained) surfaces, SEM evidenced nail root and free edge contours details. EDX measurements displayed O, S and Ca in the pigmented areas, suggesting the presence of calcium sulphate ( $\text{CaSO}_4$ ) used as a nail polish. Al, Fe and Si in the free edge of the nail, were referred to remnants from manicure devices. The partially skeletonized body of the Blessed Sante Brancorsini (1343-1394) contained multiple calcified nodules near the lumbar vertebrae. One of these nodules underwent BSM,