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Historical Article

The heart of Blessed Anne-Madeleine Remuzat: a biomedical approach of "miraculous" heart conservation

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ABSTRACT

We present here the results of our inter-disciplinary examination of the mummified heart of Blessed Anne-Madeleine Remuzat (1696–1730). This organ has been examined in the context of a canonization process. This analysis is related to important aspects of the early history of anatomy in Europe, that of "Holy autopsies", and to the relationship between anatomical investigations, Catholic theology, and religious/medical customs.
According to anatomical, genetic, toxicological, and palynological analyses, it has been shown that this organ has not been naturally ("miraculously") conserved but embalmed using myrtle, honey, and lime. Moreover, a right ventricle dilatation has been diagnosed, that may represent a post-tuberculosis condition and may have played a role in the cause of death of this religious figure.

1. Introduction

In the context of the canonization process deposed to the Vatican (Congregation for Saints Purposes), we have been asked by the Archbishopric of Marseille (France) to perform the global scientific examination of the mummified heart of the Blessed Anne-Madeleine Remuzat (AMR) kept in the basilica of Sacred Heart.

Born in Marseille on November 29, 1696, Anne-Madeleine Remuzat asked her parents to be allowed to enter the convent of the Visitation at only 9 years old: the request was granted. In 1708 she began to experience severe sufferings, which she bore patiently during her whole life. In 1709 her parents withdrew her, but in 1711 she re-entered the convent and in 1713, made her profession. At this time she devoted herself to prayer, and the *Spiritual Retreat* she wrote then is a proof of her progress in the contemplative life. On the 17th of October 1713, she experienced a «particular and extraordinary» revelation of Jesus «concerning the glory of his Sacred Heart». As her sanctity grew famous, Anne-Madeleine was consulted by many people, and was thus the mean of a spreading devotion to the Sacred Heart. Her influence convinced Mgr de Belsunce to establish the Association of Perpetual Adoration of the Sacred Heart in Marseille, an association of which she wrote the statutes. As Jansenism and a spirit of moral laxity had invaded the town by then, Anne-Madeleine suffered keenly, and in reparation inflicted on her body continual mortifications. When her superiors forbade these austerities, she begged God to mortify her himself and from that day she went into a

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painful decline. In 1720, during the plague in Marseille, God enjoined her to institute a feast in the honor of the Sacred Heart, which was established on the same year. She died on the 15th of February 1730. Since 1722, the veneration of the Sacred Heart had spread throughout the French Provence, Lyon, Rouen, Constantinople, Cairo, Spain, Louisiana, Persia, Syria, and the Indies by her endeavors. In 1888, her cause was submitted to the Sacred Congregation, whose favorable vote was given on the 18th of December 1890. Leo XIII signed on the 24th of December 1891, the introduction of the cause of the Venerable servant of God [1,2].

2. Material and methods

The reliquary was opened on December 15, 2011. Before any gross examination, just after its opening, five SPME (Solid Phase Micro-Extraction) captors have been introduced inside of the reliquary in close contact with the heart, and left for 15 minutes, then taken off and enclosed for further molecular analyses (Fig. 1).

The heart was then extracted from the reliquary, and supported macroscopic examination and further complementary analyses:

- A multi-detector row computed tomography (General Electric, VCT STD WSO, class 3–STR1 C CT Lightspeed VCT 64) without any iodinated contrast material, using the following parameters: detector configuration: 64×0,625 mm; slice thickness: 0.625 mm; tube voltage: 80 kV; automatic mAs; helicoidally snap shop in 0.4 sec of rotation; standard filter reconstruction.
- A radiocarbon dating carried out on a sample of aorta (analysis by AMS in Beta Analytic Inc., Miami, FL, USA).
- The granular substance from the inner part of the heart was submitted to a microscope examination. The methodology employed for the sampling and preparation was the exact same one that of putrefaction fluid deposits [3], and dental calculus [4]: immersion in a solution of 10% diluted acetic acid, and 10% diluted formaldehyde for 48 h, sampling of 200 µl from the supernatant, and centrifugation (800 turns per minute for 10 min) in order to obtain two spots per slide. Four slides were produced, all were colored by the technique of hematoxylin–eosin–saffron after fixing of the spots to the air.
- An elemental analysis was performed on a small sample from the heart's filling powder (2 mg). Techniques used were inductively coupled plasma mass spectrometry (ICP-MS) (Elan DRCe quadrupole spectrometer, Perkin Elmer, Les Ulis, France) and inductively coupled plasma atomic emission spectrometry (ICP-OES) (JY 24, Horiba Jobin Yvon, Longjumeau, France). For both techniques, samples were first mineralized in hot concentrated nitric acid (Nitric acid 65% Suprapur, VWR, Fontenay-sous-Bois, France) and completed with ultra pure water (MilliQ, Millipore, Molsheim, France) to obtain a final volume of 0.5 ml (500 µl). In order to detect elements of interest, a fast semi-quantitative analysis of all elements of the periodic table with the ICP-MS TotalQuant method was first effectuated. Nine elements were hereafter quantitatively measured: Pb, Sn, Sb, Cu, Bi, Hg by ICP-MS, and Fe and Ca by ICP-OES.
- Five solid-phase microextraction (SPME) fibers were analyzed by gas chromatography/mass spectrometry (GC/MS), using an adapted purge and trap technique [detail of equipment: gas chromatograph Agilent 6890 fitted with the mass spectrometer 5973; injector Gerstel Combipal with the CIS4 injector and the Alex (automated liner exchanger)]. All SPME fibers were introduced in the liner. The liner was automatically placed in the injector for analysis. The volatile materials of the sample were desorbed at 300°C on the GC column kept at low temperature.
- About 5 mg of mummified tissue were extracted and dedicated to the ancient DNA facilities at the Institute of Evolutionary Biology in Barcelona. First, a proteinase-K (100 mg/mL), Tris (50 mM) and 10% SDS lysis solution was applied overnight at 50 °C with agitation. The resulting supernatant was extracted in three consecutive steps of phenol, phenol-chloroform and chloroform-isoamilic alcohol, and the organic phase was subsequently concentrated with Amicon Ultra centrifugal filters (Millipore). Finally, the extract was purified with a silica-extraction kit (Fermentas) to remove potential polymerase chain reaction (PCR) inhibitors and eluted to a 30 µl volume. The hypervariable region 1 of the mitochondrial genome (mtDNA) was amplified by PCR in two overlapping fragments, with the primer couples L16,055-H16,218 and L16,185-H16,378. The amplification was based on a two-step PCR protocol designed to efficiently generate template in the first step with limiting primers [5]. PCR products of the expected size were cut from a 2% low-melting point agarose gel, purified with a silica-binding method and cloned into bacteria using TOPO-TA cloning kit (Invitrogen). Inserts with the right size were sequenced in a ABI3730 capillary sequencer (Applied Biosystems).
- Few grams of the embalmed heart were processed using KOH 5% without heating in order to lighten vegetal tissues and concentrate pollen grains. After centrifugations and rinsing, 50 µl of residue were deposed between a slide and cover glass. One slide has been completely examined at light microscope (×25). Each pollen grain has been observed at ×1000 magnification for the detailed examination of its morphology and its identification. Identification of pollen grains mainly refers to Reille [6–8]. Information on plant systematic and flowering refers to Coste [9].

Fig. 1. General view of the mummified heart within the reliquary, with the SPME captors in situ.

3. Results

3.1. Macroscopic examination

The heart weights 55 g and measures 8.2 cm of full vertical diameter (anatomical vertical diameter, from the basis of the aorta to the apex, being 6.2 cm), and 5.4 cm of maximal horizontal diameter (just below the basis of the aorta) (Fig. 2). It is of human morphology and constitutes a complete heart accompanied by a fragment of thoracic aorta of 1.2 cm long and 2.4 cm of circumference in anatomical continuity, and flattened. The heart is entirely mummified. The inter-ventricular artery is clearly visible, characterized by a very tortuous aspect. Diffuse clear brown granular matters are present on the surface of the organ.

Two ancient sutured openings are clearly visible: one (A) of 7.5 cm long, placed at the junction of the left and right ventricles, on the anterior face of the heart, oriented to the right side, going through the apex, then ending on the posterior side of the inter-ventricular groove, at mid-height, sutured by a large wire (Fig. 3). A second one

Fig. 2. General view of the heart (anterior part).

(B) measuring 7 cm of full length, on the left ventricle, beginning on its lateral side threw the aorta and the pulmonary artery, ending at the apex.

Finally, no atheromatous lesion was macroscopically seen on the aorta section.

3.2. Radiological analysis

Computed tomographic (CT) scan sections showed an incomplete filling of all four cavities by particles of low density comparatively to the cardiac walls (100–120 HU to 220–390 HU) (Fig. 4a). No calcification was found within this filling, or in the cardiac walls, remains of valves, and on the trajectory of coronaries. The left ventricle appeared very dilated with a grossly circular section of 24.6 mm of diameter. Comparatively, the right ventricle section has a grossly oval shape (transversal axis of 25.2 cm, anterior–posterior axis of 30.2 cm) (Fig. 4b). Ventricle walls have the following

Fig. 3. Detail of the post-embalming sewing of the ventricles.

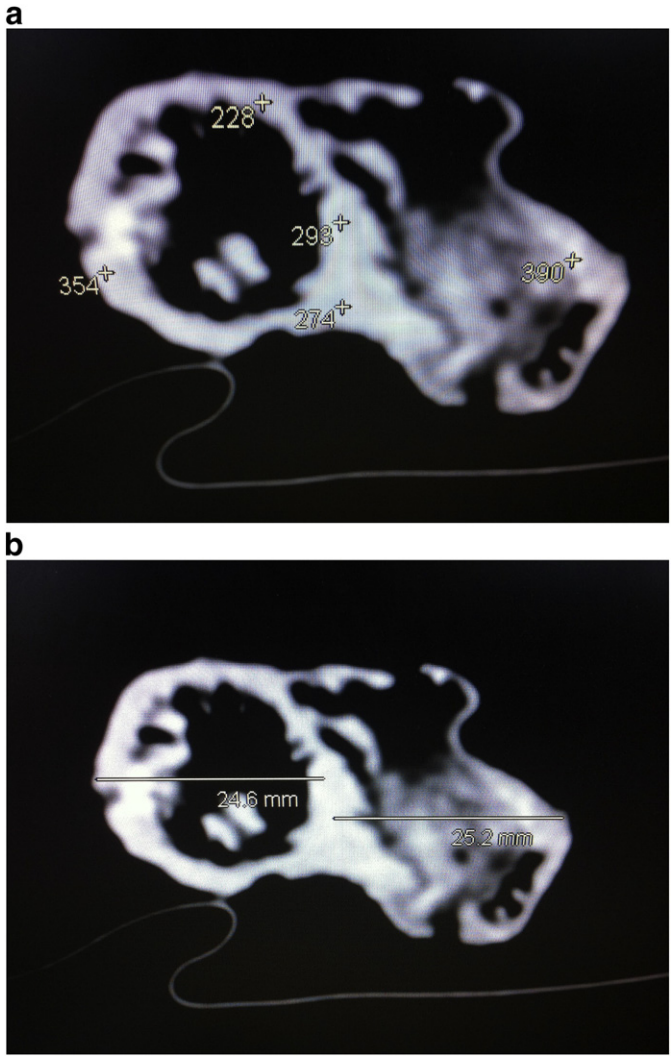

Fig. 4. CT scan horizontal section of the heart (mid-ventricular level) showing the internal filling (a) and the right ventricle dilatation (b).

thicknesses: anterior part of the left ventricle 0.4 mm, inter-ventricle septum 0.6 mm, right ventricle on its lateral part 0.2 mm. Such measures, even on a mummified material with artificial filling of internal cavities, are strongly evocative of a right ventricle dilatation, of unknown origin. No anomaly was seen at the level of the heart's major blood vessels, and heart valves.

3.3. Carbon dating

Results of radiocarbon dating with 2-sigma calibration yielded a calibrated date between A.D. 1660 and present days, nicely bracketing the year of death of the Blessed Anne-Madeleine Remuzat (1730).

3.4. Microscopic analysis

All samples showed the absence of any human cell (no red blood cells, no leucocytes) but the presence of numerous vegetal fibers and mineral formations (Fig. 5a and b)

3.5. Molecular analysis

All samples were poorly charged but some were characterized by the presence of methoxy-phenyl-oxime, a component of honey that may have been used during the embalming process (Table 1).

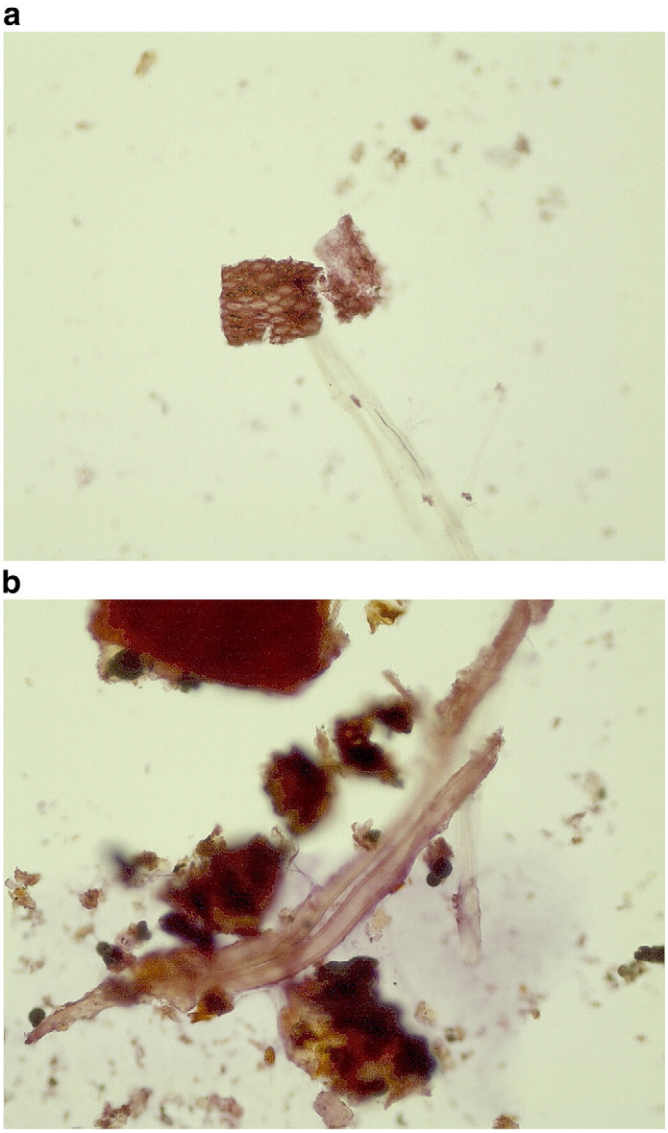

Fig. 5. Microscopic aspect of the internal filling with numerous vegetal fibers (a), and mineral formations (b).

3.6. Elemental analysis

Results of the various elemental analyses are given in the Table 2. They showed the presence of a majority of calcium (possibly from lime, according to the specimen and context), mixed with limited quantities of mead, copper and iron. Traces of tin and antimony have been highlighted, and traces of mercury.

Table 1
List of all molecules detected with GC/MS (cervical skin)

Benzenic hydrocarbure	Toluene
	Xylene
	Ethyl-benzene
Other	Methoxy-phenyl-oxime
	Trimethoxy-indanone
	Benzaldehyde
	Limonene
	Hexane-dioic acid bis 2-ethyl-hexyl-ester

Table 2
Results of elemental analyses (concentrations are given in μg/g for the white powder and in μg/L of mineralization for the white crystals and the green formation)

	Pb	Sn	Sb	Cu	Bi	Hg	Fe	Ca
Powder	1 617	33	5	529	5	4	622	31 033
White crystals	115	4	2	312	1	2	-	4 680
Green formation	3	1	2	1 159	1	2	-	371

3.7. Genetic analysis

A DNA comparison was carried out between the mummified heart and hairs said to come from the Blessed Anne-Madeleine Remuzat held by the church of Saint-Pierre d'Auriol (France) (Fig. 6a and b).

The female sex of the mummified tissue (heart) was genetically confirmed. Several mtDNA clone sequences were generated for the mummified tissue. A consensus haplotype was obtained (16126C-16294 T-16296 T-16324C) that corresponds to a T2* haplogroup. This haplotype has 31 matches in a in-house database of 22,807 mtDNA sequences (data compiled by F. Calafell, Institute of Evolutionary

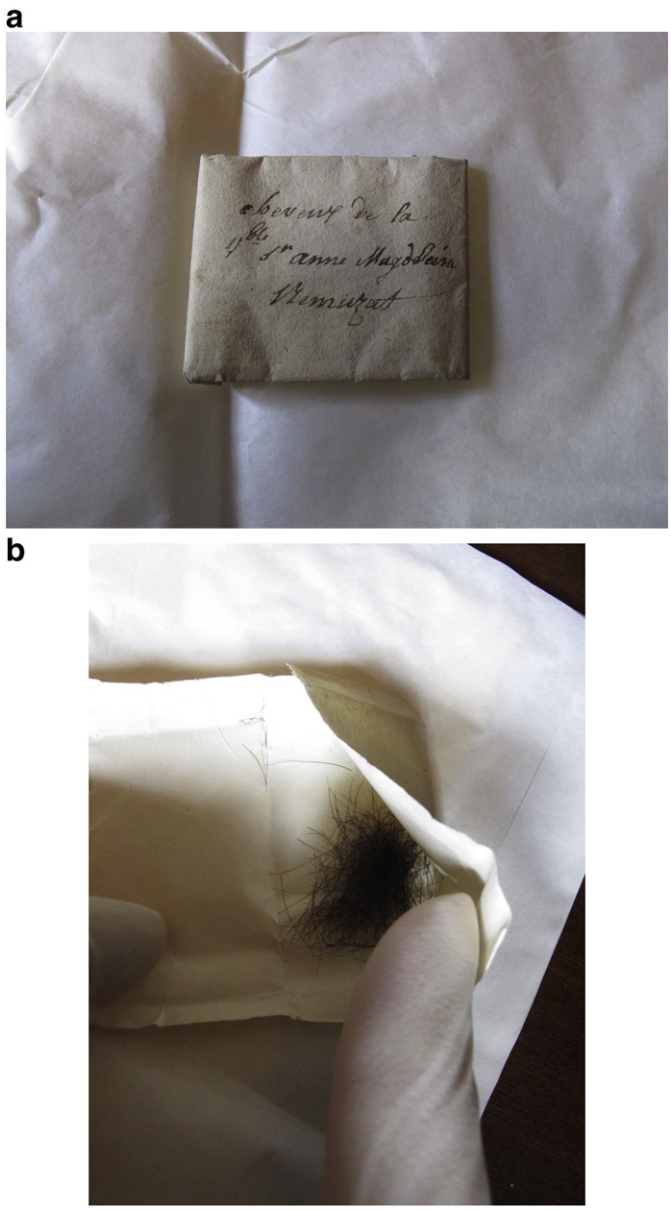

Fig. 6. Inscribed envelop (a) containing the so-called hairs (b) of Anne-Madeleine Remuzat.

Biology, Barcelona). The hairs yielded no mtDNA, so no comparison was possible between the samples.

3.8. Pollen examination

Various taxa have been recorded while counting more than 250 pollen grains (Fig. 7):

- *Pinus*, Pinaceae (3 well-preserved specimens). *Pinus* species cannot be identified using pollen grains. Four species are currently living in the Southern France, *Pinus pinea*, *P. laricio*, *P. halepensis*, and *P. pinaster*, the pollination of which occurs from April to June.
- *Cedrus*, Pinaceae (1 well-preserved specimen). This pollen, according to its morphology (large size with a marginal crest [10]), probably belongs to the species *C. libani* (cedar of Lebanon), already known in France before its extensive reintroduction in 1862 [11], blooming from July to August.
- *Quercus sp.* (deciduous oak), Fagaceae (1 well-preserved specimen). Morphological characters allow identifying this pollen grain as deciduous oaks, represented in the region by *Quercus pubescens* (white oak), which blooms from April to May.
- *Artemisia* genus, Asteraceae (3 well-preserved specimens). Five of the 18 species living in France are found in the Provence region: *Artemisia absinthium* (blooming from July to September), *A. arborescens* (blooming from June to July), *A. comphorata* (blooming from September to October), *A. vulgaris* (blooming from July to September), *A. campestris* (blooming in August and September), *A. glutinosa* (blooming in September and October), and *A. gallica* (blooming in September and October). Since no observation was possible with the scanning electronic microscope, the recorded pollens could not be identified.
- Asteraceae and asteroideae (two well-preserved specimens). These pollen grains belong to this subfamily, as showed by the structured spines and the absence of lacunae. The small size

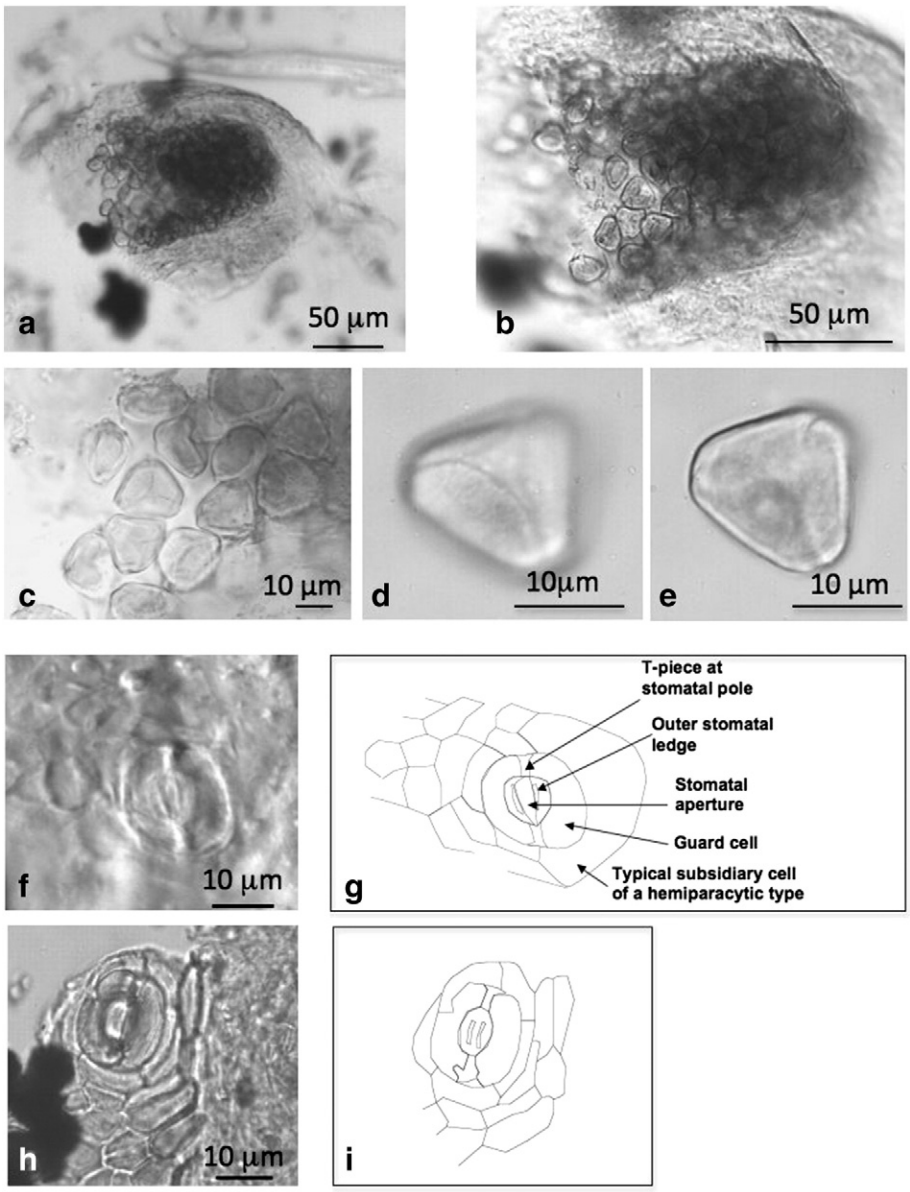

Fig. 7. Stamen of *Myrtus communis* full of pollen grains (a and b); Focus on some of these pollen grains within the stamen (c); Isolated pollen grain of *Myrtus communis* (d and e); Hemiparacytic stomata of *Myrtus communis* (f); Hypothetic hemiparacytic stomata with the right cell adjacent to the guard cell enclosing it (the other four normal epidermal subsidiary cells surrounding the left guard cell) (g); Anomocytic stomata of *Myrtus communis* (h); Hypothetic anomocytic stomata with five epidermal subsidiary cells enclosing the two guard cells (i).

(<20 μm) allows referring to the group of Anthemidae where the group of chrysanthemums seems in good agreement with such pollen morphology. One of these pollen grains is similar to that of the species *Leucanthemum vulgare* (daisy). This species is widely distributed in France and blooms from May to August.

- *Phlomis*, Lamiaceae (1 well-preserved specimen). This is a typical pollen of Lamiaceae (with a supratectate perforated reticulum) showing only three colpi, characteristic of *Phlomis*[12]. Three species are living in southern France (*P. fruticosa*, *P. lychnitis*, *P. herba-venti*), blooming from May to July. Without any observation at the scanning electronic microscope, it is difficult to distinguish between the three Mediterranean species.
- Rosaceae (1 well-preserved specimen). This tricolporate and smoothly striate pollen shows morphological characters of this highly diversified family, which includes a lot of fruit (cherry, almond, peach, plum) trees and roses. It is impossible to identify pollen of this family at the genus level. Blooming period within the family ranges from February to July.

Considering the large dissemination of pollen of these taxa and their blooming period, their rare occurrence in the heart could be caused by an airborne contamination during the embalming process.

The most frequent pollen grain (estimated more than 300 isolated specimens in the studied slide), often grouped in clusters of 2 up to 20 even more specimens, belongs to *Myrtus communis* (Myrtaceae), i.e., the sweet-smelling myrtle endemic of the Mediterranean region and frequent on the Provencal coastline (blooming period: May to July), according to its syncolpate characteristic small pollen grain (diameter: 15 μm). Richness in a monospecific pollen and occurrence of frequent residues of vegetal tissues including cuticles, stomata and stamen tissues suggest that flowers (at least stamens) and pieces of plants have been introduced into the heart. This assumption has been confirmed by the discovery of a large fragment of stamen full of pollen grains.

Though *Myrtus communis* and *Eucalyptus* display almost the same pollen, both belonging to the Myrtaceae genus, the risk of confusion can be discarded because this Australian Eucalyptus genus was introduced in France in the latest 19th century and maintained restricted to some places in the French Riviera up to about the mid 20th century [13], except if some herbalists already possessed this plant with flowers in 1730.

Leaf cuticle anatomical data, especially stomata, support morphological evidence for separating taxa of Myrtaceae family at the generic level [14]. Few stomata could be observed here and could be attributed to two different stomata morphotypes: anomocytic type (which is the current type of Myrtaceae genera) and a hemiparacytic one which is especially observed in *Myrtus communis* associated also with anomocytic and anisocytic type [15]. The occurrence of cuticles with hemiparacytic and anomocytic morphotypes, support the hypothesis of the use of *Myrtus communis* (sweet-smelling myrtle) by embalmers.

4. Discussion

All the analyses showed that the heart of the Blessed Anne-Madeleine Remuzat has been mummified artificially (i.e. no "conservative miracle"). An anatomical opening, by two longitudinal incisions, in order to extract post-mortem intra-cavity thrombus, was followed by a filling of all four cavities with honey, odoriferous plants and mineral substances (mainly lime mixed with copper, but no mercury). Traces of tin, antimony and mercury are to be interpreted as impurities originating from the contamination by lead (corresponding to a previous reliquary? or to an adjunction to lime?). The heart was then definitively sewn.

Other mummified hearts of historical origin have already been described in the medical literature. The heart of Richard the Lionheart (died 1199) showed an embalming process where mercury, lime, plants (daisy, mint, myrtle), creosote, and incense played a role [16].

The heart of Santa Rosa (Viterbo, close to Rome, Italy, 13th c. AD) has been removed from its mummified body, without any further preparation, lacking the great arteries, systemic/pulmonary veins, and the posterior wall of the atria. A diagnostic of cardiac embolism as a complication of left ventricular diverticulum has been proposed [17] in a context of sternum agenesis and Cantrell's syndrome [18]. But due to the complete absence of post-mortem opening and preparation of the organ, a differential diagnosis of post-mortem intra-cavity thrombus seems much more probable [19].

"Holy autopsies" are currently known from the early 14th c. AD in Italy, performed under ecclesiastic authorities, consisting of "the practice of inspecting the internal organs of a holy person shortly after death for corporeal signs of sanctity" that might be invoked as evidence for further beatification or canonization [20]. In 1308, the cadaver of Chiara of Montefalco (Umbria), a Franciscan abbess, was opened by the nuns from her community (without the presence of any physician), who found a small crucifix and instrument of the Passion into her heart (she indeed claimed to have Jesus in her heart); we ignore the following preparation of the organ. In 1320, the public autopsy of the Blessed Margarita of Castello was carried out in a church. Three stones engraved with images of the Holy Family were found in the heart of this virgin… (though the autopsy was performed by two surgeons and three physicians, in front of many ecclesiastics).

The goal of such openings was first to see, then to restore the body for a further presentation to pilgrims and religious communities.

The hearts of Louis XIII (died in 1643) and Louis XIV (died in 1715), both conserved in reliquaries deposed in the crypt of the basilica of Saint-Denis (close to Paris, France) have never been studied yet. Preliminary observation showed a comparable opening of all four cavities for these two hearts (with a complementary protection by textiles and covered by copper granules for Louis XIII's one). The heart of Louis XVII (died in 1795), now deposed in the crypt of the basilica of Saint-Denis, has been genetically identified [21]. It sustained no anatomical preparation, having been dehydrated in alcohol (eau-de-vie) during several years by the practitioner, doctor Pelletan [22].

Concerning the pathological background of Anne-Madeleine Remuzat, the diagnosed right ventricle dilatation may represent a post-tuberculosis condition, as classically described in the medical literature [23]. Other causes of the disease cannot be excluded (Table 3), and genetic ones could be highlighted by further DNA analyses [24].

Anyway, tuberculosis may have played a role in the mechanism and cause of death. Indeed, witnesses describe the last moments of the individual as follows: presence of stigmata (with subsequent anemia) accompanied by ecstasy since 5 years (1725), palpitation (considered as "twinges of divine love") and, for the last weeks of life (from January 1730), extreme exhaustion, hemoptysis, cold, strong thoracic oppression, then death [25]. Such clinical signs, even if nonspecific, may be considered as a final decompensation of a right heart anomaly, being either an acquired or congenital form [26–28].

Table 3
Potential causes of right ventricle dilatation compatible with the 18th century

Toxicological origin	Alcohol
Metabolic origin	Thyroid disease
	Pregnancy
Infectious origin	Coxsackie B virus
	Other enteroviruses
Post-myocardial infarction	
Auto-immune origin	
Genetic origin	
Idiopathic	

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