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## **Diatoms on the hair of Holy Mary-Magdalene relics**

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### Author contributions

PC initiated and coordinated the project, and collected the samples in the cave. RW performed the HIM analyses. SM and FS analysed the diatom and environmental samples. SM and FS wrote the manuscript and RW and PC commented on the final version of the manuscript.

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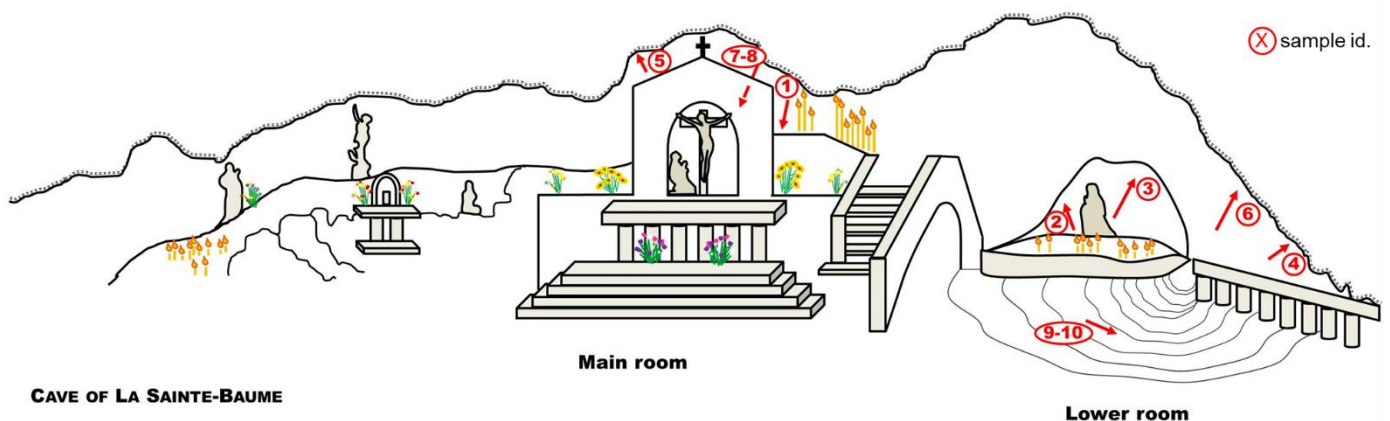
# Diatoms on the hair of Holy Mary-Magdalene relics

## Abstract

Remains of corroded diatoms were found on one strand of hair from the relics of Holy Mary-Magdalene. It was initially hypothesized that they could be tracers of her living environment, the cave of La Sainte-Baume reputed to be the place of her thirty-year retreat before she died. To validate this assumption, ten samples collected in the cave of La Sainte-Baume from moist parietal surfaces and pool waters were investigated. Some biological remnants were found in the samples. No evidence of the presence of the diatom species from the hair in the cave's environment led to reject this hypothesis.

The highly corroded status of the hair diatoms, their likely paleo-origin together with their association with mineral and organic material, rather argue for cosmetic use of diatom-rich material, such as cleaning clays traditionally used in the Mediterranean region and Africa for hair care.

Keywords: diatoms, Mary-Magdalene relics, paleochristian hair, La Sainte-Baume, cave, forensic anthropology, rhassoul.



## **1. Introduction**

Diatoms are microscopic, unicellular, brown algae that can be found in nearly all types of moist environments (waters, soils, air). Their siliceous cell walls make their remains persistent in time, characteristic that favoured their use in paleolimnological or archaeological applications. However, to our knowledge, diatoms have never been studied in relics. During the forensic examination of the relics of Holy Mary-Magdalene, remains of diatoms were found in the hair conserved with the skull attributed to the saint in the crypt of the basilica of Saint-Maximin, La Sainte-Baume (Provence, France). The context of this examination has been published with a summary of the legendary life of the saint and the history of her relics (Charlier et al. 2019; Charlier et al. 2017). Briefly, after being banned from the Holy Land and persecuted, Mary-Magdalene is said to have crossed the Mediterranean Sea to Provence (South-East France) and have retired to a cave on a hill, named La Sainte-Baume, where she lived for thirty years until she died.

The global aim of this study was to determine whether the diatoms found in Holy Mary-Magdalene's hair resulted from contamination by her environment, when the saint was living as a hermit in the cave of La Sainte-Baume. More specific objectives were: i) to illustrate all remains of diatoms found in the hair of Holy Mary-Magdalene, ii) to analyse the diatom content in several surfaces from La Sainte-Baume cave (wall seepage, water or sediments), and iii) to discuss the plausibility of the environmental origin of the diatoms present on Holy Mary-Magdalene's hair.

## **2. Sample collection and analysis**

### ***2.1. Hair diatoms***

One strand of hair from the scalp of Holy Mary-Magdalene was examined under a

helium ion microscope (HIM, Zeiss Orion NanoFab; see details in Charlier et al. 2017), which is a new technique maintaining the integrity of samples. The hair being of historic and religious value, it had to be returned to the relics without any deterioration, reason why no cleaning or gold coating was performed prior to high-resolution microscopy observations and microphotographs acquisition. Pictures of the diatoms found on the hair were taken at magnifications up to  $\times 50\,000$ , to catch relevant morphological characteristics for species identification.

## ***2.2. Cave surfaces and waters***

In the cave of La Sainte Baume (South Eastern France,  $43^{\circ} 19' 38''$  N,  $5^{\circ} 45' 53''$  E), 10 samples of wall seepage, moist mosses and lichens, clay, saltpetre, and water from small pools were sampled in January 2017. They were collected in duplicates by means of a swab for solid surfaces or direct water sampling (40 mL) in the pools (Table 1, also see Graphical abstract) and preserved with formalin. The replication of sample collection allowed for microscopy analysis based on two complementary protocols performed by two independent operators, in order to describe diatom content as well as other microorganisms present or relevant complementary descriptors.

After homogenisation of the first replicates of sample suspensions and concentration of the material in sedimentation chambers when necessary, 125  $\mu$ L of the pellet were pipetted onto a Nageotte counting chamber for enumeration under a light microscope (Olympus BX51) at a  $\times 400$  magnification. In parallel, the other duplicate samples were treated with hydrochloric acid to remove carbonates, then analysed under light microscopy (Leitz Dialux) at a  $\times 1000$  magnification.

Microscopic mineral and organic remains were identified with the catalogues of Liebmann (1962, p. 530-531) and Straub (2016, p. 21-32).

### 3. Results

#### *3.1. Microscopy analyses reveal one single diatom species on Mary-Magdalene's hair*

Diatoms adhering on the hair near mineral or organic dust were found (Figure 1A, arrows), but all remains were highly corroded and mostly fragmented. A total of 9 different valves were observed, considering both external and internal views (e.g. Figure 1B). However, no intact frustule was found in the sample. At high magnification, the fine structures (Figure 2A) of the frustules were partially hidden by a top layer of mineral or organic matters. Careful comparisons between specimens highlighted that the remains all belonged to one unique centric species, with 15-18  $\mu\text{m}$  diameter.

Valve ornamentation consisted of two parts. The central area was large and flat, and ornamented with scattered puncta (Figure 2B). The obstruction of the structures as visible on the external view (likely areolae) impeded to state whether some of them were openings of fultoportulae. Alveolar ribs with small intern simple openings (without "Schattenlinien", Figure 3A) were found on the cell margins. These chambers were also visible on external views because of the corrosion of the valves. The field of areolae appeared to be surrounded by a sort of hyaline ring, externally delimited by the alveolar structures (Figure 2B, arrows).

Traces of marginal fultoportulae were visible on internal views (Figures 3A and 1B), occurring beneath every costa, with two perceptible satellite pori. No rimoportula was observed, probably as the consequence of frustule corrosion and fragmentation. Some microphotographs showed radiate areolated flat fields (Figure 3B) or granulated elevations (Figure 1B) that were only visible on the internal view.

### ***3.2. Putative identification of the diatom***

Several morphological features of these valves were in agreement with the description of the fossil diatom *Tertiarius transilvanicus* var. *disseminatopunctatus* (Pantocsek) Håkansson & Khursevich: size, small marginal chambers with simple internal openings, scattered areolae on the central area, hyaline ring between the areolate surface and the marginal chambers, one fultoportula on the mantel in front of each costa. However, some typical structures of this species were not visible: the marginal rimoportula (expected to be located on one costa) and the central ring of fultoportulae (surrounding the areolae surface). One criterion also echoed the description of *Lindavia antiqua* (W. Smith) Nakov et al.: a central star-shaped flat field of areolae, structure which is only known in this species to our knowledge. However, this species usually exhibits a concentrically undulate valve with complex internal openings of the marginal chambers (“Schattenlinien”); these features were not visible on our specimens.

Taphonomic issues and the high degree of corrosion of the diatoms complicate its unequivocal identification. The presence of weird structures (the radial granulate elevations visible in the internal views of one valve; Figure 1B) neither known in *Tertiarius* nor in *Cyclotella*, *Stephanodiscus* or *Cyclostephanos*, even make this identification harder.

### ***3.3. Diatom content in the samples from the cave is incidental***

The samples collected in the cave were dominated by mineral fragments from the rock walls (calcite, silicates and iron oxides)(Table 1). Even after concentration of the material, no convincing evidence of biological content was found (except for Sample 2, see below). In the samples collected on solid surfaces, most organic remains were related to human activity such as the presence of paper/textile fibres (even coloured and synthetic ones) and 10 to 50 µm soot particles from candles or oil lamps.



Some zooclasts, phytoclasts or other particles arising from soils were also found, e.g. phytoliths, fragments of dead leaves, crystals of anhydrite and gypsum. Occasionally, significant amounts of mycelium and bacteria were noted, providing evidence of *in situ* organic matter decomposition activity. In Sample 2, other biological remnants were found (suggesting that birds and mammals passed through the cave) and an intense development of coccoid bacteria was observed.

On the other hand, in the water samples, suspended matter content was very poor. Regarding diatoms, only three very tiny cells were found (length below 5  $\mu\text{m}$ ), in two samples taken from the cave walls (mosses, saltpetre; Samples 5 and 6). Those likely autochthonous pennate diatoms were, however, not identifiable to the genus level at  $\times 400$  magnification. No big centric diatom as seen on the hair of the relics was found.

## **4. Discussion**

### ***4.1. Current knowledge on the distribution of the diatom species found on Holy Mary-Magdalene's hair***

Most features of the valves observed on the hair of Holy Mary-Magdalene advocate for identification as *Tertiarius transilvanicus* var. *disseminatopunctatus* (Håkansson and Khursevich 1997; Houk, Klee, and Tanaka 2010) or as *Lindavia antiqua* (Houk, Klee, and Tanaka 2010): e.g. size, small marginal chambers with simple internal openings (Figure 1B), central star-shaped flat field of areolae (Figures 1B and 3B), hyaline ring between the areolate surface and the marginal chambers (Figure 2B), traces of fultoportulae (Figure 3A). The genera *Tertiarius* and *Lindavia* are morphologically closely related (Nakov et al. 2015) and the damages on the valves did not allow to catch the morphological features allowing to separate one genus from the other. Some concerns arose due to unusual radial granulate elevations observed in some internal views. These features could have been caused by the fossilisation of the valves

(diagenesis); otherwise they can also constitute real structures from an unknown diatom species. To support this second hypothesis, more in-depth investigations of Holy Mary-Magdalene's hair (increasing numbers of strands, and careful cleaning of the diatoms from organic and mineral particles) would be needed.

*Tertiarius transilvanicus* var. *disseminatopunctatus* was initially described as a fossil lacustrine species, found in tertiary deposits from Hungary (Pantocsek 1905). It belongs to an extinct genus in Thalassiosirales (Alverson, Jansen, and Theriot 2007) which had a widespread planktonic distribution before the Pleistocene's glacial period (e.g. Karabanov et al. 2004; Israde-Alcántara et al. 2010). *Lindavia antiqua* was reported as a littoral species in lakes from several locations of the Northern Hemisphere (Houk, Klee, and Tanaka 2010). It was found in several fossil core samples around the area of the cave, including in the district of Saint-Maximin-La Sainte-Baume ([https://inpn.mnhn.fr/espece/cd\\_nom/75522/tab/archeo/dept/83](https://inpn.mnhn.fr/espece/cd_nom/75522/tab/archeo/dept/83)). According to Krammer and Lange-Bertalot (1991), this species would have a contemporary nordic-alpine distribution and could be found especially on mosses. In central Europe however, it is more typical of the Late-Glacial period (Marciniak 1981).

#### ***4.2. Putative origin of these diatom specimens***

Very little organic material was found in the samples collected in the cave, most of them suggesting human contamination, probably linked to the pilgrim activity inside the sanctuary. The fact that no planktonic diatoms were found in the water column of the pools was not surprising given the volume of water collected; more algal material would have been likely collected by scraping the bottom of those basins. Only three specimens of minute diatoms were observed by chance in the seep, moist parietal samples. In their review of cave diatoms, Falasco et al. (2014) pointed out that diatom taxa in

subterranean environments can be of smaller sizes than freshwater forms. However, no (big) centric diatom was recovered during the cave's sampling, arguing for the rejection of hair contamination during the stay of Holy Mary-Magdalene in the cave. Moreover, to our knowledge, diatoms have never been described in Jurassic limestones, suggesting that the diatoms came from elsewhere than from the cave's rock.

This is in agreement with the paleo-origin suggested by several taxonomical criteria, in particular: a highly specialized assemblage exclusively composed of one planktonic species, no intact (closed) frustule found. Moreover, the high degree of corrosion and fragmentation of the specimens, together with their mineral and organic coating obstructing areolae, argue for a fossil assemblage included in diatom-rich clay or diatomite. Diatomite, also called diatomaceous earth, is a geological deposit of fossilised fragmented diatoms, with several mechanical properties (Korunic 1998). Notably, the abrasiveness of diatom dust promoted its use as a pest-controlling agent (Quarles and Winn 1996). It is a high-efficiency natural insecticide against undesirable insects and mites (Dunn et al. 2016; Collins and Cook 2006) used to prevent infestation of livestock and pets, but also to control head lice in humans (Bessette 2005). In North-Africa and around the Mediterranean basin, silica-rich clays known under the name of "rhassoul" or "ghassoul" (Tertiary lacustrine deposits: Chahi et al. 1997; Tokarský 2018) are used since ancient times as soap and shampoo (El Fadeli et al. 2010; Faustini et al. 2018). In this type of clay, the silicates may arise from the dissolution of diatoms (Chahi et al. 1997; Robert, Gauthier, and Chamley 1984). A plausible hypothesis to explain the presence of diatoms on Holy Mary-Magdalene's hair would be this kind of cosmetic use of clay, marl or diatomite, to clean and care her hair. To validate this, an intensive investigation of clays and marls used for haircare would be necessary.

## 5. Acknowledgements

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## 6. Declaration of conflicting interests

The authors declare no conflict of interest related to this research.

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Table 1. List of the environmental samples collected in the cave, collection mode and main observation results. Information in bold highlight high amounts of material observed in the samples.

Sample ID°	Location of sampling	Collection mode	Main microscopy observations	
			Minerals	Organic material
1	Stalagmites behind the main altar, main room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Colourless sclerites, colourless and coloured cellulose fibres, soot particles <b>Edaphic tracers:</b> Phytoliths, one thecamoebian test ( <i>Diffflugia</i> sp.) <b>Environmental impacts:</b> <i>Quercus</i> sp. pollen <b>In situ decomposition evidence:</b> Unbranched mycelium
2	Clay behind Mary-Magdalene statue, lower room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Colourless and coloured sclerites and cellulose fibres, soot particles <b>Environmental impacts:</b> Bird feather barbules, mammal (non-human) hair <b>In situ decomposition evidence:</b> Colony of coccoid bacteria
3	Reddish rocks behind Mary-Magdalene statue, lower room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Colourless sclerites <b>Edaphic tracers:</b> Phytoliths <b>In situ decomposition evidence:</b> Unbranched mycelium
4	Lichens, lower room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Colourless sclerites, soot particles <b>Edaphic tracers:</b> Phytoliths, anhydrite
5	Mosses behind the main altar, main room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Colourless sclerites <b>Edaphic tracers:</b> Anhydrite, gypsum <b>Environmental impacts:</b> One minute frustule of pennate diatom
6	Saltpetre, lower room	Swab rubbing	<i>Calcite associated with iron oxides</i>	<b>Remains of human activity:</b> Coloured cellulose fibres, soot particles <b>Edaphic tracers:</b> Phytoliths, dead leaves fragments, amorphous organic matter, anhydrite <b>Environmental impacts:</b> Two tiny pennate diatom individuals, attached to organic material <b>In situ decomposition evidence:</b> Branched and calcified mycelium
7-8 (two samplings)	Pool behind the main altar, main room	Free water samplings	<i>Calcite</i>	<b>Remains of human activity:</b> Colourless cellulose fibres
9-10 (two samplings)	Pool, lower room	Free water samplings	<i>Calcite and silicates</i>	<b>Remains of human activity:</b> Colourless cellulose fibres <b>Edaphic tracers:</b> Tracheids from resinous and deciduous trees

Figure 1. Hair with diatom fragments (four distinct valves). A) Two fragments of diatoms are indicated by the arrows. B) Internal view of the centric diatom, showing radial elevations at the margins of the central area and the traces of the marginal fultoportulae on the internal side of the mantel (above); external view showing the central area with obstructed areolae and opened marginal chambers (below).

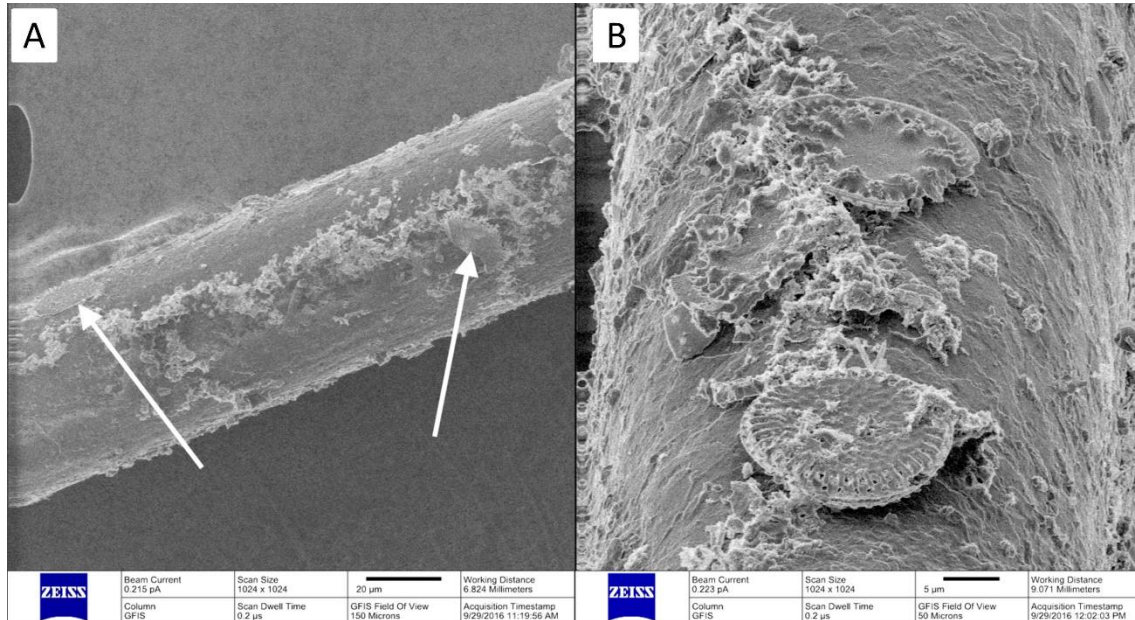


Figure 2. External views of the diatom. A) High magnification microphotograph of the valve surface showing the obstruction of areolae, likely organic fibres and numerous little depressions probably due to dissolution. B) Highly corroded fragment (also seen on Figure 1A, right arrow) showing the scattered traces of areolae of the central area and a hyaline ring (arrows) between areolae and marginal chambers.

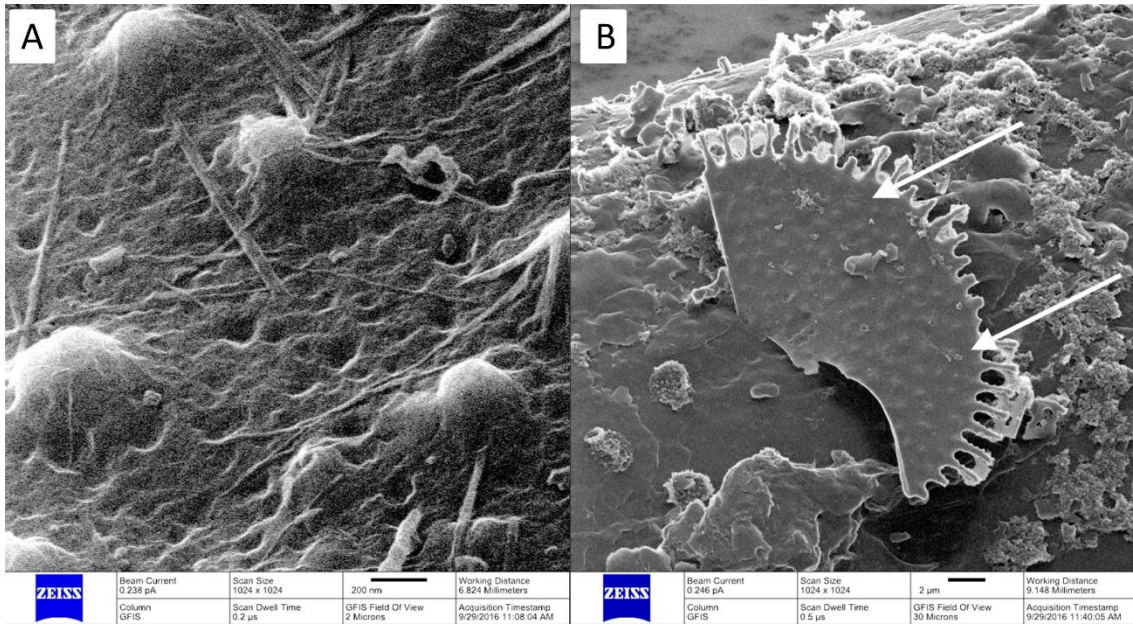


Figure 3. Internal views of the diatom. A) Simple openings of the marginal alveolar ribs and traces of fultoportulae near the valve edge between the chambers. B) Radial areolated fields in the marginal area, also seen as elevations in Figure 1B.

