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### ► To cite this version:

Avril Meffray, Yann Ardagna, Bernard Sillano, Sandy Parmentier, Benoit Pouget, et al.. Variola virus DNA in skeletal remains, 17th-18th centuries, southeastern France. *Clinical Microbiology and Infection*, In press, 27 (12), pp.1871-1872. 10.1016/j.cmi.2021.09.015 . hal-03356279

**HAL Id: hal-03356279**

**<https://hal.science/hal-03356279>**

Submitted on 14 Nov 2022

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# Journal Pre-proof

Variola virus DNA in skeletal remains, 17<sup>th</sup>-18<sup>th</sup> centuries, southeastern France

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PII: S1198-743X(21)00535-8

DOI: <https://doi.org/10.1016/j.cmi.2021.09.015>

Reference: CMI 2692

To appear in: *Clinical Microbiology and Infection*

Received Date: 18 June 2021

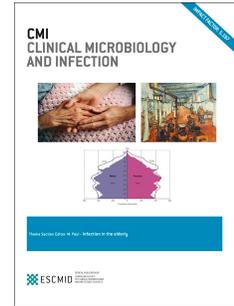
Revised Date: 6 September 2021

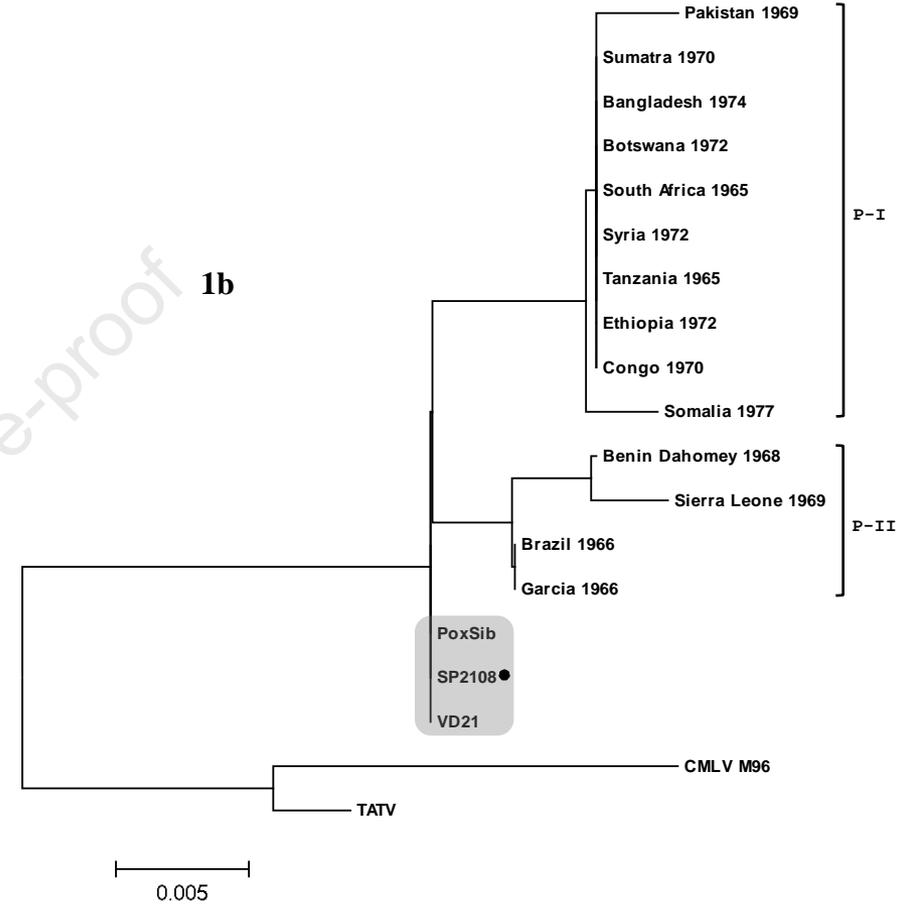
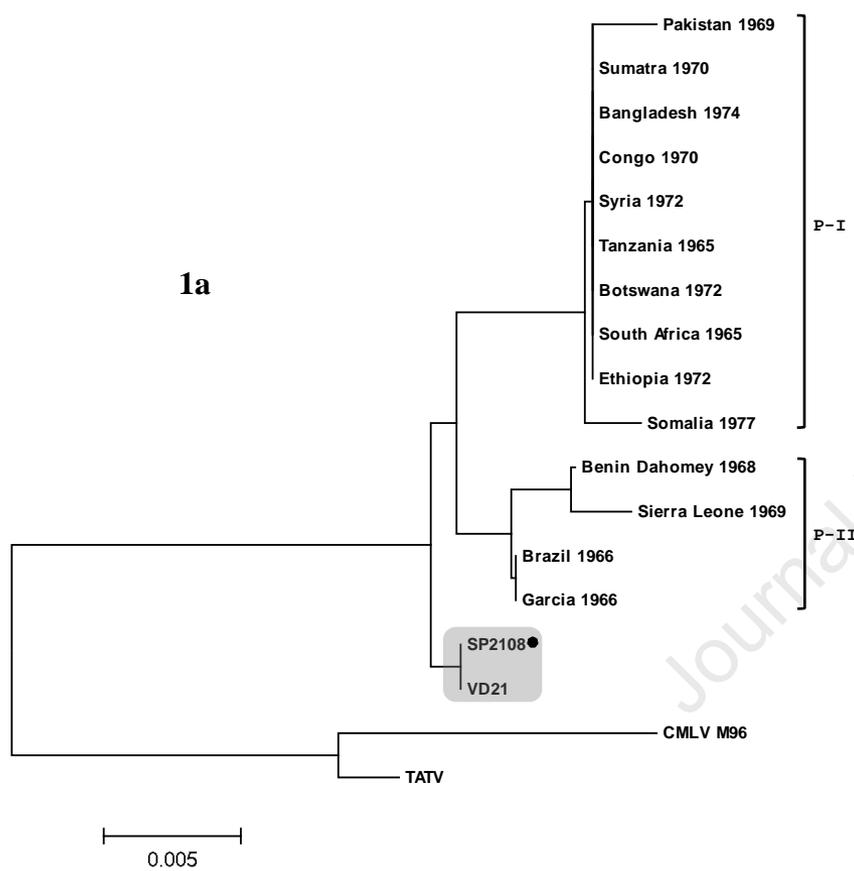
Accepted Date: 13 September 2021

Please cite this article as: Meffray A, Ardagna Y, Sillano B, Parmentier S, Pouget B, Signoli M, Biagini P, Variola virus DNA in skeletal remains, 17<sup>th</sup>-18<sup>th</sup> centuries, southeastern France, *Clinical Microbiology and Infection*, <https://doi.org/10.1016/j.cmi.2021.09.015>.

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1 Letter to the Editor

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3 **Variola virus DNA in skeletal remains, 17<sup>th</sup>-18<sup>th</sup> centuries,**  
4 **southeastern France**

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25 *To the Editor,*

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27 Smallpox is one of the most devastating diseases known to mankind. Its deadly hold,  
28 now vanished from the face of the earth thanks to a large-scale eradication campaign launched  
29 by the World Health Organization (WHO) in 1967, has since become a subject of medical,  
30 epidemiological and historical interest for the scientific community [1]. Ancient biological  
31 remains have been investigated for variola virus (VARV) DNA, broadening our knowledge  
32 on its evolutionary history in medieval / modern Northern Europe and modern Siberia [2-4];  
33 however, we are still unable to estimate efficiently its presence in ancient urban or rural  
34 populations from Western Europe.

35 Our study, stemming from previous palaeomicrobiological investigations performed  
36 on contemporary foetus and infants possibly exposed to treponemal disease [5], aimed to  
37 analyse 26 subjects excavated from the chapel of “Saint-Laurent de la Capelette” in Marseille  
38 city, southeastern France (see Supplementary material, **Historical and archaeological**  
39 **context** and **Table S1**). We carried out macroscopic and molecular analysis to evaluate the  
40 prevalence of smallpox infection among this osteoarchaeological corpus dating back to the  
41 mid-17<sup>th</sup>- early-18<sup>th</sup> centuries.

42 Bone samples (small cranial and costal elements) were collected and fully documented before  
43 proceeding to molecular investigations. VARV DNA presence was investigated using several  
44 PCR detection systems (<150 bp) targeting A38R, B7R, A30L, and E9L genomic regions; a 430  
45 bp PCR amplification (E9L) to analyze the fragmented nature of the viral genome was also  
46 performed (see Supplementary material, **Methods** and **Table S2**). Extensive precautions were  
47 taken at each step of the study to meet with the ‘authenticity criteria’ expected for ancient DNA  
48 studies [5]. No studies on modern smallpox were ever conducted in our facilities, and positive

49 controls were never used; negative controls (sterile water) were included systematically in  
50 extraction and DNA amplification procedures.

51 Remains of one individual only (SP2108, child 1-4 year-old at the time of death, fairly  
52 complete skeleton except for the skull; costal fragment tested) yielded positive PCR signals;  
53 the four detection systems used gave amplicons of expected size on agarose gel  
54 electrophoresis, whereas no signal was observed for the longer amplification assay  
55 highlighting an extensive degradation of the viral genome.

56 Sequences analysis confirmed the VARV origin of amplified material, and revealed strict  
57 identities with partial sequences obtained from Siberian [2] and Lithuanian [3] mummies  
58 dating from similar periods (17<sup>th</sup>-18<sup>th</sup> centuries; PoxSib and VD21 isolates, respectively) (see  
59 Supplementary material, **Figure S1**). Phylogenetic and temporal analysis of concatenated  
60 sequences confirmed the divergent position of these 300-year-old VARV sequences, distinct  
61 from 20<sup>th</sup> century P-I and P-II clades [2,3] (**Figure 1**, and Supplementary material, **Figure**  
62 **S2**).

63 Our findings are consistent with smallpox being a Eurasian pandemic at this time,  
64 spreading through the Ancient Continent via trade, migratory, exploratory and invasion routes  
65 [1-4]. It echoes previous results, to the extent that the French SP2108 strain could either be an  
66 ancestor of the 20<sup>th</sup> century VARV strains, or a member of an ancient lineage with no  
67 connections, however, to the recent times outbreaks strains [2].

68 Importantly, above results entail also the possibility of bone samples from  
69 archaeological context as a substrate for VARV molecular detection, the bone marrow being a  
70 known site of replication for the virus, thus freeing palaeomicrobiologists from the necessity  
71 of using mummified and/or frozen ancient remains to investigate this pathogen. Of note, the  
72 skeleton of this young child displayed no signs of pathological conditions such as periosteal  
73 remodelling or *osteomyelitis variolosa* (see Supplementary material, **Figure S3**).

74           In the present case, there is no reason to assume that this specimen was contaminated  
75 by any other than the natural ways, as opposed to voluntary transmission by inoculation, since  
76 the advent of this practice in modest populations did not occur in southeastern France until the  
77 second half of the 18<sup>th</sup> century [1,6]. On the same note, the first immunization session in  
78 Marseille took place only on March 27, 1801 at the “Hospice de la Charité”, involving  
79 cowpox virus [6]. In the deteriorated sanitary context of the 17<sup>th</sup> and 18<sup>th</sup> centuries, smallpox  
80 was a classic companion of Western populations, whether they were poor or favoured. As an  
81 endemo-epidemic disease, it affected mainly children under 5 years of age and caused a high  
82 mortality among them [6]. The parish archives of the “Saint-Laurent dite la Capelette” chapel  
83 show the recurrence of young children burials, especially in the early 18<sup>th</sup> (see Supplementary  
84 material, **Methods** and **Table S3**), with the occasional occurrence of a greater number of  
85 losses among adults, that could result from more violent epidemic outbreaks. As pointed out  
86 by P. Darmon, contrary to plague, “smallpox, in a much more diffuse way, decimated  
87 consistently and piercingly by small touches, eliminating the generations at the base without  
88 really slashing into the mass” [6]. Considered as a common and inevitable scourge, public  
89 health policies of the time struggled little against its omnipresence and did not classify it as  
90 part of quarantine diseases. The presence of smallpox throughout the year, with a mortality  
91 pattern that staggered over the four seasons, did not lead to the use of mass burials, which  
92 explains why this infected specimen was found in a normal burial context (parish church) [1,  
93 6].

94           The uncovering of conserved, though fragmented, VARV DNA in 300-year-old bone  
95 samples of a child's skeleton identified in south France adds clues to the dispersion of  
96 smallpox in past populations; subsequent investigations are now expected.

97

98 **Transparency declaration**

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100 **Author contributions**

101 All authors have made substantial contributions to the work. Study concept and design: AM, YA,  
102 PB. Data acquisition: AM, YA, BS, SP, BP, MS. Analysis and/or interpretation of data: AM,  
103 YA, PB. Drafting the manuscript: AM, PB.

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105 **Conflict of interest**

106 The authors declare that there are no conflicts of interest.

107

108 **Funding information**

109 The work was supported by the UMR 7268 ADES, the French Blood Agency PACA Corse and  
110 the French Ministry of Research (no specific grant).

111

112 **Acknowledgments**

113 The authors are grateful to ML. Deligny for excellent technical assistance.

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135 **Figure legends**

136

137 **Figure 1.** Neighbor-joining (NJ) phylogenetic trees generated using 20<sup>th</sup> and 17<sup>th</sup>-18<sup>th</sup>  
138 centuries VARV, and animal orthopoxvirus partial sequences.

139 **a)** A38R, B7R, A30L, and E9L concatenated sequences (429 nt, SP2108 reference) (PoxSib  
140 not included, no A38R sequence available).

141 **b)** B7R, A30L, and E9L concatenated sequences (325 nt, SP2108 reference).

142 20th century VARV P-I / P-II clades are shown. 17<sup>th</sup>-18<sup>th</sup> centuries VARV isolates (PoxSib-  
143 Siberia/Yakoutia; VD21-Lithuania; SP2108-southeastern France, this study) are shaded. Scale  
144 bar indicates nucleic acid substitutions per position.

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