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Natural mummification of the human gut preserves bacteriophage DNA

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One sentence summary: Gut phageome of mummified human remains.
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ABSTRACT

The natural mummification process of the human gut represents a unique opportunity to study the resulting microbial community structure and composition. While results are providing insights into the preservation of bacteria, fungi, pathogenic eukaryotes and eukaryotic viruses, no studies have demonstrated that the process of natural mummification also results in the preservation of bacteriophage DNA. We characterized the gut microbiome of three pre-Columbian Andean mummies, namely FI3, FI9 and FI12, and found sequences homologous to viruses. From the sequences attributable to viruses, 50.4% (mummy FI3), 1.0% (mummy FI9) and 84.4% (mummy FI12) were homologous to bacteriophages. Sequences corresponding to the *Siphoviridae, Myoviridae*, *Podoviridae* and *Microviridae* families were identified. Predicted putative bacterial hosts corresponded mainly to the Firmicutes and Proteobacteria, and included *Bacillus*, *Staphylococcus, Clostridium*, *Escherichia*, *Vibrio*, *Klebsiella*, *Pseudomonas* and *Yersinia*. Predicted functional categories associated with bacteriophages showed a representation of structural, replication, integration and entry and lysis genes. The present study suggests that the natural mummification of the human gut results in the preservation of bacteriophage DNA, representing an opportunity to elucidate the ancient phageome and to hypothesize possible mechanisms of preservation.

Keywords: ancient microbiomes; bacteriophages; microbiome; mummy; phageome; virome

INTRODUCTION

The human gut microbiome is home to diverse communities comprised of bacteria, archaea and eukaryotes (Yatsunenko *et al.* 2012; Hoffmann *et al.* 2013); yet, an increasing number of studies have demonstrated that the human gut is also inhabited by diverse viral communities, many of which are bacteriophages

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(Minot *et al.* 2011, 2013). Bacteriophages play important roles in biogeochemical cycles (Fuhrman 1999) and in the evolution of their bacterial hosts (Ai, Meng and Zeng 2000; Bollback and Huelsenbeck 2001; Coberly *et al.* 2009; Minot *et al.* 2013; Cvirkaite-Krupovic, Carballido-Lopez and Tavares 2015); however, we are just beginning to understand the role of bacteriophages as part of the human microbiome (Sun and Relman 2013; Abeles and Pride 2014). Previous studies have demonstrated that bacteriophages are part of the human oral (Pride *et al.* 2012; Edlund *et al.* 2015), skin (Robles-Sikisaka *et al.* 2013; Denesvre *et al.* 2015), genitourinary tract (Santiago-Rodriguez *et al.* 2015c) and gut microbiomes (Breitbart *et al.* 2003; Minot *et al.* 2011; Hofer 2013; Cadwell 2015; Ray 2015). Bacteriophages also have major impacts on human health and disease (Willner *et al.* 2009, 2011; Ly *et al.* 2014; Landini *et al.* 2015; Norman *et al.* 2015; Santiago-Rodriguez *et al.* 2015e; Wang, Gao and Zhao 2015). In diseases such as periodontitis, the relative abundance of bacteriophages belonging to the *Myoviridae* family is higher in subjects with the disease compared to subjects with good periodontal health. While results may be influenced by the representation of myoviruses in databases, these bacteriophages are believed to shape oral bacterial communities by lysing their hosts, thus, are believed to promote periodontal disease (Ly *et al.* 2014; Santiago-Rodriguez *et al.* 2015e). Other more serious diseases, including inflammatory bowel disease (IBD), have also associated bacteriophages with a dysbiosis of the gut bacterial communities, probably resulting in the disease (Norman *et al.* 2015).

Human microbiomes dating to hundreds and thousands of years are just beginning to be characterized, and have also been associated with dietary shifts, dietary habits and periodontal health and disease (Adler *et al.* 2013; Cano *et al.* 2014; Warinner, Speller and Collins 2015; Weyrich, Dobney and Cooper 2015). Yet, very few studies have focused on the viral composition of ancient human samples. Previous studies have focused on viruses in ancient human specimens including retroviruses and those from the *Flaviridae*, *Rhabdoviridae*, *Parvoviridae* families (Emerman and Malik 2010; Patel, Emerman and Malik 2011; Aswad and Katzourakis 2012; Katzourakis 2013; Lavialle *et al.* 2013; Rivera-Perez *et al.* 2015). It is feasible to hypothesize that ancient microbiomes are also home to a community of bacteriophages homologous to those present in modern human microbiomes. A previous study characterizing the virome of fossilized fecal material from the14th century found that bacteriophages comprised a good proportion of the viral communities (Appelt *et al.* 2014).

The natural mummification process is also known to preserve ancient microbial DNA due to cold temperatures and low oxygen levels (Cano *et al.* 2000; Zink *et al.* 2000; Tito *et al.* 2012). Our previous study characterizing the gut microbiome of a pre-Columbian Andean mummy identified sequences associated with bacteria, archaea, fungi, pathogenic eukaryotes and eukaryotic viruses (Santiago-Rodriguez *et al.* 2015a); yet, no studies have demonstrated that the process of natural mummification also results in the preservation of bacteriophage DNA. Bacteriophage communities are usually characterized using viral metagenomics, which consists in the enrichment of viruses by CsCl gradient ultracentrifugation (Rosario *et al.* 2009; Walker 2010; Ly *et al.* 2014; Santiago-Rodriguez *et al.* 2015c). A previous study characterized the viral communities of a 14th century coprolite using viral metagenomics, but the method needs to be tested in mummified human specimens (Appelt *et al.* 2014). Shotgun metagenomics has also shown to provide information on microbial communities in ancient human samples (Adler *et al.* 2013). While shotgun metagenomics is not selective for bacteriophage DNA, it is useful in characterizing phage communities in modern samples (Belda-Ferre *et al.* 2012; Santiago-Rodriguez *et al.* 2015e). Therefore, by using metagenomics, we aim to: (i) determine the percentage of sequences associated with bacteriophages, (ii) identify bacteriophages sharing sequence homology to modern bacteriophages and (iii) determine predicted functional categories associated with bacteriophages in the gut of naturally preserved human mummies.

MATERIALS AND METHODS

Description of mummified human remains

The specimens studied are presently stored at the Museum of Anthropology and Ethnology of the University of Florence, Italy. Autopsies were performed by paleopathologists G. Fornaciari and colleagues, and specimens were collected from internal organs by cutting the skin and the ribs. The first mummy, FI3, was an adult male dating to the 14th—15th century that showed a good preservation of the skin with the adnexa and a massive presence of fungi and ectoparasites. The presence of microscopic, non-pathological fungi, including the genus *Aspergillus* (easily identifiable with Periodic Acid Schiff staining), is a very common finding in mummies as a post-mortem invasion phenomenon. DNA was extracted from abdominal viscera. The second mummy, FI9, was a female of estimated 18–23 years of age, dating to the 11th century A.D. DNA was extracted from the descending colon, but the ascending and transverse colon, as well as paleofeces were previously characterized by our group (Santiago-Rodriguez *et al.* 2015a). The third mummy, FI12, was an adult female, estimated age 20–25 years, and autopsy showed that she was afflicted by bronchopneumonia. An exact date for the mummy is unknown, but it is evident that she belonged to the Inca culture from the fetal position found in the burial basket. DNA was extracted from the transverse colon.

Avoidance of contamination

We employed the standard precautions for ancient DNA work including the use of sterile gloves, pretreatment of mortars, pestles and homogenizers with HCl, use of UV-irradiated safety cabinets, dedicated gel trays, tanks and reagents. The autopsy was performed by paleopathologists wearing sterile surgical coats, sterile latex gloves, sterile masks, headdresses and overshoes. The outermost portions of the specimens were not used to eliminate the risk of surface contamination, and one replicate per sample was obtained for further analyses. The mummified specimens were immediately kept and sealed in sterile containers, reducing the possibility of subsequent contamination. The samples were stored aseptically in hermetic plastic containers in a dry environment with silica gel at 18°C –20°C. DNA extraction and further precautions were performed as described previously (Santiago-Rodriguez *et al.* 2015a) and are detailed in Methods (Supporting Information).

Metagenome analyses for viruses

DNA library preparation for metagenome sequencing was performed at the Next-Generation sequencing provider Molecular Research Laboratory (MRDNA) (www.mrdnalab.com; Shallowater, TX, USA) under strict procedures to eliminate cross-contamination with modern DNA as described previously (Santiago-Rodriguez *et al.* 2015a). Libraries were sequenced using Illumina MiSeq following Truseq DNA library preparation

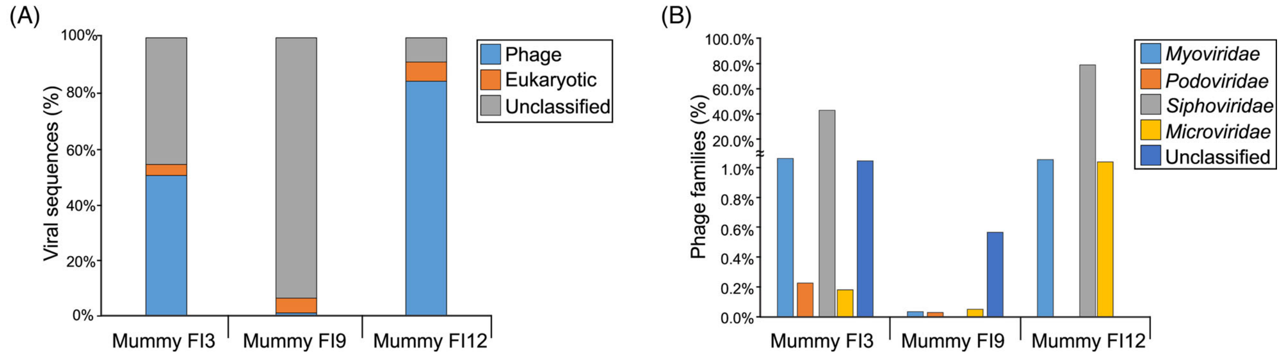

Figure 1. Panel (**A**) shows the percentage of sequences homologous to phages, eukaryotic viruses and unclassified viruses. Percentage was calculated based on the total number of sequences corresponding to viruses. Panel (**B**) shows the percentage of sequences corresponding to phage families. Families included the *Siphoviridae*, *Myoviridae*, *Podoviridae*, *Microviridae* and unclassified, and were determined based on sequence homology to known phages.

protocol, and sequence files were processed as described previously (Santiago-Rodriguez *et al.* 2015a). Data were then uploaded and annotated using the MG-RAST pipeline and taxonomic assignments were determined using the SEED database with a minimum e-value of 80% (Meyer *et al.* 2008). To determine the percentage of sequences associated with viruses and the predicted putative hosts at the phylum level, data were acquired from the Virus category. Sequences were also mapped to a virus database that included both prokaryotic and eukaryotic viruses (www.phantome.org; ftp://ftp.ncbi.nih.gov/genomes/Viruses/). Mapping was performed using CLC Genomics Workbench with the following parameters: no masking, mismatch cost = 2, insertion cost = 3, deletion cost = 3, with an 80% identity over a minimum of 50% of the read length. Mapped reads were also retrieved from CLC Genomics Workbench as a SAM file and processed using mapDamage for further ancient DNA authentication as described previously (Ginolhac *et al.* 2011). Predicted functional categories associated with bacteriophages were analyzed using MG-RAST with a minimum e-value of 80%.

16S rRNA gene analyses

16S rRNA gene data from these mummies were used to associate the phages predicted putative hosts with the bacterial taxonomy at the phylum level. SourceTracker analyses were also performed to identify possible sources of contamination (Knights *et al.* 2011; Santiago-Rodriguez *et al.* 2015a,b). 16S rRNA gene methods and analyses are described in Methods (Supporting Information).

RESULTS

Metagenome and 16S rRNA gene high-throughput sequencing data

For the metagenome analyses, a total of 16 805 260 (mummy FI3), 146 081 692 (mummy FI9) and 16 537 474 (mummy FI12) sequences, with an average length of 100 bp were analyzed. For the 16S rRNA gene analyses, a total of 79 752 (mummy FI3), 8731 (mummy FI9) and 57 979 (mummy FI12) sequences with an average length of 270 bp were acquired, but data were rarefied to 8000 sequences to minimize the effect of disparate sequence number in the results (Santiago-Rodriguez *et al.* 2015a). We utilized SourceTracker using 135 human (45 oral, 45 skin and 45 gut), and 45 soil microbiomes to identify possible sources of contamination in the mummified gut tissues (Fig. S1, Supporting Information) (Santiago-Rodriguez *et al.* 2015a). Given that the samples were obtained from the mummies' colon, it is expected some of the ancient sequences to match modern gut microbiomes, as in the case of mummy FI3. However, given that the sequences did not match the most likely sources of contamination (shown as unknown) in any of the mummies, namely skin and soil microbiomes, suggests that no external sources of contamination contributed to the findings reported in the present study (Santiago-Rodriguez *et al.* 2015a,b). We also performed mapDamage analyses for mummy FI3 mapped viral reads, but did not note the typical DNA damage pattern at the 5′ or 3′ ends as described for eukaryotic genomes (Knapp *et al.* 2012; Der Sarkissian *et al.* 2014) (Fig. S2, Supporting Information).

Natural mummification preserves bacteriophage DNA

Metagenome analyses showed that the mummified guts included in the present study had sequence homology to viral genomes, with a proportion corresponding to bacteriophages. A total of 2198 (mummy FI3), 74 052 (mummy FI9) and 275 (mummy FI12) sequences were homologous to viruses. Approximately 50.4%, 4.0% and 45.6% of the viral sequences in mummy FI3 were homologous to bacteriophages, eukaryotic viruses and unclassified viruses, respectively. Mummy FI9 had the majority of the viral sequences (93.7%) not matching bacteriophages or eukaryotic viruses (unclassified). Approximately 84.4%, 6.9% and 8.7% of the viral sequences in mummy FI12 were homologous to bacteriophages, eukaryotic viruses and unclassified viruses, respectively (Fig. 1A). Analysis of the phage families showed that 42.9% (mummy FI3), 0.29% (mummy FI9) and 79.9% (mummy FI12) of the sequences were homologous to siphoviruses. Approximately 4.6% (mummy FI3), 0.04% (mummy FI9) and 3.6% (mummy FI12) of the sequences were homologous to myoviruses. Podoviruses represented 0.2% and 0.03% of the viral sequences in mummies FI3 and FI9, respectively. No podovirus-homologous sequences were present in mummy FI12. Microviruses contributed 0.2%, 0.05% and 1.8% of the viral sequences in mummies FI3, FI9 and FI12, respectively. The remaining sequences associated with bacteriophages could not be classified (Fig. 1B).

Analyses of predicted putative hosts at the phylum level showed that the majority (80.4%) of the sequences associated with bacteriophages in mummies FI3 and FI12 were homologous to those having Firmicutes as the bacterial hosts. Other putative hosts at the phylum level included the Proteobacteria, Actinobacteria or Cyanobacteria. Notably, mummy FI9 had

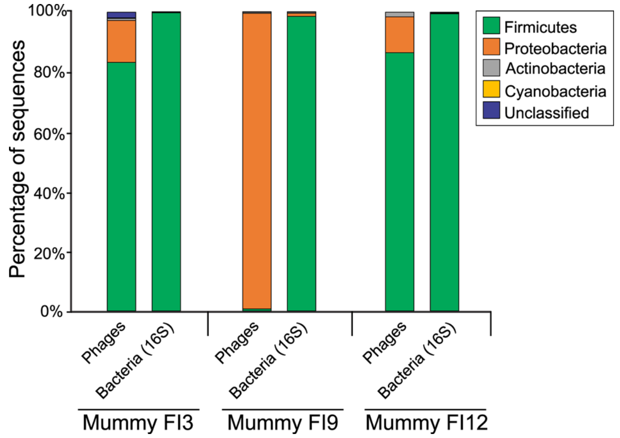

Figure 2. Bacteriophage predicted putative bacterial host at the phylum level was determined based on sequence homology. Predicted putative hosts included the Firmicutes, Proteobacteria, Actinobacteria, Cyanobacteria and unclassified. Figure also shows the bacterial phylum based on analysis of the 16S rRNA gene variable region V4.

the majority of the bacteriophage sequences sharing homology to those infecting Proteobacteria (93.6%) (Fig. 2). 16S rRNA gene analyses showed that the Firmicutes were the most represented bacterial group in mummies FI3 (99.9%), FI9 (98.5%) and FI12 (99.4%) (Fig. 2).

Mapping results also demonstrated that reads in the mummified guts corresponded to phage homologs. Table 1 shows examples of the mapping results to presumptive bacteriophages with the highest number of reads and coverage. Examples include *Staphylococcus*, *Cronobacter* and *Brochothrix* phages in mummy FI3, *Lactobacillus* and *Staphylococcus* phages in mummy FI9, and *Bacillus* and *Cronobacter* phages in mummy FI12. Reads also mapped across bacteriophage genomes, although not broadly, in mummies FI3 (Fig. 3A), FI9 (Fig. 3B) and FI12 (Fig. 3C). Examples shown include *Staphylococcus* bacteriophage StB20 (Fig. 3A), *Lactobacillus* phage AQ113 (Fig. 3B) and Enterobacteria phage phiX174 sensu lato (Fig. 3C). Regions that mapped to modern Enterobacteria phage phiX174 in mummies FI3 (Panel A), FI9 (Panel B) and FI12 (Panel C) are shown in Fig. S3 (Supporting Information). Nucleotide differences between modern and ancient sequences are shown in red, and indicate that ancient sequences do not correspond to the standard spike-in control used in Illumina sequencing.

Table 1. Examples of bacteriophage sequences mapping known bacteriophage genomes in mummies FI3, FI9 and FI12. Bacterial taxonomic classification at the family level was determined using 16S rRNA gene analyses. Family and relative abundance percentages are shown.

Presumptive bacteriophage	Phage family	Number of reads	Average coverage	Accession number	Reference length (bp)
Mummy FI3					
Acinetobacter phage 133	*Myoviridae*	48	7.51E-03	NC_015250.1	159 801
Aeromonas phage 65	*Myoviridae*	93	9.62E-03	NC_015251.1	235 229
Bacillus phage 0305phi8-36	*Myoviridae*	110	0.01	NC_009760.1	218 948
Brochothrix phage A9	*Myoviridae*	295	0.1	NC_015253.1	127 065
Campylobacter phage CP21	*Myoviridae*	101	0.02	NC_019507.1	182 833
Cellulophaga phage phiST	*Siphoviridae*	67	0.03	NC_020842.1	79 114
Clostridium phage CDMH1	*Myoviridae*	60	0.05	NC_024144.1	54 279
Cronobacter phage vB_CsaM_GAP32	*Myoviridae*	411	0.05	NC_019401.1	358 663
Cyanophage P-RSM6	*Myoviridae*	54	6.28E-03	NC_020855.1	192 497
Enterobacteria phage phiX174 sensu lato	*Microviridae*	21	0.26	NC_001422.1	5386
Erwinia phage Ea35-70	*Myoviridae*	69	5.41E-03	NC_023557.1	271 084
Klebsiella phage K64-1 DNA	*Myoviridae*	54	4.77E-03	NC_027399.1	346 602
Mycobacterium phage Myrna	*Myoviridae*	135	0.02	NC_011273.1	164 602
Pelagibacter phage HTVC008M	*Myoviridae*	77	0.01	NC_020484.1	147 284
Prochlorococcus phage P-HM2	*Myoviridae*	98	0.01	NC_015284.1	183 806
Pseudomonas phage 201phi2-1	*Myoviridae*	93	6.85E-03	NC_010821.1	316 674
Pseudomonas phage PaBG	*Myoviridae*	176	0.03	NC_022096.1	258 139
Sphingomonas phage PAU	*Myoviridae*	90	0.01	NC_019521.1	219 372
Staphylococcus phage StB20	*Siphoviridae*	1127	2.5	NC_019915.1	40 917
Staphylococcus phage Twort	*Myoviridae*	172	0.06	NC_007021.1	130 706
Synechococcus phage ACG-2014f	*Myoviridae*	219	0.04	NC_026927.1	228 143
Synechococcus phage S-SKS1	*Siphoviridae*	149	0.02	NC_020851.1	208 007
Vibrio phage KVP40	*Myoviridae*	65	5.93E-03	NC_005083.2	244 834
Yersinia phage phiR1-37	*Myoviridae*	106	0.01	NC_016163.1	262 391
Mummy FI9					
Enterobacteria phage phiX174 sensu lato	*Microviridae*	416	1.86	NC_001422	5386
Lactobacillus phage AQ113	*Myoviridae*	4215	0.05	NC_019782	36 566
Staphylococcus phage StB20	*Siphoviridae*	7695	0.04	NC_019915	40 917
Mummy FI12					
Bacillus phage G	*Myoviridae*	263	0.41	NC_023719.1	497 513
Cronobacter phage vB_CsaM_GAP32	*Myoviridae*	168	0.07	NC_019401.1	358 663
Enterobacteria phage phiX174 sensu lato	*Microviridae*	47	1.86	NC_001422.1	5386
Pseudomonas phage phiKZ	*Myoviridae*	51	0.06	NC_004629.1	280 334

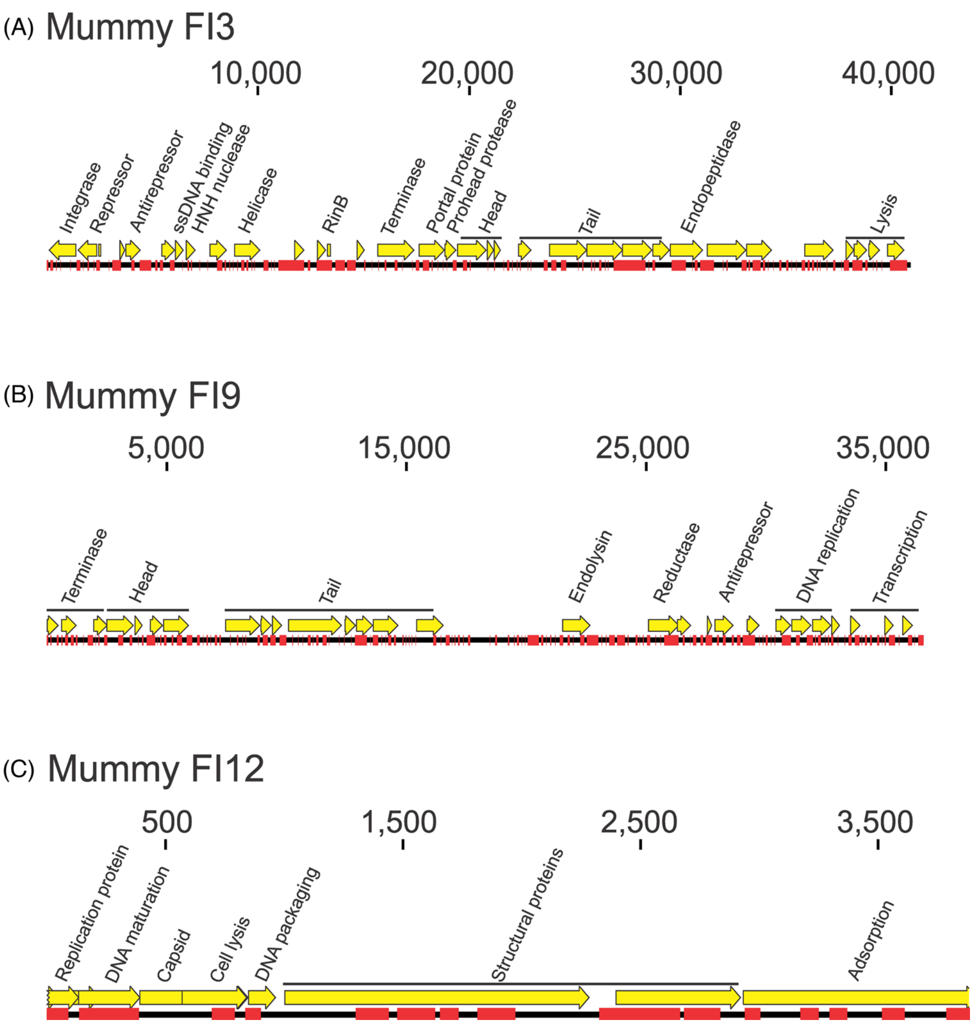

Figure 3. Examples of mapping results in mummies FI3 (Panel **A**), FI9 (Panel **B**) and FI12 (Panel **C**). Examples included presumptive *Staphylococcus* phage StB20 (mummy FI3), *Lactobacillus* phage AQ113 (mummy FI9) and Enterobacteria phage phiX174 sensu lato (mummy FI12). Reads mapping to the phage genomes are shown in red.

Predicted functional categories associated with bacteriophage genes were divided into structure (head and tail), entry and lysis, integrases, replication, packaging, antirepressors, repressors, virulence, introns and hypothetical proteins. The majority of the bacteriophage categories in mummy FI3 corresponded to packaging (35.3%), and entry and lysis (31.2%) (Fig. 4A). Mummy FI9 had the majority of the bacteriophage categories (98.7%) corresponding to integrases (Fig. 4B). Most of the bacteriophage categories in mummy FI12 corresponded to the entry and lysis (65.1%) (Fig. 4C).

DISCUSSION

Our data reconsistent with authentic ancient DNA, as shown with the high level of fragmentation (Ubaldi *et al.* 1998) and the SourceTracker analyses (Santiago-Rodriguez *et al.* 2015a). We also performed the mapDamage analyses to assess patterns of DNA damage that could be consistent with ancient DNA (Der Sarkissian *et al.* 2014), but did not note these patterns with the phageomes tested. While mapDamage has proven to be useful in determining DNA damage in eukaryotic genomes (Knapp *et al.* 2012), the method still needs to be further tested in microbiomes. Damage artifacts are often used to authenticate ancient DNA, but these may represent a challenge when characterizing ancient microbiomes. DNA damage analyses may not always provide reliable information of ancient microbiomes as nucleotide differences could also represent a novel microorganism (Warinner, Speller and Collins 2015). In addition, ancient DNA originating from microorganisms or eukaryotes would possibly need to be analyzed differently as different degrees of damage may be associated with specific taphonomic conditions.

Little is known about bacteriophage DNA preservation in ancient human specimens (Appelt *et al.* 2014). Our study adds to the knowledge of ancient viruses by showing that the natural mummification process of the human gut results in the preservation of bacteriophage DNA. While the recovering and sequencing methods differed, the relative abundances of siphoviruses, myoviruses, podoviruses and microviruses were virtually similar to previously reported viromes in extant human guts and coprolites (Breitbart *et al.* 2003; Appelt *et al.* 2014). Of interest was also the presence of sequences that were not homologous to known bacteriophages, consistent with their rapid evolution in modern human guts (Minot *et al.* 2013). These results are also consistent with previous studies showing that a proportion of viral sequences in the human gut usually cannot be assigned to existing reference genomes (Breitbart *et al.* 2003; Minot *et al.* 2011; Muniesa and Jofre 2014).

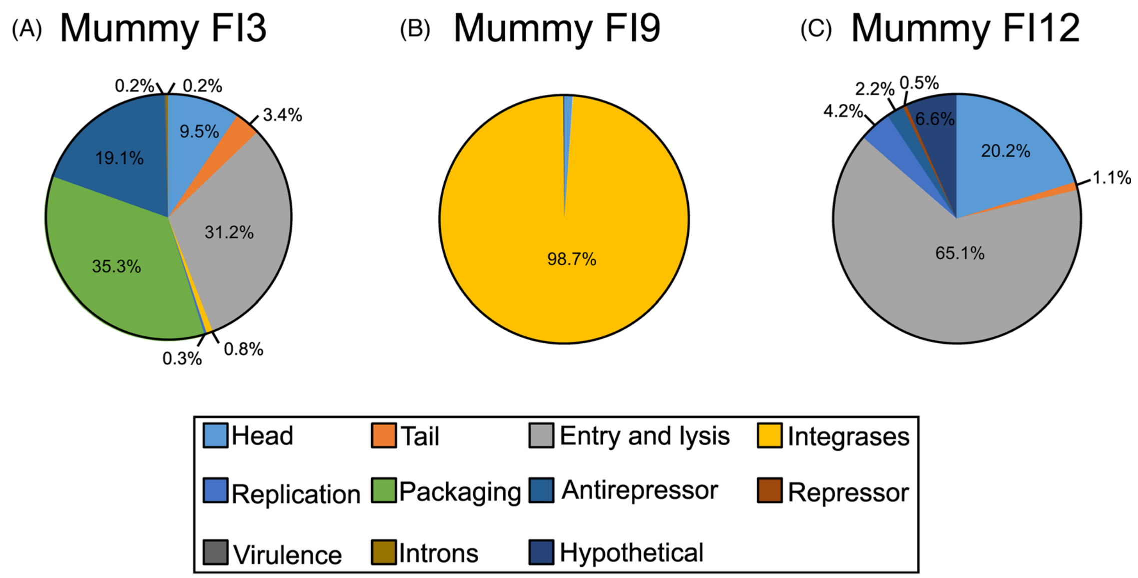

Figure 4. Functional categories attributed to bacteriophages. Categories included structure (head and tail), entry and lysis, integrases, replication, packaging, antirepressors, repressors, virulence genes, introns and hypothetical proteins.

While we have demonstrated that phage DNA is preserved in mummified gut tissue, the preservation processes remain to be elucidated. A possible explanation for bacteriophage DNA preservation in mummified gut tissue may include the replication cycles. A proportion of the sequences associated with bacteriophages in the mummies guts corresponded to integrases, antirepressors and repressors. While these genes are known to be markers of lysogeny and may suggest the presence of prophages, it is difficult to demonstrate with our current data that temperate bacteriophages were in a prophage state in the mummified gut tissues. However, it is reasonable to hypothesize that prophages were preserved along with their bacterial host genomes. Of interest is also the possibility that the process of natural mummification resulted in the induction of prophages due to desiccation, which is known to trigger the lytic cycle (Brovko 2007).

The detection of strictly virulent bacteriophage DNA is intriguing as it may suggest that there may be other mechanisms supporting bacteriophage preservation during the process of natural mummification of the human gut. It is known that lytic bacteriophages are persistent members of the human microbiome and can be detected up to 60 days in the oral cavity, suggesting that they may attach to mucous layers (Barr *et al.* 2013; Abeles and Pride 2014; Edlund *et al.* 2015). Although similar studies have not been carried out in ancient samples, it is feasible that mucous layer(s) in mummified human specimens may also act as a reservoir of lytic bacteriophages (Fornaciari 1993; Corthals *et al.* 2012). Intact capsids may aid in the preservation of bacteriophage DNA, but this still needs to be demonstrated with mummified gut tissue using electron microscopy (Appelt *et al.* 2014). Another possible explanation for the detection of bacteriophages in the mummified guts is their seemingly high proportions in the human gut, where concentrations may range between 10^7 and 10^{10} per gram of feces (Muniesa and Jofre 2014). This relatively high initial concentration may aid in the detection of bacteriophage DNA even if some inactivation has occurred during natural mummification.

Given that lysogenic bacteriophages co-evolve with their bacterial hosts, culture-independent methods using sequence homology have shown to be relatively accurate in predicting putative bacterial hosts up to the genus level in modern human viromes (Minot *et al.* 2011; Ly *et al.* 2014). While the same techniques have shown to not possess this same specificity with strictly lytic bacteriophages, they are still useful in providing insights into predicted putative bacterial hosts (Ly *et al.* 2014). Previous studies have also associated predicted phage putative hosts with 16S rRNA gene data (Pride *et al.* 2012; Ly *et al.* 2014; Abeles *et al.* 2015; Santiago-Rodriguez *et al.* 2015c,d), but phageome relative abundances do not always mirror those of their bacterial hosts (Edlund *et al.* 2015). This may be due to different dynamic relationships present for different host/phage pairs (Abeles *et al.* 2014, 2015; Ly *et al.* 2014; Santiago-Rodriguez *et al.* 2015c). While associations between the phageome and 16S rRNA gene data exhibit limitations, results may still provide insights into phage–host interactions.

Other possible reasons for specific bacteriophages being preserved in ancient gut phageomes may include differences in gender (Abeles *et al.* 2014), dietary habits (Minot *et al.* 2011), and health status (Cadwell 2015), which are known to affect the phageome in extant human guts. We can only speculate that differences in the mummies gender, dietary habits, culture and health status may influence their phageomes (Santiago-Rodriguez *et al.* 2015a). This may have been the case for mummy FI9, where, although the metagenome analyses generated >140 million sequences (compared to >16 million sequences for mummies FI3 and FI12), we can only hypothesized that differences in her phageome may be associated to the mentioned factors. Ideally, virome metagenomics would better capture these trends when compared to shotgun metagenomics. While we are in the process of developing a method to study bacteriophages and other viruses in ancient specimens using viral metagenomics, from the data it is evident that shotgun metagenomics provide insights on bacteriophage sequences in ancient human gut microbiomes. Results also provide insights into bacteriophage community structure and composition in the gut of naturally preserved mummies.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

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Conflict of interest. None declared.

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