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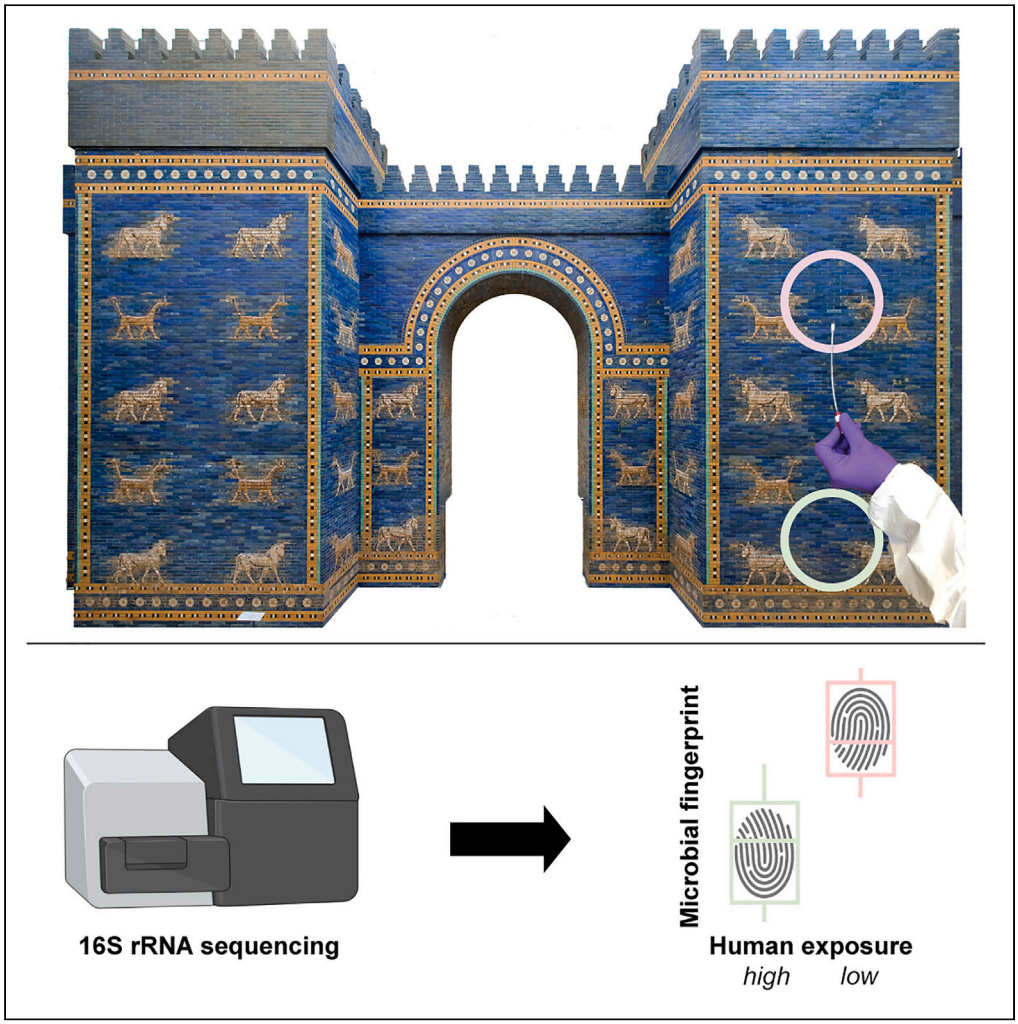

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Highlights

Microbial 16S rRNA profiling of museum objects of natural and cultural heritage

Microbial fingerprints quantitatively capture exposure to human contact

Exposure to human contact may overwrite preceding microbial profile of object

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Microbial fingerprints reveal interaction between museum objects, curators, and visitors

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SUMMARY

Microbial communities reside at the interface between humans and their environment. Whether the microbiome can be leveraged to gain information on human interaction with museum objects is unclear. To investigate this, we selected objects from the Museum für Naturkunde and the Pergamonmuseum in Berlin, Germany, varying in material and size. Using swabs, we collected 126 samples from natural and cultural heritage objects, which were analyzed through 16S rRNA sequencing. By comparing the microbial composition of touched and untouched objects, we identified a microbial signature associated with human skin microbes. Applying this signature to cultural heritage objects, we identified areas with varying degrees of exposure to human contact on the Ishtar gate and Sam'al gate lions. Furthermore, we differentiated objects touched by two different individuals. Our findings demonstrate that the microbiome of museum objects provides insights into the level of human contact, crucial for conservation, heritage science, and potentially provenance research.

INTRODUCTION

In light of the global debate on ownership in collections, both of cultural and natural heritage, the significance of provenance research has dramatically increased over the past years. Furthermore, the authenticity of art objects and the ability to reliably distinguish fakes from originals, is equally important.[1] More and more, in the light of the post-colonial discourse, museum visitors and the wider public take a growing interest in these questions as well.[2] The interaction between object and environment is reflected in the composition of the associated microbial communities. Although different in specific aspects, the main challenges for cultural and natural heritage are the same.[3] A better knowledge of this relation will potentially advance the research of provenance in natural history and cultural collections, open avenues to new knowledge on various population dynamics, co-evolution and symbiosis, and geographical and climatic conditions.

Microbiomes, which refers to the community of microorganisms that live in a defined habitat, for example, on or within an object, act as indicator for interactions — between organisms,[4,5] organisms and objects,[6] and objects and their environment.[7] Microbiome analysis meanwhile is a well-established approach in biology and medical studies.[6] On the level of the human individual, microbiomes provide insights in the personal health status and possible causes of dysfunctions. On the community level, the microbial landscape and microbial load on public places are used as an indication for the health risk given through the identification of certain bacteria or other microbes.[8] Cities possess a consistent "core" set of non-human microbes, which reflect important features of cities and city-life.[9] With the growing potential to detect a "molecular echo" of an individual's microbiome on touched surfaces,[10,11] which could lead to substantial progress in the scientific analysis of forgery and illicit traffic cases in the field of cultural heritage,[1] new ethical and legal aspects need to be considered.[12]

In the field of heritage science, microbiome analyses are used to identify and assess deterioration processes in porous building materials or cellulose-based substrates, parchment, and paintings.[13] So, for example, halophilic microorganisms, involved in the biodeterioration of historic building materials, such as brick and paint coating, have been identified.[14] Specific genera such as Aeromonas present on canvas and panel paintings can be potentially responsible for deterioration and fading of paint layers.[15] Microbial biofilms are known to cause health issues and also to catalyze deterioration processes.[16]

Studies of microbiomes on smaller, mobile heritage objects are still rare. Some are e.g., dealing with health risk of handling heritage objects[17] and the microbial profile on historical books and archives (Mikrobib project). In a study on smuggled marble sculpture of unknown

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Table 1. Overview of sampled objects

Location	Collection	Object	# samples	Contrast
Museum für Naturkunde	Fossil Collection	Tendaguru (dinosaur bone)	29	Touched/untouched
Museum für Naturkunde	Mollusc Collection	Triton horn, mussel shells, chiton	12	Touched/untouched
Pergamonmuseum	Vorderasiatisches Museum	Ishtar gate	27	Height profile, material, exposed/unexposed to visitors
Pergamonmuseum	Vorderasiatisches Museum	Procession Street	8	Height profile, material, exposed/unexposed to visitors
Pergamonmuseum	Vorderasiatisches Museum	Sam'al gate lions	27	Material, exposed/unexposed to visitors
Pergamonmuseum	Antikensammlung	Zeus Sosipolis Tempel, Hellenistic Hall	8	Height profile, material
Rathgen- Forschungslabor	Office items	Computer mouse/keyboard	4	Touched by different, identifiable individuals

origin, microbiome analysis was performed with the aim to reconstruct the history of the storage of the objects, to infer a possible relationship among them, and to elucidate their geographical shift.[18] In another study microbial isolates, commonly found in several environmental matrices of Korea, China, Thailand, and Japan, were confirming the eastern provenance of Kinkarakawa-gami wallpapers, unique works of art produced in Japan between 1870 and 1905.[19] In each of these cases, the microbiome being sampled is the product of ongoing or recent processes with the environment.

Here, we analyzed and assessed the microbiome on natural and cultural heritage objects in two museums, the Berlin Museum für Naturkunde (MfN) and the Pergamonmuseum (PM), part of the Stiftung Preussischer Kulturbesitz (SPK) and UNESCO World Heritage since 1999, at the crossroad of art and science, to answer the following questions:

Is there a robust microbial signature of the environment in which the objects are stored? How long does this signal perdure in the face of handling efforts, or visitor exposure, which may produce different biological signals? Are there any indications for interactions between the general public or (living) curators with these objects? By combining data science techniques with microbial sampling approaches, we obtained and interpreted the microbial profile from heritage objects to generate a reliable baseline for decoding new knowledge in the field of heritage science.

RESULTS

16S rRNA sequencing captures microbial profile of museum objects

To assess the feasibility of detecting interactions between museum objects and their environment, minimally invasive microbial analysis of museum objects from the Pergamonmuseum (PM) and Museum für Naturkunde (MfN) in Berlin was conducted through swabbing. We selected museum objects of varying size and materials and in various locations (exhibition, collection, workshop), which allowed sampling of areas with variable exposure to human contact (Table 1).

Samples were collected during two sampling rounds in October 2020 and 2021. The microbial biomass residing on the surface of the heritage objects was sampled using aseptic technique and a swabbing method compatible with the conservation of heritage objects.[20] A total of 126 samples from unique museum objects and controls were collected.

Samples were subsequently subjected to 16S rRNA amplicon sequencing. To keep bioinformatic discovery parameters consistent, raw sequencing data from both sampling rounds were merged and pre-processed together (Figures 1A and S1). The resulting processed count tables quantify the abundance of Amplicon Sequence Variants (ASV), which represent DNA sequences detected in each sample. Each ASV feature may or may not be assigned to a phylogenetic tree with variable resolution.

On average, each sample contained a total of 31,446 reads with 194 unique ASVs detected. Given that many samples contained a relatively low DNA content, we used a set of negative controls to define quality control filtering criteria. These negative controls included samples taken of air or included only various library preparation reagents. These negative control samples are particularly important in low yield settings because of concepts such as the 'kitome,' which represents the microbial contamination of commonly used lab reagents and may produce false signal biasing downstream analysis.[21]

While the negative control samples showed a large range of total reads, only a single negative control sample showed more than 100 unique ASV features detected (Figure 1B). Therefore, we used the number of unique ASV features detected in combination with total reads as filtering criteria. A total of 47 samples were removed because they had less than 500 total reads or less than 100 unique ASV features detected. Downstream analysis was focused on a total of 79 samples excluding negative controls.

To explore the data, we applied unsupervised dimension reduction using principal component analysis to the 79 samples passing quality control filtering (Figure 1C). We observed that the first principal component separated samples taken during the first and the second sampling round indicating the presence of 'batch effects.' Batch effects represent nuisance factors commonly observed in molecular data.[22] Therefore, we limited most comparisons to within each sampling round. Investigating the phylogenetic makeup of the microbial features revealed that the majority of reads originated from phyla of *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Figure 1D).

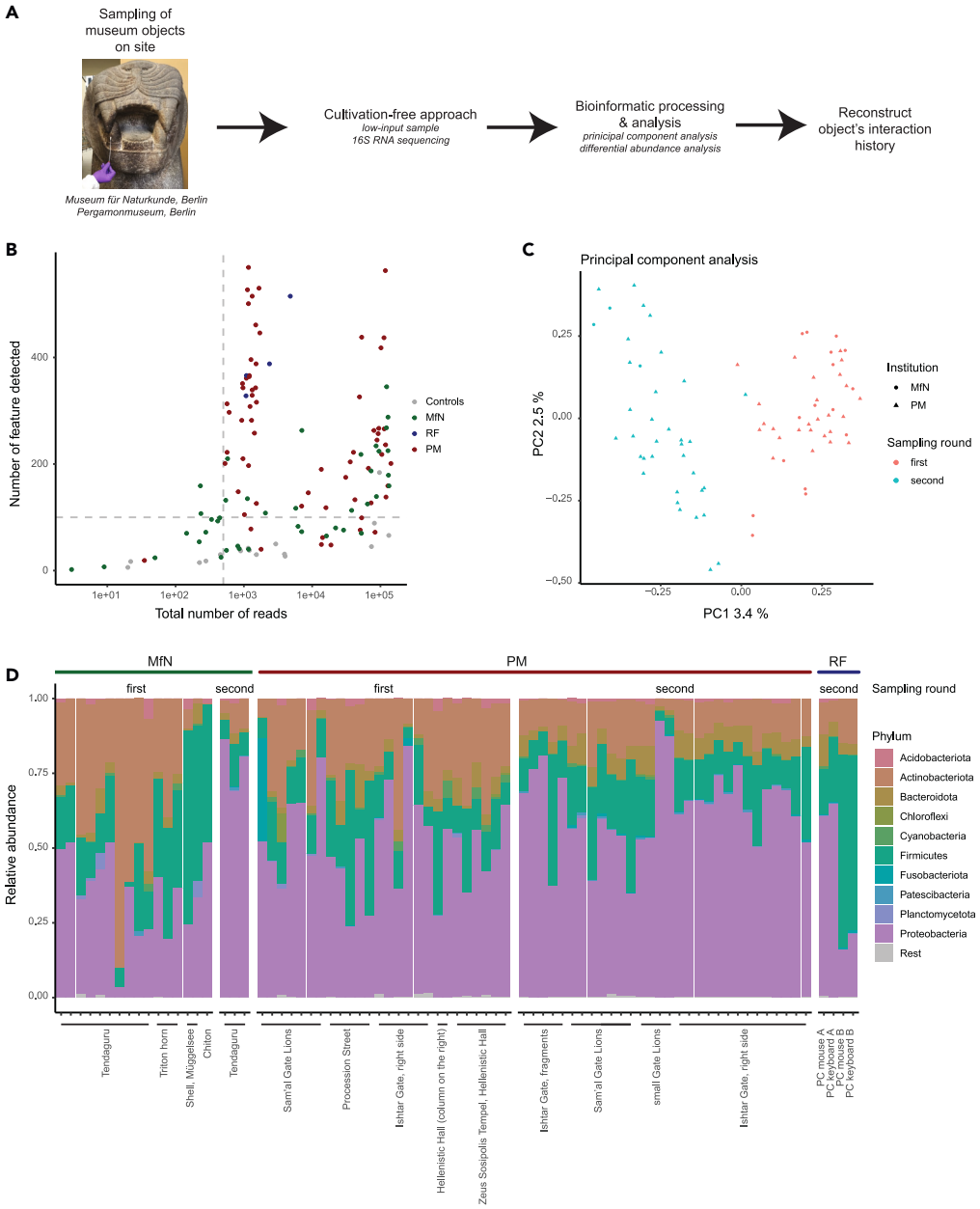

Figure 1. 16S rRNA gene sequencing captures microbial profile of museum objects

16S rRNA gene sequencing captures microbial profile of museum objects.

(A) Workflow overview.

(B) Scatterplot shows the total number of reads (x axis) and number of unique ASV features detected (y axis) across all samples and controls. Samples taken at the MfN, PM, and RF are colored green, red, and blue, respectively. Negative control samples are colored gray. Gray dashed lines indicate quality control thresholds.

(C) Principal components 1 (x axis) and 2 (y axis), and their respective percentage of explained variance, stratify samples and controls. Samples taken during the first and second sampling rounds are colored red and blue, respectively.

(D) Barplot shows relative abundance of microbial phyla (y axis) across all samples passing quality control filtering (x axis). Colors represent the ten most frequent phyla. See also Figure S1 and Table S1.

Microbial fingerprint separates touched and untouched natural history objects

We first analyzed natural history objects from the MfN obtained during the first sampling round. These objects consisted of three dinosaur bones from Tendaguru (Lindi region, Tanzania) from the fossil collection. In addition, one shell of a Triton ("Triton horn," Ranellidae), one Green Chiton (*Chiton olivaceus*), and one mussel shell collected in the Lake Müggelsee near Berlin were selected from the mollusk collection

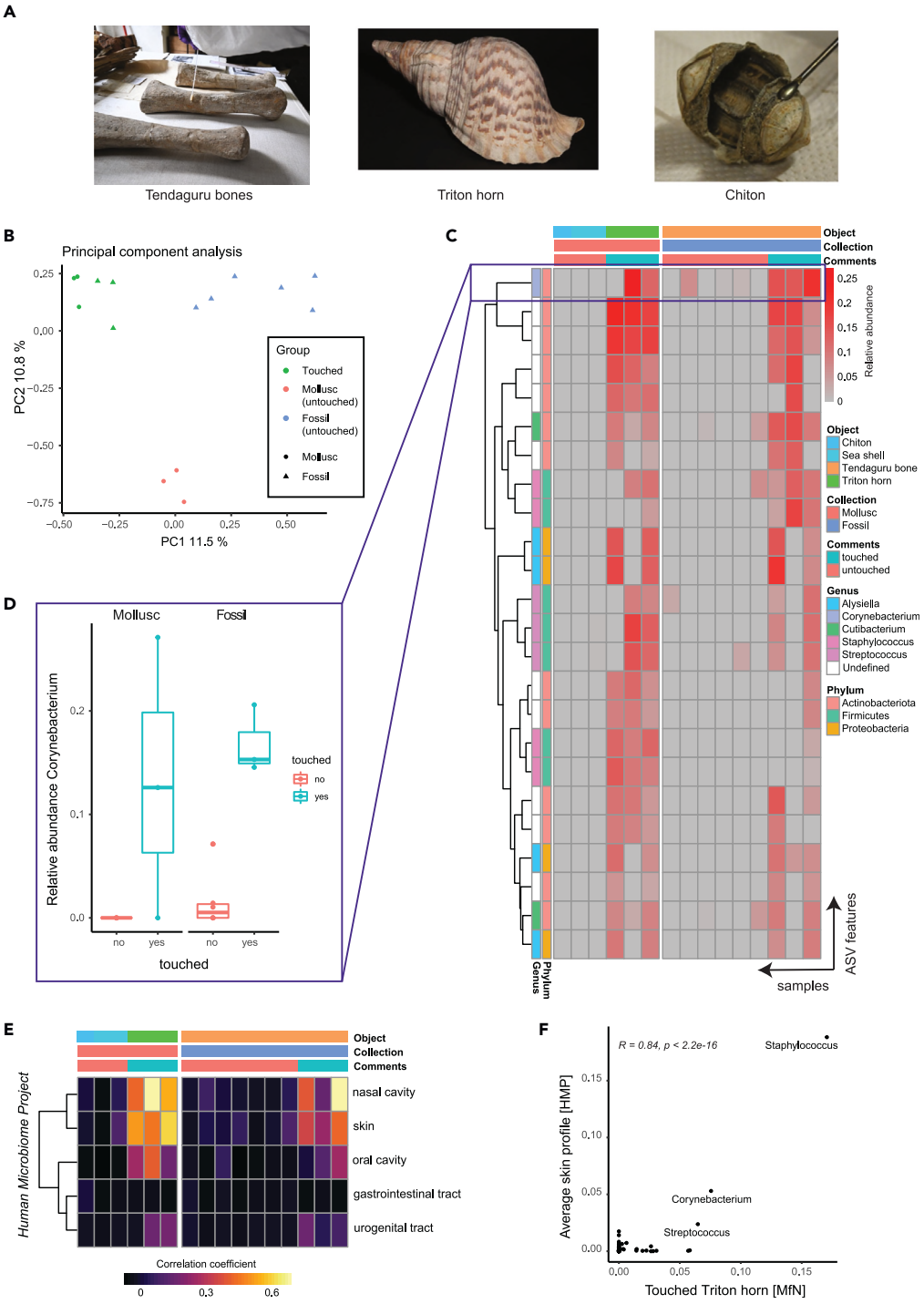

Figure 2. Microbial fingerprint separates touched and untouched natural history objects

(A) Photos shows sampled natural science museum objects.

(B) Principal components one (x axis) and two (y axis) separate touched (green) from untouched mollusks (red) and fossils (blue). Numbers represent the proportion of variance explained by each component. Colors and shapes represent touch status and collection, respectively.

(C) Heatmap illustrates abundance of ASV features (rows) significantly positively associated with touch status across samples (columns). Gray and red colors represent low and high abundance values.

(D) Boxplot displays the abundance of an ASV feature assigned to *Corynebacterium* at the genus level across touched (blue) and untouched (red) samples for both mollusks (left) and fossils (right). This genus is known to be highly abundant in human skin. The box represents the interquartile range, the horizontal line in

Figure 2. *Continued*

the box is the median, and the whiskers represent 1.5 times the interquartile range. Purple boxes mark data in heatmap panel C corresponding to data shown in panel D.

(E) Heatmap illustrates average correlation of genus level abundance between HMP body location (rows) and mollusk and fossil samples (columns). Colors represent Pearson correlation.

(F) Scatterplot displays strong correlation of genus level abundance of touched Triton horn (x axis) and the average HMP skin profile (y axis). Statistical significance was assessed using Pearson correlation. See also Figures S2–S4.

(Figure 2A). A total of 15 samples passed quality control filtering and were included in this analysis. Some of these objects were commonly used as hands-on examples for visitors, while others were stored in the archives away from human contact. Thus, some objects were frequently handled (touched) by visitors and curators while others remained largely without direct human interaction (untouched). Therefore, these museum objects provided the opportunity to ask questions about the relationship between the object's environment and its microbiome composition.

Unsupervised dimension reduction using principal component analysis revealed that frequently touched objects from both mollusk and fossil collections converged into a single cluster while untouched mollusk and fossil objects clustered separately (Figure 2B). These results suggested that the microbial profiles of untouched mollusk and fossil objects were distinct but upon touch converged onto an identical microbial profile.

Next, we set out to discover the taxonomic identities of the ASV features driving the separation between touched and untouched objects. To achieve this goal, we performed a differential abundance analysis comparing the abundance of each ASV feature between the objects that were touched and those that were left untouched. A total of 24 ASV features were significantly positively associated with touch status (multivariate regression, FDR <25%, Figure 2C). For example, an ASV feature assigned to *Corynebacterium* at the genus level was almost exclusively detected in both touched mollusk and fossil objects compared to untouched samples (Multivariate regression, $p < 0.001$, Figure 2D). We validated our statistical analysis by comparison with a commonly used differential abundance method,[23,24] which demonstrated high levels of correlation (Figure S2, Pearson Rho 0.8, $p < 2.2e\text{-}16$).

Given that we observed a strong effect of touch on the composition of the microbiome of these museum objects, we derived a touch signature by calculating the proportion of reads assigned to any of these 24 ASV features. This signature robustly separated touched from untouched mollusk and fossil objects (Figure S3). We refer to this signature, and analogous derivations, which distinguish two groups of samples as microbial fingerprints throughout this manuscript.

Importantly, several features in this touch signature were previously linked to the human skin microbiome.[25] In particular, *Corynebacterium*, *Staphylococcus*, and *Streptococcus* were among the most abundant bacterial genera of the human skin. Our taxonomic classifier was able to assign genus level information for 14 out of the 24 ASV features included in our signature. Eight of those 14 ASV features were derived from one of these three genera associated with human skin.

To further validate the hypothesis that the microbial touch signature was linked to the microbiome of human skin, we analyzed publicly available data from the Human Microbiome Project (HMP). We downloaded processed 16S rRNA data spanning 2,970 samples taken from six major human body sites. While sampling sites did not include the hand, human skin was represented by sampling at the left and right antecubital fossa (inner elbow) and retroauricular crease (behind the ear). To enable comparison between our own dataset and the HMP data, raw counts were aggregated at the genus level and normalized. Next, we calculated the correlation between normalized abundance across 79 genera that were detected in both datasets for all pairwise comparisons. The correlation between mollusk and fossil objects and the HMP data was greater in touched compared to untouched objects (Figure S4). Indeed, the strongest average correlations were observed for HMP skin and nasal cavity samples (Figure 2E). For example, the genera *Staphylococcus*, *Corynebacterium*, and *Streptococcus* showed high abundance levels in both the average HMP skin profile and a touched Triton horn sample (Figure 2F). Taken together, these results suggest that the microbial touch signature we identified on the museum objects may indeed represent direct, e.g., skin, or indirect, e.g., breath, exposure to human interaction.

Microbial fingerprint reflects exposure to human contact in cultural artifacts

We next sought out to investigate the microbial profile of cultural heritage objects from the Pergamonmuseum. The Ishtar Gate was a grand entrance to the ancient city of Babylon. The gate was built during the reign of King Nebuchadnezzar II in the 6th century BCE and was decorated with glazed brick reliefs depicting dragons, bulls, and lions. Only small fragments were found during the excavation by the Deutsche Orient-Gesellschaft (German Oriental Society) from 1899 to 1917 in Babylon. Friedrich Rathgen, first Director of the Rathgen-Forschungslabor, planned and carried out an ambitious desalination campaign for the fragments, before the animal reliefs were re-assembled, supplemented with modern glazed bricks, and presented to the public when the Pergamonmuseum opened in 1930.

We first compared a total of 10 samples, taken of ceramic tiles at varying heights ranging from 1.2 to 7.6 m (Figure 3A). Following principal component analysis of this subset of samples, we discovered a significant association between the first principal component and height, indicating that the microbial profile of the tiles varied with height in a continuous manner (Pearson correlation, Rho: 0.98, P: 5.8e-7) (Figure 3B). Of note, no significant association was observed between principal components one and two with either color (blue versus yellow) or illustration of the tiles (dragon or bull) (Figure S5). This is of special interest, as often blue tiles are not original, but so-called "Nachbrandziegel," dating to the 20th century time of reconstruction of the gate.

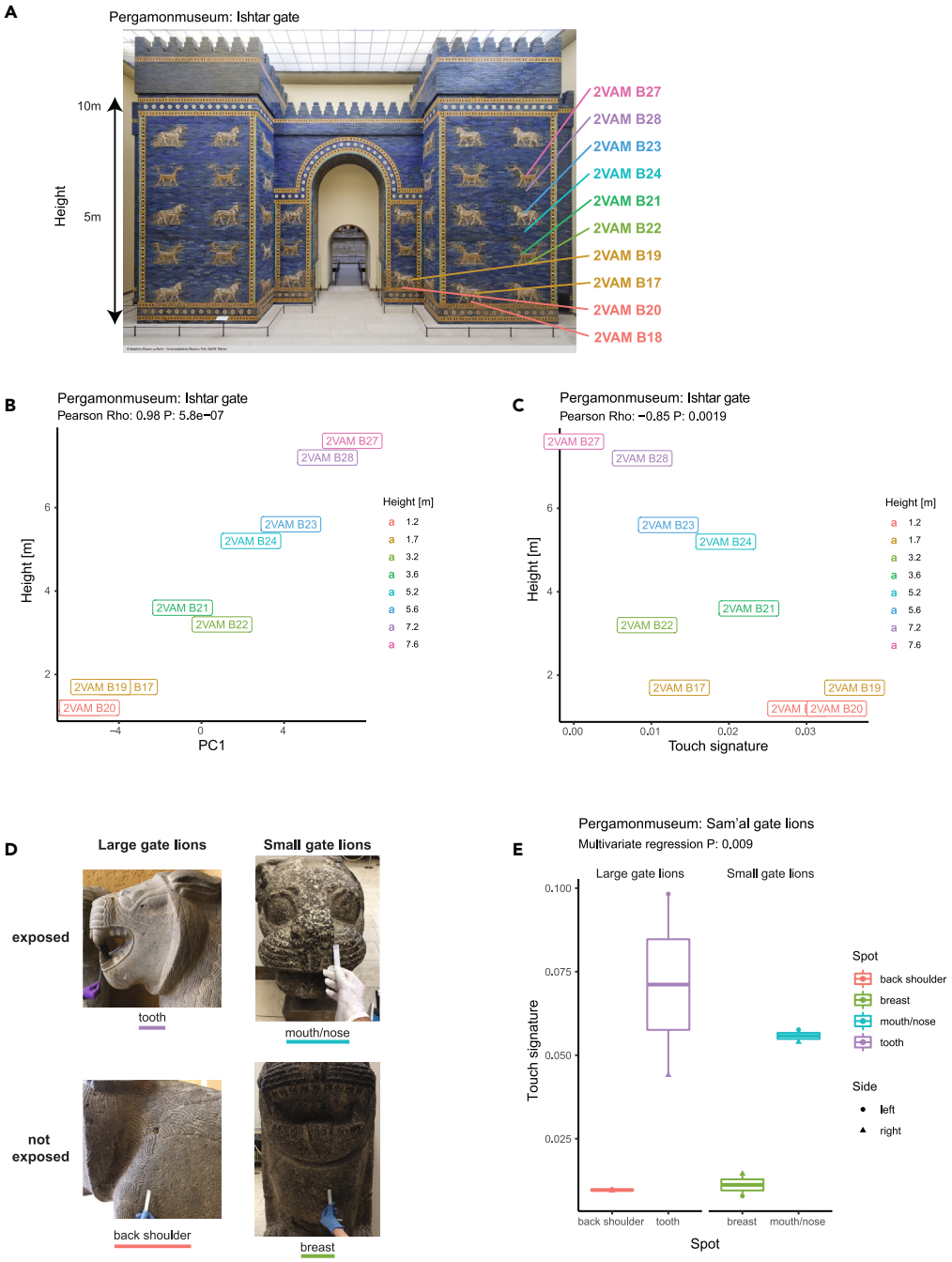

Figure 3. Microbial fingerprint reflects exposure to human contact in antiquity objects

Microbial fingerprint reflects exposure to human contact in antiquity objects.

(A) Photo shows sampling locations on Ishtar gate. Photo credit: Staatliche Museen zu Berlin - Vorderasiatisches Museum, Olaf M. Teβmer.

(B) Principal component one (x axis) correlates with height (y axis).

(C) Touch signature (x axis) correlates negatively with height (y axis). For B) and C), Samples are colored by height and statistical significance was assessed using Pearson correlation.

(D) Photos depict sampling spots with varying degree of exposure to human contact for both large and small gate lions Photo credit: Rathgen-Forschungslabor, Stefan Simon.

(E) Boxplot illustrates differences in touch signature levels (y axis) across various sampling areas of gate lion sculptures (x axis). Color represents sampling areas and shape represents the side of the museum hallway where the sculpture is located. The box represents the interquartile range, the horizontal line in the box is the median, and the whiskers represent 1.5 times the interquartile range. Multivariate linear regression model was used to assess statistical significance. See also Figures S5–S7.

While no single ASV feature passed significance filtering after multiple testing correction, the top 30 most strongly associated ASV features showed differential abundance with height (Figure S6). This observation suggests that the signal of any single ASV feature may not be strong enough to reach statistical significance given the limited sample size. However, the signal is enhanced and reaches statistical significance when many ASV features are aggregated together as captured by principal component one.

Given that lower tiles are within reach of the museum visitors, we hypothesized that there is an association between height and the exposure to human contact. As a proxy for exposure to human contact we used our touch signature derived from the analysis of mollusk and fossil objects. Indeed, the touch signature significantly decreased with height (Pearson correlation, Rho: −0.85, P: 0.002) (Figure 3C), indicating that lower tiles had greater exposure to human contact compared to higher tiles.

Additionally, we applied a Bayesian statistical approach called sourcetracker[26] to quantify the contribution of the touch signature to samples taken from the Ishtar gate. While unknown factors made up a large fraction, the relative contribution of touched microbial profiles was greater in lower tiles, and that of untouched microbial profiles greater in higher tiles. (Figures S6A and S6B).

Having identified an association with the touch signature and height of the Ishtar gate, we asked if we could identify variable exposure to human contact on a smaller antiquity object. Therefore, we analyzed a total of four stone sculptures of the Sam'al gate lions, a pair of small and large gate lions on each side of the museum hallway. The mouth/nose and the tooth area of the small and large gate lions, respectively, are exposed and may get touched by visitors. In contrast, the breast and back shoulder areas of the small and large gate lions, respectively, are less exposed and less attractive to physical interaction with visitors. (Figure 3D). Indeed, the touch signature was significantly higher for areas with greater exposure to visitors across both small and large gate lions (Multivariate regression, P: 0.009) (Figure 3E). Sourcetracker analysis[26] additionally confirmed increased relative contribution of microbial profiles associated with touched mollusk and fossil objects in areas with greater exposure to human contact (Figure S7C).

Microbial fingerprint separates objects touched by different persons

Taken together, these observations suggested that one of the major sources of variation in the microbial profiles of museum objects is the exposure to human contact. Next, we set out to test whether the microbial profile could be used to differentiate objects touched by two different persons. Previous studies have shown that personal possessions can be identifiable by their owner based on microbial signatures.[27–29] Therefore, we sampled personal office items including computer mouse and keyboard frequently and solely touched by two different subjects, called subjects A and B. These office items served as positive controls because we expect to find a high load of personal microbes on them which increases the sensitivity for distinguishing two subjects. In addition, each subject touched the surface of glazed ceramic tiles from the Ishtar gate with lower levels of exposure to visitors and the back shoulder of the Sam'al gate lions made of stone immediately prior to sampling for a period of 60 s. As an additional control, one tile from the Ishtar gate with high levels of exposure was touched by subject B prior to sampling.

Independent principal component analysis of these 11 samples revealed that the first principal component explained 16% of variance and separated objects based on whether it was touched by subject A or subject B (Figures 4A and 4B). Additionally, we applied Sourcetracker analysis[26] using the personal office items as source and the museum objects as sinks. Our analysis confirmed that the relative contribution of subjects A and B was greater in museum objects handled by subjects A and B, respectively (Figure S7). Moreover, the tile with exposure to visitors and touched by subject B, clustered with objects touched by subject B.

This observation suggests that the microbial signal associated with exposure to visitors was lost or overwritten by the microbial signature of subject B. The second principal component explained only 14% of variance and separated the office items from the museum objects (Figure 4C). Of note the office items consist of a cast polymer while the Ishtar gate tiles are made of glazed ceramic and the Sam'al gate lions are made of stone. Moreover, the museum objects are housed in the exhibition hall of the Pergamonmuseum while the office items come from a different location.

Next, we performed differential abundance analysis. We compared the abundance of ASV features between subject A and B while accounting for the material of each object using multivariate regression. Indeed, several ASV features were identified with significantly altered abundance in subject A compared to subject B (Multivariate regression, FDR <25%) (Figure 4D). For example, two ASV features assigned to *Corynebacterium* and *Enhydrobacter* at the genus level showed lower and higher abundance in subject A compared to B, respectively (Figures 4E and 4F). Of note, the differential abundance was robustly detected across multiple materials for the objects from the Ishtar gate, gate lion sculptures, and office items.

DISCUSSION

Taken together, our results demonstrate that human touch or exposure to human contact are major contributors to the variance in microbial composition observed on the surfaces of museum objects. Moreover, human touch impacts the microbial composition of museum objects to a degree that may enable distinction between objects touched by two different individuals in a short time correlation. Our results suggest that other variables including material, surface state paint, and location explain relatively smaller proportions of variance. These findings are in agreement with previous work which demonstrated that home surfaces harbor a microbial signature which can be predicted by their occupants and that supersedes inter-surface differentiation within the home.[30]

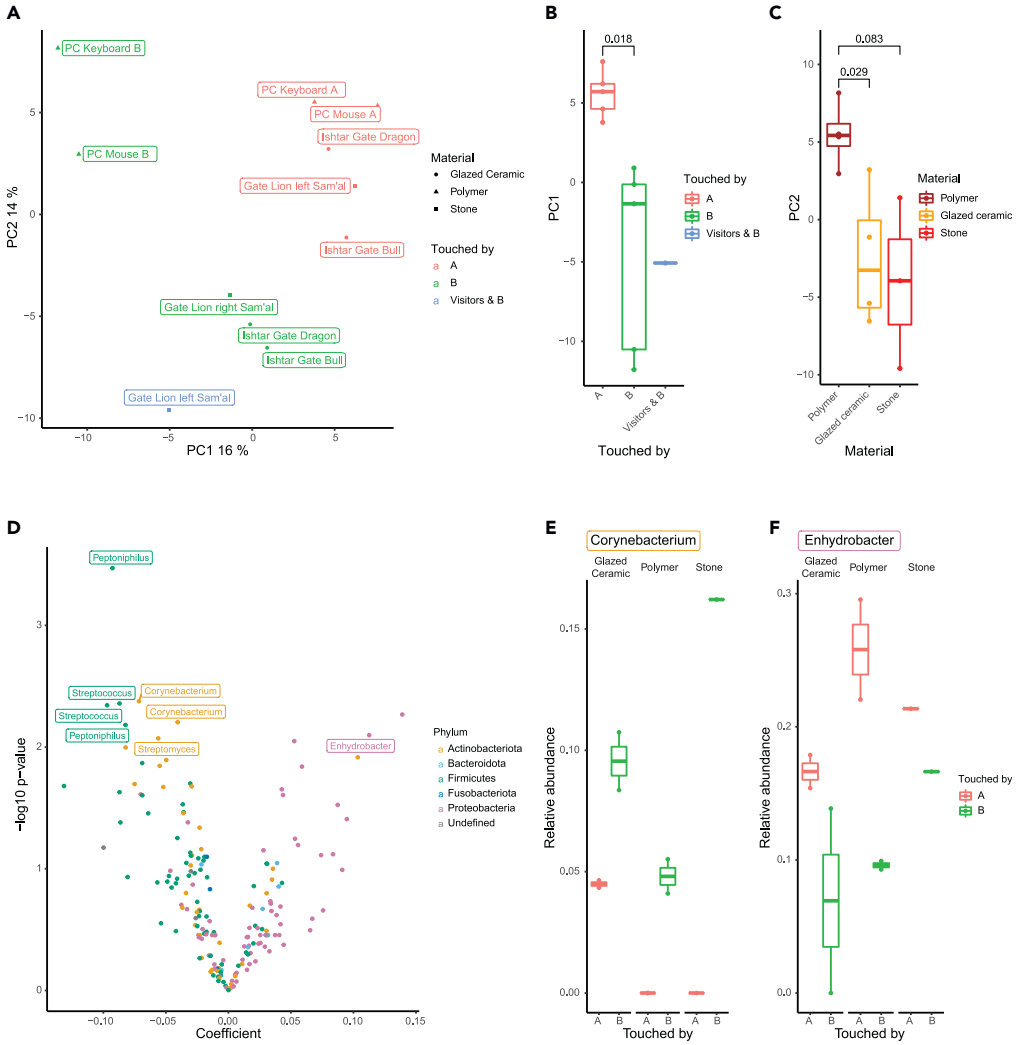

Figure 4. Microbial fingerprint separates objects touched by different persons

Microbial fingerprint separates objects touched by different persons.

(A) Principal components 1 (x axis) and 2 (y axis) separate objects touched by different subjects. Colors represent which subject touched the object. Shape represents the material of the object.

(B) Boxplot shows the principal component one (y axis) stratified by which subject touched the object (x axis). For all boxplots, the box represents the interquartile range, the horizontal line in the box is the median, and the whiskers represent 1.5 times the interquartile range.

(C) Boxplot shows principal component two (y axis) stratified by the material of the object (x axis). For B) and C), p values are derived from two-sided T-tests.

(D) Volcano plot shows the coefficient (x axis) derived from the multivariate regression model and the -log10 transformed p value (y axis) for ASV features. Colors represent Phylum. Available Genus information for top 10 ASV features is displayed. Positive coefficient values indicate higher abundance of ASV features in subject A compared to B.

(E and F) Boxplots depict differences in abundance of exemplary ASV features. Abundance of individual exemplary ASV features assigned to *Corynebacterium* and *Enhydrobacter* at the Genus level is greater and lower in subject B compared to subject A, respectively. See also Figure S7.

Limitations of the study

Given the requirement of minimally invasive sampling methodology, we applied dry nylon swabs to sample the objects. Thus, the amount of DNA obtained from each sample was at the low yield range near the limit of detection. Indeed, we removed 47 (38%) samples which did not pass our relatively lenient filtering criteria. Therefore, the biological signal captured is most likely also limited by the sampling methodology.

The natural heritage collections comprising both touched and untouched mollusk and fossil specimens have provided an opportunity for us to determine a microbial signature that is associated with human skin and nasal cavity profiles. Of note, the HMP dataset did not include samples taken of the human hand, the most likely source of direct contact between museum objects and human skin. Additionally, previous studies have demonstrated high levels of microbial variability on the human hand, particularly between individuals.[31] As a result, the derivation of hand-specific microbial signatures is a challenging task without clear indications of who handled the objects and limited sample size.

Consequently, our signature is not exclusively indicative of touch by the human hand, but rather serves as a proxy for exposure to human contact. Increase in this signature was detected on areas of cultural heritage objects with greater exposure to visitors, confirming its validity. Of note, the touch signature demonstrated robustness across heterogeneous objects, materials, locations, as well as multiple sampling and sequencing rounds.

On an architectural monument like the Ishtar gate in the PM, these results suggest that the microbiome composition reflects the exposure to human interaction. We cannot be certain whether, and if, how excessively, visitors actually touch the monument surface, but a greater level of exposure in lower compared to higher tile levels is obvious. However, even at a smaller physical scale, differentially exposed areas carry distinct microbial profiles as demonstrated at the Sam'al gate lions.

These results suggest that microbial fingerprints can be used to measure, monitor, and eventually control exposure to human contact on museum objects.

Interestingly, both the mollusk and fossil objects lost their original microbial fingerprint upon touch. A similar observation was made with the Ishtar gate tiles exposed to visitors that gained the personal microbial fingerprint of subject B upon touch. These observations suggest that upon touch the previous interaction history of the object may get partially overwritten or even lost. In summary, our findings may have implications relevant to heritage provenance or forensic and criminological studies.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Materials availability
  - Data and code availability
- METHOD DETAILS
  - Sample collection
  - DNA extraction, 16S rRNA gene amplification and sequencing
  - Bioinformatic processing and quality control
  - Human Microbiome Project data
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Statistical analysis
  - Human Microbiome Project analysis
  - Sourcetracker analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107578.

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AUTHOR CONTRIBUTIONS

J.V. and S.S. obtained funding for the project. C.F., C.Q., and S.S. sampled heritage objects. C.F. and J.O. supervised wet lab procedures and contributed to microbiological data discussion. F.B. and A.M. performed wet lab experiments. C.M., D.H., L.R., and L.S. performed bioinformatic processing and data analysis. L.S. and S.S. wrote the manuscript. All authors read and contributed to the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the authors used ChatGPT in order to improve editing and writing style. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
16s rRNA sequencing	Human Microbiome Project[6]	https://www.hmpdacc.org/hmp/overview/
16s rRNA sequencing	This manuscript	BioPreoject: PRJNA975603
Software and algorithms		
R	R Core Team	4.0.5
QIIME2	Bolyen et al.[32]	2021.4.0
DESeq2 R package	Love et al.[23]	1.30.1
sourcetracker R package	Knights et al.[26]	https://github.com/danknights/sourcetracker
qiime2R R package	Bisanz	0.99.6
ggplot2 R package	Wickham et al.[33]	3.3.6
Analysis code	This paper	https://github.com/lkmklsmn/microbiome_in_museum

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Lukas Simon (lukas.simon@bcm.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- 16S rRNA amplicon sequencing data have been deposited at Sequencing Read Archive and are publicly available as of the date of publication. This paper analyzes existing, publicly available data. Accession numbers are listed in the key resources table.
- All original code has been deposited at Github and is publicly available as of the date of publication. DOIs are listed in the key resources table.

METHOD DETAILS

Sample collection

Sampling was carried out using a minimally invasive methodology previously optimized by Flocco et al.[20] at the Leibniz Institute DSMZ. All objects under study were surface-sampled on site with dry, nylon flocked swabs (Copan 552 C Brescia Italy). Although the use of wet swabs may increase the microbial biomass yield of the sampling procedure, such approach was excluded for this work, since moistening is not compatible with the conservation of several cultural heritage object selected for our study (Quye, A. and Strlič, M., 2019). In addition, the swab-moistening procedure adds one manipulation step which may increase the risk of introducing contamination.[20]

A series of objects exhibited or stored at three cultural heritage institutions in Berlin, Germany (Museum für Naturkunde, MfN; Pergamon-museum, PM; Rathgen- Forschungslabor, RF) were sampled. The objects were selected to reflect different degrees of exposure to human contact (Table 1). The objects from the MfN were classified into "touched" and "untouched" following the frequency of their use. "Touched objects" are those that have been handled frequently by researchers (e.g., specific significant fossils key for many different kinds of analyses) and/or touched by the public during "behind the scene" dedicated tours or open door days. "Untouched objects" are those that have only been touched once (to the best of our knowledge), during the event of their collecting. From that moment they stayed in their original package or in their cabinet until they were sampled for this project.

Briefly, for the first sampling round, 2 by 2 cm areas were swabbed for approximately 90 s. During the second sampling round at the PM areas of 0.3m^2 were swabbed for approximately 3 min. After each sampling, swabs were immediately placed in vials and transported to the DSMZ laboratories in Braunschweig and stored frozen at −20°C till further processing. The same procedure was applied to swabs without sample, which were used as negative controls. Also, controls for materials and reagents used in downstream processes were included.

DNA extraction, 16S rRNA gene amplification and sequencing

A workflow 'from swabs to sequence' for biomolecular analyses of cultural heritage objects, previously optimized by DSMZ researchers (Flocco et al. in preparation), was employed. The main steps and reagents are briefly described here. For the extraction of nucleic acids, swab samples were brought out of the freezer and allowed to thaw at room temperature. DNA was extracted using the QIAamp DNA Micro Kit, (Qiagen) selecting the protocol for isolation of genomic DNA from tissues and proceeding according to manufacturer's instructions. DNA was quantified using the Quant-iT™ Picogreen dsDNA reagent and kit (Invitrogen) according to the manufacturer's instructions. The 2-step PCR amplification targeted the V3–V4 region of the 16S rRNA gene with specific primers which also contained an adaptor and barcode sequence. PCR products were purified using the Nucleospin® Gel and PCR clean up kit (Machery-Nagel), according to manufacturer instructions, followed by quantification using the Qubit™ dsDNA high sensitivity assay kit (Invitrogen) and pooled equimolarly. The 16S amplicon library pool was cleaned and concentrated using the AMPure XP magnetic beads (Beckman Coulter) and re-quantified using the mentioned Qubit assay. Sequencing was performed using the MiSeq sequencing technology (Illumina Inc., LA Jolla, CA) generating paired-end sequences with 300 base pairs per read.

Bioinformatic processing and quality control

Next generation sequencing reads were processed using the QIIME2 software.[32] A QIIME2 object combining reads from all samples from both sampling rounds was created using the *qiime tools import* command. Next, paired-end reads were joined using the *qiime vsearch join-pairs* command.[34] Reads were filtered based on quality scores and the presence of ambiguous base calls using the *qiime quality-filter q-score* command.[35] Data was subsequently denoised using the *qiime deblur denoise-16S* command.[36] Taxonomic classification was performed using the SILVA database (release 132)[37] and the *qiime feature-classifier classify-sklearn* command.

Samples with less than 500 total reads or less than 50 unique ASV features detected as well as negative controls were removed from subsequent analysis. The final Qiime2 object after quality control filtering contained a total of 3,184,281 reads across 5,787 unique ASV features and 79 samples. Data was normalized by calculating the square-root of the proportion of total reads assigned to each ASV feature.

Human Microbiome Project data

Data was downloaded from the HMP website (www.hmpdacc.org). The count matrix consisted of 45,383 ASV features and 2,970 samples. Samples were taken from 18 different body sites divided into 5 major categories. Data was normalized and the proportion of reads classified at genus level was calculated. A total of 79 genera were present in both the HMP dataset and our dataset of museum objects.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Statistical analysis was performed using the R programming language making use of the qiime2R and phyloseq[38] packages. Qiime2 data was loaded into R using the *read_qza* function of the qiime2R package.

We applied independent dimension reduction for various subsets of samples using principal component analysis. For each principal component analysis, ASV features were subset to those detected in at least two samples. Principal component analysis was performed using the *prcomp* function and the scale parameter set to "TRUE."

Differential abundance analysis was performed using multivariate regression as implemented in the *lm* function. The normalized ASV abundance was used as the dependent variable of the model, while the independent variables were derived from the metadata. p-values were corrected for multiple testing using the *p.adjust* function with the method parameter set to "BH."

The touch signature was calculated as the proportion of reads derived from the 24 ASV features that were significantly positively associated with touch status in the mollusc and fossil objects.

Data visualizations were generated using the ggplot2,[33] pheatmap, and gridExtra R packages.

Human Microbiome Project analysis

Data was downloaded from the HMP website (www.hmpdacc.org). The count matrix consisted of 45,383 ASV features and 2,970 samples. Samples were taken from 18 different body sites divided into 5 major categories. Data was normalized and the proportion of reads classified at genus level was calculated. A total of 79 genera were present in both the HMP dataset and our dataset of museum objects. Human Microbiome Project analysis. Pearson correlation was calculated across these 79 genera between all HMP samples and museum objects. For visualization purposes correlations were averaged with each major body site category.

Sourcetracker analysis

Sourcetracker[26] was downloaded from Github (https://github.com/danknights/sourcetracker). We followed the analysis workflow described in the example.R script. Briefly, *sourcetracker* predicts the source of microbial communities in a set of input samples, called sinks. Touched and untouched natural heritage objects from the MfN were defined as sources and the cultural heritage objects from the Pergamonmuseum as sinks. In addition, we trained *sourcetracker* on personal office items and applied it to cultural heritage objects touched by two different subjects to predict which subject had touched the object. For this analysis, the personal office items touched by subject A and B were defined as sources and the cultural heritage objects as sinks.