RECONSTRUCTION OF ORAL MICROBIOMES FROM EXTINCT AND EXTANT ANTHROPOIDS THROUGH ANCIENT DNA

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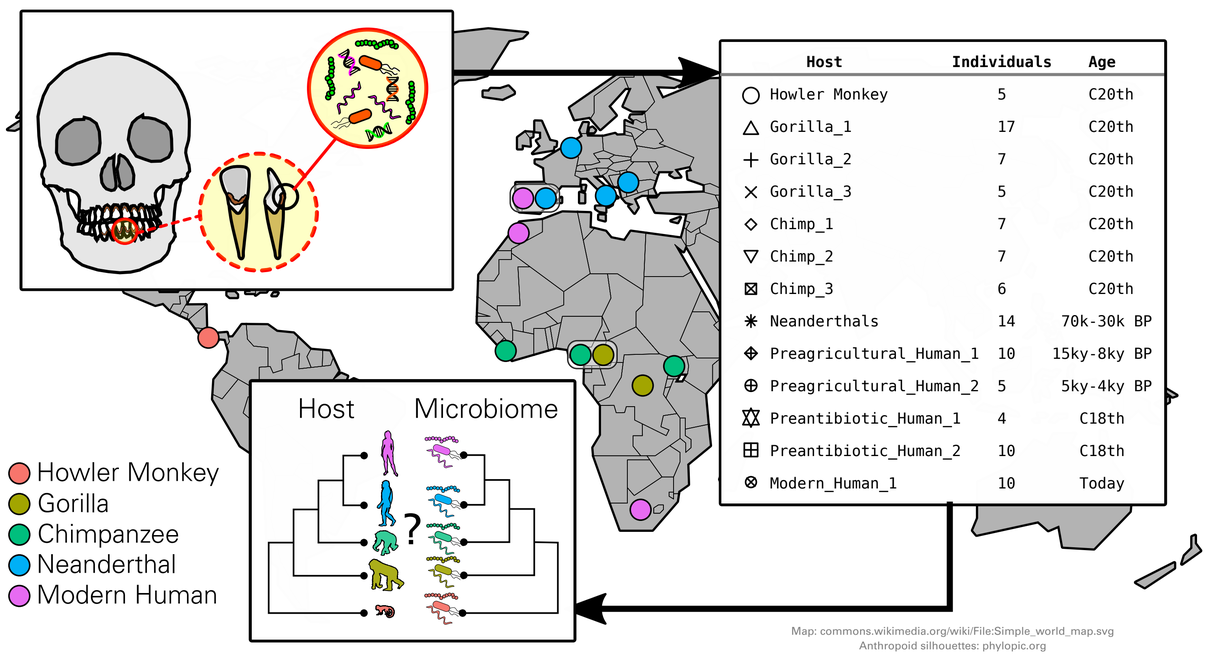
1. Background

Modern microbiome research has shown the importance of our microbial communities in health and disease, yet has mainly focused on the evolution of the gut microbiome of either Western industrialised societies or captive animals. In contrast to sampling from live individuals, **dental calculus from archaeological remains,** which has been shown to be a rich source of well preserved bacterial DNA**, presents an opportunity to 'non-invasively' study the oral microbiome from a wider diversity of species** and populations. To explore the potential of calculus microbiomes to **study the co-evolution of anthropoids and their microbiomes**, we assessed the following:

1. What is the range of preservation of oral microbiomes in different archaeological individuals?
2. Are there any phylogenetic correlations between the plaque microbiomes of different anthropoid taxon at both compositional and genomic levels?
3. What are the main taxonomic drivers of any observed differences between each host taxon?



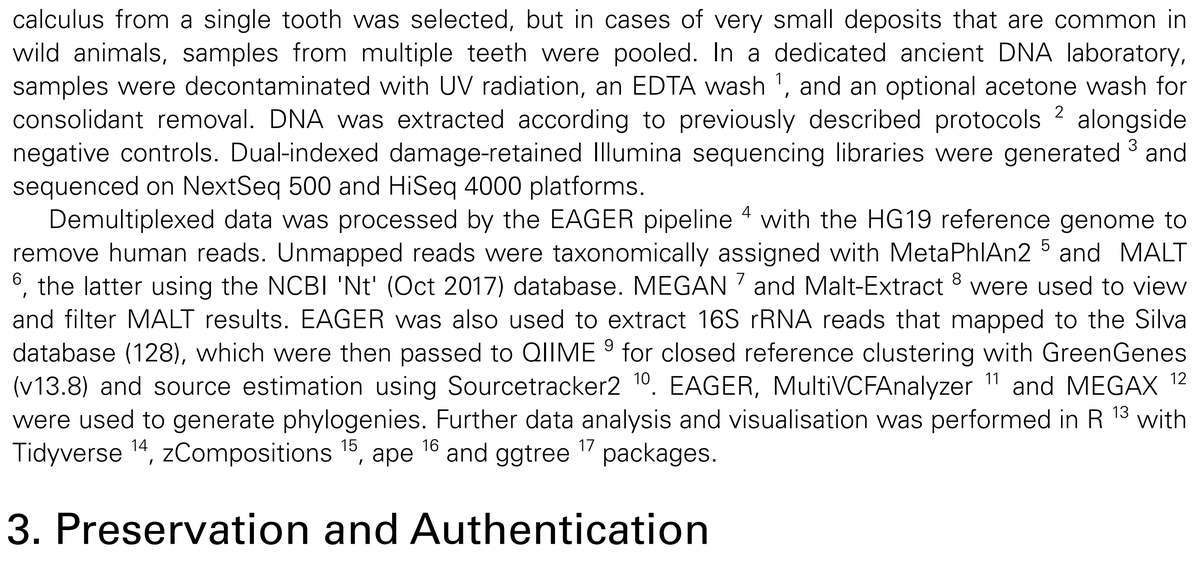
2. Methods

Dental calculus samples were removed from teeth using a sterile dental scaler. The largest dental calculus from a single tooth was selected, but in cases of very small deposits that are common in wild animals, samples from multiple teeth were pooled. In a dedicated ancient DNA laboratory, samples were decontaminated with UV radiation, an EDTA wash [1], and an optional acetone wash for consolidant removal. DNA was extracted according to previously described protocols [2] alongside negative controls. Dual-indexed damage-retained Illumina sequencing libraries were generated [3] and sequenced on NextSeq 500 and HiSeq 4000 platforms.

Demultiplexed data was processed by the EAGER pipeline [4] with the HG19 reference genome to remove human reads. Unmapped reads were taxonomically assigned with MetaPhlAn2 [5] and MALT [6], the latter using the NCBI 'Nt' (Oct 2017) database. MEGAN [7] and Malt-Extract [8] were used to view and filter MALT results. EAGER was also used to extract 16S rRNA reads that mapped to the Silva database (128), which were then passed to QIIME [9] for closed reference clustering with GreenGenes (v13.8) and source estimation using Sourcetracker2 [10]. EAGER, MultiVCFAnalyzer [11] and MEGAX [12] were used to generate phylogenies. Further data analysis and visualisation was performed in R [13] with Tidyverse [14], zCompositions [15], ape [16] and ggtree [17] packages.

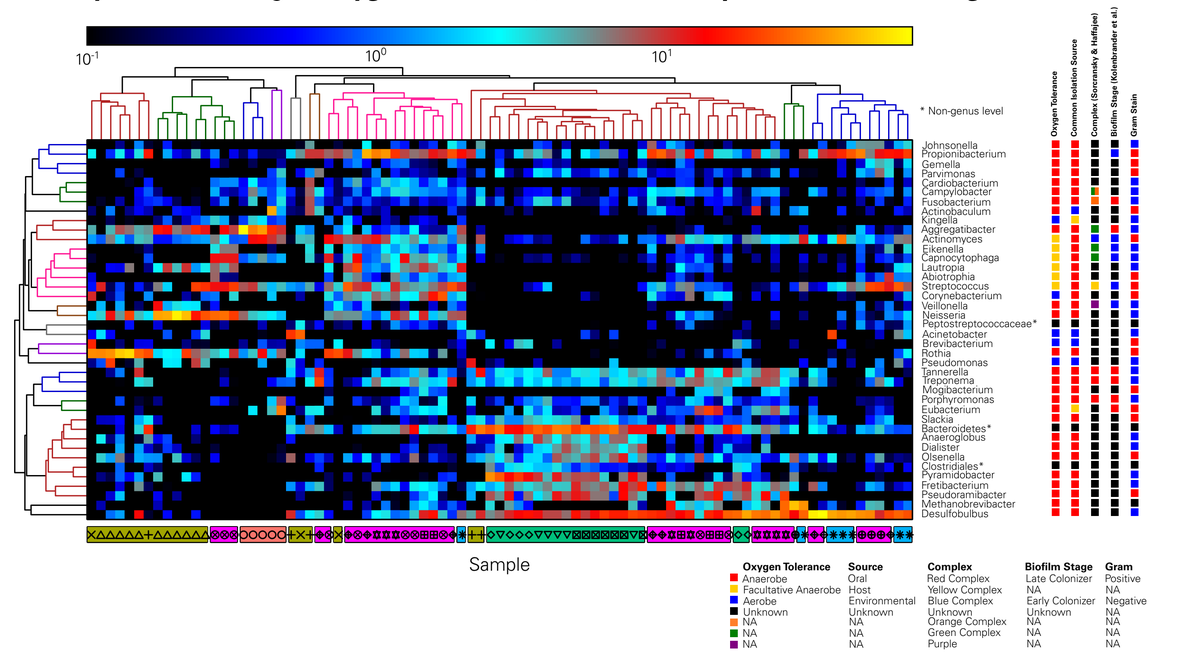
3. Preservation and Authentication

*Mappings to a 16S rRNA gene database show **consistent assignment to human plaque genera at varying amounts, in different hosts** and ancient samples. **Middle Palaeolithic samples can yield oral taxa with** characteristic patterns of **authentic aDNA:** damage, short reads, and low edit distances.*

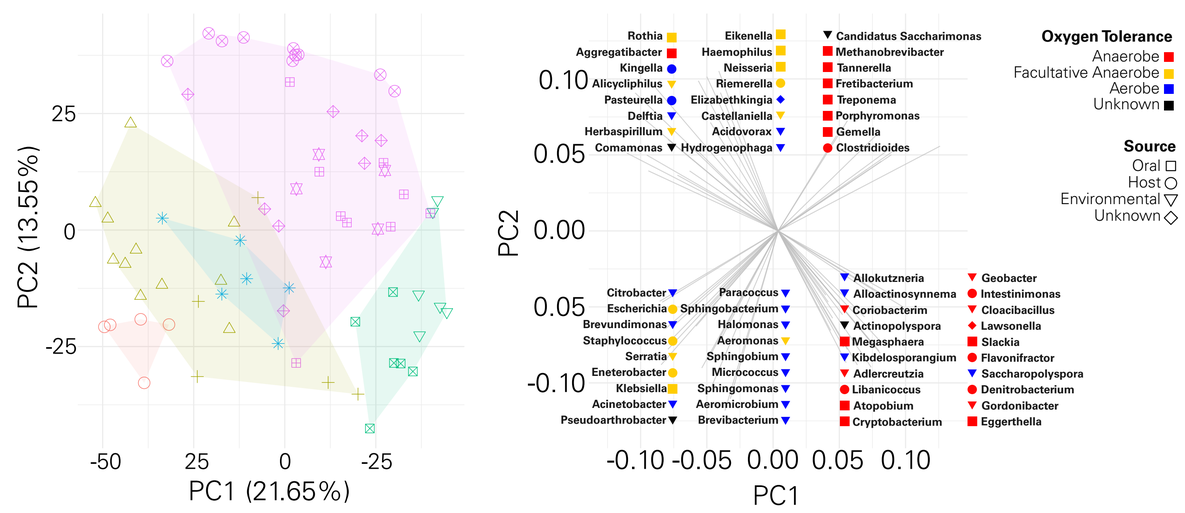


4. Composition

*Shotgun-derived marker gene heatmap shows a **compositional tendency to cluster by host**, but **also by** factors relating to **oxygen tolerance, microbial complex and biofilm stage**.*

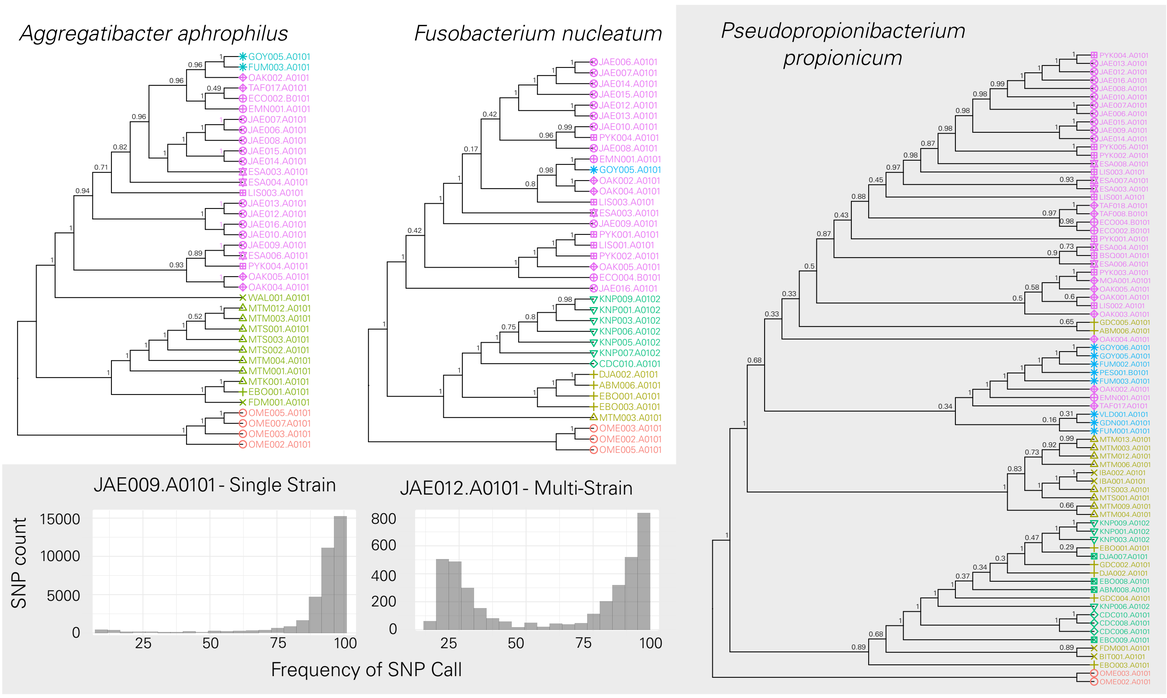


***PCA** of CLR transformed data [18] **shows clustering by host genus**. PC1 variance is also described **by oxygen tolerance**. PC2 is **partially driven by environmental vs oral taxa**.*



5. Phylogeny

*Low coverage SNP-based whole-genome **phylogenies of oral taxa often recapitulate relationships of host genera. Cross-mapping from related strains** makes more **sophisticated analysis difficult**.*



6. Implications

1) Authentic remnants of the **oral microbiota can be recovered in dental calculus from a 70,000 year old Neanderthal** individual, despite variable preservation across archaeological individuals.
2) Compositional analysis shows **clustering of oral microbiomes by host genus**. Oxygen tolerance and source factors require investigation into environmental contamination and reference sequence bias.
3) Low resolution (>=1x, >10=%) oral taxa **genome phylogenies appear to recapitulate that of the host genera**. Development of strain separation techniques from short read data is required.

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