**Supporting Data for “The challenges of reconstructing tropical biodiversity with sedimentary ancient DNA: a 2200-year-long metagenomic record from Bwindi Impenetrable Forest, Uganda”**

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**The “Bwindi\_Samples” folder contains summary data for all sediment samples and negative controls.**

**AgeDepthModel.pdf:** Age-depth model from BACON age model software. Hiatus was enforced at 642 cm.

**DataMatrix\_Core4A\_elephantDungSite.xlsx:** Complete elemental profiles for core 4A (elephant dung site).

**DataMatrix\_Core4A\_elephantDungSite\_selectedElements.xlsx:** Profiles of selected critical elements for core 4A (elephant dung site).

**DataMatrix\_Core5A\_elephantWallowSite.xlsx:** Complete elemental profiles for core 5A (elephant wallow site).

**DataMatrix\_Core5A\_elephantWallowSite\_SelectedElements.xlsx:** Profiles of selected critical elements for core 5A (elephant wallow site).

**DNA\_Degradation\_vs\_Environment.pdf:** Relationships between DNA read length, 5´ C🡪T, and 3´ G🡪A and environmental parameters for core MUB17-2C/D. The strength (Spearman’s ρ) and statistical significance (uncorrected *p*-values) of non-parametric correlations are given for each comparison.

**MasterCore\_DataMatrix.xlsx:** Complete elemental profiles for the master core.

**MasterCore\_DataMatrix\_selectedElements.xlsx:** Profiles of selected critical elements for the master core.

**Sediment\_length\_distributions.pdf:** Histograms of unique, merged sequence lengths from sediment and negative control samples. Negative controls with no sequencing surviving filtration (BWINDI-B2, BWINDI-LB1, BWINDI-LB3) were excluded. The low counts of reads between 132 and 148 bp is an artifact of the trimming and merging procedures.

**SedimentShotgun\_SummaryTable.xlsx:** Table summarizing metagenomic and DNA taphonomic results from all sediment sample and negative controls.

**The “MEGAN\_nt” folder contains output from the MEGAN analyses using the non-redundant nucleotide database (“nt”).**

**Normalized\_BWINDI\_Shotgun\_nt\_NeighbourNet.\*:** Neighbor-net analysis of normalized MEGAN results at ordinal level using Bray-Curtis distances. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Shotgun\_nt\_PCoA.\*:** Principle Coordinates Analysis (PCoA) of normalized MEGAN results at ordinal level using Bray-Curtis distances. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_nt\_Shotgun.megan:** Normalized MEGAN comparison of sediment megaBLAST identifications. Unidentified sequences were excluded. At least one sequence per taxon per sample was retained.

**Raw\_MEGAN\_nt:** MEGAN results (RMA6 format) for sediment and negative control megaBLAST identifications for each individual sample. Negative controls with no unique, merged reads surviving filtration (BWINDI-B2, BWINDI-LB1, BWINDI-LB3) were excluded. Due to the size of these files, they are only available on the Smithsonian Figshare repository (DOI 10.25573/data.10072625: <https://smithsonian.figshare.com>.).

**The “MEGAN\_Refseq” folder contains output from the MEGAN analyses using the Refseq Genomic database.**

**Normalized\_BWINDI\_Shotgun\_Refseq\_NeighbourNet.\*:** Neighbor-net analysis of normalized MEGAN results at ordinal level using Bray-Curtis distances. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Shotgun\_Refseq\_PCoA.\*:** Principle Coordinates Analysis (PCoA) of normalized MEGAN results at ordinal level using Bray-Curtis distances. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Refseq\_Shotgun.megan:** Normalized MEGAN comparison of sediment megaBLAST identifications. Unidentified sequences were excluded. At least one sequence per taxon per sample was retained.

**Raw\_MEGAN\_Refseq:** MEGAN results (RMA6 format) for sediment and negative control megaBLAST identifications for each individual sample. Negative controls with no unique, merged reads surviving filtration (BWINDI-B2, BWINDI-LB1, BWINDI-LB3) were excluded. Due to the size of these files, they are only available on the Smithsonian Figshare repository (DOI 10.25573/data.10072625: <https://smithsonian.figshare.com>.).

**The “MetaPhlAn2” folder contains output from the MetaPhlAn2 analyses.**

**MetaPhlAn2\_abundance\_heatmap\_order.\*:** Heatmap of sediment taxa abundances identified at the ordinal level. Image available in PNG and PDF formats.

**MetaPhlAn2\_abundance\_heatmap\_species.\*:** Heatmap of sediment taxa abundances identified at the species level. Image available in PNG and PDF formats.

**The “QIIME2” folder contains visualizations of the output from the QIIME 2 analyses.**

Due to the size of these files, the QIIME2 data are only available on the Smithsonian Figshare repository (DOI 10.25573/data.10072625: <https://smithsonian.figshare.com>.).

**core-metrics-50-bray-curtis.qzv:** PCoA using Bray-Curtis distances using phylogenetic information and rarefaction to 50 sequences.

**core-metrics-50-jaccard.qzv:** PCoA using Jaccard distances using phylogenetic information and rarefaction to 50 sequences.

**core-metrics-500-bray-curtis.qzv:** PCoA using Bray-Curtis distances using phylogenetic information and rarefaction to 500 sequences.

**core-metrics-500-jaccard.qzv:** PCoA using Jaccard distances using phylogenetic information and rarefaction to 500 sequences.

**core-metrics-nonphylo-50-bray-curtis.qzv:** PCoA using nonphylogenetic Bray-Curtis distances and rarefaction to 50 sequences.

**core-metrics-nonphylo-50-jaccard.qzv:** PCoA using nonphylogenetic Jaccard distances and rarefaction to 50 sequences.

**core-metrics-nonphylo-500-bray-curtis.qzv:** PCoA using nonphylogenetic Bray-Curtis distances and rarefaction to 500 sequences.

**core-metrics-nonphylo-500-jaccard.qzv:** PCoA using nonphylogenetic Jaccard distances and rarefaction to 500 sequences.

**taxabarplots.qzv:** Taxonomic compositions of samples using VSEARCH-clustered sequences.

**The “Taxonomic Profiles” folder contains taxonomic profiles from the sediment samples.**

**Normalized\_BWINDI\_Shotgun\_nt\_Archaea.\*:** MEGAN archaeal taxonomic profiles of sediment samples using the “nt” database. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Shotgun\_nt\_Bacteria.\*:** MEGAN bacterial taxonomic profiles of sediment samples using the “nt” database. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Shotgun\_Refseq\_Archaea.\*:** MEGAN archaeal taxonomic profiles of sediment samples using the Refseq Genomic database. Image available in SVG and PDF formats.

**Normalized\_BWINDI\_Shotgun\_Refseq\_Bacteria.\*:** MEGAN bacterial taxonomic profiles of sediment samples using the Refseq Genomic database. Image available in SVG and PDF formats.

**SedimentComposition\_MEGAN\_nt.pdf:** Sedimentary taxonomic profiles at domain-level of sediment samples versus sample depth. Analysis performed using MEGAN and the “nt” database. The category “Other” includes sequences assigned to clades including “cellular organisms” and “viruses”.

**SedimentComposition\_MEGAN\_Refseq.pdf:** Sedimentary taxonomic profiles at domain-level of sediment samples versus sample depth. Analysis performed using MEGAN and the Refseq Genomic database. The category “Other” includes sequences assigned to clades including “cellular organisms” and “viruses”.

**SedimentComposition\_QIIME2.pdf:** Sedimentary taxonomic profiles at domain-level of sediment samples versus sample depth. Analysis performed using QIIME 2.

**TaxaPresence\_Plants\_Metazoa.pdf:** Tables of presence/absence of plant (=Embryophyta) and animal (=Metazoa) taxa in the sedimentary DNA samples using the nt and Refseq databases.

**TaxaPresence\_Plants\_nt\_Refseq.pdf:** Comparison of presence/absence of plant (=Embryophyta) taxa in the sedimentary DNA samples using the nt (cyan) and Refseq (red) databases.