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Metabarcoding of Wildlife Fecal Samples from Air Force Installations to Inform Public Health Risk Assessments

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Wildlife feces of unknown host origin were sampled from three Air Force Bases in the U.S. Samples were subsequently processed and sequence analyses were performed in the entomology laboratory. The intent of the study was to determine if the sequencing approaches used might produce actionable Public Health information at installations, to support risk assessments that are often based on presumed living hazard and threat distributions but which are rarely ground-truthed and lack functional granularity for response actions. Findings are presented and discussed.

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Metabarcoding of Wildlife Fecal Samples from Air Force Installations to Inform Public Health Risk Assessments

DTIC Report

USAFSAM – Public Health and Preventive Medicine Department – Medical Entomology Team

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PREFACE

This report details efforts by the Medical Entomology Program at Wright-Patterson AFB, OH to test the effectiveness of opportunistic fecal sampling of wildlife and pests to identify potential public health threats to personnel on the installation. Samples were collected across three bases (Wright-Patterson, Moody, and Davis-Monthan AFBs) and analyzed via DNA metabarcoding and a viral probe-capture panel to determine whether this approach was viable to detect living hazards in the environment. This proof-of-concept study was intended to provide evidence for a scaled up approach to additional and internationally located installations if successful.

1 SUMMARY

1.1 Fecal Sampling

Fecal samples (n = 83) were collected across three Air Force Bases from November 2020 through April 2021. Wright-Patterson AFB (Fairborn, OH) was sampled in November 2020 (n = 10), December 2020 (n = 2), January 2021 (n = 10), and March 2021 (n = 2). Moody AFB (Moody, GA) was sampled December 2020 (n = 22). Davis-Monthan AFB (Tucson, AZ) was sampled April 2021(n = 37). Samples were collected in both the built environment (i.e., in/around buildings) and from natural areas within the respective AFBs.

1.2 Nucleic Acid Extraction Kit Comparison

A subset of fecal samples was selected to test the effectiveness of various DNA and RNA isolation kits. A total of six kits were tested and from those the Qiagen PowerFecal Pro kit was selected as most effective for DNA extractions and the Qiagen RNeasy PowerMicrobiome kit was selected for RNA extractions. Please see accompanying publication for further details on this study.

1.3 DNA Metabarcoding

Cytochrome oxidase I (COI) metabarcoding was conducted on a selection of fecal samples (n = 52) to identify the host species for the feces, and to detect any additional public health threats that could be present in the broader environment. From the metabarcoding data a total of 40 hosts were identified and two instances of parasitic infection were detected. A total of 30 distinct species were detected.

16S rRNA metabarcoding was also conducted on a subset of samples (n = 38) to identify fecal bacteria that could be of public health interest. 16S metabarcoding was only successful at identifying bacteria down to the genus level which was not specific enough to detect specific public health threats. Attempts were made to further deduce species level identities by assembling bacterial sequences into larger contigs for identification. A total of 16 nearly complete 16S gene fragments were constructed, however, even this level of analysis was not able to discern pathogenic bacterial strains from common environmental microbes for most of the sequences. Despite this challenge, one 16S gene fragment assembled from sequences from a spiny lizard scat was identified as *Salmonella enterica* and represents the most likely instance of identifying a potential pathogen in this dataset.

1.4 Viral Probe Panel

A hybrid probe capture approach was used to identify potential viruses within a subset of samples (n = 16). A viral probe panel was designed based off genome fragments from over 200 viruses that could be found in North America. The probe panel was able to successfully detect five of six viruses from a mixed control sample, however, no viruses were detected within the fecal samples that were tested. Large segments of the positive control pool were able to be reassembled into a near complete genome for LACV.

2 INTRODUCTION

2.1 Background

Genetic analysis of wildlife fecal samples has expanded across a variety of disciplines. Collection of wildlife fecal samples represents a potential avenue to track animal populations within an area or perform routine disease surveillance. Previous studies have used fecal analysis to track animal diets (Casper et al., 2007; Deagle et al., 2005; Iwanowicz et al., 2016; Kaunisto et al., 2017; Pompanon et al., 2012; Thuo et al., 2019), measure parasite load (Avramenko et al., 2015), conduct biomonitoring (Heyde et al., 2020) and population genetics surveys (Bellemain et al., 2005; Chetri et al., 2019; Janečka et al., 2008), and monitor host-microbiome interactions (Ingala et al., 2018; Stappenbeck & Virgin, 2016). Genetic analysis of wildlife fecal samples represents an unexplored avenue for monitoring wildlife and potential public health threats at AFBs both within the natural and built environment. This methodology would be critical for OCONUS installations where wildlife or feral animals may harbor pathogens, yet by virtue of their behavior (nocturnal) or biology (size, harborage) not be readily detectable in the environment to Public Health or Pest Management personnel. At present, public health data often lacks granularity and requires decision makers to make risk assessments at the country or regional level instead of using local data sources.

2.2 Approach

Three AF installations were chosen on the basis of differing climates and sampled at the listed dates (WPAFB: Nov 2020-Jan 2021; Moody AFB: Dec 2020; DMAFB: Apr 2021). Targeted sampling of known pest areas around the installations as well as surveys of highly trafficked human/nature interfaces resulted in the collection of 83 fecal samples. A subset of those samples was then analyzed via COI metabarcoding (n = 52), 16S metabarcoding (n = 38), and/or a viral probe capture panel (n = 16).

2.3 Methodology

2.3.1 Field Collections

Fecal/scat samples were collected in the environment from the ground or adjacent surfaces, and were from unknown animal hosts. The condition of the material varied being in some cases intact, but some samples appeared somewhat degraded. In the field, scat samples were handled with sterile gloves, disposable forceps, and collected in sterile urine cups. After collection samples were returned to the Medical Entomology Lab at Wright-Patterson AFB and either kept at room temperature until extraction (~1 week) or stored at -80°C until extractions could be done.

2.3.2 Nucleic Acid Extractions

Two different nucleic acid kits were used in the processing of samples. The PowerFecal Pro kit (Qiagen Product #51804) was used for the extraction of only DNA to be used downstream in metabarcoding applications. The RNeasy Power Microbiome kit (Qiagen Product #26000-50) was used to elute both RNA and DNA to be used downstream in the viral probe panel. Both kits were selected based on their performance in comparison to 5 other commercially available kits in an initial methods comparison.

2.3.2.1 Qiagen QIAmp PowerFecal Pro DNA Extraction

DNA extractions proceeded as described by the manufacturer (Qiagen) in the kit handbook. A bead-beading step using 25hz for 10 minutes on the TissueLyzer II was included in the lysis state. The starting material for each extraction ranged from 70 - 225 mg. Samples with a fibrous consistency or an abundance of hair were extracted at the lower end of that range. The final elution volume for all samples was normalized to 70 µl and after extraction DNA aliquots were stored at -20°C.

2.3.2.2 Qiagen RNeasy Power Microbiome Extraction

RNA extractions proceeded as describe in the Qiagen kit handbook. A bead-beading step using the TissueLyzer II was again included, and the starting material followed the same range as DNA extractions. Of note, no DNase treatment step was included in the RNA extraction so that both RNA and DNA were co-eluted. The final elution volume was 70 µl and after extraction RNA/DNA aliquots were stored at -80°C.

2.3.3 Metabarcoding Sample Prep, Sequencing, and Bioinformatics

Two separate approaches were utilized for the metabarcoding portion of this project. The main concept of metabarcoding is to generate PCR amplicons that are then sequenced. The first approach focused on using degenerate COI primers (Leray et al., 2013) that amplify across a broad range of eukaryotic taxa. This amplicon would be used to identify the host, analyze diet, and detect any potential parasites or other public health threats. The second approach focused on using 16S primers (Zhang et al., 2020) that amplify across bacteria and archaea to analyze the microbial communities within the fecal material and detect any known pathogens.

To generate the COI amplicons, a reaction setup consisting of 12.5µl KAPA HiFi Hotstart Uracil+ DNA Polymerase (Roche Product# 07959052001), 2.5µl forward primer mICOIintF, 2.5µl reverse primer jgHCO2198, 2.5µl ddH_2O, and 5µl DNA template was used. Cycling conditions were used as described in Table 1. To generate the 16S amplicons, an identical reaction setup was used except for substituting the 26ABF forward primer and 1492R reverse primer. Cycling conditions were used as described in Table 1.

Table 1: Metabarcoding Primers and PCR Conditions

COI Primers			16S Primers		
Name:	**Orientation:**	**Sequence:**	**Name:**	**Orientation:**	**Sequence:**
mICOIintF	Forward	GGWACWGGWTGAACWGTWTAYCCYCC	26ABF	Forward	GSVYACTGCTATCGGMTT
jgHCO2198	Reverse	TAIACYTCIGGRTGICCRAARAAYCA	1492R	Reverse	GGTTACCTTGTTAYGACTT
COI PCR Cycling			**16S PCR Cycling**		
Step	**Temp**	**Time**	**Step**	**Temp**	**Time**
Hot Start:	95°C	3min	**Hot Start:**	95°C	3min
16 cycles:			**35 cycles:**		
Denature	95°C	10s	Denature	95°C	10s
Anneal	62°C	30s (touchdown -1°C each cycle)	Anneal	62°C	30s
Extend	72°C	60s	Extend	72°C	60s
25 cycles:			**Final Extend:**	72°C	3min
Denature	95°C	10s			
Anneal	46°C	30s			
Extend	72°C	60s			
Final Extend:	72°C	3min			

Following amplification, amplicons were cleaned using a 1.8X ratio of AMPure XP Beads (Beckman Coulter #A63881) before moving onto the library prep stage. Amplicons were then prepped using the Nextera XT DNA Library Preparation Kit (Illumina FC-131-1024). Samples were then sequenced on an Illumina MiSeq using the v2 300 cycle sequencing kit (Illumina MS-102-2002).

Raw FASTQ files were exported from the Illumina MiSeq and preprocessed using Geneious Prime Version 2022.1.1. The BBDUK add-in was used to remove adapters, indexes, and primer sequences from the amplicons. Processed FASTQ files were then imported into R version 4.0.5 and merged into exact sequence variants (ESVs) using the DADA2 package (Callahan et al., 2016, 2017).

For the 16S metabarcoding data, taxonomic IDs were made using the IdTaxa function within the DECIPHER package at a threshold of 45 (Wright, 2016). The Silva SSU r138 database (Glöckner et al., 2017; Pruesse et al., 2007) was used as the classification reference. To further investigate

potential public health threats, sequences from families with known pathogens were pooled together by sample and exported to Geneious. In Geneious, those potentially pathogenic ESVs were assembled into larger contigs using the Geneious Assembler at a similarity threshold of 99%.

For the COI metabarcoding data, ESVs were exported back to Geneious and blasted against a reference database of sequences. The BIN database (Ratnasingham & Hebert, 2013) from Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007) was downloaded in June 2021 and used to identify hosts, diet, and potential parasites.

2.3.4 Viral Probe Panel Sample Prep, Sequencing, and Bioinformatics

To identify the presence of viral sequences in the DNA/RNA extracts, a viral probe panel workflow was employed consisting of double-stranded cDNA synthesis, library preparation, probe pulldown, and finally sequencing. In addition to the scat sample extracts, a combined pool consisting of La Crosse (American Type Culture Collection [ATCC] VR-1834), Colorado Tick Fever (strain Florio N-7180; ATCC VR-1233), West Nile (USAFSAM Entomology Laboratory positive), Bourbon (BEI Resources [BEI-RRP] NR-50146), Heartland (strain MO-4; BEI Resources NR-50078), and California Encephalitis (Melao strain TRVL-9375; ATCC VR-761) viruses was extracted and used as a control. Starting from the extracts, samples were first synthesized into double-stranded cDNA using the Maxima H Minus Double-Stranded cDNA Synthesis Kit (Thermo Scientific K2562). cDNA reactions were run in duplicate, pooled, and cleaned using a 1.8x ratio of AMPure XP Beads. Cleaned cDNAs then proceeded to a library prep step using the Illumina DNA Prep (Illumina 20018704).

Prior to sequencing, an xGen hybridization capture was performed using a custom probe panel from IDT (Integrated DNA Technologies, Inc.). This probe panel was designed using NCBI GenBank supplied FASTA files of viral genomes expected to be present in North America. Additionally, 8 bacteriophage genomes were selected to serve as a potential positive control group as bacteriophages would be expected to be present in environmental samples. The probe panel approach was selected as a means to remove host background sequences which could drown out the signal from any viral sequences in the samples. Prior to the capture, the hybridization probes were dried down using the IDT's AMPure XP Bead DNA concentration protocol (appendix A of IDT protocol) with Salmon sperm DNA instead of human COT DNA. The xGen hybridization capture was then performed in accordance with IDT's tube-protocol with pooled samples of cDNA (8 samples/reaction). After completion of the capture, samples went through 10 cycles of post-capture PCR. Samples were then sequenced on an Illumina MiSeq using the v2 300 cycle sequencing kit (Illumina MS-102-2002).

Raw FASTQ files were exported from the Illumina MiSeq and preprocessed using Geneious Prime. The BBDUK add-in was used to remove adapters and indexes from the raw files. Processed sequences greater than 124bp were then blasted against a custom reference database containing viral genomes from the probe panel design using the BLASTN (Basic Local Alignment Search Tool Nucleotide; NCBI) function. Any BLAST hits were pulled and assembled into larger contigs using the Geneious de novo assembler at default settings. Geneious assembly was still run in samples that returned no BLAST hits, and the resulting contigs were blasted against a broader reference database containing potential vertebrate host genomes as well

as the BOLD COI database. These contigs were also blasted against the Silva 16S database to look for potential off-target sequencing of microbial genomes.

Additionally, for analysis of the control pool sample, sequences were initially blasted against the custom reference database containing viral genomes and then pooled by virus. The pooled hits were then mapped by to the reference genome segments of each virus and assembled into larger contigs. The resulting contigs were blasted against the entire NCBI GenBank nucleotide database to assess the accuracy of any assembled contigs.

3 RESULTS AND DISCUSSION

3.1 Results

A total of 82 wildlife fecal samples were collected across three AFBs. Of those samples, 52 underwent COI metabarcoding to identify host, prey items, parasites, and any other non-microbial organisms of interest. Additionally, 38 samples underwent 16S metabarcoding to identify bacterial or fungal pathogens of interest. Finally, 16 samples were sequenced via the hybrid probe capture panel for viruses.

3.1.1 COI Metabarcoding Results

COI metabarcoding was successful for 50 of the 52 samples sequenced. Sequences were processed and aggregated into ESVs resulting in 2611 ESVs per sample. Next ESVs were blasted against the BOLD reference database which resulted in 402 ESVs with assigned taxonomy per sample. Across all samples a total of 30 unique species were identified consisting of 17 mammals, 5 birds, 2 amphibians, 1 fish, 1 reptile, 2 species of insect (cockroach and silverfish) and 2 species of intestinal parasite. Of the 50 successfully sequenced samples, a total of 38 hosts were identified from the sequencing data. An additional two samples were inconclusive but had a tentative host inferred from the sequencing and the remaining 10 samples yielded no usable data on host identity.

Figure 1 : COI Metabarcoding of Owl Sample

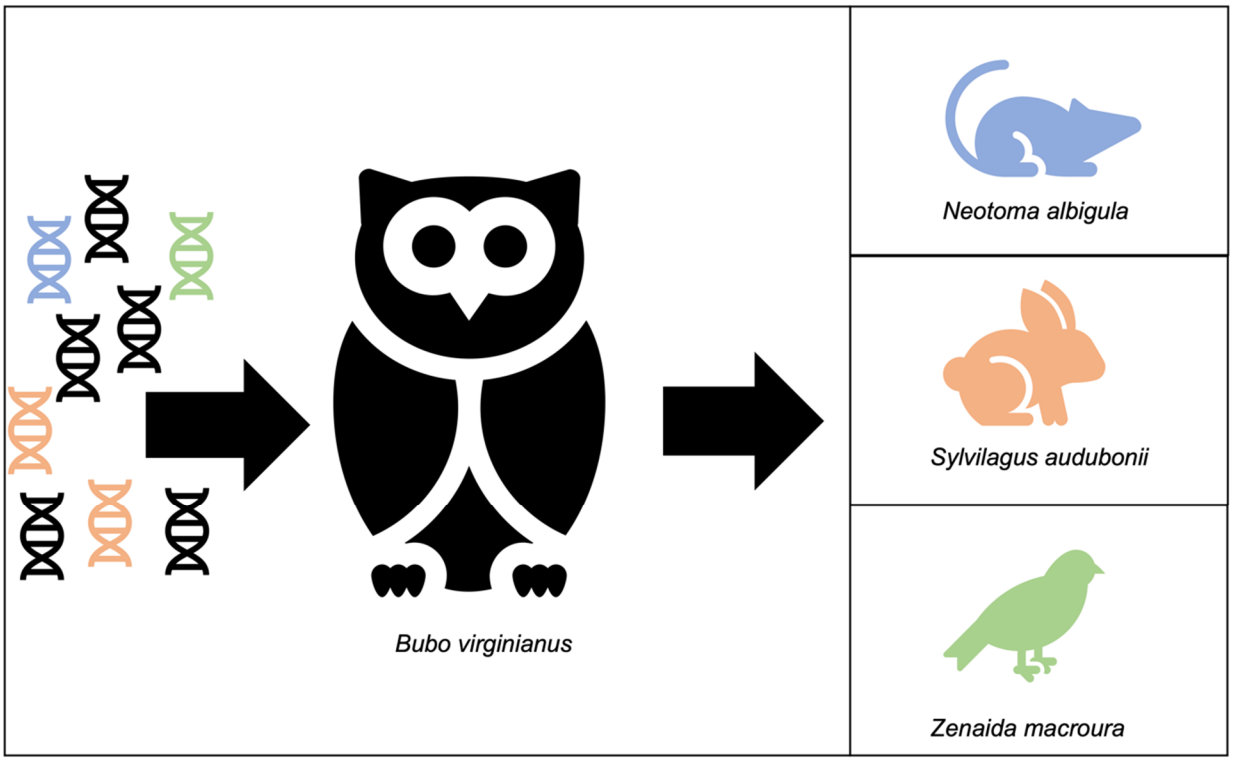

Fig. 1 - Schematic of metabarcoding approach and resulting data for a fecal sample collected from a great horned owl (*Bubo virginianus*). Color of DNA fragment (left) is traced back to a taxonomic assignment and yields data on the host as well as potential prey items.

A small subset of COI samples yielded additional information beyond host identification. Some samples showed potential prey items such as a coyote (*Canis latrans*) sample that contained DNA from a raccoon (*Procyon lotor*) while another coyote sample contained DNA from a desert cottontail (*Sylvilagus audubonii*). A great horned owl (*Bubo virginianus*) sample (Fig. 1) yielded the most prey items with DNA traces from desert cottontail, white-throated woodrat (*Neotoma albigula*), and mourning dove (*Zenaida macroura*) present. Additionally, a sample identified as striped skunk (*Mephitis mephitis*) showed infection with the intestinal roundworm parasite *Baylisascaris columnaris* while a separate dog (*Canis familiaris*) sample carried DNA from the hookworm *Ancylostoma caninum*. Finally, the most impactful sample from a public health perspective came from a mouse (*Mus musculus*) dropping collected within a food service building. The mouse sample (Fig. 2) contained DNA traces from two insects, the American cockroach (*Periplaneta americana)* and the long-tailed silverfish (*Ctenolepisma longicaudata*) as well as turkey (*Meleagris gallopavo*) and pig (*Sus scrofa*) which presumably came from mouse consumption of food items in the nearby area.

Figure 2 : COI Metabarcoding of Mouse Sample

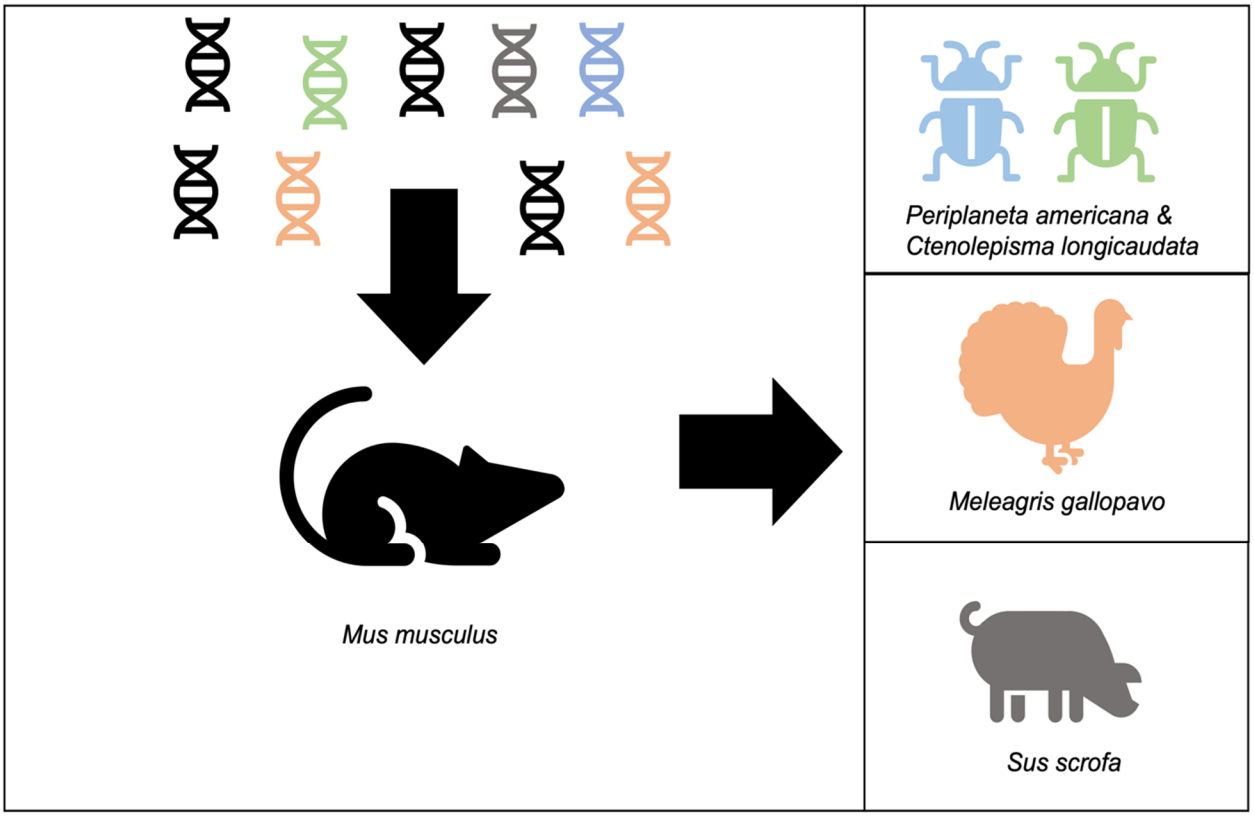

Fig. 2 - Schematic of metabarcoding approach and resulting data for a fecal sample collected from a mouse (*Mus musculus*). Color of DNA fragment (left) is traced back to a taxonomic assignment and yields data on the host as well as potential prey/food items.

3.1.2 16S Metabarcoding Results

16S metabarcoding was successful for all 38 of the samples sequenced. Sequences were processed and aggregated into ESVs resulting in 3709 ESVs per sample. ESVs were assigned

taxonomy using the SILVA 16S reference database resulting in 571 ESVs per sample with taxonomic data. No sequences were identified down the species level and only 40.7% of ESVs had genus level assignments (Appendix C).

Due to the nature of 16S metabarcoding, it is often impossible to identify sequences past the genus level (Kim et al., 2011), as was the case in this dataset. However, to identify public health threats it is necessary to make species or even strain level calls on an organism. To further investigate potential public health threats, sequences from families with known pathogens were pooled together by sample and exported to Geneious. In Geneious, those potentially pathogenic ESVs were assembled into larger contigs using the Geneious Assembler at a similarity threshold of 99%. There were 76 families identified across all the samples, 18 of which had known bacterial pathogens. Additionally, 10 potentially pathogenic fungal genera were investigated. A total of 16 contigs (all bacterial) from 12 samples were identified as potentially pathogenic (Table 2).

Table 2: Potential Public Health Threats from 16S Metabarcoding Assemblies

Sample ID	Host	Contig length	ESVs	Read Total	% Match	Potential ID
21-084-001Z	*B. canadensis*	1139	45	197	100.00	*Bacillus cereus*
21-116-003	*S. audubonii*	896	14	78	98.88	*Providencia rettgeri*
21-116-025	*C. latrans*	701	87	1133	100.00	*Escherichia coli*
21-116-025	*C. latrans*	931	85	813	100.00	*Escherichia coli*
21-116-025	*C. latrans*	464	5	15	100.00	*Providencia rettgeri*
21-116-032	*S. magister*	1475	108	237	100.00	*Salmonella enterica*
21-117-001	*M. musculus*	1265	154	1136	100.00	*Escherichia coli*
21-116-020	*X. tereticaudus*	1098	85	657	99.36	*Salmonella enterica*
21-116-020	*X. tereticaudus*	852	39	204	99.77	*Escherichia coli*
21-116-024	*P. tajacu*	987	82	488	99.29	*Salmonella enterica*
21-116-024	*P. tajacu*	977	41	549	100.00	*Escherichia coli*
20-343-020	*M. musculus*	1262	223	5511	99.76	*Escherichia coli*
21-020-009	*S. floridanus*	1246	109	1207	99.92	*Yersinia sp*
20-322-002	*S. carolinensis*	1225	179	1568	100.00	*Escherichia coli*
20-322-003	*M. mephitis*	1186	104	947	100.00	*Escherichia coli*
20-342-002	*Inconclusive*	858	102	1531	100.00	*Escherichia coli*

Table 2: Potential pathogenic bacteria assembled from 16S metabarcoding data. Columns describe host species, length of assembled contig, number of ESVs used in assembly, total reads used in assembly, and the percent of matching bases when the contig was blasted against the NCBI nucleotide database.

3.1.3 Viral Probe Panel Results

Seventeen of the 19 total samples were successfully prepped via the Illumina DNA. The remaining 17 samples (which included the control pool) were successfully prepped via the IDT hybridization probe capture and sequenced on the Illumina MiSeq. Reverse reads from this sequencing run were of low quality and therefore discarded. All subsequent analysis was conducted using only the forward sequencing reads which totaled 71,749 reads per sample.

The 16 fecal samples yielded no significant hits when blasted against a reference database containing viral sequences using in the probe panel design. Additionally, the samples were blasted against the BOLD COI database and a separate database containing host genome sequences which still yielded no significant hits. Finally, a subsample of sequences from each sample were blasted against the 16S SILVA database which resulted in several high-quality hits.

In contrast, the control pool sample yielded significant hits for 5 of the 6 viruses used. The sequences that produced significant hits were then pooled by virus and mapped back to the reference genome for that virus using the Geneious assembler. Assembled contigs varied greatly in length with the largest contigs coming from the La Crosse and Colorado Tick Fever viral sequences (Table 3).

Table 3: Largest Assembled Contigs from Virus Control Pool Sample

Control Pool	Segment	Reads	Longest Contig	NCBI Best Match
Bourbon Virus	1	1	151	150/151
Heartland Virus	Large	3	151	151/151
Heartland Virus	Medium	3	151	145/151
Heartland Virus	Small	3	150	148/150
CTFV	1	1	151	149/151
CTFV	2	5	151	151/151
CTFV	3	3	281	281/281
CTFV	4	11	219	217/219
CTFV	5	71	2290	2290/2290
CTFV	6	3	151	151/151
CTFV	7	26	734	734/734
CTFV	8	2	150	150/150
CTFV	9	20	1168	1145/1168
CTFV	10	2	151	151/151
CEV (Melao)	Large	7	151	148/151
CEV (Melao)	Medium	4	299	298/299
CEV (Melao)	Small	7	404	404/404
La Crosse Virus	Large	396	6744	6740/6744
La Crosse Virus	Medium	287	4196	4195/4196
La Crosse Virus	Small	40	825	800/825
WNV	0	0	0	0

Table 3: Summary of assembly results for the six viruses in the control pool sample. The column NCBI Best Match refers to the number of exact matching nucleotides for the specific virus when blasted against the entire NCBI Nucleotide database. (CTFV – Colorado Tick Fever Virus, CEV – California Encephalitis Virus (Melao), WNV – West Nile Virus).

3.2 Discussion

Sequencing of wildlife fecal samples from around Air Force bases showed mixed success across this proof-of-concept study. Results from the COI metabarcoding of fecal samples were promising and in line with other studies which have shown that even highly degraded samples can provide useful information on animal populations (Poggenburg et al., 2018; van der Heyde et al., 2021). The COI primer pair (Leray et al., 2013) also provided appropriate taxonomic resolution of vertebrate hosts while still being able to detect other taxa of interest like hookworms and roundworms. Samples from predators such as *Bubo virginianus* (Fig. 2) and *Canis latrans* highlighted how fecal sampling can quickly provide information on taxa throughout a region based on the prey items detected by metabarcoding. This is in line with several studies showing the effectiveness of metabarcoding in reconstructing predatory diets (Casper et al., 2007; Pompanon et al., 2012; Rytkönen et al., 2019; Thuo et al., 2019). It also supports the premise that a single fecal sample can potentially provide insight across biological domains to include large and small, nocturnal and diurnal host animals, evidence for predation and scavenging, and detection of parasitism. From a public health perspective, the most impactful samples collected came from mouse droppings inside of base buildings. In particular, a sample from Moody AFB (Fig. 3) was successfully identified as host mouse DNA, and also contained American cockroach and long-tailed silverfish DNA. More crucially, this sample also showed traces of turkey and pig DNA suggesting that mice in the area were either actively consuming food items or were at a minimum in the immediate vicinity of food preparation areas.

Results from the 16S metabarcoding were less successful in providing meaningful data for public health managers. The pitfalls of 16S rRNA sequencing for microbial communities is well documented (di Bella et al., 2013; Janda & Abbott, 2007; Schloss, 2010). In particular, information beyond the genus level is difficult to infer from partial fragments of the 16S rRNA gene (Kim et al., 2011). This was true in our dataset as well as not sequences were identified beyond the genus level. However, other studies have shown success in using full-length 16S rRNA sequences in obtaining species-level resolution (Dueholm et al., 2020; Earl et al., 2018; Numberger et al., 2019).

In this study, the 16S primers used were designed to amplify full-length 16S sequences which were subsequently fragmented during the library prep phase. Due to this workflow, the Geneious de novo assembler showed utility in recreating a handful of near full-length contigs (Table 2). Despite the length of these contigs and the high percent matching to known pathogens, it is impossible to say any of the listed organisms were present. For example, *Bacillus cereus* is a known food pathogen, however, its contig also matched 100% to *Bacillus toyonensis* (a marine microbe) and *Bacillus thuringiensis* (soil microbe) which are not pathogenic. The *Providencia rettgeri* contigs also similarly matched *Providencia vermicola* (insect pathogen) while the *Yersinia* contig matched the multiple non-pathogenic strains like *Yersinia kristensenii* and *Yersinia intermedia*. This pattern was especially relevant in *Escherichia coli* where it was impossible to distinguish near full-length 16S gene fragments down to the necessary strain level.

The most likely pathogen identified was the 100% *Salmonella enterica* contig isolated from a spiny desert lizard (*Sceloporus magister*). Reptiles are potential reservoirs of *Salmonella enterica* so it is entirely plausible the observed pathogen was present. The contig also matched most

closely to only the pathogenic strains as opposed to the other *Salmonella* contigs which matched equally with other plausible environmental species.

Finally, the hybrid probe panel to assess viral communities also showed mixed results. No viral signatures were detected in any of the 16 samples sequenced. Other studies have shown success in detecting viruses from feces(Chen et al., 2018; Chong et al., 2019; Duarte et al., 2019), however, these studies focused on fresh samples in populations with known infections. Possibly the DNA or RNA in the samples was too degraded to allow for detection, or it is entirely plausible that our effort was not sufficient to collect a sample from a recently infected individual. Despite this lack of evidence, the probe panel did show success in eliminating host background from all the samples.

Additionally, the probe panel showed success in detecting five of the six viruses in the control pool sample (Table 3). Beyond just detection, it was possible to assemble the viral components into much larger fragments by mapping back to the known reference genomes in Geneious. This mapping was successful even for an admixture of closely related sequences such as La Crosse virus (LACV) and California Encephalitis virus (CEV, strain Melao). The majority of reads mapped to LACV and generated a near complete genome. The CEV sample (strain Melao) aligned most closely with Melao virus, allowing its presence in the positive pool to be confirmed.

4 CONCLUSIONS

Sequencing of fecal samples showed utility in identifying species of origin and as a broader biomonitoring tool of animal taxa around AFBs via COI metabarcoding. Screening of samples for pathogens via 16S metabarcoding was not successful and deeper analysis by reassembly of full-length fragments only revealed one compelling instance of a potential pathogen. Viral screening of samples via a custom hybrid probe panel was also unsuccessful in field samples. The probe panel was successful in identifying viral sequences in a control sample and showed utility in creating near full-length genomes from a mixed viral sample.

5 RECOMMENDATIONS

Based on the analysis of the current study the following recommendations for future work are offered. COI metabarcoding of wildlife fecal samples represents a potential avenue for monitoring animal taxa at AFBs. This may be especially useful in areas with limited local knowledge of fauna present such as OCONUS installations. 16S metabarcoding to detect microbial pathogens is not recommended as current sequencing technology does not easily identify sequences at the necessary species or even strain level. Analysis of fecal samples for viral sequences also shows limited utility. However, our custom hybrid probe panel was successful in identifying and assembling viral genomes. Further study is warranted on this approach, particularly in samples that are more likely to come from infected individuals or in different sample types such as water outflows.

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7 REFERENCES

Avramenko, R. W., Redman, E. M., Lewis, R., Yazwinski, T. A., Wasmuth, J. D., & Gilleard, J. S. (2015). Exploring the Gastrointestinal "Nemabiome": Deep Amplicon Sequencing to Quantify the Species Composition of Parasitic Nematode Communities. *PLOS ONE*, *10*(12), e0143559. https://doi.org/10.1371/JOURNAL.PONE.0143559

Bellemain, E., Swenson, J. E., Tallmon, D., Brunberg, S., & Taberlet, P. (2005). Estimating Population Size of Elusive Animals with DNA from Hunter-Collected Feces: Four Methods for Brown Bears. *Conservation Biology*, *19*(1), 150–161. https://doi.org/10.1111/j.1523-1739.2005.00549.x

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Casper, R. M., Jarman, S. N., Deagle, B. E., Gales, N. J., & Hindell, M. A. (2007). Detecting prey from DNA in predator scats: A comparison with morphological analysis, using Arctocephalus seals fed a known diet. *Journal of Experimental Marine Biology and Ecology*, *347*(1–2), 144–154. https://doi.org/10.1016/J.JEMBE.2007.04.002

Chen, Q., Wang, L., Zheng, Y., Zhang, J., Guo, B., Yoon, K.-J., Gauger, P. C., Harmon, K. M., Main, R. G., & Li, G. (2018). Metagenomic analysis of the RNA fraction of the fecal virome indicates high diversity in pigs infected by porcine endemic diarrhea virus in the United States. *Virology Journal 2018 15:1*, *15*(1), 1–9. https://doi.org/10.1186/S12985-018-1001-Z

Chetri, M., Odden, M., Sharma, K., Flagstad, Ø., & Wegge, P. (2019). Estimating snow leopard density using fecal DNA in a large landscape in north-central Nepal. *Global Ecology and Conservation*, *17*, e00548. https://doi.org/10.1016/j.gecco.2019.e00548

Chong, R., Shi, M., Grueber, C. E., Holmes, E. C., Hogg, C. J., Belov, K., & Barrs, V. R. (2019). Fecal Viral Diversity of Captive and Wild Tasmanian Devils Characterized Using Virion-Enriched Metagenomics and Metatranscriptomics. *Journal of Virology*, *93*(11), 205–224. https://doi.org/10.1128/JVI.00205-19

Deagle, B. E., Tollit, D. J., Jarman, S. N., Hindell, M. A., Trites, A. W., & Gales, N. J. (2005). Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Molecular Ecology*, *14*(6), 1831–1842. https://doi.org/10.1111/j.1365-294X.2005.02531.x

di Bella, J. M., Bao, Y., Gloor, G. B., Burton, J. P., & Reid, G. (2013). High throughput sequencing methods and analysis for microbiome research. *Journal of Microbiological Methods*, *95*(3), 401–414. https://doi.org/10.1016/J.MIMET.2013.08.011

Duarte, M. A., Silva, J. M. F., Brito, C. R., Teixeira, D. S., Melo, F. L., Ribeiro, B. M., Nagata, T., & Campos, F. S. (2019). Faecal Virome Analysis of Wild Animals from Brazil. *Viruses 2019, Vol. 11, Page 803*, *11*(9), 803. https://doi.org/10.3390/V11090803

Dueholm, M. S., Andersen, K. S., McIlroy, S. J., Kristensen, J. M., Yashiro, E., Karst, S. M., Albertsen, M., & Nielsen, P. H. (2020). Generation of Comprehensive Ecosystem-Specific Reference Databases with Species-Level Resolution by High-Throughput Full-Length 16S rRNA Gene Sequencing and Automated Taxonomy Assignment (AutoTax). *MBio*, *11*(5), 1–14. https://doi.org/10.1128/mBio.01557-20

Earl, J. P., Adappa, N. D., Krol, J., Bhat, A. S., Balashov, S., Ehrlich, R. L., Palmer, J. N., Workman, A. D., Blasetti, M., Sen, B., Hammond, J., Cohen, N. A., Ehrlich, G. D., & Mell, J. C. (2018). Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *Microbiome*, *6*(1), 190. https://doi.org/10.1186/s40168-018-0569-2

Glöckner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., & Ludwig, W. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of Biotechnology*, *261*, 169–176. https://doi.org/10.1016/J.JBIOTEC.2017.06.1198

Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N. E., & Nevill, P. (2020). Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. *Molecular Ecology Resources*, *20*(3), 732–745. https://doi.org/10.1111/1755-0998.13148

Ingala, M. R., Simmons, N. B., Wultsch, C., Krampis, K., Speer, K. A., & Perkins, S. L. (2018). Comparing Microbiome Sampling Methods in a Wild Mammal: Fecal and Intestinal Samples Record Different Signals of Host Ecology, Evolution. *Frontiers in Microbiology*, *0*(MAY), 803. https://doi.org/10.3389/FMICB.2018.00803

Iwanowicz, D. D., Vandergast, A. G., Cornman, R. S., Adams, C. R., Kohn, J. R., Fisher, R. N., & Brehme, C. S. (2016). Metabarcoding of Fecal Samples to Determine Herbivore Diets: A Case Study of the Endangered Pacific Pocket Mouse. *PLOS ONE*, *11*(11), e0165366. https://doi.org/10.1371/journal.pone.0165366

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/JCM.01228-07

Janečka, J. E., Jackson, R., Yuquang, Z., Diqiang, L., Munkhtsog, B., Buckley-Beason, V., & Murphy, W. J. (2008). Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. *Animal Conservation*, *11*(5), 401–411. https://doi.org/10.1111/j.1469-1795.2008.00195.x

Kaunisto, K. M., Roslin, T., Sääksjärvi, I. E., & Vesterinen, E. J. (2017). Pellets of proof: First glimpse of the dietary composition of adult odonates as revealed by metabarcoding of feces. *Ecology and Evolution*, *7*(20), 8588–8598. https://doi.org/10.1002/ece3.3404

Kim, M., Morrison, M., & Yu, Z. (2011). Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. *Journal of Microbiological Methods*, *84*(1), 81–87. https://doi.org/10.1016/J.MIMET.2010.10.020

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(1), 1–14. https://doi.org/10.1186/1742-9994-10-34/FIGURES/5

Numberger, D., Ganzert, L., Zoccarato, L., Mühldorfer, K., Sauer, S., Grossart, H. P., & Greenwood, A. D. (2019). Characterization of bacterial communities in wastewater with enhanced taxonomic resolution by full-length 16S rRNA sequencing. *Scientific Reports 2019 9:1*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-46015-z

Poggenburg, C., Nopp-Mayr, U., Coppes, J., & Sachser, F. (2018). Shit happens … and persists: decay dynamics of capercaillie (Tetrao urogallus L.) droppings under natural and artificial conditions. *European Journal of Wildlife Research*, *64*(3), 29. https://doi.org/10.1007/s10344-018-1187-9

Pompanon, F., Deagle, B. E., Symondson, W. O. C., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology*, *21*(8), 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, *35*(21), 7188–7196. https://doi.org/10.1093/NAR/GKM864

Ratnasingham, S., & Hebert, P. D. N. (2007). bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/10.1111/J.1471-8286.2007.01678.X

Ratnasingham, S., & Hebert, P. D. N. (2013). A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLOS ONE*, *8*(7), e66213. https://doi.org/10.1371/JOURNAL.PONE.0066213

Rytkönen, S., Vesterinen, E. J., Westerduin, C., Leviäkangas, T., Vatka, E., Mutanen, M., Välimäki, P., Hukkanen, M., Suokas, M., & Orell, M. (2019). From feces to data: A metabarcoding method for analyzing consumed and available prey in a bird-insect food web. *Ecology and Evolution*, *9*(1), 631–639. https://doi.org/10.1002/ece3.4787

Schloss, P. D. (2010). The effects of alignment quality, distance calculation method, sequence filtering, and region on the analysis of 16S rRNA gene-based studies. *PLoS Computational Biology*, *6*(7), 19. https://doi.org/10.1371/JOURNAL.PCBI.1000844

Stappenbeck, T. S., & Virgin, H. W. (2016). Accounting for reciprocal host–microbiome interactions in experimental science. *Nature*, *534*(7606), 191–199. https://doi.org/10.1038/nature18285

Thuo, D., Furlan, E., Broekhuis, F., Kamau, J., MacDonald, K., & Gleeson, D. M. (2019). Food from faeces: Evaluating the efficacy of scat DNA metabarcoding in dietary analyses. *PLoS ONE*, *14*(12). https://doi.org/10.1371/JOURNAL.PONE.0225805

van der Heyde, M., Bateman, P. W., Bunce, M., Wardell-Johnson, G., White, N. E., & Nevill, P. (2021). Scat DNA provides important data for effective monitoring of mammal and bird biodiversity. *Biodiversity and Conservation*, *30*(12), 3585–3602. https://doi.org/10.1007/s10531-021-02264-x

Wright, E. S. (2016). Using DECIPHER v2.0 to analyze big biological sequence data in R. *R Journal*, *8*(1), 352–359. https://doi.org/10.32614/RJ-2016-025

Zhang, R.-Y., Zou, B., Yan, Y.-W., Jeon, C. O., Li, M., Cai, M., & Quan, Z.-X. (2020). Design of targeted primers based on 16S rRNA sequences in meta-transcriptomic datasets and identification of a novel taxonomic group in the Asgard archaea. *BMC Microbiology*, *20*(1), 25. https://doi.org/10.1186/s12866-020-1707-0

8 APPENDIX

8.1 Appendix A: Sample Table

8.1.1 Table A-1: Fecal samples and genetic analyses

TABLE A-1. TABLE OF COLLECTED FECAL SAMPLES AND SUBSEQUENT GENETIC ANALYSES

Specimen ID	Tentative ID	Collect date	Installation	Collection site	COI	16S	Viral	Sequencing Confirmed Host
20-308-050	Avian	11/3/2020	WPAFB	USAFSAM				
20-308-051	Unknown	11/3/2020	WPAFB	USAFSAM				
20-308-052	Goose	11/3/2020	WPAFB	Navy Building				
20-308-053	Unknown	11/3/2020	WPAFB	Navy Building	Yes	Yes		*M. monax*
20-322-001	Squirrel	11/17/2020	WPAFB	Building 10 Area C	Yes			*S. carolinensis*
20-322-002	Squirrel	11/17/2020	WPAFB	Building 10 Area C	Yes	Yes	Yes	*S. carolinensis*
20-322-003	Groundhog	11/17/2020	WPAFB	Kitty Hawk Shed	Yes	Yes	Yes	*M. mephitis & B. columnaris*
20-322-004	Groundhog	11/17/2020	WPAFB	Kitty Hawk Shed				
20-322-005	Groundhog	11/17/2020	WPAFB	Area B Softball			Yes	
20-322-006	Groundhog	11/17/2020	WPAFB	Area B Softball				
20-342-001	Unknown	12/7/2020	Moody	Comm Squad	Yes	Yes		**Inconclusive**
20-342-002	Unknown	12/7/2020	Moody	Build 217	Yes	Yes		**Inconclusive**
20-342-003	Canine	12/7/2020	Moody	Build 336	Yes			**Inconclusive**
20-342-004	Canine	12/7/2020	Moody	Build 336				
20-342-005	Unknown	12/7/2020	Moody	Moody Gym Park	Yes	Yes		**Inconclusive**
20-343-007	Mouse	12/8/2020	Moody	Ento Build				
20-343-008	Frog	12/8/2020	Moody	Build 4 NR	Yes	Yes	Yes	*D. squirellus*
20-343-009	Armadillo	12/8/2020	Moody	820 Unit Area	Yes			*A. fowleri*
20-343-010	Armadillo	12/8/2020	Moody	Ammo Depot				
20-343-011	Armadillo	12/8/2020	Moody	Ammo Depot	Yes	Yes	Yes	*P. lotor & G. holbrooki*
20-343-012	Armadillo	12/8/2020	Moody	Ammo Depot				
20-343-013	Armadillo	12/8/2020	Moody	Ammo Depot	Yes			*A. fowleri*
20-343-014	Armadillo	12/8/2020	Moody	Ammo Depot				
20-343-015	Armadillo	12/8/2020	Moody	Ammo Depot	Yes			*A. fowleri*
20-343-016	Armadillo	12/8/2020	Moody	Ammo Depot	Yes			*A. fowleri*
20-343-017	Frog	12/8/2020	Moody	Mission Lake	Yes			**Inconclusive**
20-343-018	Unknown	12/8/2020	Moody	Mission Lake	Yes			**Failed**
20-343-019	Unknown	12/8/2020	Moody	Ento Build	Yes	Yes		**Inconclusive**
20-343-020	Mouse	12/8/2020	Moody	BEX	Yes	Yes	Yes	*M. musculus*

Specimen ID	Tentative ID	Collect date	Installation	Collection site	COI	16S	Viral	Sequencing Confirmed Host
20-343-021	Mouse	12/8/2020	Moody	Chow Hall	Yes	Yes	Yes	*M. musculus, S. scrofa, M. gallopavo, P. americana, C. longicaudata*
20-343-022	Mouse	12/8/2020	Moody	Field Club	Yes	Yes	Yes	*P. americana*
20-343-023	Omnivore	12/8/2020	Moody	Mission Lake	Yes		Yes	*S. hispidius*
20-357-001	Goose	12/22/2020	WPAFB	Navy Building				
20-357-002	Goose	12/22/2020	WPAFB	Navy Building				
21-20-001	Unknown	1/19/2021	WPAFB	CDC South				
21-20-002	Unknown	1/19/2021	WPAFB	CDC South	Yes		Yes	*C. lupus & A. caninum*
21-20-003	Unknown	1/19/2021	WPAFB	CDC South				
21-20-004	Unknown	1/19/2021	WPAFB	CDC South	Yes			**Inconclusive**
21-20-005	Avian	1/19/2021	WPAFB	852 Natural Area	Yes			**Failed**
21-20-006	Avian	1/19/2021	WPAFB	852 Natural Area	Yes	Yes	Yes	*Z. macroura*
21-20-007	Carnivore	1/19/2021	WPAFB	852 Natural Area	Yes	Yes		*C. latrans & P. lotor*
21-20-008	Carnivore	1/19/2021	WPAFB	852 Natural Area	Yes			**Inconclusive**
21-20-009	Deer	1/19/2021	WPAFB	852 Natural Area	Yes	Yes		*S. floridanus*
21-20-010	Deer	1/19/2021	WPAFB	852 Natural Area	Yes		Yes	*S. floridanus*
21-084-001	Goose	3/25/2021	WPAFB	Building 840	Yes	Yes		*B. canadensis*
21-104-001	Rat	4/14/2021	DMAFB	Building 3210	Yes	Yes	Yes	*N. albigula*
21-105-001	Rat	4/15/2021	DMAFB	Building 5010	Yes	Yes	Yes	*N. albigula*
21-116-001	Mouse	4/26/2021	DMAFB	Building 8030				
21-116-002	Mouse	4/26/2021	DMAFB	Building 8030	Yes	Yes		*N. albigula*
21-116-003	Mouse	4/26/2021	DMAFB	Tent City	Yes	Yes		*S. audubonii*
21-116-004	Rabbit	4/26/2021	DMAFB	Tent City	Yes	Yes		*S. audubonii*
21-116-005	Rabbit	4/26/2021	DMAFB	Oil Aggregate			Yes	
21-116-006	Unknown	4/26/2021	DMAFB	Oil Aggregate				
21-116-007	Squirrel	4/26/2021	DMAFB	Oil Aggregate	Yes	Yes	Yes	*X. tereticaudus*

Specimen ID	Tentative ID	Collect date	Installation	Collection site	COI	16S	Viral	Sequencing Confirmed Host
21-116-008	Owl	4/26/2021	DMAFB	707 Jet 1	Yes	Yes		**Inconclusive**
21-116-009	Rabbit	4/26/2021	DMAFB	707 Jet 1			Yes	
21-116-010	Unknown	4/26/2021	DMAFB	707 Jet 1	Yes	Yes		*Z. macroura*
21-116-011	Rabbit	4/26/2021	DMAFB	707 Jet 1				
21-116-012	Unknown	4/26/2021	DMAFB	707 Jet 2				
21-116-013	Unknown	4/26/2021	DMAFB	707 Jet 2	Yes	Yes		*Z. macroura*
21-116-014	Unknown	4/26/2021	DMAFB	707 Jet 3				
21-116-015	Owl	4/26/2021	DMAFB	A10 Hangar				
21-116-016	Owl	4/26/2021	DMAFB	A10 Hangar				
21-116-017	Owl	4/26/2021	DMAFB	A10 Hangar				
21-116-018	Owl	4/26/2021	DMAFB	A10 Hangar				
21-116-019	Owl	4/26/2021	DMAFB	A10 Hangar	Yes	Yes		*B. virginianus, N. albigula, S. audubonii, Z. macroura*
21-116-020	Unknown	4/26/2021	DMAFB	Airfield North	Yes	Yes	Yes	*X. tereticaudus*
21-116-021	Owl	4/26/2021	DMAFB	Airfield North	Yes	Yes		*E. traillii*
21-116-022	Rabbit	4/26/2021	DMAFB	Landfill	Yes	Yes	Yes	**Inconclusive** *(S. audubonii)*
21-116-023	Unknown	4/26/2021	DMAFB	Landfill				
21-116-024	Javelina	4/26/2021	DMAFB	Landfill	Yes	Yes		*P. tajacu*
21-116-025	Coyote	4/26/2021	DMAFB	Airfield South	Yes	Yes		*C. latrans & S. audubonii*
21-116-026	Coyote	4/26/2021	DMAFB	Airfield South				
21-116-027	Hawk	4/26/2021	DMAFB	Comm Tower				
21-116-028	Coyote	4/26/2021	DMAFB	Comm Tower				
21-116-029	Coyote	4/26/2021	DMAFB	Comm Tower				
21-116-030	Mouse	4/26/2021	DMAFB	Immigration	Yes	Yes	Yes	*N. albigula*
21-116-031	Owl	4/26/2021	DMAFB	Golf Course	Yes	Yes		**Inconclusive** *(O. virginianus)*
21-116-032	Bat	4/26/2021	DMAFB	Golf Course	Yes	Yes	Yes	*S. magister*

Specimen ID	Tentative ID	Collect date	Installation	Collection site	COI	16S	Viral	Sequencing Confirmed Host
21-116-033	Deer	4/26/2021	DMAFB	DM South	Yes	Yes		*Inconclusive*
21-117-001	Mouse	4/27/2021	DMAFB	Commissary	Yes	Yes	Yes	*M. musculus & P. merriami*
21-117-002	Rat	4/27/2021	DMAFB	Old Chow Hall	Yes	Yes	Yes	*R. norvegicus & S. magister*

8.2 Appendix B: Maps of Sampling Effort

8.2.1 Wright-Patterson AFB

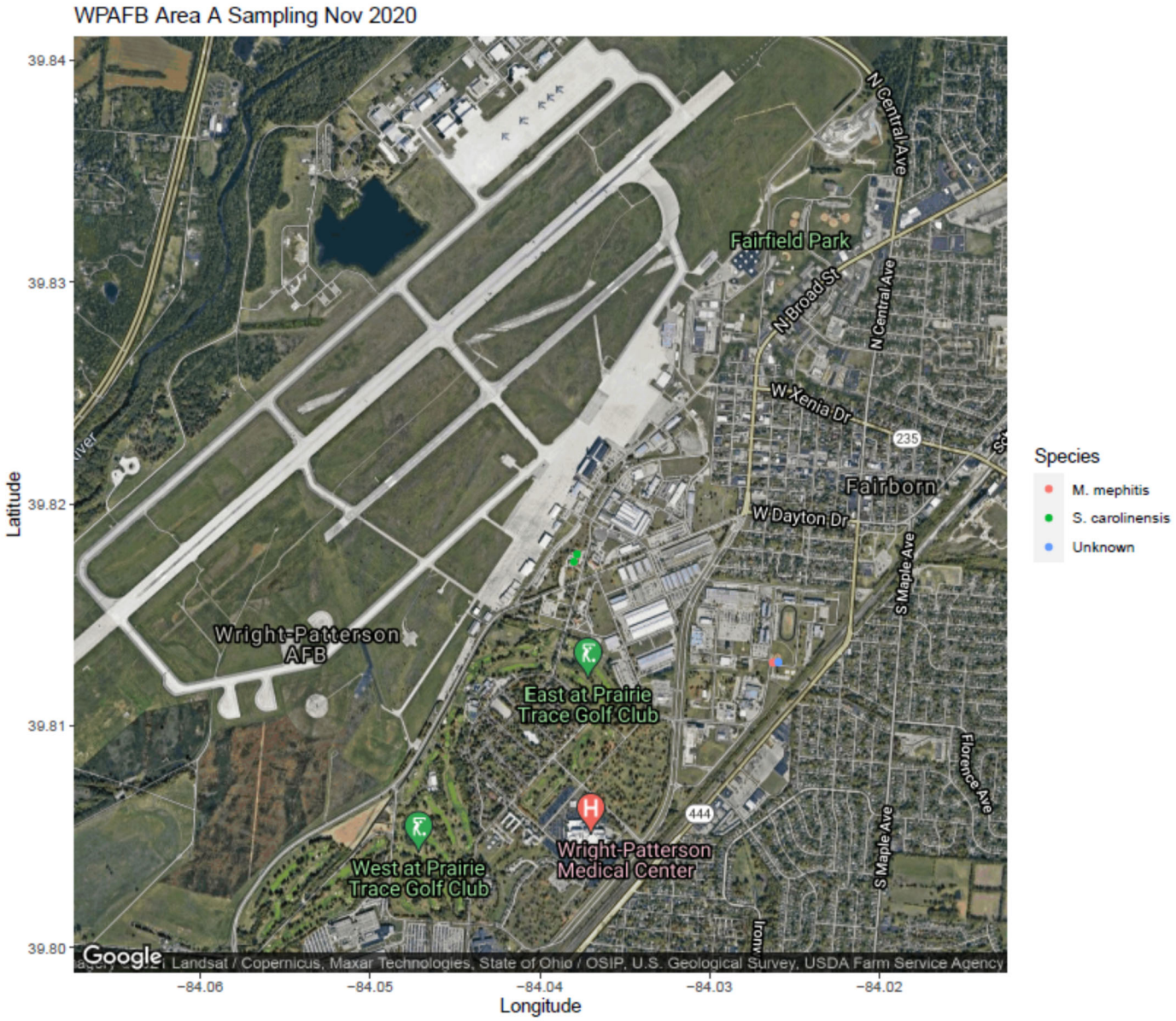

Figure B-1: Wright-Patterson AFB Area A scat sampling with sequencing confirmed IDs

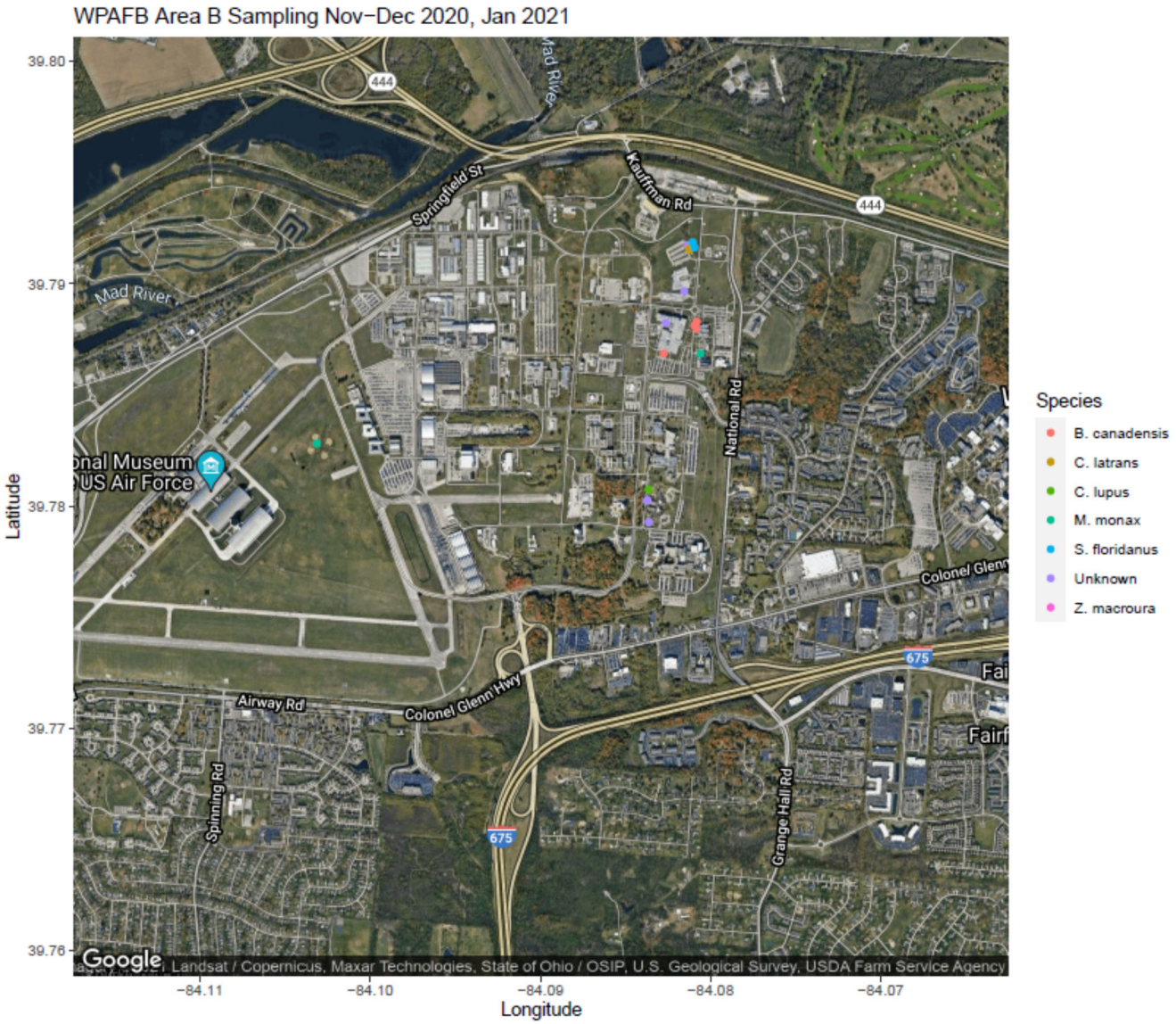

Figure B-2: Wright-Patterson AFB Area B scat sampling with sequencing confirmed IDs

8.2.2 Moody AFB

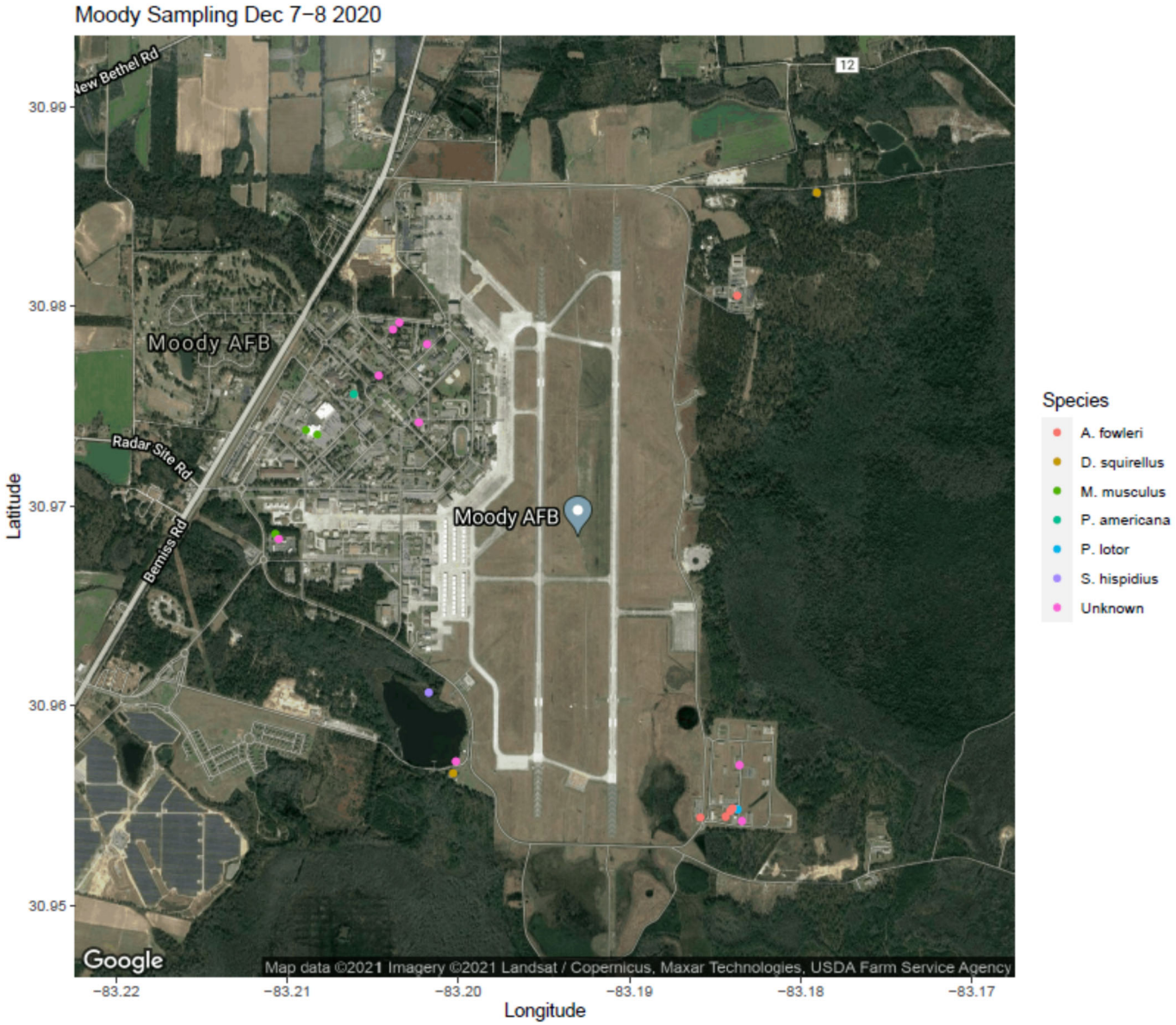

Figure B-3: Moody AFB scat sampling with sequencing confirmed IDs

8.2.3 Davis-Monthan AFB

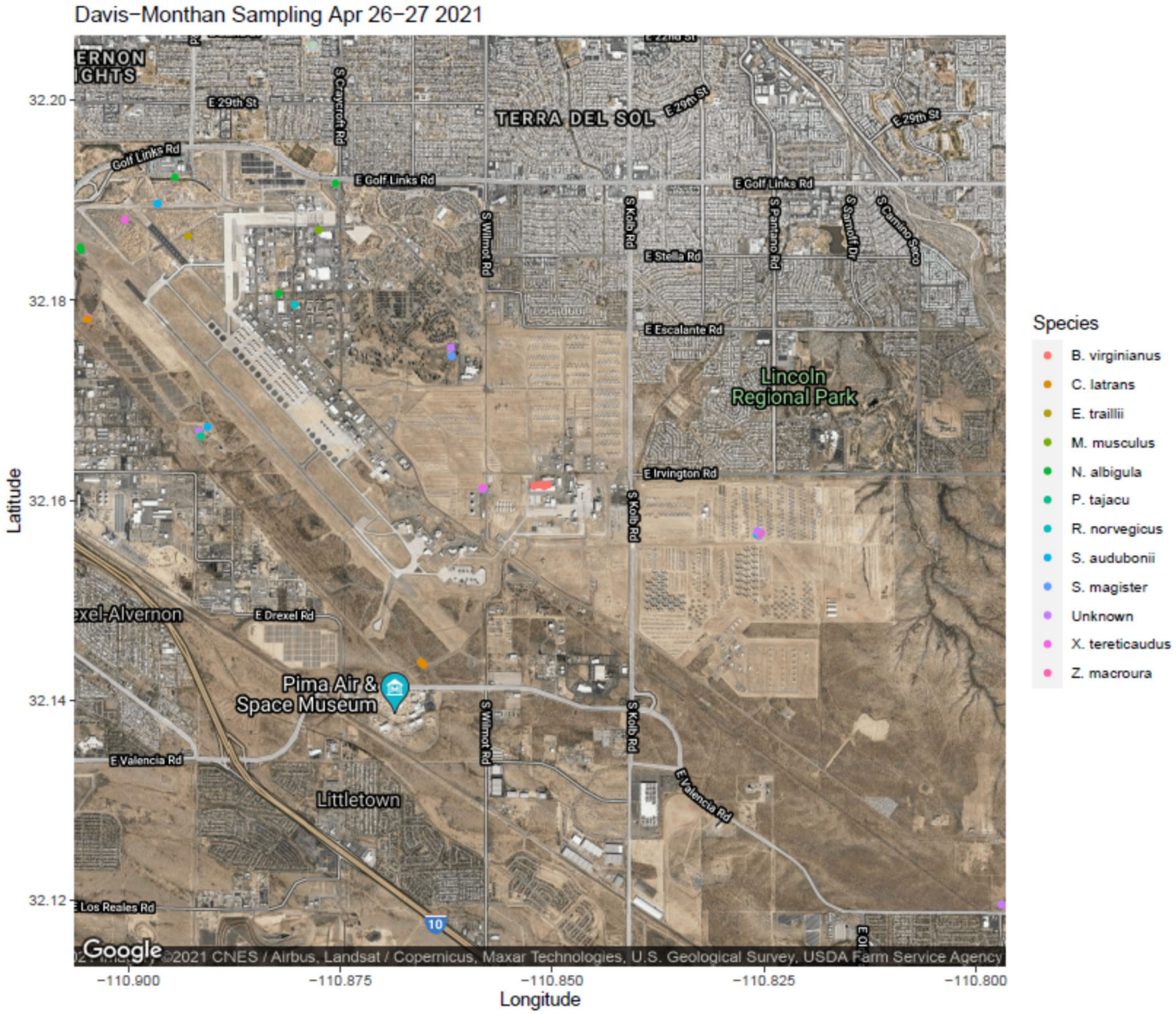

Figure B-4: Davis-Monthan AFB scat sampling with sequencing confirmed IDs

8.3 Appendix C: 16S Metabarcoding Genus Results per Sample

8.3.1 Wright-Patterson AFB

Table C1 : 20-308-053 Marmota monax (Groundhog)

Genus	Sequences	ESVs	ratio
NA	5919	454	0.86
Pseudomonas	345	24	0.05
Eleutherascus	270	8	0.04
Hyphozyma	145	13	0.02
Limnobacter	147	52	0.02
Altererythrobacter	2	2	0.00
Bresslauides	6	3	0.00
Chaetosphaeridium	10	1	0.00
Colpoda	5	4	0.00
Conexibacter	1	1	0.00
Edaphobaculum	2	1	0.00
Heteromita	11	7	0.00
IMCC26207	1	1	0.00
Lautropia	3	2	0.00
Marinobacter	9	2	0.00
Novosphingobium	1	1	0.00
Pajaroellobacter	1	1	0.00
Pir4 lineage	18	7	0.00
Planctomicrobium	6	2	0.00
Saccobolus	1	1	0.00
Thiopseudomonas	10	2	0.00
Total	6913	589	0.99

Table C2 : 20-322-02 Sciurus carolinensis (Eastern gray squirrel)

Genus	Sequences	ESVs	ratio
NA	4218	623	0.69
Defluviitaleaceae UCG-011	1220	131	0.20
Marvinbryantia	345	86	0.06
NK4A214 group	214	63	0.03
Escherichia-Shigella	119	11	0.02
[Eubacterium] oxidoreducens group	2	1	0.00
Lachnospiraceae UCG-006	20	5	0.00
Total	6138	920	1.00

Table C2: 20-322-03 Mephitis mephitis (Striped skunk)

Genus	Sequences	ESVs	ratio
NA	5964	738	0.59
Paenibacillus	3393	204	0.34
Aspergillus	215	27	0.02
Escherichia-Shigella	221	18	0.02
Fontibacillus	65	2	0.01
Lachnospiraceae UCG-006	79	10	0.01
Sphingobacterium	125	46	0.01
Citrobacter	21	4	0.00
Enterobacillus	1	1	0.00
Mucor	15	2	0.00
Peziza	16	3	0.00
Rhizomucor	4	1	0.00
Total	10119	1056	1.00

Table C3: 21-20-006 Zenaida macroura (Mourning dove)

Genus	Sequences	ESVs	ratio
Paenibacillus	3838	173	0.47
NA	3300	480	0.40
Cohnella	800	17	0.10
Lachnospiraceae NC2004 group	51	10	0.01
Pantoea	117	19	0.01
Aneurinibacillus	15	1	0.00
Bacillus	14	4	0.00
Domibacillus	4	1	0.00
Lachnoclostridium	19	8	0.00
Pilaira	21	3	0.00
Pseudogracilibacillus	4	2	0.00
Streptomyces	1	1	0.00
Total	8184	719	0.99

Table C 4: 21-20-007 Canis latrans (Coyote)

Genus	Sequences	ESVs	ratio
NA	10443	642	0.62
Arthrobacter	3325	165	0.20
Thelebolus	2152	46	0.13
Collinsella	687	162	0.04
Blautia	6	1	0.00
Mucor	6	2	0.00
Peptoclostridium	1	1	0.00
Pseudarthrobacter	30	4	0.00
Pseudomonas	65	22	0.00
Slackia	25	4	0.00
Total	16740	1049	0.99

Table C5 : 21-20-009 Sylvilagus floridanus (Eastern cottontail)

Genus	Sequences	ESVs	ratio
NA	10861	802	0.65
Paenibacillus	2416	191	0.14
Rheinheimera	950	143	0.06
Yersinia	1004	92	0.06
Escherichia-Shigella	638	22	0.04
Akkermansia	87	5	0.01
Candidatus Saccharimonas	131	50	0.01
Chryseobacterium	124	32	0.01
Cohnella	129	3	0.01
Lachnospiraceae UCG-006	150	6	0.01
Alishewanella	17	2	0.00
Cyniclomyces	38	10	0.00
Defluviitaleaceae UCG-011	4	1	0.00
Erysipelatoclostridium	17	5	0.00
Harryflintia	37	2	0.00
Lachnospiraceae NC2004 group	5	1	0.00
Paludicola	7	1	0.00
Pantoea	19	2	0.00
Prevotellaceae UCG-001	1	1	0.00
Rhodotorula	3	1	0.00
Rikenellaceae RC9 gut group	2	2	0.00
Ruminococcus	1	1	0.00
Salmonella	2	1	0.00
UCG-004	32	16	0.00
Total	16675	1392	1.00

Table C6 : 21-084-001 Branta canadensis (Canada goose)

Genus	Sequences	ESVs	ratio
Paenibacillus	1572	143	0.58
NA	873	82	0.32
Bacillus	81	26	0.03
Ammoniibacillus	54	11	0.02
Cohnella	49	8	0.02
Solibacillus	63	12	0.02
Fontibacillus	5	1	0.00
Jeotgalibacillus	7	3	0.00
Lysinibacillus	5	1	0.00
Total	2709	287	0.99

Table C7 : 21-084-001Z Branta canadensis with Zymo storage

Genus	Sequences	ESVs	ratio
Paenibacillus	1985	161	0.52
NA	1206	104	0.32
Cohnella	212	11	0.06
Bacillus	194	46	0.05
Ammoniibacillus	148	14	0.04
Solibacillus	45	5	0.01
Fontibacillus	2	1	0.00
Jeotgalibacillus	7	2	0.00
Pantoea	2	1	0.00
Total	3801	345	1.00

8.3.2 Moody AFB

Table C8 : 20-341-001 Inconclusive Host

Genus	Sequences	ESVs	ratio
NA	3804	441	0.49
Paenibacillus	2890	335	0.37
Mucor	358	19	0.05
Cohnella	211	3	0.03
Streptomyces	188	77	0.02
Ammoniibacillus	74	11	0.01
Brevibacillus	101	37	0.01
Citrobacter	78	8	0.01
Bacillus	28	12	0.00
Fontibacillus	20	2	0.00
Jeotgalibacillus	7	2	0.00
Kirkomyces	2	2	0.00
Rhodococcus	1	1	0.00
Solibacillus	4	1	0.00
Total	7766	951	0.99

Table C9 : 20-342-002 Inconclusive Host

Genus	Sequences	ESVs	ratio
NA	7579	600	0.58
Paenibacillus	3440	221	0.26
Mucor	785	31	0.06
Cohnella	455	12	0.03
Escherichia-Shigella	422	29	0.03
Lachnospiraceae UCG-006	123	11	0.01
Sphingobacterium	154	76	0.01
Acinetobacter	11	8	0.00
Ammoniibacillus	15	2	0.00
Citrobacter	39	4	0.00
Total	13023	994	0.98

Table C10 : 20-342-005 Inconclusive Host

Genus	Sequences	ESVs	ratio
Candidatus Udaeobacter	3407	242	0.44
NA	1283	282	0.17
Candidatus Solibacter	1168	237	0.15
Microvirga	821	145	0.11
LD29	776	32	0.10
Kouleothrix	65	18	0.01
Paenibacillus	74	9	0.01
Rhynchobodo	40	5	0.01
Sphingomonas	66	28	0.01
Acidicaldus	8	5	0.00
Bacillus	2	1	0.00
Bryobacter	1	1	0.00
Cohnella	2	2	0.00
Fontibacillus	11	2	0.00
Luteitalea	1	1	0.00
Total	7725	1010	1.01

Table C11 : 20-343-008 Dryophytes squirellus (Squirrel tree frog)

Genus	Sequences	ESVs	ratio
NA	11996	546	0.60
Paenibacillus	3906	155	0.20
Mucor	2980	32	0.15
Rhodotorula	692	66	0.03
Hydrogenoanaerobacterium	125	13	0.01
Acinetobacter	29	8	0.00
Colacogloea	22	2	0.00
Escherichia-Shigella	13	2	0.00
Fontibacillus	26	2	0.00
Pseudoplatyophrya	56	17	0.00
Ruminococcus	4	2	0.00
Sphingobacterium	2	1	0.00
Total	19851	846	0.99

Table C12 : 20-343-011 Procyon lotor (Racoon)

Genus	Sequences	ESVs	ratio
NA	6380	485	0.85
Escherichia-Shigella	269	13	0.04
Paenibacillus	309	78	0.04
Caproiciproducens	253	47	0.03
Clostridium sensu stricto 1	46	14	0.01
Lachnospiraceae UCG-006	83	9	0.01
Microbacter	46	8	0.01
Aspergillus	2	1	0.00
Citrobacter	34	3	0.00
Cohnella	15	2	0.00
Herbinix	31	14	0.00
Lachnospiraceae NC2004 group	1	1	0.00
Paludibacter	1	1	0.00
Sarcina	4	2	0.00
Total	7474	678	0.99

Table C13: 20-343-019 Inconclusive Host

Genus	Sequences	ESVs	ratio
NA	7220	743	0.65
Paenibacillus	2850	234	0.26
Acinetobacter	214	78	0.02
Bresslauides	168	39	0.02
Azospirillum	135	40	0.01
Colpoda	128	29	0.01
Escherichia-Shigella	76	14	0.01
Phaselicystis	134	36	0.01
Pseudomonas	81	22	0.01
Alkanindiges	2	1	0.00
Candidatus Soleaferrea	11	2	0.00
Cohnella	16	1	0.00
Fontibacillus	6	2	0.00
Hesseltinella	7	3	0.00
Lachnospiraceae UCG-006	10	4	0.00
Mucor	45	6	0.00
Rhizomucor	8	1	0.00
Rhodotorula	23	4	0.00
Tatumella	20	9	0.00
Total	11154	1268	1.00

Table C14 : 20-343-020 Mus musculus (House mouse)

Genus	Sequences	ESVs	ratio
NA	6940	313	0.88
Escherichia-Shigella	541	23	0.07
Bacteroides	253	69	0.03
Lachnospiraceae UCG-006	102	7	0.01
Hafnia-Obesumbacterium	10	3	0.00
Helicobacter	2	1	0.00
Hungatella	24	10	0.00
Total	7872	426	0.99

Table C15: 20-343-021 Mus musculus (House mouse)

Genus	Sequences	ESVs	ratio
NA	6124	978	0.78
Christensenellaceae R-7 group	891	82	0.11
Lactobacillus	749	133	0.09
Leminorella	60	10	0.01
Anaerotruncus	13	3	0.00
Bacteroides	7	4	0.00
Caproiciproducens	23	2	0.00
Desulfofarcimen	1	1	0.00
Pediococcus	25	4	0.00
Providencia	1	1	0.00
Xenorhabdus	1	1	0.00
Total	7895	1219	0.99

Table C16: 20-343-022 Mus musculus (House mouse)

Genus	Sequences	ESVs	ratio
NA	5114	494	0.85
Paenibacillus	373	72	0.06
Caproiciproducens	319	12	0.05
Chryseobacterium	42	19	0.01
Cohnella	39	6	0.01
Lactobacillus	57	12	0.01
Ammoniibacillus	8	2	0.00
Bergeyella	28	10	0.00
Candidatus Symbiothrix	2	2	0.00
Christensenellaceae R-7 group	26	5	0.00
Paludicola	16	4	0.00
Parabacteroides	7	7	0.00
Pir4 lineage	1	1	0.00
Robinsoniella	10	3	0.00
Sporolactobacillus	10	4	0.00
Total	6052	653	0.99

8.3.3 Davis-Monthan AFB

Table C17: 21-104-001 Neotoma albigula (White-throated woodrat)

Genus	Sequences	ESVs	ratio
Prevotellaceae Ga6A1 group	1125	40	0.41
Lactobacillus	989	109	0.36
NA	375	53	0.14
Alistipes	189	22	0.07
Ileibacterium	66	19	0.02
Allobaculum	2	1	0.00
Bifidobacterium	11	4	0.00
Lachnospiraceae UCG-008	2	1	0.00
Prevotellaceae UCG-004	9	1	0.00
Total	2768	250	1.00

Table C18 : 21-105-001 Neotoma albigula (White-throated woodrat)

Genus	Sequences	ESVs	ratio
NA	1110	252	0.60
Heteromita	425	177	0.23
Acinetobacter	72	40	0.04
Lactobacillus	65	18	0.04
Alistipes	56	13	0.03
Ileibacterium	49	20	0.03
Allobaculum	30	11	0.02
Bifidobacterium	20	8	0.01
Alkanindiges	3	1	0.00
Bacillus	3	1	0.00
Muribaculum	1	1	0.00
Pseudomonas	4	3	0.00
Total	1838	545	1.00

Table C19 : 21-116-002 Neotoma albigula (White-throated woodrat)

Genus	Sequences	ESVs	ratio
NA	398	39	0.74
Alistipes	80	11	0.15
Lactobacillus	61	15	0.11
Prevotellaceae UCG-004	1	1	0.00
Total	540	66	1.00

Table C20 : 21-116-003 Sylvilagus audubonii (Desert cottontail)

Genus	Sequences	ESVs	ratio
NA	2006	235	0.67
Providencia	905	93	0.30
Proteus	71	18	0.02
Xenorhabdus	3	1	0.00
Total	2985	347	0.99

Table C21 : 21-116-004 Sylvilagus audubonii (Desert cottontail)

Genus	Sequences	ESVs	ratio
NA	153	50	0.89
Alistipes	8	1	0.05
Akkermansia	5	1	0.03
Hydrogenoanaerobacterium	4	1	0.02
Monoglobus	2	1	0.01
Total	172	54	1.00

Table C22 : 21-116-007 Xerospermophilus tereticaudus (Round-tailed ground squirrel)

Genus	Sequences	ESVs	ratio
NA	464	66	0.50
Alistipes	259	19	0.28
Cokeromyces	206	34	0.22
Lachnospiraceae FCS020 group	3	2	0.00
Total	932	121	1.00

Table C23 : 21-116-008 Inconclusive host

Genus	Sequences	ESVs	ratio
NA	204	73	0.75
Rhodotorula	68	9	0.25
Massilia	1	1	0.00
Total	273	83	1.00

Table C24 : 21-116-010 Zenaida macroura (Mourning dove)

Genus	Sequences	ESVs	ratio
Lactobacillus	1663	245	0.56
NA	1253	197	0.43
Faecalibaculum	20	8	0.01
Paenibacillus	5	1	0.00
Pediococcus	3	1	0.00
Total	2944	452	1.00

Table C 25 : 21-116-013 Zenaida macroura (Mourning dove)

Genus	Sequences	ESVs	ratio
NA	2403	424	0.63
Heteromita	748	204	0.20
Acinetobacter	210	103	0.05
Alistipes	118	22	0.03
Lactobacillus	110	23	0.03
Ileibacterium	95	39	0.02
Allobaculum	30	9	0.01
Bifidobacterium	34	17	0.01
Desemzia	30	21	0.01
Alkanindiges	10	3	0.00
Bacillus	9	6	0.00
Muribaculum	3	1	0.00
Neurospora	5	1	0.00
Pisciglobus	1	1	0.00
Pseudomonas	15	9	0.00
Sordaria	2	1	0.00
Total	3823	884	0.99

Table C26: 21-116-019 Bubo virginianus (Great-horned owl)

Genus	Sequences	ESVs	ratio
NA	1886	332	0.55
Lactobacillus	914	149	0.27
Bifidobacterium	334	85	0.10
Allobaculum	163	35	0.05
Ileibacterium	136	20	0.04
Lachnospiraceae NK4A136 group	3	2	0.00
Total	3436	623	1.01

Table C27: 21-116-020 Xerospermophilus tereticaudus (Round-tailed ground squirrel)

Genus	Sequences	ESVs	ratio
NA	6569	746	0.86
Alistipes	581	28	0.08
Caproiciproducens	325	20	0.04
Escherichia-Shigella	112	9	0.01
Blautia	1	1	0.00
Lachnospiraceae AC2044 group	1	1	0.00
Lachnospiraceae FCS020 group	23	4	0.00
Lachnospiraceae NK4A136 group	4	1	0.00
Lachnospiraceae UCG-006	25	4	0.00
Lachnospiraceae UCG-008	5	2	0.00
Prevotellaceae UCG-004	17	2	0.00
Total	7663	818	0.99

Table C28 : 21-116-021 Empidonax traillii (Willow flycatcher)

Genus	Sequences	ESVs	ratio
NA	558	150	0.59
Naganishia	262	96	0.28
Enterococcus	117	49	0.12
Alternaria	11	7	0.01
Total	948	302	1.00

Table C29 : 21-116-022 Inconclusive – Sylvilagus audubonii (Desert cottontail)

Genus	Sequences	ESVs	ratio
NA	3263	192	0.91
Christensenellaceae R-7 group	122	20	0.03
Naganishia	103	59	0.03
Peziza	60	6	0.02
Alistipes	45	12	0.01
Total	3593	289	1.00

Table C30: 21-116-024 Pecari tajacu (Javelina)

Genus	Sequences	ESVs	ratio
NA	3516	655	0.81
Alistipes	320	24	0.07
Escherichia-Shigella	120	17	0.03
Lysinibacillus	110	38	0.03
Pyramidobacter	81	16	0.02
Christensenellaceae R-7 group	35	14	0.01
Lachnospiraceae UCG-006	51	8	0.01
Phascolarctobacterium	37	25	0.01
Bacillus	15	4	0.00
CAG-352	4	1	0.00
Catenibacterium	1	1	0.00
Cellulosilyticum	7	3	0.00
Citrobacter	3	1	0.00
Coprobacter	6	2	0.00
Lachnospiraceae UCG-008	2	1	0.00
Marvinbryantia	3	1	0.00
Paenibacillus	11	2	0.00
Prevotella	4	3	0.00
Prevotellaceae UCG-004	7	1	0.00
Rikenellaceae RC9 gut group	6	4	0.00
Solibacillus	3	1	0.00
UCG-005	13	2	0.00
Total	4355	824	0.99

Table C31 : 21-116-025 Canis latrans (Coyote)

Genus	Sequences	ESVs	ratio
Alloprevotella	3687	61	0.42
NA	3629	490	0.41
Prevotellaceae Ga6A1 group	535	4	0.06
Negativibacillus	443	34	0.05
Escherichia-Shigella	227	23	0.03
Anaerobiospirillum	96	35	0.01
Lachnospiraceae UCG-006	95	7	0.01
Bacteroides	24	10	0.00
Cyniclomyces	3	1	0.00
Fusobacterium	2	1	0.00
Oscillibacter	39	5	0.00
Phascolarctobacterium	3	1	0.00
Providencia	15	5	0.00
Succinivibrio	2	1	0.00
Total	8800	678	0.99

Table C32 : 21-116-030 Neotoma albigula (White-throated woodrat)

Genus	Sequences	ESVs	ratio
Lactobacillus	1423	245	0.39
Prevotellaceae Ga6A1 group	991	39	0.27
NA	851	192	0.23
Allobaculum	361	146	0.10
Ileibacterium	40	15	0.01
Alistipes	11	4	0.00
CAG-873	1	1	0.00
Faecalibaculum	1	1	0.00
Lachnospiraceae UCG-008	8	2	0.00
Roseburia	2	1	0.00
Total	3689	646	1.00

Table C33 : Inconclusive – Odocoileus virginianus (White-tailed deer)

Genus	Sequences	ESVs	ratio
NA	1443	364	0.90
Planomicrobium	96	37	0.06
Planococcus	49	20	0.03
Microvirga	13	11	0.01
Total	1601	432	1.00

Table C34 : 21-116-032 Sceloporus magister (Desert spiny lizard)

Genus	Sequences	ESVs	ratio
NA	8776	694	0.83
Caproiciproducens	1488	28	0.14
[Eubacterium] nodatum group	118	48	0.01
Desulfovibrio	132	43	0.01
Bilophila	1	1	0.00
Blautia	6	2	0.00
Lachnospiraceae UCG-008	1	1	0.00
Lactonifactor	2	1	0.00
Odoribacter	26	4	0.00
Salmonella	18	13	0.00
Total	10568	835	0.99

Table C35 : 21-116-033 Inconclusive Host

Genus	Sequences	ESVs	ratio
NA	3200	263	0.80
Arthrobacter	384	82	0.10
Zymoseptoria	221	15	0.06
Leptosphaeria	104	5	0.03
Preussia	43	6	0.01
Pseudarthrobacter	29	9	0.01
Janthinobacterium	5	1	0.00
Massilia	3	2	0.00
Total	3989	383	1.01

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

ACRONYM	DESCRIPTION
16S	16S ribosomal RNA gene
AFB	Air Force Base
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Tool
BEI	Biological and Emerging Infections [BEI Resources]
BEI-RRP	Biological and Emerging Infections Research Resources Program, designated BEI Resources
CEV	California Encephalitis Virus
COI	Cytochrome Oxidase Subunit I
LACV	La Crosse Virus
NCBI	National Center for Biotechnology Information
WNV	West Nile Virus