

Article title

16S rRNA Gene Sequencing of Microbiota from the Preen Oil and Cloaca of Chipping Sparrows (*Spizella passerina*)

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Running title

Chipping Sparrow Microbiome Analysis

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Abstract

We present the results of 16S rRNA gene amplicon sequencing of the microbiota from preen oil and the cloaca of chipping sparrows (*Spizella passerina*) collected near Mountain Lake Biological Station (MLBS) in Pembroke, VA.

Announcement

New world sparrows (Passerillidae), specifically non-migratory dark-eyed juncos (*Junco hyemalis carolinensis*), are at the forefront of avian microbial ecology studies (1). These birds harbor symbiotic bacteria used for chemical communication through preen oil (2). In the same habitat is one overlooked species, the migratory chipping sparrow (*Spizella passerina*). Here, we describe microbial communities of the preen oil and cloaca of chipping sparrows to provide information for future interspecific comparative studies.

This study was conducted in compliance with Indiana University Bloomington Institutional Animal Care and Use Committee guidelines (15–026), US Fish and Wildlife Service (MB093279-1), and Virginia Department of Game and Inland Fisheries (058772). Birds were sampled as previously described (2). Microbial communities from the preen oil and cloaca were collected using a pre-moistened swab with sterile buffer (20 mM Tris pH 8; 2 mM EDTA; 1.2% Triton X-100). We extracted DNA with the QIAGEN DNeasy Powerlyzer PowerSoil DNA Isolation Kit with the following modifications: 1) Swabs were soaked in 500 µL bead solution and 200 µL phenol:chloroform:isoamyl alcohol for 10 min before bead-beating. 2) Samples received 100 µL each of solutions C2 and C3, plus 1 µL RNase A, and incubated at 4°C for 5 min before one-step centrifugation. 3) Lysates were mixed with 650 µL solution C4 and 650 µL 100% ethanol instead of using 1200 µL solution C4 alone. 4) DNA was eluted in 60 µL of solution C6, reduced from 100 µL (1). We amplified bacterial DNA using nested PCR as described previously (2). The amplified V4 region of the 16S rRNA gene was prepared using the V2 500 cycle MiSeq Reagent Kit (Illumina MS102-2003) and sequenced on the Illumina MiSeq platform by the Michigan State University Research Technology Support Facility's Genomics Core generating 2 x 250 bp reads.

Analyses were performed using R Statistical Software v4.3.3 (3). We used DADA2 v1.30.0 (4) to process sequencing reads. Forward and reverse reads were trimmed 10bp at the 5' end and truncated at 240bp and 200bp at the 3' end respectively. Paired-end reads were merged and chimeric sequences

were removed. Table 1 tracks reads through the DADA2 pipeline. We assigned taxonomy using the SILVA 138.1 data set with species information (5). Contaminating sequences from blank and water extractions were removed using decontam v1.22.0 (6). We used phyloseq v1.46.0 (7) to analyze alpha and beta diversity metrics. Finally, we used vegan v2.6.6.1 (8) for statistical analyses and ggplot2 v3.5.1 (9) for generating figures.

A column chart comparing relative order abundance between the preen oil and cloaca showed no noticeable differences (Fig. 1A). This was confirmed by the Similarity Percentages function (simper) in vegan which did not identify any statistically significantly different taxa in preen oil when compared to cloaca. Observed amplicon sequence variants (ASVs), Shannon diversity, and Simpson's diversity index, showed that the preen oil community was less diverse than that of the cloaca, but the data were not significant (Fig. 1B). We saw no significant difference in Bray-Curtis dissimilarity between the preen oil and cloaca communities (Fig. 1C).

Data availability statement

The 16S rRNA gene amplicon sequences have been deposited in the GenBank Sequence Read Archive (SRA) under the BioProject accession number [PRJNA1117373](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1117373) under the SRA accession numbers [SRR29202434](https://www.ncbi.nlm.nih.gov/sra/SRR29202434)-[SRR29202455](https://www.ncbi.nlm.nih.gov/sra/SRR29202455).

Acknowledgments

This work was supported by the BEACON Center for the Study of Evolution in Action (National Science Foundation DBI-0939454). We thank the University of Virginia, Mountain Lake Biological Station, and the Mountain Lake Lodge.

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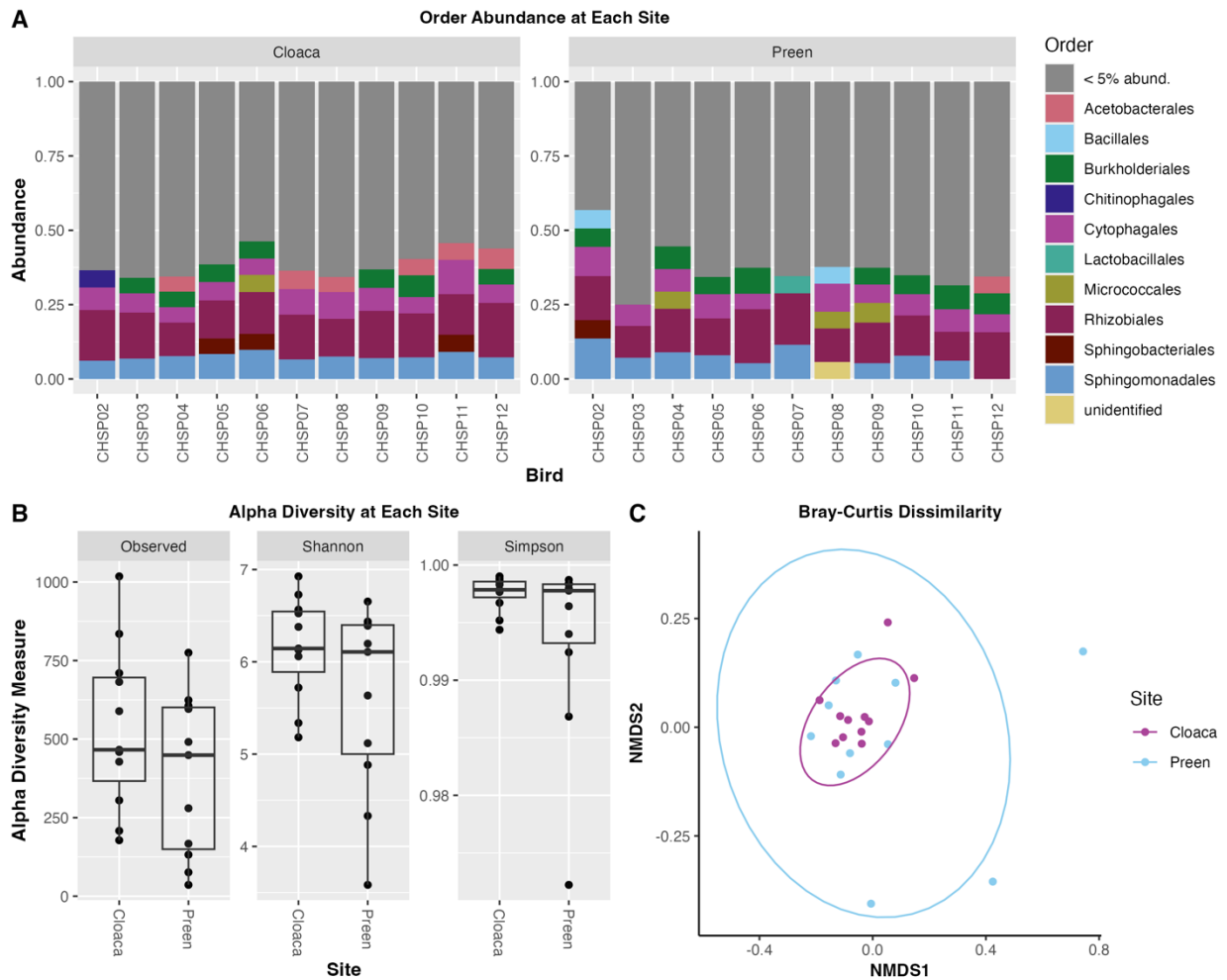


Figure 1. Microbial Diversity and Community Composition in Cloaca and Preen Gland Samples from chipping sparrows. A) Relative abundance of orders obtained from 16S rRNA gene sequencing of preen oil and the cloaca. Orders with less than 5% abundance were grouped together as were orders that were unidentified. B) Alpha diversity of cloaca and preen oil communities. C) NMDS plot of Bray-Curtis dissimilarity.

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| Sample | Site | Input | Filtered | Denoised F | Denoised R | Merged | Non-Chimera | NCBI Accession |
|--------|--------|--------|----------|------------|------------|--------|-------------|-----------------------------|
| 237 | Preen | 38532 | 34316 | 33689 | 33706 | 32041 | 30803 | SRR29202434 |
| 22 | Preen | 59017 | 53469 | 52934 | 52982 | 48990 | 48558 | SRR29202435 |
| 214 | Cloaca | 19470 | 18049 | 17818 | 17853 | 17483 | 17483 | SRR29202436 |
| 20 | Cloaca | 54567 | 45840 | 44686 | 44668 | 40451 | 39019 | SRR29202437 |
| 207 | Preen | 8057 | 7037 | 6860 | 6878 | 6591 | 6542 | SRR29202438 |
| 186 | Cloaca | 29577 | 25491 | 25041 | 25060 | 23672 | 22923 | SRR29202439 |
| 184 | Cloaca | 55020 | 48926 | 48163 | 48153 | 46358 | 44916 | SRR29202440 |
| 93 | Preen | 11302 | 10028 | 9851 | 9844 | 9332 | 9012 | SRR29202441 |
| 8 | Cloaca | 53394 | 48310 | 46990 | 47125 | 43746 | 42312 | SRR29202442 |
| 180 | Cloaca | 112134 | 100851 | 100044 | 100117 | 89998 | 88560 | SRR29202443 |
| 39 | Cloaca | 11372 | 9649 | 9344 | 9322 | 8763 | 8707 | SRR29202444 |
| 377 | Cloaca | 58412 | 53359 | 52344 | 52381 | 50307 | 49818 | SRR29202445 |
| 372 | Cloaca | 45801 | 42088 | 41404 | 41524 | 40076 | 39548 | SRR29202446 |
| 329 | Preen | 40065 | 36088 | 35652 | 35645 | 34274 | 33466 | SRR29202447 |
| 326 | Preen | 44570 | 40618 | 39818 | 39740 | 37751 | 37589 | SRR29202448 |
| 319 | Preen | 20659 | 18642 | 18256 | 18305 | 17466 | 17133 | SRR29202449 |
| 2 | Cloaca | 40953 | 35685 | 34840 | 34926 | 33020 | 31999 | SRR29202450 |
| 298 | Preen | 9871 | 9269 | 9186 | 9208 | 9144 | 6304 | SRR29202451 |
| 283 | Preen | 1655 | 1461 | 1379 | 1374 | 1308 | 1308 | SRR29202452 |
| 262 | Cloaca | 23692 | 21166 | 20744 | 20850 | 19894 | 18729 | SRR29202453 |
| 123 | Preen | 3984 | 3585 | 3452 | 3488 | 3244 | 3202 | SRR29202454 |
| 103 | Preen | 43007 | 38430 | 37808 | 37882 | 36601 | 35407 | SRR29202455 |

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