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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 using Biospec Products MiniBeadBeater-16 run 2X for 30 sec. 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 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 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. Default parameters for DADA2 were used except reads were trimmed 10bp

at the 5’ end and truncated at 240bp (F) and 200bp (R) at the 3’ end. 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 (Observed amplicon sequence variants (ASVs), Shannon diversity, and Simpson’s diversity index) and beta diversity (Bray-Curtis dissimilarity). 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 preen oil and cloaca showed no noticeable differences (Fig. 1A). The Similarity Percentages function (simper) did not identify any statistically significantly different taxa in preen oil compared to cloaca. Alpha diversity analysis showed that the preen oil community was less diverse than that of the cloaca, though not significantly (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 under the SRA accession numbers SRR29202434- SRR29202455.

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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.

Table 1: Sample information for sequencing reads.

Bird	Sample	Site	Input	Filtered	Denoised F	Denoised R	Merged	Non-Chimera	NCBI Accession
CHSP02	262	Cloaca	23692	21166	20744	20850	19894	18729	SRR29202452
CHSP03	8	Cloaca	53394	48310	46990	47125	43746	42312	SRR29202442
CHSP04	39	Cloaca	11372	9649	9344	9322	8763	8707	SRR29202444
CHSP05	2	Cloaca	40953	35685	34840	34926	33020	31999	SRR29202438
CHSP06	377	Cloaca	58412	53359	52344	52381	50307	49818	SRR29202445
CHSP07	372	Cloaca	45801	42088	41404	41524	40076	39548	SRR29202446
CHSP08	20	Cloaca	54567	45840	44686	44668	40451	39019	SRR29202437
CHSP09	184	Cloaca	55020	48926	48163	48153	46358	44916	SRR29202440
CHSP10	214	Cloaca	19470	18049	17818	17853	17483	17483	SRR29202435
CHSP11	180	Cloaca	112134	100851	100044	100117	89998	88560	SRR29202443
CHSP12	186	Cloaca	29577	25491	25041	25060	23672	22923	SRR29202439
CHSP02	123	Preen	3984	3585	3452	3488	3244	3202	SRR29202454
CHSP03	298	Preen	9871	9269	9186	9208	9144	6304	SRR29202450
CHSP04	93	Preen	11302	10028	9851	9844	9332	9012	SRR29202441
CHSP05	103	Preen	43007	38430	37808	37882	36601	35407	SRR29202455
CHSP06	237	Preen	38532	34316	33689	33706	32041	30803	SRR29202453
CHSP07	207	Preen	8057	7037	6860	6878	6591	6542	SRR29202436
CHSP08	283	Preen	1655	1461	1379	1374	1308	1308	SRR29202451
CHSP09	319	Preen	20659	18642	18256	18305	17466	17133	SRR29202449
CHSP10	326	Preen	44570	40618	39818	39740	37751	37589	SRR29202448
CHSP11	22	Preen	59017	53469	52934	52982	48990	48558	SRR29202434
CHSP12	329	Preen	40065	36088	35652	35645	34274	33466	SRR29202447