Cold and lonely: low microbial abundance and no core microbiota in mosquito populations across Greenland

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Abstract

Microbes are key players in insect ecology and evolution, but host-microbe interactions vary widely in stability and predictability across and within host species. The microbiota of insect clades such as high-latitude mosquitoes remain understudied, despite their high abundance and potential economic and medical relevance. Here, we implemented a novel approach combining amplicon-based species identification, microbial quantification and community composition reconstruction, and joint species distribution modelling, to resolve temporal and spatial variation in the abundance and diversity of bacteria and fungi associated with *Ochlerotatus* (formerly *Aedes*) mosquitoes across Greenland. COI amplicon data for 573 mosquitoes collected from five regions of Greenland over 15 years enabled the identification of two species, *O. nigripes* and *O. impiger*, revealing an overlap in their distributions and limited within-species genetic structure. Quantitative 16S-V4 rRNA amplicon sequencing showed low bacterial densities compared to other insect species, alongside highly variable microbiota composed of environmentally versatile genera, including *Janthinobacterium*, *Pseudomonas*, *Serratia*, and *Escherichia-Shigella*. Interestingly, several mosquitoes yielded DNA reads of local vertebrates (likely from blood meals), along with putative vertebrate pathogens such as *Bartonella.* Fungal communities were low in density and variable in composition, with much of the diversity comprising putative phytopathogens. In contrast, *Cladosporium* was consistently detected across mosquitoes and controls, indicating likely contamination. Notably, bacterial and fungal communities showed pronounced variation across geographic locations and sampling years, but not between sexes. Together, these findings suggest that Greenland *Ochlerotatus* mosquitoes do not rely on core microbiota, but engage in flexible associations with diverse, low-abundance microbial communities. They also point to mosquitoes as likely vectors of bacterial and fungal pathogens in the Arctic.

Keywords: symbiosis, high-throughput sequencing, 16S rRNA metabarcoding, microbiota absolute abundance, mosquito

Introduction

Symbiotic microorganisms have played crucial roles in the ecology and evolution of insects, often influencing their hosts' biology in multiple ways (Douglas, 2022). For example, microbes can affect insects' ability to survive on specific diets, provide resistance to toxic chemicals, protect against pathogens, parasites, parasitoids, and predators, or influence reproduction (Kaltenpoth et al., 2025; Łukasik & Kolasa, 2024). On the other hand, while many insects rely on symbionts, others appear to thrive with little or no microbial assistance. In some species, host-adapted microbial communities are sparse or apparently absent (Hammer et al., 2017, 2019; Sanders et al., 2017). Furthermore, even within a single species, microbial diversity and abundance can vary dramatically (Lange et al., 2023), highlighting the greater dynamics and context-dependency of host-microbiome relationships.

During the last decade, the development of sequencing techniques and a decrease in sequencing costs have led to a rapid increase in the number of studies on microbial community composition across diverse insect clades. Unfortunately, many of these studies have struggled with methodological challenges such as contamination, casting doubt on their outcomes. Such challenges are particularly pronounced in systems with overall low microbial abundances (Knight et al., 2018; Salter et al., 2014; Williamson et al., 2025). Across insects in general, we are still far from understanding how microbial diversity and abundance vary across and within species, across populations, sexes, or over time and space (Adair et al., 2020; Malacrinò, 2022; Thompson et al., 2017). This lack of comprehensive knowledge limits our conclusions on insect biology, including the factors that govern their behaviour, ecological roles, and significance to the human economy and health.

The nutritional imbalance of the diet of blood-feeding insects has promoted associations with microbial symbionts that supplement deficient nutrients (Cornwallis et al., 2023; Husnik, 2018). Since blood-feeding has evolved independently in different insect clades that rely on blood to different extents (Lehane, 2005), their associations with symbiotic bacteria are also rather variable. Some species may form loose associations, especially those that can source nutrients from alternative foods, while others are strictly dependent on symbionts (Husnik, 2018; Manzano-Marín et al., 2015; Nováková et al., 2015). Furthermore, besides nutrient-providing symbionts or opportunistic microbes, blood-feeding insects can also vector pathogenic microbes, thereby posing a health threat. Given the persisting debate around the significance of microorganisms for these insects, understanding the distribution and predictability of such associations remains topical and essential. This is certainly the case for mosquitoes (family Culicidae), a diverse clade that is a nuisance in nearly every region of the world, but also critical vectors of many viruses (Hernandez-Valencia et al., 2023; Janjoter et al., 2024; Moonen et al., 2023; Olmo et al., 2023), of pathogenic micro-eukaryotes (Guttery et al., 2022), and of other disease-causing agents (Batson et al., 2021). While the specific roles of many of the members of the microbial community in mosquitoes are generally unknown, some of them have shown great potential in hampering host reproduction and in reducing vectorial capacity. Specifically, the introduction of specific strains of the endosymbiont *Wolbachia* has been shown to lower disease transmission efficiency and to improve public health (Alomar et al., 2023; Hoffmann et al., 2024; Minwuyelet et al., 2023). Nevertheless, much of the mosquito microbiome research has focused on a few model species linked to the transmission of the most significant human disease agents. Moreover, most studies have been conducted on laboratory populations, leaving the natural microbiome of the vast majority of the more than 3,000 mosquito species described to date underexplored (Alomar et al., 2023; Hoffmann et al., 2024; Minwuyelet et al., 2023). As a result, it remains unresolved how often diverse mosquito species rely on specific microbial clades for nutrition, defense, or reproduction, or whether the mere presence of any bacteria may suffice.

Greenland is known for its overwhelming number of mosquitoes in the summer. The two species reported from the island, *Ochlerotatus nigripes* (Fig. 1a,b) and *Ochlerotatus impiger* (both formerly in the genus *Aedes)* (Böcher et al., 2015; Reeves et al., 2013) feed on the blood of a wide range of local mammals and birds (Corbet & Downe, 1966). However, they can also consume plant nectar, and females are able to mature their egg batches without a blood meal (Corbet, 1967). This plasticity in diet is likely contributing to great population sizes even when vertebrate hosts are scarce. Although the transmission of disease-causing viruses by mosquitoes and other vectors has been recorded in polar regions, the vectorial capacity of *O. nigripes and O. impiger* in Greenland is generally considered limited (Müllerová et al., 2018; Schilling et al., 2025), with the exception of the single report of Jamestown Canyon encephalitis virus in a Nuuk population (Snyman et al., 2024). Nevertheless, vectorial capacity can vary geographically and be influenced by both abiotic drivers, such as climate change, and biotic factors, including the host's microbiota. Understanding the factors shaping the microbiota of Greenland mosquitoes is thus of high importance.

In this study, we comprehensively characterise the microbial communities of mosquitoes across Greenland. Our aim is to understand the patterns and likely drivers of microbial abundance, diversity, and distribution across mosquito species, populations, haplotypes, and between sexes, and to investigate whether these common insects carry symbiotic bacteria and fungi with a known functional impact on host reproduction, nutrition, or defense.

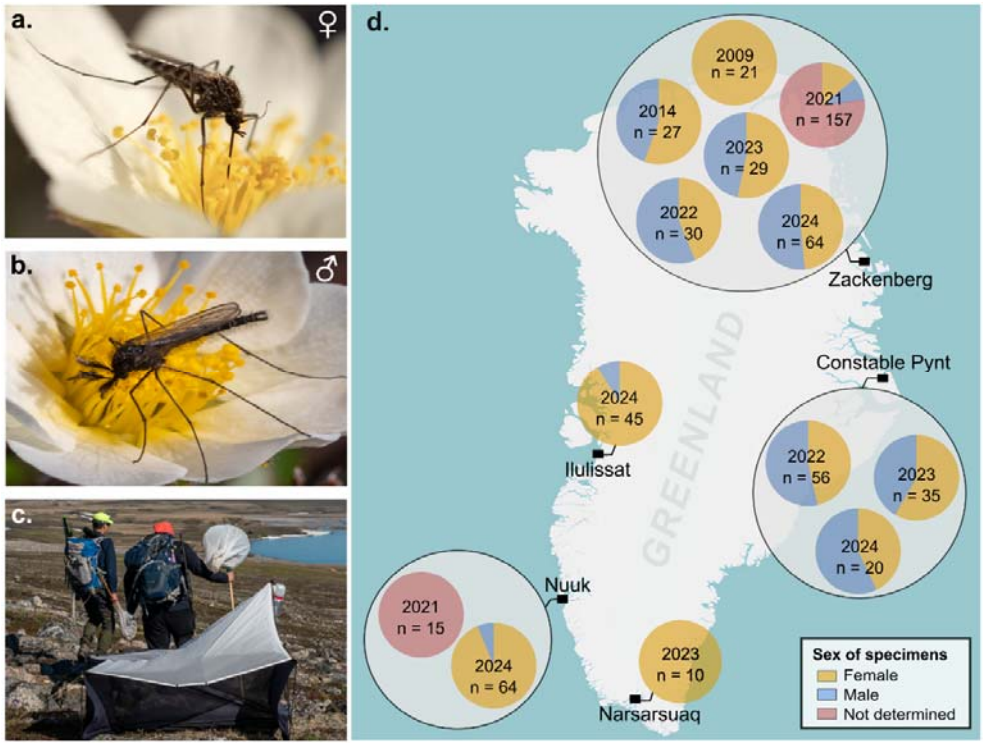

Fig. 1. *Ochlerotatus nigripes* a) female and b) male, feeding on nectar of *Dryas* flowers in Zackenberg Valley, NE Greenland. c) Two of the study authors (TR & MK) in Zackenberg Valley, demonstrating the sampling tools: a Malaise trap and a sweep net. Photos: PŁ. d) Mosquito sampling sites, years, and sampling depth. Colors within pie charts indicate the proportion of successfully processed females, males, and individuals whose sex was not recorded, in each site/year combination.

Methods

Sampling and specimen selection

Insects were collected by sweep netting or Malaise traps (Fig. 1c) in five areas of Greenland: Zackenberg Valley, Constable Pynt, Narsarsuaq, Nuuk, and Illulissat, between 2009 and 2024 (Fig. 1d, Table S1). Specimens were preserved in 96% ethanol and stored at -20°C until processing.

From our Zackenberg 2021 and Nuuk 2021 collections, we processed diverse insects, including mosquitoes (Buczek et al., 2024), without their prior identification or sexing, and later we selected mosquito specimens based on the barcode sequence (see below). From the remaining multi-species community samples, we specifically sexed and selected mosquitoes based on morphology (following Dahl, 2015). As a reference for microbial abundance comparisons, we selected libraries corresponding to four additional species from the Zackenberg 2021 collection based on barcode assignment and microbial load estimation. As additional references for these comparisons, we used *Wolbachia*-infected and *Wolbachia*-free *Aedes aegypti* adult mosquitoes from a long-term laboratory culture at Jagiellonian University and overwintering *Culex pipiens* mosquito females collected in a cottage basement in the village of Przybysławice near Miechów, South Poland (Table S1).

Amplicon library preparation and sequencing

Mosquitoes were processed individually, in five batches (Table S1), following the DNA extraction and library preparation protocol detailed recently (Buczek et al., 2024). In short, the mosquitoes were placed in 2ml tubes with 200 µl lysis buffer, homogenised using ceramic beads, and then incubated at 55 °C for two hours. To 40 µl of the homogenate aliquot, we added 20,000 copies of a linearised plasmid carrying an artificial 16S rRNA amplification target, either Ec5502 or Ec5001 (Tourlousse et al., 2016), to enable subsequent quantification, and purified the DNA using SPRI beads (Rohland & Reich, 2012). For each batch of 88 samples, we used a positive extraction control and two negative extraction controls.

Amplicon libraries were prepared using a two-step PCR approach (Table S2). During the first step, we simultaneously amplified a 418 bp portion of insect cytochrome c oxidase I (COI) gene, the 16S rRNA V4 region (16S hereafter) for bacteria, and the Internal Transcribed Spacer region 2 (ITS2) for fungi, using a mixture of primers BF3-BR2 (Elbrecht et al., 2019), 515F-806R (Parada et al., 2016), and ITS1F-ITS2 (Buée et al., 2009; White et al., 1990), respectively, with Illumina adapter tails. During the second step, the first-step PCR products were cleaned using SPRI beads; subsequently, they were used as templates for second PCR reactions with primers that completed the adapters and added a unique combination of indexes for each sample. Two positive PCR controls and two blank PCR controls were included along with mosquito samples for PCR I, and an additional blank control was included for the second round of PCR II reactions. Amplification success and amplicon size were verified by agarose gel electrophoresis after each PCR. All amplicon libraries were pooled together based on gel band intensity, cleaned with SPRI beads, and submitted for sequencing on either NovaSeq 6000 SPrime 500-cycle flow cell or NextSeq 2000 P2 600-cycle flow cell (Table S1).

Data analysis

Demultiplexed fastq files for all samples were run through our customized bioinformatic pipeline, described in detail at https://github.com/Symbiosis-JU/Bioinformatic-pipelines. Initially, reads were split into bins corresponding to different targeted regions (COI, 16S rRNA or ITS2) based on primer sequences at the beginning of each read. For each marker region, paired-end reads were quality-filtered and merged using PEAR (Zhang et al., 2014). Representative sequences were then

dereplicated, denoised, taxonomically classified, and clustered into Operational Taxonomic Units (OTUs) based on 97% similarity threshold, using VSEARCH (Rognes et al., 2016), USEARCH (Edgar, 2010) and UNOISE (Antich et al., 2021). Taxonomy assignment was based on the MIDORI reference database version GB239 (Leray et al., 2022) for COI, the SILVA database v138 (Quast et al., 2012; Yilmaz et al., 2014) for 16S rRNA and the UNITE database v10.0 (Abarenkov et al., 2024) for ITS2 amplicon sequences. For each of the regions, the pipeline provided tables with two levels of classification: ASVs (Amplicon Sequencing Variant) and OTUs (Operational Taxonomic Unit). The resulting data tables (https://doi.org/10.6084/m9.figshare.c.8012695.v1) were then used as input for a series of marker region-specific custom steps, as described below.

Mitochondrial data analysis

The COI ASV table was used to determine mosquito population structure across the sampled locations and to reconstruct potential biotic interactions. Libraries with fewer than 100 total COI reads were excluded from analysis. For all others, we selected the most abundant COI variant among ASVs taxonomically assigned to the genus *Ochlerotatus* as the specimen barcode.

COI ASVs taxonomically assigned to taxa other than mosquitoes were separated and used to reconstruct the presence/absence of biological signal of associated species (i.e., putative sources of blood meal, or parasitic mites). Using a conservative approach, we selected 10 reads as the threshold for reporting presence.

Microbial data analysis

The ASV table for the 16S rRNA marker gene sequences was used as a template for a custom reference-based decontamination process combined with template quantification (Łukasik et al., 2017; Nowak et al., 2025). We excluded any ASVs taxonomically assigned as mitochondria, chloroplasts, Eukaryota, Archaea, or chimeras. We then compared the maximum relative abundance attained by each unique 16S rRNA ASV to its relative abundance in any negative control. Unless the maximum relative abundance attained in at least one experimental library was more than ten times the maximum relative abundance attained in any blank library, the ASV was classified as a ‘contaminant’. The remaining sequences were classified as corresponding to ‘non-contaminants’. Additionally, we screened for ASVs classified taxonomically as *Polaromonas* sp., *Brachybacterium* sp. or *Variovorax* sp., which are known contaminants in the reagents used in our laboratory (Fig. S1).

Next, we estimated the absolute abundance of bacterial 16S rRNA gene copies in each processed sample based on the spike-in reference (Tourlousse et al., 2016). Specifically, we multiplied the ratio of decontaminated reads to spike-in reads by the number of spike-in copies added (20,000) and by 5, to account for using only one-fifth of the insect homogenate during the DNA extraction. To assess whether total microbial load varied significantly across mosquito populations from various Greenland locations sampled over multiple years, and between sexes and mosquito species, we applied a generalised linear model (GLM) to log-transformed absolute 16S rRNA copy counts. Since we were primarily interested in the consistency of abundances between mosquito species, sexes, sites and years, we included the main effects of these factors, and their interactions. Models were fitted using the stats package (Venables & Ripley, 2002) in RStudio v4.4.1. Samples for mosquito individuals with undetermined sex were excluded from this analysis.

ITS2 ASV table was decontaminated as described for 16S rRNA data. As no ITS spike-ins were included during library preparation, we followed our previous approach (Płoszka et al., 2025) and used the insect COI read number as a baseline and reference for comparing fungal amounts among individual insects. To this effect, for all libraries with fungal amplicon data, we computed ITS2/COI read number ratios as a means of assessing relative abundance of fungi.

ASV/OTU richness and abundance distribution were estimated for the full bacterial and microbial decontaminated datasets. Non-metric Multidimensional Scaling (NMDS) was performed using the ordinate function in the Phyloseq package (McMurdie & Holmes, 2013) with Bray–Curtis dissimilarity, four dimensions (k = 4), and up to 200 iterations per run (try = 200), allowing a maximum of 500 steps per iteration (maxit = 500). The algorithm was allowed up to 1000 random starts (trymax = 1000). Ordinations included only samples for which indivual sex was determined. ComplexPheatmap (Gu, 2022; Gu et al., 2016) and ggplot2 (Wickham, 2016) were used for visualisation. Core microbiome analysis and UpSet plots were performed with mia (Borman et al., 2025) and microbiome (Lahti & Sudarshan, 2017).

Hierarchical Modelling of the Microbial Species Communities

To assess how host factors and environmental settings influence the composition of *Ochlerotatus* microbial communities, we used generalised linear mixed modelling of multivariate responses, applied through the joint species distribution model of Hierarchical Modelling of Species Communities (HMSC) (Ovaskainen et al., 2017; Ovaskainen & Abrego, 2020). For bacteria, we focused on variation at the level of 16S rRNA OTUs, quantified in terms of their absolute abundance and their identity and distribution across 400 individual mosquitoes. For fungi, we focused on variation at the level of ITS2 OTUs as successfully quantified across 364 mosquitoes.

Due to the zero-inflated nature of the data (i.e. the many absences of individual taxa, corresponding to counts of zero), we modelled microbial abundances through a hurdle approach: we first modelled presence-absences, then abundance conditional on presence – in each case fitting separate models to bacteria and fungi. In modelling presence-absences, we assumed a Bernoulli distribution and a probit link function; in modelling abundances conditional on presence, we log-transformed and scaled the response, assuming a normal distribution. For bacteria, we used the estimated total copy numbers per individual of 16S-V4 OTUs as our metric of abundance (see previous section for how these estimates were derived). For fungi, we lacked a spiked-in benchmark for the ITS2 marker and, therefore, used the raw abundances. (For how this was accounted for in model structures, see below).

To measure the extent to which the mosquito microbiota composition is predictable, we used the species of mosquito, sex of individuals (excluding those with undetermined sex), and the interaction between sex and species as fixed effects. Since the sequencing depth of each individual (defined as decontaminated read counts) is likely to affect the detectability of microbial taxa, we included this covariate in the presence/absence models of both bacteria and fungi. In abundance models, we used different approaches for bacteria and fungi. For the former, the effect of variation in sequencing depth was carefully adjusted for by relating the number of reads of each taxon to the reads from a known quantity of spike-in (see previous section). Thus, no further correction for read counts was called for. However, for fungi, we lacked an appropriate benchmark in terms of a spike-in. Thus, read counts was included as a covariate in the model of fungal abundances. As random effects, we included the COI haplotype of the mosquitoes, location and year of collection, as well as host individual, to account for variation specific to each sampled mosquito.

We assumed the default prior distribution of HMSC (Ovaskainen & Abrego, 2020). We fitted the model using the Markov Chain Monte Carlo (MCMC) approach implemented in the R-package Hmsc (Tikhonov et al., 2017). The posterior distribution was sampled with four chains, with 250 thinned MCMC iterations per chain. We ignored the first 125 thinned iterations as burn-in to yield 250 samples per chain and hence 1,000 samples in total. The thinning parameter was increased as thin = 1, 10, 100, … until satisfactory MCMC convergence was reached. We considered MCMC convergence satisfactory if the third quartile of the potential scale reduction factors over the parameters measuring the

responses of the taxa to sex, species, and the interaction between both was at most 1.1; this was reached with thin = 100 for 16SV4 OTU and ITS2.

To evaluate model fit, we focused on explanatory and predictive powers – the former based on fitting the models to all data and the latter through two-fold cross-validation. As metrics for evaluation, we used AUC for presence-absence models and R^2 for models on abundance conditional on presence. To examine the importance of the fixed and random effects in structuring microbiome variation, we applied a variance partitioning approach to the explained variation. In other words, we examined what proportion of the overall variation explained by the model could be attributed to the individual factors and covariates. To examine the effects of sex and species, we evaluated the proportion of taxa that showed either a positive or negative response with at least 95% posterior probability. To examine residual patterns of co-variation not explained by host sex or species, we examined which pairs of bacterial taxa showed a positive or negative association for each random effect (location, sampling year, haplotype and individual) with at least 90% posterior probability. The latter approach will reveal what microbial taxa covary in their presence and abundance beyond what can be expected based on the fixed effects of sex and species.

Results

Following multitarget amplicon sequencing for 609 adult mosquitoes, raw reads for each sample were split into amplicon datasets corresponding to COI, 16S rRNA, and ITS2 markers. Samples for which either COI or 16S rRNA amplification was unsuccessful were discarded. Ultimately, we retained amplicon libraries for a total of 573 mosquitoes. Of these, 328 were collected from Zackenberg, 111 from Constable Pynt, 10 from Narsarsuaq, 45 from Ilulissat, and 79 from Nuuk across different years (Fig. 1).

COI data reveal the geographic distribution and biotic interaction patterns of two species of *Ochlerotatus* mosquitoes across Greenland

We first assessed the species diversity and distribution across the sampled mosquito populations and across years, based on mitochondrial COI amplicon sequencing data. After filtering out rare and noisy COI amplicon sequence variants (ASVs), we retained a total of 27,996,651 COI reads, 48,859 per library on average (range 104-48,859). Most of these COI reads (99.59%) represented mosquitoes. Specifically, 569 ASVs were classified into 15 operational taxonomic units (OTUs), all assigned to the genus *Ochlerotatus*.

Haplotype analysis based on these barcode sequences showed the presence of 53 COI variants among mosquito populations, of which 35 were assigned as *O. nigripes* and 18 as *O. impiger*. *O. nigripes* was found at all sampled sites, whereas *O. impiger* was only detected in South and West Greenland. In both species, we identified the main haplotypes dominant across all populations where the species was present (Fig. 2a). In each population, this haplotype was accompanied by less common haplotypes, many of which were represented by one individual only, and often separated by a single nucleotide from the dominant haplotype.

Among the 0.41% of COI reads not corresponding to *Ochlerotatus* (Fig. 2c), the majority were assigned to the water mite genus *Arrenurus*, found across 16 mosquito individuals, while reads assigned to the mite genus *Trombidium,* were found in one individual. Further, in thirteen mosquitoes we detected likely traces of blood meals, i.e. COI reads matching locally present mammals. This included the northern collared lemming (*Dicrostonyx groenlandicus)* detected in seven mosquito individuals, the muskox (*Ovibos moschatus*) detected in two individuals, the Greenland dog (*Canis*

lupus) in one, and human (*Homo sapiens*) in three. The mammalian signal was always found in female mosquitoes, or in individuals for which sex was not determined, agreeing with the expectation that only mosquito females feed on blood.

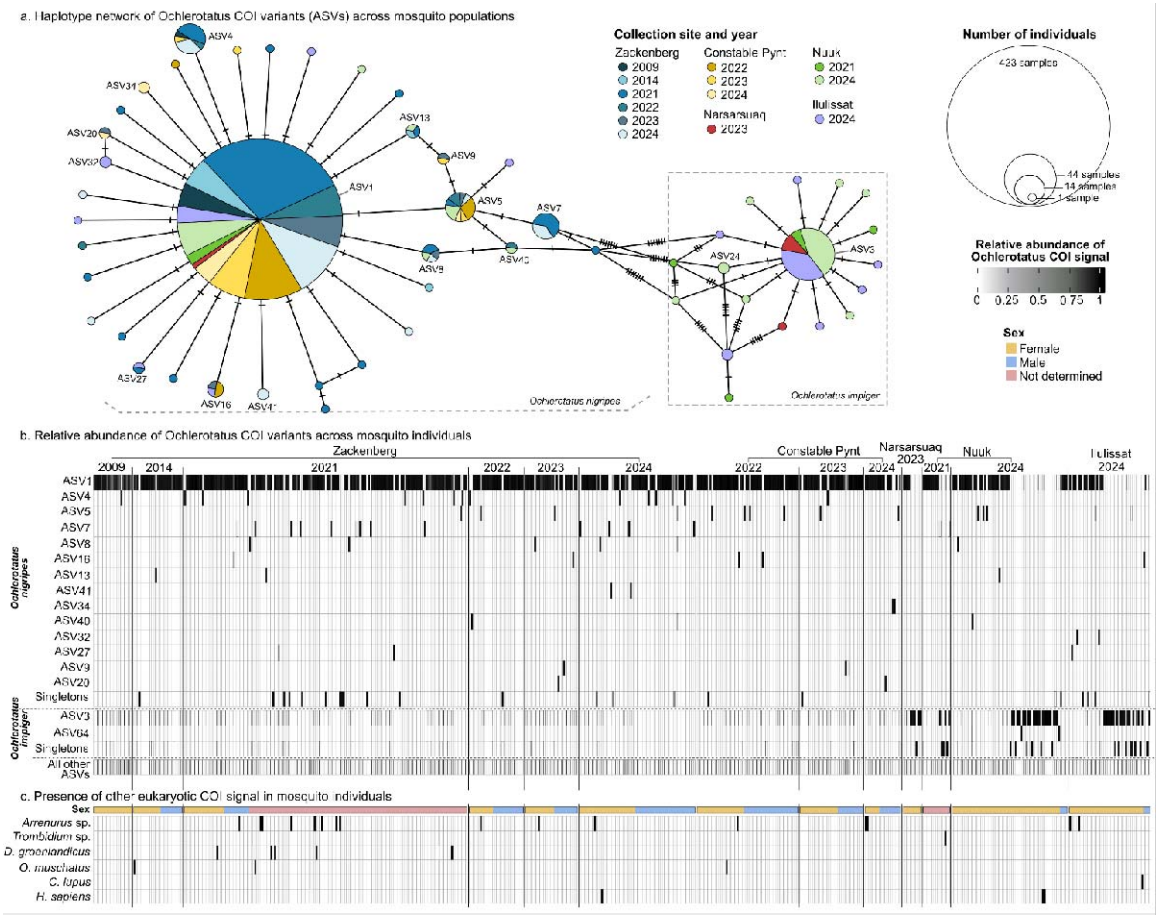

Fig. 2. Mitochondrial diversity of *Ochlerotatus* mosquitoes and their biotic interactions. a) Minimum spanning network (epsilon = 0) of *Ochlerotatus* barcodes. Circles represent unique haplotypes, scaled by the number of individuals, hatch marks indicate 1 bp differences, and colors denote sampling site and year. b) Relative abundance of the most common *Ochlerotatus* COI variants (ASVs) among *Ochlerotatus* mosquitoes from different sampling locations and years. Variants that were found in a single library were grouped and are presented jointly as 'singletons'. c) The presence of reads representing non-mosquito animals in mosquito COI libraries. The sex of each individual is displayed by color in the top row. In panels b and c, columns represent individual mosquitoes.

Quantitative analysis of 16S rRNA copies show low microbial load in *Ochlerotatus* mosquitoes

For the 573 *Ochlerotatus* mosquitoes confidently classified into species, we obtained a total of 9,123,448 reads for the V4 region of the 16S rRNA gene, with an average of 15,922 reads per library (range 550–128,919). A large share of these reads represented OTUs abundant in negative controls or corresponding to plasmid sequences from the quantification spike-in (Fig. S1). Such reads were bioinformatically filtered using negative controls as references. After this decontamination procedure, individual libraries retained only 1,239 reads on average (range 0–47,627). Using information on the representation of spike-in plasmids in the amplicon data to estimate the absolute abundance of 16S

rRNA copies in *Ochlerotatus* mosquitoes, we concluded that the median number of bacterial rRNA copies in a mosquito individual was 8,546 (range 0 - 19,000,000, average 153,315).

We compared these values against other insect species processed in the laboratory at the same time and using identical methods: cultured *Aedes egypti* mosquitoes (lines with or without the *Wolbachia* symbiont), overwintering wild *Culex pipiens* mosquitoes from Poland, and four other insect species of comparable size from our Zackenberg 2021 collection (Fig. 3). In these other mosquito samples, the estimated median numbers of bacterial rRNA copies were between one and two orders of magnitude greater than in *Ochlerotatus* spp.: in *Wolbachia*-free *A. egypti,* the median was 173,284.3 (average 373,445, range 11,829–2,145,833), in *Wolbachia*-infected *A. egypti*, 892,208 (average 1,926,381.7, range 489,900–8,926,860), and in *C. pipiens* it was 760,626 (average 1,881,956, range 21,133–10,360,000). As a further, indirect indication of low bacterial abundance in *Ochlerotatus* libraries, we found a low ratio of 16S rRNA reads to COI reads; this ratio was 0.33 when combining reads for all libraries and 0.76 when averaged across individuals, respectively. Identical PCR reaction conditions and primer mixes resulted in much greater ratios for other mosquitoes: for *C. pipiens* it was 1.30 for all libraries combined, 2.87 when averaged; for WB-free *Ae. aegypti* it was 2.33 for all libraries combined, 13.52 when averaged; and for WB-infected *Ae. aegypti* it was 1.73 for all libraries combined, 2.6 when averaged. Interestingly, other Greenland showed less intra-population variation and varying degrees of microbial abundance. Median 16S rRNA copy abundances estimated for dipterans *Phytomyza puccinelliae* (n = 8) and *Trichotanypus posticalis* (n = 14) were several-fold lower, but for *Metriocnemus ursinus* flies (n = 56) and the hemipteran *Nysius groenlandicus* (n = 37603), more than two orders of magnitude greater than for *Ochlerotatus* individuals (Fig. 3).

The GLM model revealed that total bacterial abundance varied in different ways across years in different sites (Table S3: interaction site × year) and between sexes (interaction sex × year). No significant effects of sex, species, or their interaction were detected.

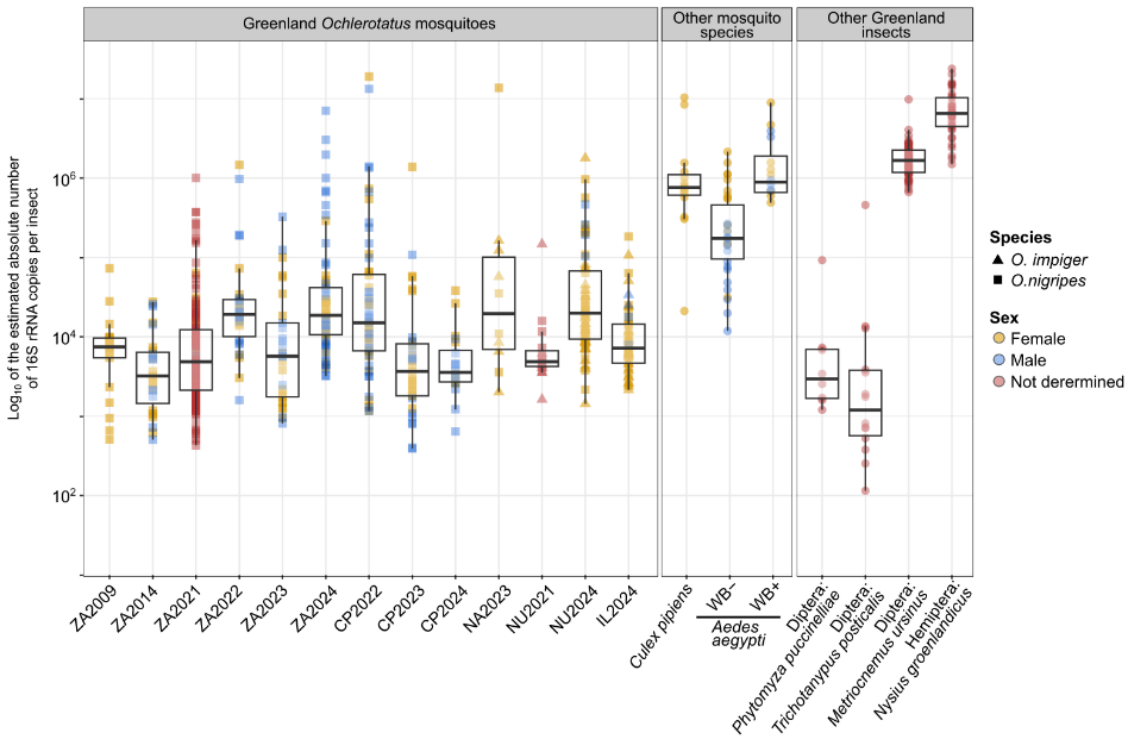

Fig.3. Absolute 16S rRNA abundance in *Ochlerotatus* mosquitoes from different sites and years, estimated based on quantification spike-in plasmids. Reference species include wild *Culex* mosquitoes captured in Poland, cultured *Aedes aegypti* either infected (WB+) or non-infected (WB-) by *Wolbachia*, and four additional insect species collected in Zackenberg in 2021. Of the latter four species (*Phytomyza puccinelliae* (Diptera: Agromyzidae), *Trichotanypus posticalis* (Diptera: Chironomidae), *Metriocnemus ursinus* (Diptera: Chironomidae) and *Nysius groenlandicus* (Hemiptera: Lygaeidae)) each was only distantly related to mosquitoes, but of comparable body size.

High diversity and inconsistent bacterial community composition

Across the 573 *Ochlerotatus* libraries, we detected a total of 13,597 non-contaminant ASVs clustered into 4,346 OTUs, 945 genera, and 278 orders. On average, individual mosquitoes carried 26 OTUs (ranging from 1 to 366 OTUs in total) (Fig. 4a). The difference between the overall OTU count and the OTU count across individual mosquitoes attests to remarkable variation in microbiome richness among individuals. When examining bacterial composition across mosquito populations, we noted large variation in taxa distribution across individuals. Only 39 out of 278 bacterial orders and 32 out of 945 genera occurred in more than 10% of individual mosquitoes. Among the orders that were both highly prevalent (present in >50% of the libraries), and abundant (comprising >10% reads across libraries) were Burkholderiales (73.6% prevalence, 31.3% relative abundance), Enterobacteriales (87.8%, 19.6%) and Pseudomonadales (63%, 12.9%) (Fig. 4b). At the OTU level, the genera with the highest prevalence and abundance across libraries were *Janthinobacterium* (28.5% prevalence, 25.7% average relative abundance), *Pseudomonas* (47.5%, 11.8%) and *Serratia* (41.2%, 11.1%). Some other OTUs, such as *Escherichia/Shigella* or *Enterobacter*, were highly prevalent (52.4% and 33.2%, respectively) but represented a small proportion of reads across libraries (0.2% and 2.8%, respectively) (Fig. 4c). These clades are known for their broad environmental distribution, including the ability to colonise diverse hosts, and four of the five have been listed among common contaminants in reagents (Salter et al., 2014). However, the strict contamination control applied in our study and the high genotypic diversity observed in our data supports a biological basis for these host-microbe associations (Fig. S1, S2). A distinct category comprises the bacterium *Bartonella,* which was detected in only seven mosquitoes (1.2% prevalence), but proved highly abundant in the libraries where it was present, with an average of 526,772 16S rRNA copies per library (median = 351,795). Interestingly, in three of the mosquitoes carrying it, we detected COI signal of the northern lemming, a likely source of a bloodmeal (Fig. S3).

To understand the structure of the bacterial communities across mosquito populations, we sought to identify a core set of microbes that could be consistently associated with *Ochlerotatus* mosquitoes in Greenland. A non-metric multidimensional scaling (NDMS) based on Bray–Curtis dissimilarity matrix (stress value = 0.102) did not show clustering patterns by microbial composition across sampling locations or years (Fig. 4d). However, based on the relative abundance threshold of 0.1% and a 75% prevalence, we were unable to detect a core microbiota within or among populations in any of the sampling years, locations, or sample-location combinations (Fig. S4). On the other hand, through a survey based on OTU presence/absence, we detected a set of 10 OTUs (encompassing 7 orders and 10 genera) that were present in at least one individual in each collection (defined as site/year combination) (Fig. 4e).

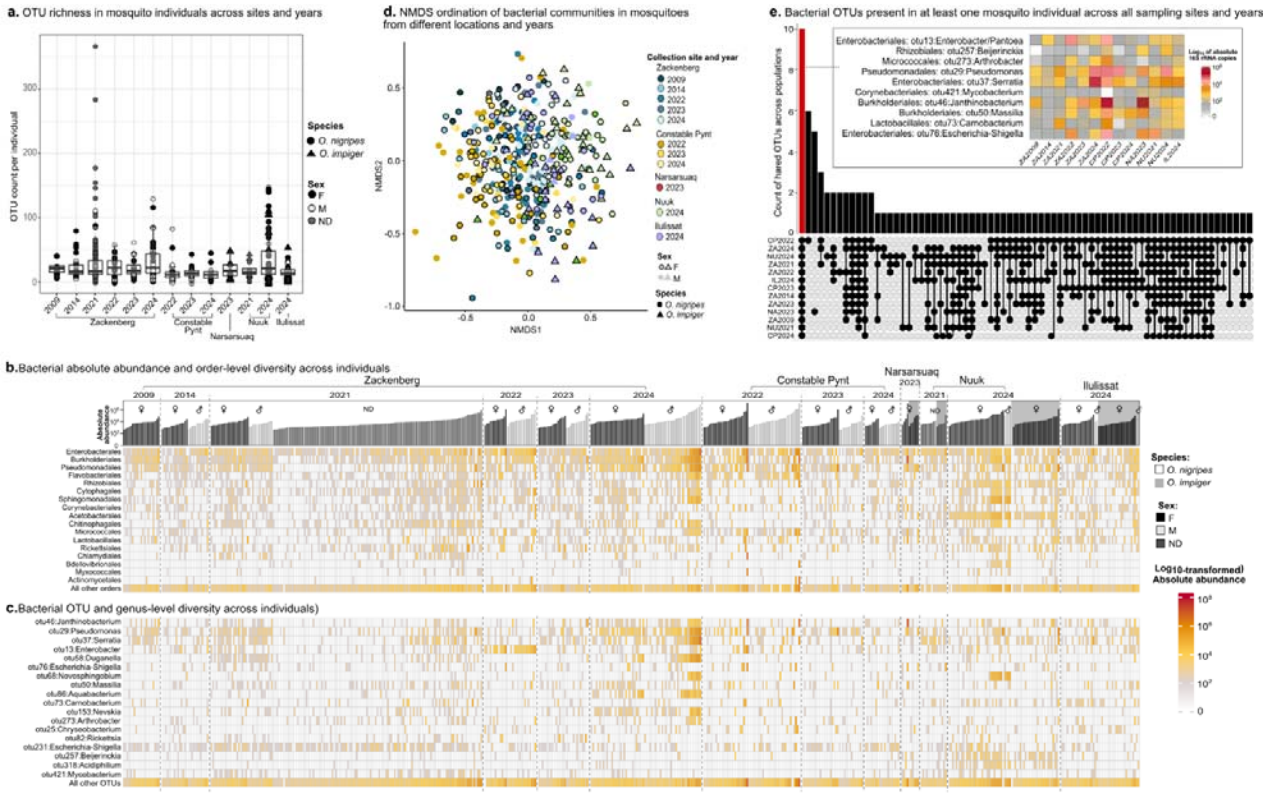

Fig. 4. Bacterial diversity in mosquito individuals across sampling locations and years. a) OTU richness across populations. Each point represents a number of OTUs in an individual mosquito, with the color indicating its sex and shape indicating species. (b-c). Variation in estimated absolute bacterial abundance (total 16S rRNA copy count) among individual mosquitoes collected across locations and years; bar colors indicate the sex of specimens. The plots show the most prevalent bacterial b) orders and c) OTUs, respectively, across 573 mosquito individuals. Only taxa with a prevalence >35% across libraries are shown. d) NMDS ordination of community dissimilarity, as based on Bray-Curtis distances, with females and males of the two mosquito species across sampled populations (site×year combinations). e) UpSet plot showing the presence/absence distribution of the top 100 most abundant bacterial OTUs across the dataset. The bars indicate the number of shared and unique OTUs, and the connected dots below the x-axis show which populations share each set. Population names are given as the first two letters of the site name and the year. The red bar highlights the number of taxa shared by all groups, and the inset heatmap displays the assigned taxonomy and the average absolute abundance of each OTU per sampled location/year.

Low abundance and inconsistent community composition in fungal microbiota

For 403 *Ochlerotatus* libraries with fungal data, we obtained 6,280,582 total ITS2 reads (15,318 per library on average, range 550 - 128,919), corresponding to 1,773 OTUs. Approximately 88% of these reads matched OTUs that were highly abundant in extraction and PCR blanks, indicating reagent-derived contamination (Fig. S5). These contaminants included the most abundant OTU in the dataset, *Cladosporium*, a fungus previously associated with insects including mosquitoes (Accoti et al., 2021; Flores et al., 2022; Hegde et al., 2024) (Fig. S6). After decontamination, we retained 508 OTUs, classified into 77 orders and 320 genera. Individual libraries retained a median of 234 reads (average 1,240; range 0–29,963), corresponding to an average of 7 OTUs (median 6; range 0–33) (Fig. 5a).

To account for variable sequencing depth, we used the ITS2/COI read number ratio as a semi-quantitative measure of fungal load (Fig. 5b). Ratios were generally low (average = 0.04, rarely exceeding 0.5), suggesting limited fungal DNA in mosquito samples and being consistent with the substantial contribution of contaminants. Community composition was highly variable across individuals, locations and sampling years. The most abundant taxon, *Mrakia* (Basidiomycota:Cystofilobasidiales), accounted for only 8.2% of total fungal reads and was present in 14% of *Ochlerotatus* libraries. The most frequently detected genera were *Monilinia* (Ascomycota:Helotiales) (prevalence 21.7%), and *Phaeosphaeria* (Ascomycota:Pleosporales) (prevalence 20.6%) (Fig. 5b).

NMDS ordination did not fully converge despite 1,000 iterations, which we interpret as further evidence of the high variability and lack of consistent community structure among mosquito individuals. Nonetheless, the ordination revealed moderate differences in fungal communities across locations and years (Fig. 5c), with minimal temporal variation at Constable Pynt. In terms of spatial differences, there was a high degree of overlap in microbial community composition between sites, but fungal communities from East Greenland differed markedly from those in the South/West populations (compare patterns in Fig. 5c to the geographic distribution of sites in Fig. 1). Among the potential taxa contributing to these differences were diverse plant pathogens, *including Monilinia* (De Miccolis Angelini et al., 2022)*, Phaeosphaeria* (Gonçalves et al., 2013)*, Cryptomyces* (Ascomycota:Rhytismatales) (Lantz et al., 2011)*, and Septoriella* (Crous et al., 2015)*.* These taxa were more common in Zackenberg and Constable Pynt, while *Godronia* (Ascomycota:Helotiales) and *Colpoma* (Ascomycota:Rhytismatales) were more frequent in Nuuk and Ilulissat, respectively. Thus, while some geographic trends were detected, communities within sites remained inconsistent over time and lacked stable taxa (Fig. S7).

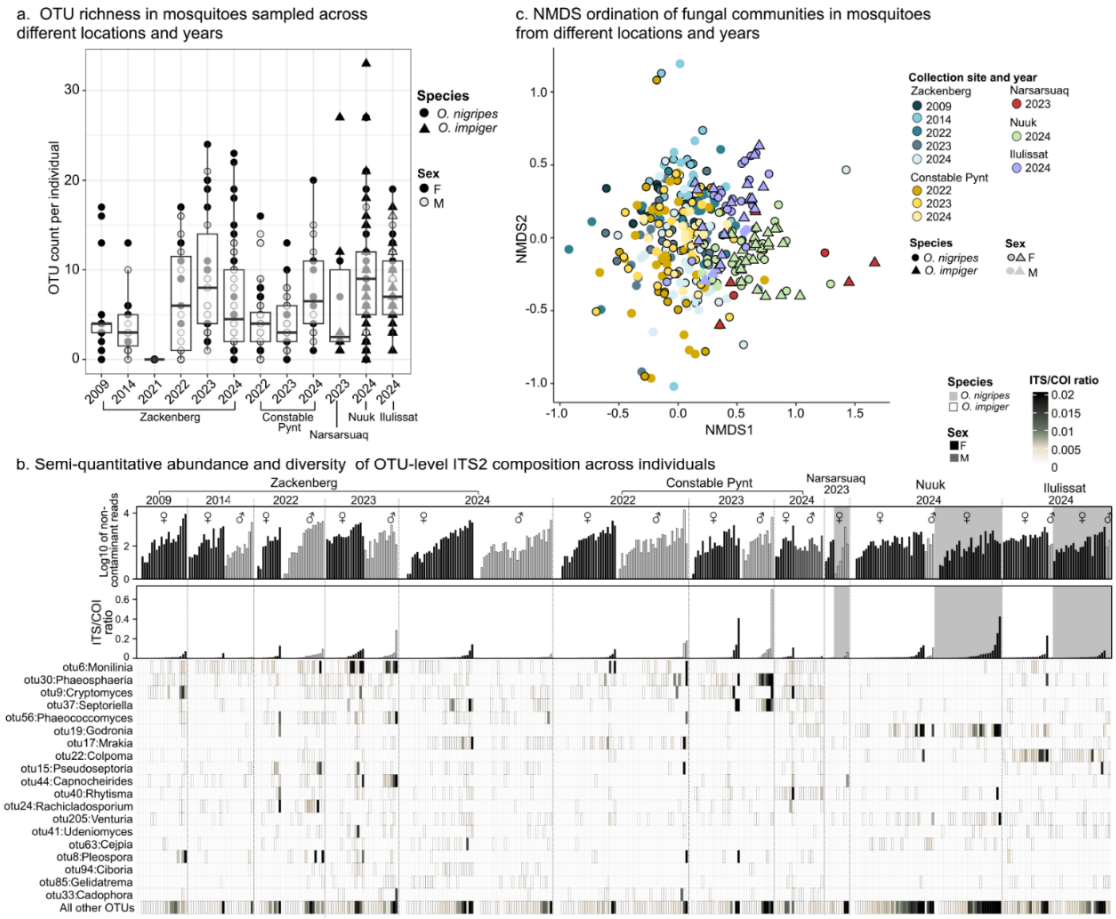

Fig. 5. Fungal diversity and distribution across *Ochlerotatus* individuals and populations. a) Fungal OTU richness across sampled populations. Each point represents the number of OTUs in an individual mosquito, with color indicating its sex and shape indicating its species. b) The distribution of fungal abundance and most common OTUs in individual mosquitoes. Barplots indicate the decontaminated total number of ITS2 copies across individuals from the sampled populations (upper panel) and the ITS2/COI ratio per library (bottom panel). The heatmap indicates the abundance of selected fungal OTUs in individual mosquitoes, expressed as ITS2/COI ratio. c) NMDS based on Bray-Curtis distances showing females and males of both *Ochlerotatus* species across sampled populations.

Drivers of variation in bacterial communities

The HMSC model of the presence or absence of bacterial taxa demonstrated moderate predictive (mean AUC = 0.778) and explanatory power (mean AUC = 0.616). The greatest proportion of variance was explained by random effects related to host individual (mean 40.5%), followed by year of sampling (mean 15.8%) and location (mean 11.3%). Host-specific traits such as sex, haplotype and species each explained less than 10 % of the variance (Table. S4-S5; Fig. S8).

The HMSC model of bacterial abundance conditional on presence reached high explanatory power (mean R^2 = 0.80), but no predictive power (mean R^2 = 0.00). Matching the model of bacterial occurrence (above), the greatest proportion of variance was explained by the host individual (mean 77.3%), while other covariates contributed less than 10% each (Table S4; Fig. S8).

To assess the systematic drivers of microbial presence/absence, we examined the species-specific responses of microbes to sequencing depth, and to the sex and the species of the individual (Fig. 6a;

Fig S9a, Table S6). Most taxa were significantly more likely to be present (or detected) the higher the sequencing depth (Fig. 6a; Fig. S6a). A low proportion of taxa (4.7%) occurred with a significantly higher probability in males than in females (Fig. 6a; Fig. S9a), and a small minority of common taxa (listed in Fig. 6a and Fig. S9; i.e., *Aurelimonas*, *Escherichia-Shigella* and *Spiroplasma*) differed in occurrence among mosquito species. Notably, the effect of mosquito sex differed between mosquito species, so that the species-specific occurrence was lower in males of *O. impiger* than males of *O. nigripes* (Fig. 6a).

For abundance conditional on presence, effects were highly consistent (Fig. 6b; Fig. S9b): when present, a majority of microbial species occurred at higher abundances in *O. impiger* than in *O. nigripes.* No other driver showed a detectable imprint.

Associations between bacteria

To better understand the drivers of bacterial community structure, we probed for signs of interactions between bacterial taxa. Considering that interactions could be a result of taxa responding similarly to shared environmental or host-related factors, and from direct or indirect interactions among taxa themselves, our HMSC models first accounted for joint responses to extrinsic factors (fixed and random effects). After controlling for these effects, we examined the residual species-species associations that cannot be attributed to the measured covariates. These associations were examined at multiple hierarchical levels: host individual, host haplotype, location, and year (Fig. S10).

In terms of species occurrence, relatively few taxa showed statistically supported associations, but within the 30 most abundant and prevalent OTUs, several statistically supported interactions emerged (Fig. S10a). At the host-individual level, interactions were mostly positive, except for *Escherichia-Shigella*, which tended to co-occur negatively with environmentally associated taxa like *Zooglea*, *Aquabacterium*, and *Duganella*. At the location level, most of the statistically supported interactions were negative; for example, *Pseudomonas* and *Massilia* were negatively associated with *Carnobacterium*, despite all three being present across populations. At the year level, members of the prevalent set (e.g., *Escherichia-Shigella, Janthinobacterium, Massilia, Pseudomonas*) showed strong positive associations. No statistically supported associations were observed at the haplotype level.

In terms of abundance conditional on presence, statistically supported residual associations tended to be positive but rare at all levels save the host individual (Fig. S10b). Notably though, most members of the prevalent set of microbial taxa showed residual associations with each other across different levels in the occurrence model (Fig. S10b). At the location level, only *Novosphingobium* and a specific *Nevskia* genotype showed a positive association. At the year level, *Massilia* showed a residual association with *Pseudomonas* and *Novosphingobium* in terms of both occurrence and abundance conditional on presence. However, at the host-individual level, nearly all taxa showed positive associations in terms of abundance. Thus, if an individual mosquito showed high abundances of one microbe, it tended to show high abundances of other taxa, too. At the level of mosquito haplotypes, no significant associations were detected for either the presence/absence or abundance conditional on presence of microbial taxa (Fig. S10b).

Drivers of variation in fungal communities

Like bacterial models (see Drivers of variation in bacterial), the HMSC model of presence/absence of individual fungal taxa reached high explanatory power (mean AUC = 0.88) and moderate predictive power (mean AUC = 0.75). Akin to the results for bacteria, the greatest proportion of variance was explained by random effects related to host individual (mean 25.1%), followed by sampling location (mean 21.5%), year (mean 14.5%) and COI haplotype (mean 10.2%). Sex, species and their interaction explained less than 10 % of the variance each (Table. S4-S5; Fig. S11).

Again, matching the results for bacteria, the HMSC model of fungal abundance conditional on presence achieved high explanatory power (mean R^2 = 0.70) but low predictive power (mean R^2 = 0.16). As in the model of fungal occurrence, the greatest proportion of variance was explained by the host individual (mean 21.7%), following sampling location (mean 11.3%), COI haplotype (mean 10.8) and sampling year (10.2%). Sex and species contributed less than 10% each (Table. S4-S5; Fig. S11).

Over half of the taxa (55%) were more likely to be detected as the sequencing depth increased (Fig. 6a; Fig. S12a). For the majority of the most abundant fungal taxa (OTUs occurring in at least 5% of samples), occurrence was similar in females of both species. Compared to the reference group (females of *O. impiger*), only a small proportion (17.6%) of these taxa (Fig. 6c) showed a significant difference in incidence. In these cases (9.8% of taxa), incidences were higher in males than in females of *O. impiger*, but lower in males of *O. nigripes*.

For abundance conditional on presence, the taxon-specific abundances recorded typically increased with the sequence yield from the sample (note a significant, positive effect of sequencing depth in 73% of OTUs; Fig. 6b; Fig. S12b). Effects were overall highly consistent, with no driver showing a detectable imprint.

Associations between fungi

In terms of occurrence, several fungal taxa showed statistically supported residual associations across all the 51 analysed OTUs (Fig. S13). Following the most prevalent OTUs across mosquitoes (e.g., *Mrakia*, *Monilinia, Cryptomyces and, Phaeosphaeria*; see Fig. 5c), most detected associations were positive at the level of the host individual. *Monilinia* appeared to interact positively with site-specific taxa such as *Colpoma* and *Godronia*, while these three taxa showed negative associations with other environmental fungi like *Poseodiscus* and *Arrhenia* (Fig. S13a). At the location level, interactions were more mixed, with *Monilinia* and *Mrakia* positively associated with each other and with *Cryptomyces*, but negatively or not associated with *Colpoma* and *Godronia*. Notably, none of these taxa displayed statistically supported interactions at the level of years. At the haplotype level, we found no associations at all.

In terms of abundance conditional on presence, statistically supported residual associations tended to be scarce at any level (Fig. S13b).

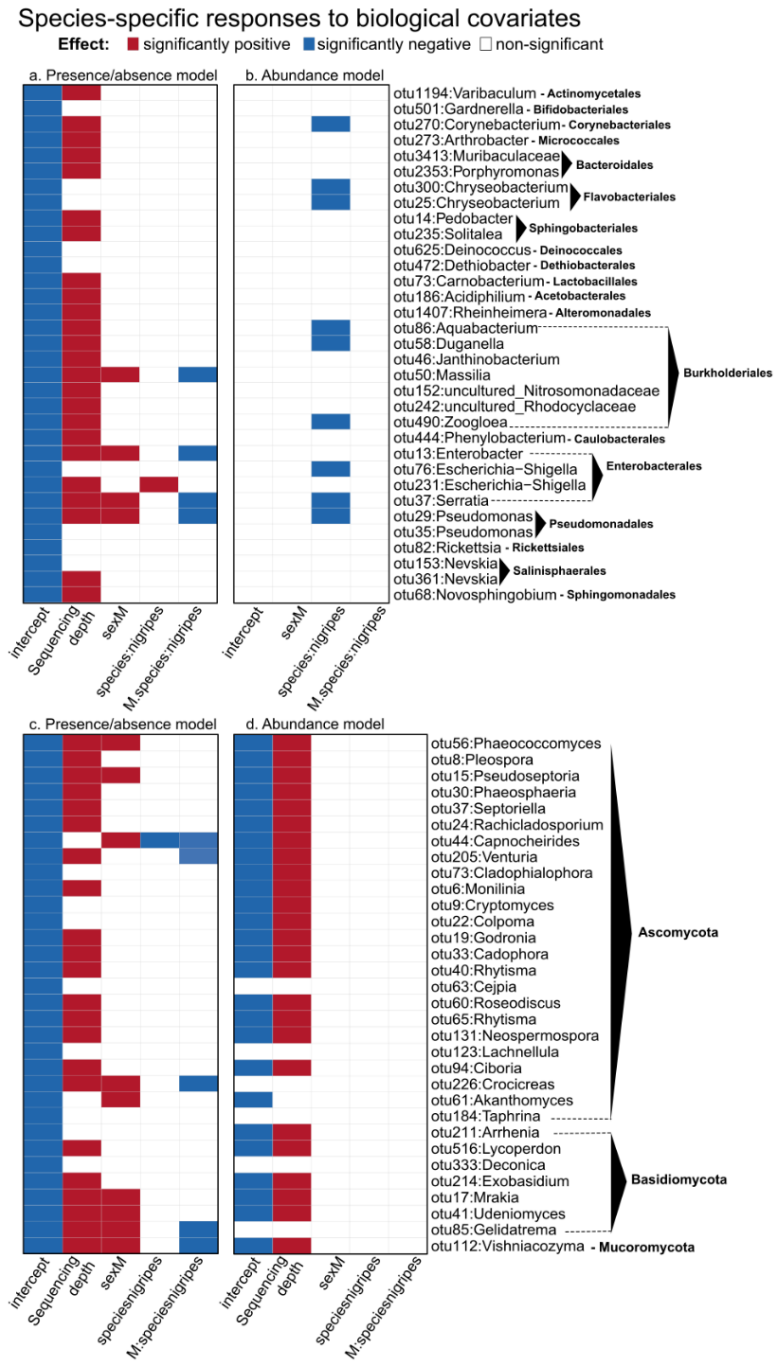

Fig. 6. Species-specific responses to biological covariates. The upper panels (a-b) show bacterial OTUs occurring in at least 10% of samples, while the lower panels (c-d) show fungal OTUs occurring in at least 5% of samples. For results across all species, see Fig. S6 and Table S5 for bacteria, and Fig. S11 for fungi. Panels a) and c) show results for the presence/absence model, panels b) and d) for the model of abundance conditional on presence. The columns show the posterior probability that the effect in question deviates from zero, with red tiles indicating positive effects, blue tiles indicating negative effects, and white tiles indicating responses that did not gain posterior support with at least 0.95 probability to their direction. Note that given how the model is parameterized, parameter estimates relate to the difference between the factor in question and the reference group (intercept, i.e., females of *O. impiger*). Thus, the colours refer to differences between this group and males of *O. impiger*

(sexM); females of *O. nigripes* (species:nigripes), and the interaction sex×species (sexM.species:nigripes).

Discussion

In this study, we characterised the bacterial and fungal communities across wild populations of *Ochlerotatus* mosquitoes in Greenland. In doing so, we sought to pinpoint microbial clades of significance to the biology of these mosquitoes, and to identify the drivers of variation in the mosquito microbiome. Our study sheds light on the geographic structure and mitochondrial diversity of *O. nigripes* and *O. impiger* across Greenland, while also showing that they harbour low and inconsistent microbial communities likely shaped by environmental factors, suggesting a dynamic but minor role of microbes in their biology.

Two mosquito species in Greenland – but many COI haplotypes

We found two mosquito species in Greenland: *O. impiger* and *O. nigripes.* Based on our sampling efforts at five sites, these species may differ in their geographical distribution: *O. nigripes* was detected in all sampled areas, while *O. impiger* was found only in South and South-West Greenland. Given their phylogenetic proximity and morphological similarity, the two species are hard to distinguish by morphological means. Thus, reliable information about the distribution of these species within Greenland has been lacking (Dahl, 2015; Villeneuve et al., 2024). While GBIF broadly reports both species across Northern North America and Scandinavia (Hebert et al., 2016; Villeneuve et al., 2024), our results highlight a mismatch between morphology-based identifications and mitochondrial genotypes. Nevertheless, our 418 bp COI barcode sequences allowed clear distinction between the species, and while serving as a framework for microbiota analysis, can also be used to map species distribution in further detail.

Outside of the barcode (which accounted for a median of 98% of *Ochlerotatus* reads), mosquito individuals typically carried lower-abundance COI variants. In 14 cases, one of these variants represented >10% of total reads, suggesting heteroplasmy – that is, the presence of distinct COI haplotypes in an individual (Paduan & Ribolla, 2008). Typically, however, several variants were present but scarce (<1% of *Ochlerotatus* reads) per library. We consider them to be likely numts, i.e., sequences of mitochondrial origin introgressed into the nuclear genomes (Mastrantonio et al., 2016; Toews & Brelsford, 2012). Interestingly, in several cases, these numts appear to match the other *Ochlerotatus* species, suggesting potential incomplete lineage sorting or recurrent hybridisation. These findings suggest that many factors may contribute to shape the evolutionary history of *O. nigripes* and *O. impiger.* Since the genus *Ochlerotatus* (Kirik et al., 2020; Namin et al., 2014) is only one of several mosquito groups forming cryptic species complexes (Fontaine et al., 2015; Villeneuve et al., 2024), further analyses incorporating genomic resources will be essential to illuminate the biological processes shaping mosquito evolution.

A limited role of bacteria in adult mosquito biology?

Where the microbiome has proven important for many blood feeding insects (Bing et al., 2017; Husnik, 2018; Zhong et al., 2007), our findings from Greenland mosquitoes suggest a limited role of bacteria and fungi. Compared to *Aedes aegypti*, *Culex pipiens* and *Anopheles gambiae, Ochlerotatus* mosquitoes from Greenland harbour remarkably low microbial loads (Habtewold et al., 2016; Villegas et al., 2023). The microbial abundances uncovered were also orders of magnitude lower than those of some other insects from the same environment. The estimated median number of ~8,000 bacterial 16S rRNA copies per mosquito may correspond to only ~1,000 bacterial cells, depending on the number of

rRNA operons per genome, or the number of genome copies per cell. As both numbers can vary substantially between bacterial taxa and physiological states (Klappenbach, 2001; Louca et al., 2018; Pecoraro et al., 2011), our estimates might still overrepresent true bacterial abundance.

While surprisingly low, these numbers are not unprecedented among insects (Andriienko et al., 2024; Hammer et al., 2019). For instance, bacterial 16S rRNA copy numbers in individual phorid flies range from the upper hundreds to tens of millions (Nowak et al., 2025), while some ants harbor bacterial levels near background contamination (Sanders et al., 2017). Among mosquitoes, quantitative studies typically report gut loads in the hundreds of thousands, with the highest values usually dominated by a single genus (Habtewold et al., 2016; Njogu et al., 2024; Villegas et al., 2023). These studies, however, have largely focused on laboratory-reared mosquitoes and specific symbionts, leaving wild populations underexplored. Given the vast variation in bacterial abundance across insects and individuals, this aspect of host-microbe interactions clearly requires systematic attention in future studies.

In many insects, high microbial abundance often reflects specialised, heritable associations with microbes that produce essential nutrients or influence important life history traits, often endosymbionts (Nowak et al., 2025; Villegas et al., 2023). Indeed, we observed such patterns in our reference species (Fig. S14), but rarely in Greenland *Ochlerotatus*, where bacterial loads were highly variable among individuals (see Table S7 for taxon-specific observations). The absence of a detectable core microbiota supports the view of a stochastic nature of these associations. Although some mosquito taxa, particularly in laboratory colonies, have been reported to share stable core members (David et al., 2016), Greenland *Ochlerotatus* showed no such pattern. Similar variability has been reported from natural mosquito populations (Coon et al., 2016; Njogu et al., 2024). Potentially, the core identified in lab-reared mosquitoes may then be a result of controlled environment conditions, rather than a general pattern across wild mosquitoes.

An even smaller role for fungi?

Paralleling the patterns observed in bacteria (above), fungal communities in Greenland mosquitoes also showed large variation among individuals. However, this individual-level variation was not quite as pronounced as observed for bacteria: of the overall variation in presence/absence of fungi, a mean of 25.1% occurred among individuals, as compared to 40.5% among bacteria. For abundance conditional on presence, the corresponding numbers were 21.7% for fungi vs. 77.3% for bacteria. Thus, mosquito-to-mosquito -level variation in the fungal communities is not quite as pronounced as in the bacterial microbiota. Nonetheless, fungal abundances were markedly low overall, with ITS/COI ratios consistently below 0.5. Given that fungi, similar to bacteria, retain many rRNA gene copies per cell (Lofgren et al., 2019), these low ratios likely suggest that actual fungal cell abundances are even lower than indicated by the read counts. These findings align with reports from *Ae. eagypti*, *Cx. quinquefasciatus,* and *Ae. albopictus*, where fungi account for less than half of sequencing reads when both host and fungal signal are co-amplified (Hegde et al., 2024).

Low numbers of fungal OTUs we observed in *Ochlerotatus* mosquitoes (a median value of 7, range 0-30) are comparable to those observed in other species. For example, wild *Cx. quinquefasciatus* have been reported to harbor ~10-20 OTUs, *Ae. aegypti* and *Ae. albopictus* ~10-15 (Hegde et al., 2024; Thongsripong et al., 2018). The richness observed in *O. nigripes* and *O. impiger* was closer to values seen in laboratory-reared *Cx. quinquefasciatus,* where the number of OTUs ranged between 5 and 22 (Flores et al., 2022).

Community composition was dominated by a handful of taxa detected across sites but reaching higher abundances at certain locations, with no OTU consistently shared among populations. Thus, as with bacteria, we found no evidence for a core fungal microbiome. Similar patterns have been noted in *Cx. pipiens* and *Culiseta incidens*, where only a few genera recur without clear patterns in prevalence

(Chandler et al., 2015). In *Ochlerotatus* mosquitoes, most OTUs appeared to be of environmental origin, with the most prevalent taxa representing genera reported as plant pathogens. For example, *Cryptomyces* is known to infect willows (Lantz et al., 2011), while *Septoriella* is associated with grasses (Crous et al., 2015). Some genera, however, have also been recorded in animals. This includes *Phaeosphaeria, which* encompasses species infecting maize (Gonçalves et al., 2013) and has been detected on amphibian skin (Alexiev et al., 2021), and M*onilinia, a* fruit tree pathogen that has also been found in pollinators (De Miccolis Angelini et al., 2022; McArt et al., 2016). Moreover, several of these fungi (i.e. *Mrakia*, *Phaeosphaeria*, *Godronia*) have been identified in environmental DNA in Greenland (Ovaskainen et al., 2020), thus supporting the notion that these fungi are acquired from the environment – potentially during adulthood.

The mosquito microbiome: patterns, drivers, and likely consequences

Despite the lack of a clear-cut microbiome, we found large variation in the occurrence and abundance of individual bacterial and fungal taxa. Interestingly, most of this variation occurred at the level of host individuals, with less variation between mosquito species, sexes, sites, and years. Mosquito individuals varied massively in overall microbial abundances and in the composition of the individual microbiome. These patterns contrasted strongly with findings for known heritable symbionts *Wolbachia* and *Buchnera*, where microbial communities remain consistent across populations (Donner et al., 2024; Nowak et al., 2025). Nonetheless, patterns similar to the ones here uncovered for mosquitoes have earlier been found in the microbiome of Swedish scuttle flies (Diptera: Phoridae). Among phorids, too, most variation occurred among host individuals rather than species (of which there are hundreds among scuttle flies), sexes, or sites (Nowak et al., 2025). Again, such patterns attest to a largely environmental origin of the individual microbiome. Among the few mosquito-associated bacteria showing any clear-cut responses to the environmental covariates measured, *Moheibacter*, *Duganella*, *Neochlamydia*, *Nevskia*, *Shewanella*, and members of the order Acetobacterales are typically associated with soil and aquatic environments (Choi et al., 2015; Liu et al., 2021; Roman et al., 2025). Similarly, the fungi *Cryptomyces*, *Ciboria*, *Cladophialophora*, *Juncaceicola*, and *Everhartia* are primarily environmental taxa, commonly associated with plant material, soil, or other terrestrial substrates (Badali et al., 2008; Becarelli et al., 2021; Lantz et al., 2011; Ovaskainen et al., 2020)**.** Interestingly, a joint, environmental origin for multiple microbes was also suggested by patterns of residual covariation among microbial taxa. When one of these taxa occurred or reached high abundances, then so did others, too – in ways not reflecting into the incidence or abundance of other microbes. Environmental impacts on their presence and abundance in the mosquito microbiome may then reflect effects driving their availability across times and seasons. What habitat an individual lives in and what other taxa it encounters may then be the main determinants of its microbiome composition. Moreover, the wild-caught adult individuals we surveyed must have varied in their age since eclosion, and many may have fed on mixed diets (i.e. nectar or blood meal). It is thus tempting to postulate that adult age and diet may also be contributing factors influencing the complexity of the mosquito microbiota. Nonetheless, some individuals appeared to be “bacterial magnets” and thus laden with microbes of multiple types. Whether this reflects individual differences in immune competence or true mutualistic cooperation is then an interesting question to solve.

Conclusions

While blood-feeding insects will often rely on a core microbiome to survive on their odd diet, our study offers no evidence for such a core community – and even less for any host-specific associations. Quite the contrary, it offers much evidence for stochastic variation at the individual level. From the

perspective of mutualistic interactions, Arctic mosquitoes can thus be described as 'cold and lonely' – a fitting characterisation of insects that are not only adapted to thrive in a frigid, inhospitable environment but also lack symbiotic microbes, surviving in isolation on a challenging diet. Needless to say, we cannot rule out the possibility that some of these microbes will still play a significant role in insect performance (see Table S7) – but what we can say is that mosquitoes live in mixed company associations, and most likely acquire their interaction partners from the environment. We hope that our study will serve as a template for a multitude of studies exploring similar patterns and processes across different mosquito species and other blood-feeding insects across the world.

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