

ORIGINAL RESEARCH OPEN ACCESS

Microbiome Dynamics During Translocation of the Critically Endangered Frog *Craugastor ranoides*

Xavier A. Harrison¹  | Katherine Herborn² | Gilbert Alvarado³ | María Marta Chavarría⁴ | Kirsty J. Marsh¹  | Alexander D.M. Wilson²  | Alexandra C. North² | Victoria E. P. Wakeham² | Roberto Fernández⁵ | Héctor Zumbado-Ulate⁶  | Jon Bielby⁷  | Robert Puschendorf² 

¹Centre for Ecology & Conservation, University of Exeter, Exeter, UK | ²School of Biological and Marine Sciences, University of Plymouth, Plymouth, UK | ³Escuela de Biología, Universidad de Costa Rica, San Pedro, Costa Rica | ⁴Programa de investigación, Área de Conservación Guanacaste, Liberia, Costa Rica | ⁵Guanacaste Dry Forest Conservation Fund, Philadelphia, Pennsylvania, USA | ⁶Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica | ⁷School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, UK

Correspondence: Xavier A. Harrison (x.harrison@exeter.ac.uk) | Robert Puschendorf (robert.puschendorf@plymouth.ac.uk)

Received: 29 August 2025 | **Revised:** 13 July 2026 | **Accepted:** 19 August 2026

Editor: Elina Rantanen | **Associate Editor:** Corinne L. Richards-Zawacki

Keywords: amphibian declines | *Batrachochytrium dendrobatidis* | conservation translocation | microbiota | skin bacterial community

ABSTRACT

Amphibian populations are declining, and species are going extinct due to habitat loss, climate change, and emerging infectious disease, prompting the increasing use of translocations as a conservation tool. Skin-associated bacterial communities play a key role in amphibian health, particularly in protecting hosts from pathogenic infection. However, to date we know relatively little about the impact of translocation on the dynamics of skin-associated bacteria, or the consequences of translocation-mediated disturbance of the microbiome for amphibian health. We investigated the skin microbiome of the critically endangered frog *Craugastor ranoides*, now restricted to a fraction of its historical range in Costa Rica. We first conducted a multi-population survey to characterise among-population variation in skin bacterial communities. We then translocated individuals to a new site, using radio tracking to monitor them over time and assess individual-level changes in body mass, microbiome composition, and predicted microbial function. We found limited among-population variation in skin microbiome composition across three different populations of *C. ranoides*. Response of the microbiome to translocation was highly individual-specific; some frogs demonstrated relatively stable community richness and composition, whilst others exhibited marked turnover in bacterial membership and predicted function. Body mass showed similar dynamics, but several of the recaptured frogs had gained mass after several days in the translocation site. Our findings highlight the importance of considering microbiome dynamics in threatened species translocations to optimise individual health. This includes considering whether individuals should be prioritised for translocation based on their initial microbiome composition, and understanding how shifts in microbiome post-translocation may alter the overall success of translocation efforts. These insights extend beyond amphibians, offering a broader framework for integrating long-term monitoring of host microbiomes and health into conservation efforts.

1 | Introduction

Amphibians have experienced population collapses and even extinctions across the globe. Over 40% of species are

threatened due to habitat loss, climate change, invasive species, and emerging infectious diseases (Lips et al. 2006; Scheele et al. 2019; Stuart et al. 2004). These declines are especially severe in tropical and high-elevation regions, where

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Animal Conservation* published by John Wiley & Sons Ltd on behalf of Zoological Society of London.

entire amphibian communities collapsed within a few years, often in pristine, protected habitats. Habitat protection remains fundamental to amphibian conservation as some species that suffered enigmatic declines were first presumed extinct, but have since been rediscovered and recovered in places in which suitable habitat protection has been afforded (García-Rodríguez et al. 2012; Scheele et al. 2017). However, these small and relictual populations remain at significant risk of extinction due to their limited size, which increases vulnerability to environmental change, demographic stochasticity, and disease outbreaks (Smith et al. 2009).

Translocation, moving individuals from captive or source populations to restored or safer habitats, has become an increasingly important conservation strategy to mitigate the high risk of extinction faced by small, isolated amphibian populations (Knapp et al. 2024). However, optimising the health of translocated individuals to maximise survival in the destination habitats is challenging (Kueneman et al. 2022; West et al. 2019). In amphibians, skin-associated bacterial communities can contribute to defence against pathogens, particularly *Batrachochytrium dendrobatidis* (Bd), the fungal pathogen responsible for chytridiomycosis (e.g., Bates et al. 2018, 2022), producing antifungal and antiviral compounds that inhibit the growth of pathogens (Harrison et al. 2020). Previous work suggests that captivity can alter the composition of skin microbiotas (Bates et al. 2019; Kueneman et al. 2022; Risely et al. 2024), and disruption of stable host-microbe associations may shape downstream susceptibility to disease. Understanding how microbiomes contribute to disease resistance and post-translocation survival is essential for optimising both animal health and the efficiency of resource allocation towards translocation-based conservation programs (Dallas and Warne 2023).

The success of amphibian translocation remains variable, with reported success rates hovering around 50%. Failures are often linked to small release numbers, poor site selection, and lack of long-term monitoring (Hossack et al. 2023) or environmental persistence of pathogens that may have caused extirpation in the first instance (Scheele et al. 2021). To date, the impact of translocation on host microbiomes remains poorly resolved, though previous work has shown that translocation can reduce gut microbiome diversity in birds (Worsley et al. 2026). This is perhaps not surprising given that microbial communities are notoriously highly dynamic (Marsh et al. 2024), shifting in response to environmental factors such as temperature, humidity, and substrate composition (Longo et al. 2015). A key outstanding question is how do we integrate the microbiome into translocation programs? Environmental variation in the destination habitat is likely to drive major changes in the host microbiome (Risely et al. 2024); some translocated individuals are likely to retain core microbial taxa that provide consistent protection from pathogens, whilst others experience turnover that could either enhance or diminish their defences (Loudon et al. 2014). For wild-caught individuals moved from one location to another (rather rewilded from captivity), we know little about how initial microbiome state predicts subsequent community composition in the destination habitat, especially for amphibians. The interplay between microbiome stability, disease risk, and environmental change is a critical but poorly understood

factor in amphibian conservation, especially when implementing conservation-based translocations (West et al. 2019).

Here we address these knowledge gaps using the critically endangered frog *Craugastor ranoides* as a case study. Once widely distributed across lowland dry forests and high-elevation cloud forests in Costa Rica, this species suffered extensive declines during the global amphibian crisis, disappearing entirely from montane habitats (Puschendorf et al. 2005, 2006). The surviving populations now persist only in isolated tropical dry forest refugia from chytridiomycosis, where high temperatures and lower humidity may have limited pathogen prevalence (Puschendorf et al. 2009; Zumbado-Ulate et al. 2014). However, these habitats are now facing unprecedented environmental stress due to climate change. During the 2014–2015 El Niño event, the region experienced extreme drought, with rainfall falling to just 39% of the long-term mean in some areas, with a very narrow distribution, most of this in just a few weeks at the start of the wet season followed by a prolonged drought (Campos et al. 2020). This climatic anomaly triggered ecosystem-wide impacts, including a 42% decline in gross primary productivity (Castro et al. 2018), and widespread tree mortality driven by species-specific hydraulic failure. In the dry forest of the Área de Conservación Guanacaste (ACG), 13% of 1577 individually monitored trees died, with some species such as *Quercus oleoides* experiencing up to 34% mortality (Powers et al. 2020). These changes in forest structure and canopy cover likely altered microhabitats critical for amphibian survival. At the same time, *C. ranoides* populations in the dry forest of ACG collapsed entirely following the complete drying of a breeding stream, and no individuals were observed at the site after the event (Puschendorf et al. 2019).

The aims of this study are to (i) quantify among-population variation in skin bacterial community composition in *C. ranoides*; and (ii) investigate the effect of translocation on *C. ranoides* skin microbiota dynamics and body mass. We monitored skin microbiome composition in frogs before and after experimental translocation to premontane rainforest and compared them to individuals sampled on stream transects in their native dry forest habitat.

2 | Methods

2.1 | Field Sites and Sampling

Fieldwork took place across four periods: multi-population microbiome surveys in January 2022 (all three sites) and April 2022 (Pedregal and Brazo del Potrero Grande); a separate January 2023 field campaign; and the March 2023 translocation. Only the 2022 surveys and the translocation contributed data to this study (per-site sample sizes and dates in Table S1). All survey sampling fell within the dry season.

Background sampling for microbiome characterisation was conducted at Murciélago, Pedregal, and Brazo del Potrero Grande (Figure 1), Área de Conservación Guanacaste, in the northwest of Costa Rica. Frogs were located during nocturnal surveys along stream habitats at each site. We used active visual searches of stream margins, rocks, and adjacent riparian vegetation to locate individuals, with searches focused on

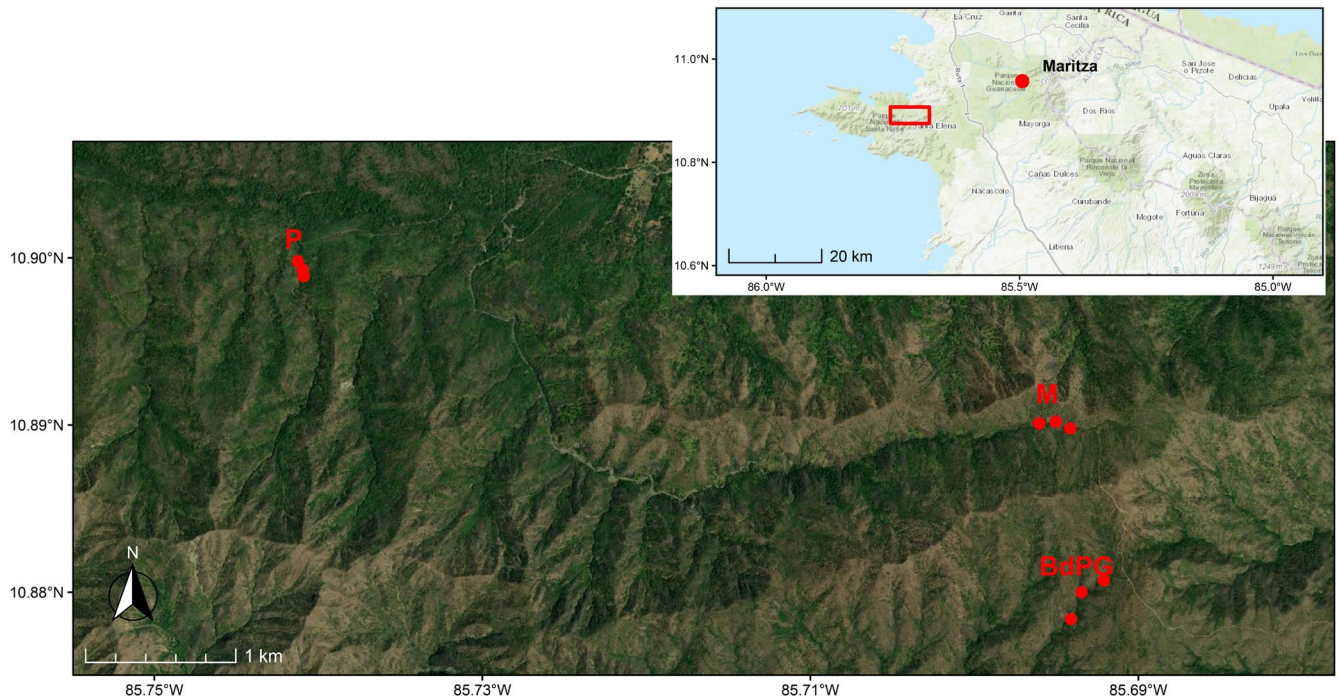


FIGURE 1 | Sampling locations in northwestern Costa Rica. The main panel shows high-resolution satellite imagery of the study area, with sites labelled as Murciélago (M), Pedregal (P), and Brazo del Potrero Grande (BdPG). Red circles indicate start and finish points of transects where amphibian skin swabs were collected for microbial community characterisation in Santa Elena Peninsula. Base imagery: Esri World Imagery basemap (Esri, Maxar, Earthstar Geographics, USDA, USGS, AeroGRID, IGN, and the GIS User Community).

capturing larger frogs rather than quantifying abundance. Searches were unconstrained by fixed transects and targeted known habitat used by *Craugastor ranoides*. The same observers used similar search approaches across all sites and periods, although the number of individuals sampled varied (Table S1).

Skin microbiome samples were collected by swabbing both the dorsal and ventral surfaces of each frog using MW100 DrySwabs (Medical Wire Equipment), and swab tips were stored in absolute ethanol at ambient temperature. We did not rinse frogs prior to swabbing. Snout–vent length (SVL) was measured with digital callipers to the nearest millimetre, and mass was recorded using Pesola Light-Line Precision Spring Scales. All individuals were released at their exact point of capture following sampling. At each frog capture location, stream water pH was measured using a YSI Pro1030 pH and Conductivity Meter (YSI Inc., Yellow Springs, OH, USA). Measurements were taken in situ at the point of frog capture within the stream. For analyses presented here, we used pH values recorded from frog pools (FP), corresponding to the immediate microhabitat of each sampled individual.

Although Bd infection status is thought to be relevant to the conservation history of *Craugastor ranoides*, this study was designed to quantify bacterial skin microbiome dynamics rather than to diagnose active *Batrachochytrium dendrobatidis* infection. Skin swabs and DNA extracts were prioritised for V4 16S rRNA microbiome profiling, and we did not collect parallel diagnostic swabs or run Bd-specific qPCR assays on the translocated individuals. Consequently, we cannot report individual Bd infection status before or after translocation.

2.2 | Translocation and Radio Tracking

To characterise the climatic position of the translocation site, we compiled georeferenced historical and current records of *Craugastor ranoides* from the Museum of Zoology, University of Costa Rica, and classified localities as persisting or missing. Mean annual temperature (BIO1) and annual precipitation (BIO12) were extracted for each locality and for the translocation site from the CHELSA v2.1 1981–2010 bioclimatic climatology (Karger et al. 2017) using the terra package in R (Hijmans et al. 2026). We plotted these localities in bivariate climate space and calculated minimum convex polygons and arithmetic centroids separately for persisting and missing localities. Maritza Biological Station occupied an intermediate position, being cooler and wetter than the currently persisting dry-forest localities but warmer and drier than most localities from which the species has disappeared. This descriptive climate-space comparison informed selection of the site; it was not intended as a formal species distribution model.

Before translocation, we conducted a 6-day pilot radiotelemetry study in the dry forest using seven adult *Craugastor ranoides*. Frogs were fitted with 0.27-g transmitters, allowed a 24-h acclimatisation period and subsequently relocated nightly. The pilot confirmed that the attachment method and telemetry equipment enabled repeated relocation of individuals, including frogs concealed within streamside crevices. It was designed to validate the tracking protocol and did not provide systematic estimates of movement distance or home range.

On March 10, 2023, 14 individuals of *Craugastor ranoides* (> 5 g mass) were translocated to Maritza Biological Station, situated

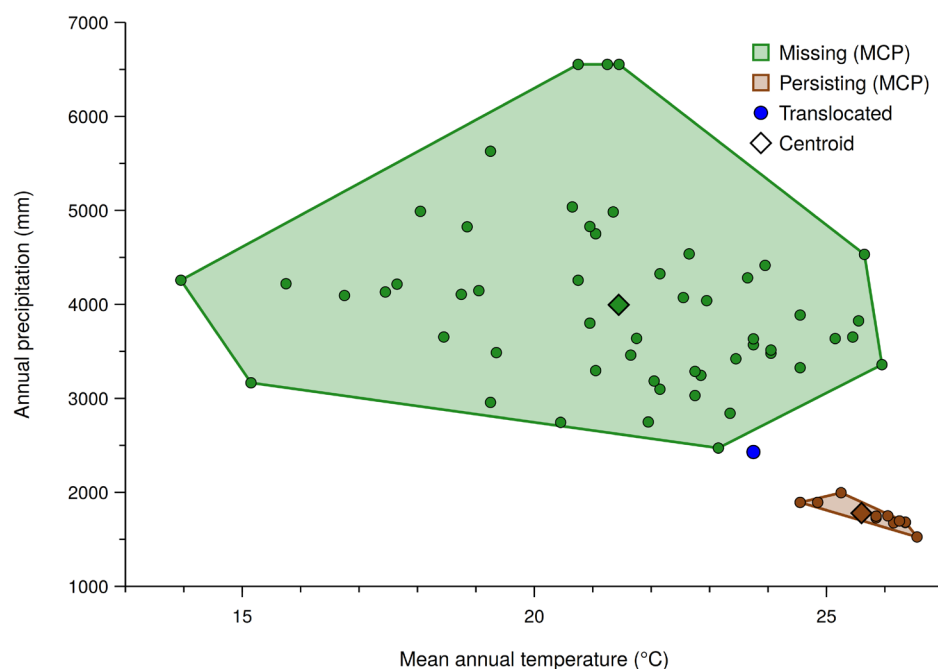


FIGURE 2 | Climate space occupied by *Craugastor ranoides* presence sites across Costa Rica. Points represent persisting localities (brown circles), missing historical localities (green circles), and the translocation site at Maritza Biological Station (blue circle). Semi-transparent polygons show minimum convex polygons encompassing persisting and missing localities, and solid diamonds indicate group centroids. Climate values represent mean annual temperature (BIO1) and annual precipitation (BIO12) extracted from the CHELSA v2.1 1981–2010 climatology. Occurrence records were compiled from the Museum of Zoology, University of Costa Rica.

at the foothills of Volcán Orosí, an area where the species historically occurred. Records from nearby Volcán Cacao document its presence before the mass declines of the late 1980s, likely caused by chytridiomycosis outbreaks (Puschendorf et al. 2019). This site was selected because it occupies an intermediate climatic position between the currently persisting dry-forest localities and the generally cooler, wetter localities from which the species has disappeared (Figure 2). To facilitate short-term post-translocation monitoring and recapture of individuals, we employed radio tracking—the only feasible method for assessing individual movement and allowing recapture for longitudinal microbiome sampling. Prior to translocation, we confirmed that the transmitter attachment and tracking method functioned on wild *C. ranoides* under local field conditions. Only frogs weighing more than 5 g were considered suitable for radio tracking, ensuring the transmitter weight did not exceed 10% of the individual's body mass, in line with ethical guidelines for amphibian telemetry (Muths 2003). Our sample of individuals selected comprised 9 females and 5 males. Frogs were weighed using Pesola Light-Line Precision Spring Scales (Pesola AG, Baar, Switzerland) and measured for snout-vent length (SVL) using Proster digital calipers (Model VC4102, Shenzhen, China) both prior to and following transport. Translocated frogs were swabbed at the site of origin prior to transport.

After sampling, each frog was placed in an individual sterile polyethylene bag (Whirl-Pak, Nasco, USA) and transported in a Coleman 30-Quart Performance Cooler (Newell Brands, USA) directly to Maritza Biological Station, approximately a 1-h drive from the capture location. Upon arrival, frogs were reweighed, remeasured, and fitted with LB-2X radio transmitters (0.27 g, Holohil Systems Ltd., Ontario, Canada; battery life

21 days standard, range 13–22 days) using waist-belt harnesses constructed from silicone tubing, designed to minimise restriction of movement. All 14 translocated frogs were fitted with transmitters.

We used radio tracking (Lotek Biotracker VHF receiver, 138–174 MHz, with a Lotek LiteFlex 3-element Yagi antenna) to relocate individuals after release and facilitate recapture for post-translocation swabbing and body mass measurements over a 7-day monitoring period. Tagged individuals were located up to twice daily during this period, with efforts focused on confirming presence and enabling eventual recapture rather than recording detailed movement trajectories. We recaptured and swabbed 9 individuals, allowing us to compare microbiome composition pre- and post-translocation. Longer-term systematic monitoring was not feasible because the destination site is remote and access is limited during the wet season, and transmitter battery life constrained tracking duration. Frogs were released on the same night within an approximately 30 m section of stream.

2.3 | Sequencing and Bioinformatics

We extracted DNA from swabs using Zymobiotics Miniprep kits (Zymo, USA) using the standard kit protocol after removing ethanol from samples using a SpeedVac. We sent normalised DNA extracts to the Exeter sequencing service where they prepared V4 16S rRNA libraries using primers 515F and 806R and sequenced them on an Illumina NovaSeq using 250 bp Paired End reads. We used *dada2* (Callahan et al. 2016) to identify Amplicon Sequence Variants (ASVs) and assigned

taxonomy using SILVA v138.1 (Quast et al. 2013). We removed potential contaminant sequences using *decontam* (Davis et al. 2018) prior to downstream analysis. Final post-QC library sizes ranged from 15,189–176,202 reads per sample across both the Spatial and Translocation samples (mean 102,663).

2.4 | Statistical Analysis

We used *phyloseq* (McMurdie and Holmes 2013) and *vegan* (Oksanen et al. 2025) for downstream processing of cleaned microbiome data, alongside *ggplot2* (Wickham 2011), *ggordiplots* (Quensen et al. 2026), and *cowplot* (Wilke 2025) for visualisation of microbiome data. We used *vegan* for PERMANOVA and ordinations, *brms* (Bürkner 2021) to fit Bayesian generalised linear models, and *glvm* (Niku et al. 2019, 2024) to fit Generalized Linear Latent Variable models (GLLVs). We used *ComplexUpset* (Krassowski 2020; Lex et al. 2014) to visualise shared and unique ASVs by site ID, and *NetCoMi* (Peschel 2025) and *SpiecEasi* (Kurtz et al. 2026) to perform network analyses by quantifying (dis)similarities among both samples and ASV abundance data.

2.4.1 | Spatial Dataset

We rarefied samples to 15,000 reads prior to alpha diversity analysis. Rarefaction curves indicate that species discovery had saturated by this sequencing depth (Figure S1). To examine differences in alpha diversity (richness), we fitted a Negative Binomial GLM in *brms* with number of ASVs as the response, and SVL (mm) and site ID as predictors, where site is a 3-level factor describing key sampling sites: Brazo del Potrero Grande (BdPG), Murciélagos (M), & Pedregal (P); Figure 1. To examine differences in beta diversity, we used PERMANOVA and PCA ordination on CLR-transformed relative abundances to account for the compositionality of sequencing data (Gloor et al. 2017). We used GLLVM fitted to the top 50 most abundant bacterial genera to examine change in individual bacterial genera by site (category) and SVL (continuous). We built a sample similarity network based on Aitchison distance using *SpiecEasi* and *NetComi* using CLR as a normalisation method, K-Nearest-Neighbour to account for sparsification, followed by greedy clustering following methods in Peschel (2025).

2.4.2 | Translocation Dataset

We visualised changes in beta diversity using a PCA ordination on CLR-transformed unrarefied abundances, and a heatmap of the top 50 most abundant ASVs using *heatmap* (Kolde 2025). We fitted a PERMANOVA with timepoint (Pre- and Post-Translocation) and ID as fixed effects, specifying a constrained permutation structure by ID to control for repeated measures. We built a sample similarity network from the CLR-transformed ASV abundances using the same specifications as the spatial dataset. To investigate differences in individual ASVs associated with translocation, we fitted a GLLVM with time point as a fixed effect and individual ID as a random effect, with 2 latent variables.

2.4.3 | Predicted Functional Data

To investigate the metabolic functional potential of the *C. ranoides* skin bacterial communities, we used two approaches. First, we used the program *MicFunPred* (Mongad et al. 2021) to predict functional profiles from our 16S data for the translocated individuals, and visualised differences among individuals and translocation timepoints using *heatmap*. Next, we used the bioinformatic tool 'AmphiBac' (v2025.1; Woodhams et al. 2015) to predict the richness and abundance of ASVs with putative ability to inhibit the growth of *Batrachochytrium dendrobatidis* (Bd), using the alignment of ASVs to the 'strict' database for downstream analysis. The AmphiBac database classifies bacterial taxa as *Putative Bd-inhibitory* on the basis of published in vitro challenge assays against Bd; these classifications therefore indicate putative inhibitory potential rather than activity tested directly in our samples. We refer to these throughout as *Putative Bd-inhibitory* bacteria.

3 | Results

3.1 | Spatial Patterns in Skin Bacterial Community Structure

We generated microbiome community data from a total of 48 *C. ranoides* individuals sampled across three discrete populations. We found limited evidence for variation among populations in average bacterial richness (alpha diversity), instead finding substantial within-population variation (Figure 3A). There was also no evidence for an effect of pH on patterns of bacterial richness ($\text{cor} = -0.15$, $p = 0.3$, Figure S2).

We detected significant variation among populations in bacterial community beta diversity (PERMANOVA effect of site $F_{2,45} = 1.68$, $p = 0.001$, $r^2 = 7\%$; Figure 3B,C). This effect seemed to be largely driven by divergence of several Pedregal individuals from the other sampling locations (Figure 3B). Though all sites shared a large number of ASVs (Figure 4A), Pedregal has the highest number of unique ASVs that explain in part its dissimilarity to samples from other sites (Figures 3B and 4A). We found no support for significant variation among sites in dispersion around their centroids, suggesting all three sites had similar variance (betadisper test, $F_{2,45} = 3.03$, $p = 0.053$). We detected variation in community composition among individuals within sites; at the Phylum level; most individuals were dominated by Proteobacteria (50%–95% of ASVs) with Firmicutes and Actinobacteriota comprising the majority of the remaining community composition (Figure 3C). We found no effect of SVL on beta diversity (PERMANOVA $F_{1,43} = 1.19$, $p = 0.13$, $r^2 = 2.5\%$) or richness of bacterial communities (Bayesian GLMM; no support for a main effect of SVL, nor a site:SVL interaction). However, there was a positive trend for the effect of SVL on bacterial richness in two sites (Figure S3).

The sample similarity network did not reveal any systematic clustering by population (Figure 4B). Individuals from BdPG and Murciélagos were identified as 'hub' nodes with high centrality in the network, and strong similarity to other samples. Though the network analysis identified 3 distinct statistical clusters, samples from all 3 main populations were divided among

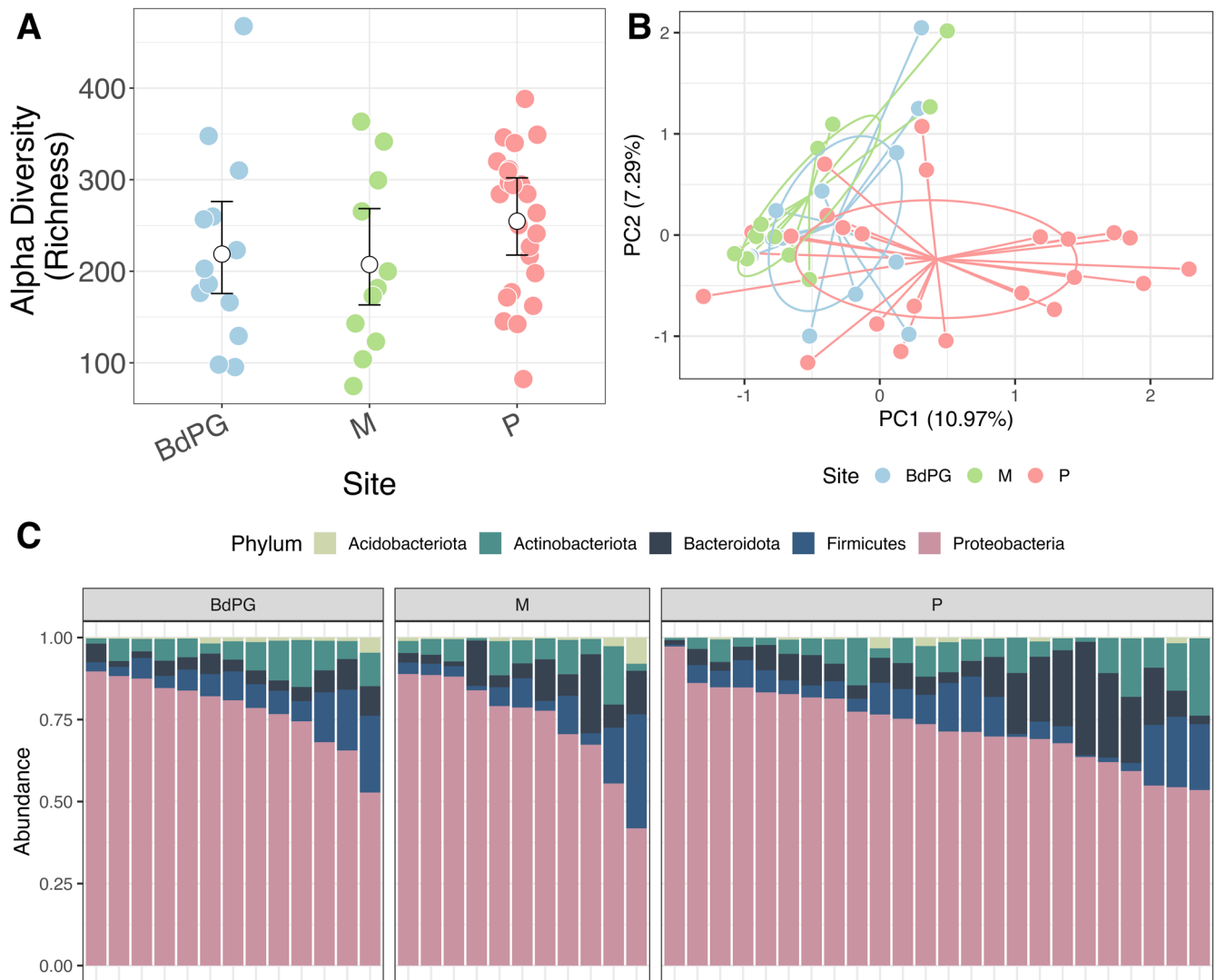


FIGURE 3 | Bacterial community composition variation in space (A) No evidence of variation in mean bacterial richness among sites. Coloured points are raw data means. White points are posterior model means from a Bayesian GLM. Bars represent 95% credible intervals. (B) PCA of Centered log-ratio bacterial community composition, coloured by site. Ellipses represent 95% confidence intervals of the mean and lines connect raw data (points) to site-specific centroids (weighted averages). (C) Stacked bar plots of the relative abundance of the top 5 bacterial Phyla, ordered by proportion of Proteobacteria and site ID (facets).

them. However, Pedregal samples were mostly found on the periphery of the network, with lower similarities to fewer samples, on average (Figure 4B). Collectively, these data support the analyses of beta diversity showing marked within-population heterogeneity in composition alongside a large core microbiome of shared ASVs (Figure 4A)—both of which are expected to disrupt the emergence of strong separation of samples by population of origin.

Using a GLLVM we found several changes at the genus level linked to site ID (Figure 4C). Using site BdPG as the intercept, frogs from Pedregal showed increases in the relative abundance of the genera *Sphingomonas*, *Pedobacter*, *Paracoccus*, and *Brevundimonas*, and decreases in multiple genera including *Yersinia* and *Microbacterium* (Figure 4C). Site M had comparably fewer differentially abundant ASVs, supporting the patterns seen in whole-community ordination (Figure 3B). Larger frogs (greater SVL) had higher relative abundances of *Streptococcus*,

Staphylococcus, and *Corynebacterium*, and lower abundances of *Pedobacter* and *Paucibacter* (Figure 4C).

3.2 | Temporal Dynamics Linked to Translocation

Of the 14 *Craugastor ranoides* individuals translocated, all contributed microbiome samples at the pre-translocation timepoint, but only nine were recaptured 7 days after translocation and resampled for bacterial community profiling post-translocation. Of the five frogs not recaptured, one individual's transmitter was detected under a large boulder approximately five meters from the stream, which may reflect predation, although tag detachment or the frog slipping the harness cannot be excluded. The remaining four were intermittently detected earlier in the study but could not be located at the final time point because they could not be accessed (frog hiding and inaccessible), or transmitter failure during the course of the radio tracking period.

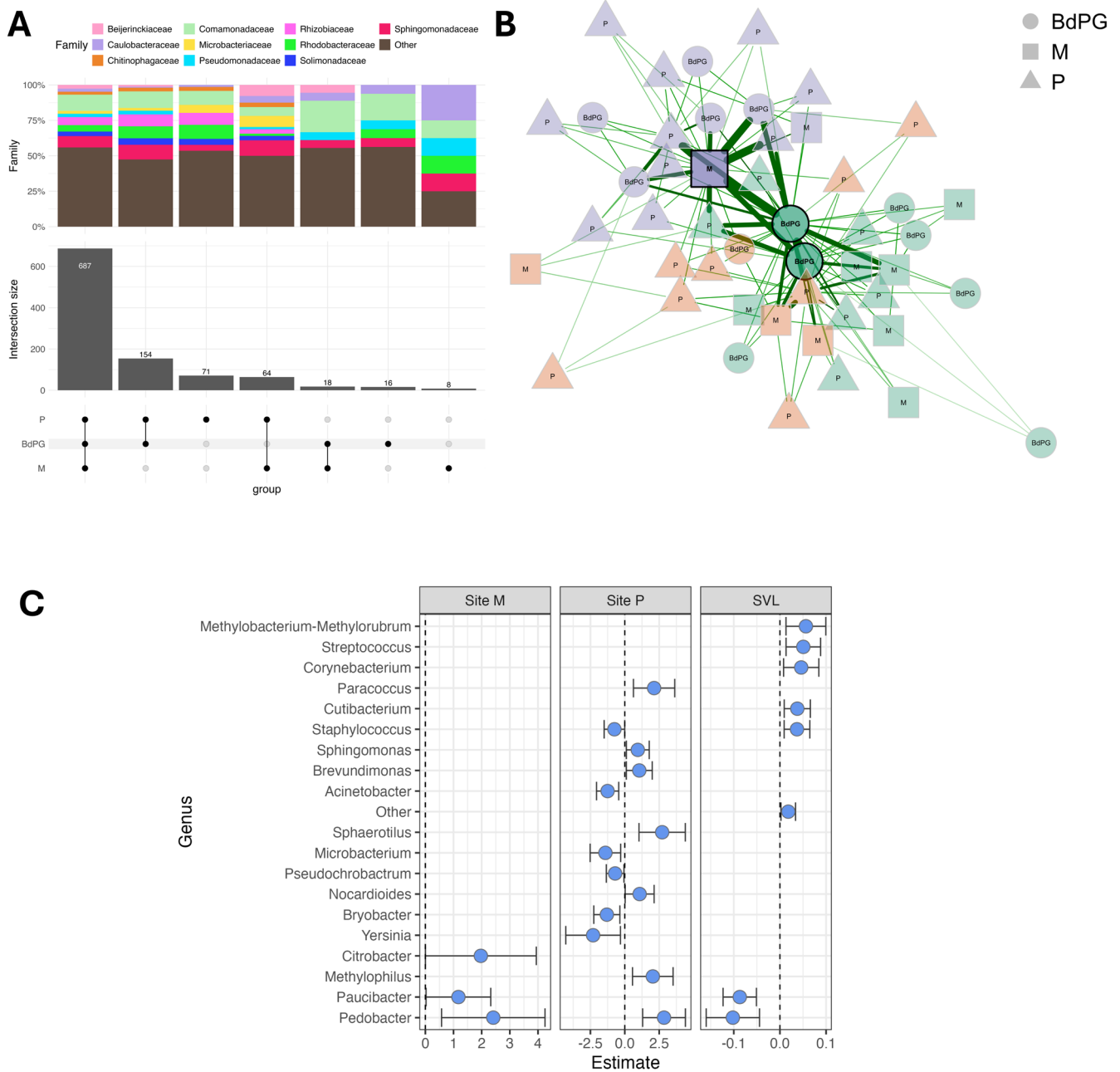


FIGURE 4 | Difference in skin bacterial composition by Site and SVL. (A) Upset plot showing overlapping and unique ASVs per site. The middle plot shows how many ASVs are shared among groups (sites) and the lower plots show which groups these ASVs occur in e.g., there were 687 ASVs that occurred in all 3 sites (Bar 1). The upper stacked barplot shows taxonomic composition at the Family level of shared ASVs for each group. Colours of bars indicate bacterial Family. (B) Sample similarity network for spatial study. Each sample is a node; connections between nodes indicate stronger covariance in ASV abundances between that pair of samples, with thicker edges showing higher relative similarity. Highlighted nodes are ‘hub’ nodes with high eigenvector centrality—connected to nodes that themselves are well connected. Node colours indicate statistical cluster/membership (C) Output of a GLLVM showing significantly different ASVs in sites P and M (compared to BdPG as the intercept), and SVL.

Body weight changes for repeat-sampled frogs were variable: five frogs gained weight, three lost weight, and one remained unchanged, with a mean weight change of +0.39 g (95% credible interval—0.27 to 1.1 g).

We found that no consistent change in community composition (Figure 5A) or community richness linked to translocation (Bayesian Negative Binomial GLMM effect of time (logs) mean 0.09; 95% credible interval—0.2 to 0.39). Individuals

pre- and post-translocation exhibited increases, decreases, and no change in richness (Figure 5B). Both the sample similarity network (Figure 5C) and heatmap of community composition (Figure 5D) supported these patterns, with no clear clustering of samples by time point or individual ID. However, some sets of samples did show similar compositional profiles and clustered together, including samples from different individuals from different timepoints (Figure 5C,D). In the sample similarity network, most of the pre-translocation samples

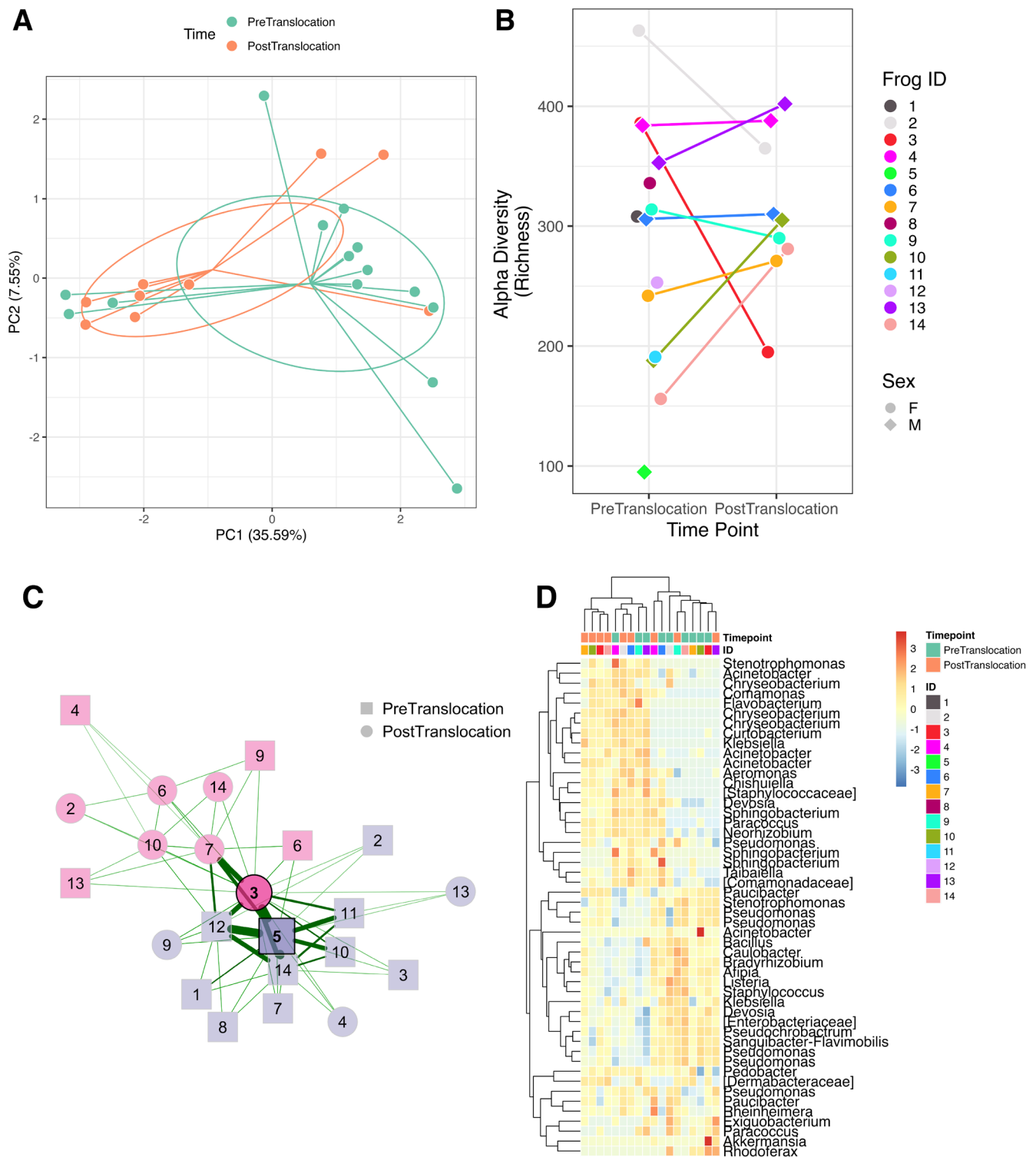


FIGURE 5 | Temporal microbiome dynamics following translocation (A) Ordination of skin bacterial communities pre- and post-translocation. (B) Changes in bacterial richness (alpha diversity). Lines link repeat sampled frogs caught 7 days after translocation. Some individuals were not recaptured and so are not connected to other points. (C) Sample similarity network based on microbiome composition for translocated individuals. Shape indicates time point (pre- vs. post-translocation). Colour denotes statistical cluster, and edge weight indicates sample similarity. The two highlighted 'hub' nodes have the highest eigenvector centrality. Some repeat-sampled individuals had no network connections (no edges with other samples) and so were removed from this network. (D) Heatmap of relative abundances of the top 50 most abundant ASVs, clustered by similarity.

belonged to the statistical cluster (Figure 5C), but strong connections between pairs of samples from the same individual were rare, suggesting strong within-individual divergence post-translocation. The fact that some individuals maintained

similar levels of richness through translocation (Figure 5B) but remain poorly connected in the network (Figure 5C) is indicative of significant turnover in membership of their bacterial microbiota.

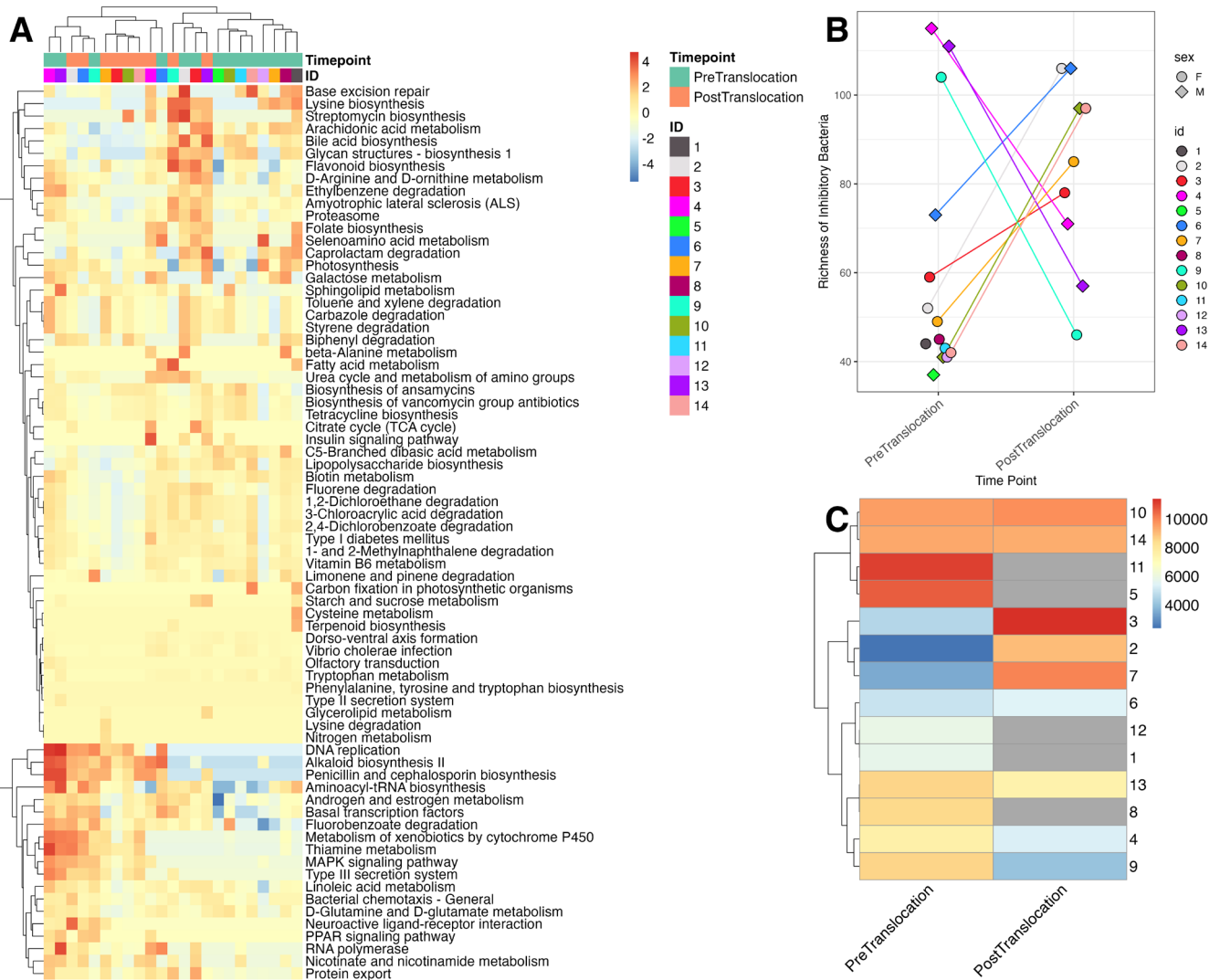


FIGURE 6 | Changes in microbiome composition and predicted function associated with translocation. (A) Heatmap of predicted functional pathways from bacterial communities of translocated frogs. Columns (frogs) are clustered by similarity in functional profile. (B) Output from AmphiBac showing richness of Putative *Bd*-inhibitory bacteria for each frog before and after translocation. (C) Heatmap of AmphiBac output showing total abundance of Putative *Bd*-inhibitory bacteria before and after translocation (each individual is a row). Grey cells indicate that individual was not recaptured/no data.

Using a GLLVM we investigated changes in the relative abundance of specific ASVs linked to translocation whilst controlling for repeated measures on individuals. We found that after translocation, there were increases in ASVs from the genus *Curvibacter*, and decreases in *Devosia*. Using the 16S sequences to predict the functional profiles of skin-associated bacterial communities, we found no consistent shifts in functional profile associated with translocation (PERMANOVA; $F_{1,8}=0.79$, $p=0.78$, $r^2=3.8\%$). There was a strong signal of individual ID in predicted functional profile ($r^2=57.2\%$), though the effect of ID was not significant ($F_{13,8}=0.9$, $p=0.24$). We found metabolic pathways with putative roles in host immunity to pathogens both prior to translocation (e.g., terpenoid biosynthesis) and after translocation (glycan synthesis) (Figure 6A).

We used the AmphiBac database to predict the richness and abundance of ASVs with inhibitory action against the growth of *Bd*. We found that the individuals were equally as likely to lose richness of Putative *Bd*-inhibitory ASVs as they were to gain

them (Figure 6B). However, when looking at total abundance of these inhibitory ASVs, most individuals either maintained or gained abundance following translocation (Figure 6C).

4 | Discussion

Translocations are an important tool for species management and conservation, especially for amphibians in the context of mitigating disease-driven biodiversity declines (Scheele et al. 2021). Performing translocations is logistically challenging, and only recently has the potential importance of host-associated microbial communities as critical agents of translocation success been recognised (Chong et al. 2019; Dallas and Warne 2023; West et al. 2019; Worsley et al. 2026). Few studies have integrated metrics of microbiome composition into decisions of which populations or individuals to translocate, nor studied the impact of translocation on subsequent microbiome dynamics (but see Worsley et al. 2026). We found substantial

within-population variation in the skin microbiota of *C. ranoides*, and no evidence of a consistent directional shift in bacterial community composition associated with translocation. Instead, we detected strong ‘individuality’ of microbial dynamics; some individuals increased the richness of their microbiomes, whilst others exhibited a stable alpha diversity with marked turnover in the identity of bacterial membership. Radio telemetry in this study was used primarily as a relocation tool to maximise recapture probability for repeat sampling (microbiome swabs and body mass) immediately after release, rather than to quantify fine-scale movement or dispersal. Given the dense streamside structure, crevice use, and the practical constraints of working at a remote site, we did not design the field protocol to generate high-resolution movement trajectories. As a result, we focus our inference on short-term persistence and repeat-measures outcomes (recapture, body mass, and microbiome), which align with the study aims.

4.1 | Limited Among-Population Variation in Skin Bacterial Communities

All three populations of *C. ranoides* exhibited equal or greater within- rather than among-population variation in traits of bacterial community composition. Limited among-population divergence in alpha diversity has been observed in the gut microbiomes of passerine birds (Liukkonen et al. 2024), but for many species spatial structure is associated with marked among-population divergence in microbiomes (reviewed in Couch and Epps 2022), especially for environmentally-labile amphibian skin microbiomes (e.g., Griffiths et al. 2018; Kueneman et al. 2014). In our study, the water courses at all three sampling sites had similar pH values in the range of 7.8–8.3, which may constrain variation in skin bacterial communities (Muletz Wolz et al. 2018). For example, several amphibian studies have linked variation in environmental pH with differences in skin microbiome composition (Harrison et al. 2025; Varela et al. 2018), whilst others have found temperature and rainfall to be important (Estrada et al. 2019; reviewed in Hernández-Gómez and Hua 2023). Despite a seemingly narrow pH envelope across sites, Pedregal showed the largest variability in skin microbiota composition, with the highest number of unique bacterial ASVs and greatest dispersion in microbial community composition in ordination space. This elevated variability may reflect the distinctive geology of Pedregal, which lies within the Santa Elena Ophiolite, where active serpentinisation produces spatially localised alkaline springs and chemically distinct surface waters (Sánchez-Murillo et al. 2014). Although the pH measured at Pedregal was only mildly alkaline, rapid dilution of serpentinising fluids within receiving streams may obscure highly localised geochemical variation. Targeted geochemical and environmental microbiome sampling would be required to test whether such variation contributes to the distinctive frog skin microbiota observed at this site.

A key unanswered question is what drives the observed *within*-population variation in skin microbiome. We found no clear relationship between SVL and *average* community composition, suggesting no consistent wholesale change in microbiome structure as hosts grow and age (Campbell et al. 2019). Using GLLVMs, we did detect subtle shifts in the relative abundance of bacterial

genera when controlling for differences among sites. These included increases in the relative abundance of *Streptococcus* and *Staphylococcus* and *Corynebacterium*, all of which are common residents of the amphibian (Niederle et al. 2019) and wider vertebrate skin microbiome (Ross et al. 2019). We also detected decreases in the relative abundance of *Paucibacter* and *Pedobacter* with increasing SVL. *Paucibacter* is common in freshwater environments in the tropics, and has been found in both the nest microbiota of foam-nesting frogs (Monteiro et al. 2023) and skin bacteriome of cascade frogs (Roth et al. 2013). Changes in the relative abundance of these ASVs in larger frogs are likely to reflect differences in the way frogs of different body size interact with the environment (Kelleher et al. 2017). For example, larger frogs may reduce time spent within or interacting directly with the water course, and so undergo a corresponding decrease in freshwater-associated bacteria.

4.2 | Temporal Dynamics of the Microbiome Linked to Translocation

We predicted that translocation would be associated with a consistent turnover in microbiome composition, and expected that individuals post-translocation would have more similar microbiomes to one another than to their pre-translocation counterparts. Our data did not support this prediction; for some individuals we observed both gain and loss of bacterial community richness over time, whilst others showed stable patterns of richness but underwent significant changes in microbial community membership. There was also no evidence of strong individual stability of the microbiome, which would manifest as resistance to changes in community membership following translocation. We also found no clear signal of a shift in predicted functional repertoire of the microbiome either side of translocation, both at the level of metabolic pathway and richness of ASVs with predicted ability to inhibit the growth of the fungal pathogen *Bd*. However, when looking at total abundance of bacteria with (predicted) *Bd*-inhibiting capability, most recaptured individuals either maintained or increased in the abundance of these bacteria following translocation. This pattern may reflect the fact that they were translocated into an ecologically more complex habitat with a greater pool of potential microbial colonists that are subsequently able to invade skin bacterial communities. The extent to which this is a passive process or represents active selection by the host for microbes with host-defensive capabilities remains unknown. Microbiomes are highly dynamic (Marsh et al. 2024), and understanding the forces shaping that variability in microbiome structure and function remains a major research goal (Worsley et al. 2024). Previous work on translocated Seychelles warblers found consistent reductions in alpha diversity associated with translocation (Worsley et al. 2026), a pattern that contrasts with our data. Most prior research on the effect of translocation on the amphibian skin microbiome tends to focus on individuals that have first been captive-reared (Korpita et al. 2023; Kueneman et al. 2022), and so drawing comparisons to individuals moved from the wild is difficult given that captivity tends to shape microbiome in a unique manner (Kueneman et al. 2022). Previous work on limosa Harlequin frogs (*Atelopus limosa*) found that housing frogs in more ecologically complex mesocosms give rise to skin microbiotas that more closely resemble ‘wild’ skin microbiomes than that of captive counterparts

(Estrada et al. 2022). Mesocosm systems may therefore offer a compelling modelling for understanding the magnitude of microbiome dynamics links to both translocation and conservation 'head starting'.

We suspect the most likely explanation for variation in the rate and extent of skin microbiota turnover we observed is individual variation in behaviour. Behaviour can shape the amphibian skin microbiome by changing how, when and for how long individual frogs interact with terrestrial and aquatic substrates (Xu et al. 2020). Though we relocated individuals through nightly radio tracking in this study, our data are not at the resolution required for tracking fine-scale among-individual variation in behaviour that we can link to microbial profiles. Future work should combine such fine-scale data with contemporary environmental samples that permit quantification of the microbial communities capable of colonising the host in the novel translocation environment. Though we detected and recaptured the majority of our study animals within 1 week of translocation, data from other systems suggest rapid dispersal may mean re-encounter probability of individuals declines swiftly over longer time scales (e.g., >4 weeks, Klocke et al. 2024). These patterns may therefore complicate attempts to quantify long-term stability and resilience of the amphibian skin microbiome linked to translocation.

4.3 | Implications for *C. ranoides* and Wider Amphibian Conservation

Our data have several implications for our understanding of the importance of the microbiome in animal translocations. Recently there has been interest in the idea of optimising the microbial communities of translocated hosts to match the destination environment (Seddon and Redford 2025; Trevelline et al. 2019); this can be achieved either by 'bioaugmentation' where beneficial bacteria are installed into resident communities from in vitro cultures, or by choosing populations or individuals that are closely matched in microbial composition to the communities found at the destination. Bioaugmentation can be challenging because the benefit imparted by the novel microbe can change depending on context (e.g., pathogen genotype; Antwis and Harrison 2018). For *C. ranoides*, all our sampled populations had broadly similar bacterial profiles. This could be viewed as a benefit for translocation in that we are able to draw on multiple separate pools of individuals that *on average* will all possess similar 'microbial toolkits' when placed in the novel environment, though of course local microhabitat variations in the destination may also shape *Bd*-inhibitory capability of the skin microbiota (Rebollar et al. 2019). These data underscore the importance of thorough surveys of wild donor populations prior to translocation (Seddon and Redford 2025); depending on the system and nature of the destination, among-individual variability in the microbiome may be seen as either beneficial or suboptimal for the translocation program. The exact balance of these two depends on how plastic the host microbiome is following translocation; if microbiomes are relatively stable then one might value strong divergence in initial microbiome composition as a bet-hedging strategy against future, unknown challenges in the novel habitat, such as variable pathogen genotypes. Our data show that some changes in microbiota composition

post-translocation might be beneficial for host health and/or resilience to challenges such as pathogenic infection. Going forward, resolving the effects of both among-site variation and translocation on the functional properties of the skin microbiome requires that we use tools like metagenomics to validate the predicted functional profiles we present here (Rebollar et al. 2018).

Beyond the microbiome, *C. ranoides* offers a compelling model for amphibian resilience. Persistence of the species in the Santa Elena Peninsula has previously been hypothesised to reflect a climatic refuge from *Bd* (Puschendorf et al. 2005, 2009, 2019; Zumbado-Ulate et al. 2014). Active serpentinisation occurs within parts of the Santa Elena Ophiolite, where discrete spring outflows can reach pH values above 11. However, Sánchez-Murillo et al. (2014) showed that these hyperalkaline fluids are rapidly diluted in receiving streams, whose mean surface-water pH was approximately 8.0. This is consistent with the mildly alkaline values of 7.8–8.3 recorded at our study sites. Our data therefore do not demonstrate that serpentinisation influences frog skin microbiota or *Bd* viability, although this remains a hypothesis that could be tested using targeted geochemical and environmental microbiome sampling.

These serpentinisation-driven habitats may thus represent not only climatic but geochemical refugia for chytrid-sensitive species like *C. ranoides*. Understanding the interplay of local environmental chemistry and microbial dynamics is crucial for amphibian conservation strategies, particularly as climate change continues to shift the distribution of both hosts and pathogens (Neely et al. 2023).

Notably, 3.5 months after the experiment concluded, six *C. ranoides* individuals were found in the immediate vicinity where they had been tracked during the study, suggesting short-term survival and possibly site fidelity. While informal, this follow-up reinforces the possibility that translocated frogs can persist in suitable montane environments. Taken together, our findings highlight the value of incorporating both individual-level variation (e.g., Klocke et al. 2023) and microbiome dynamics into amphibian translocation planning.

Author Contributions

R.P., X.A.H., K.H., V.E.P.W., A.D.M.W., A.C.N., H.Z., M.M.C., J.B., G.A. and R.F. designed and conducted the research, fieldwork and collected samples. K.J.M. and X.A.H. performed the laboratory work for DNA extraction. X.A.H. performed bioinformatic and statistical analysis. X.A.H. and R.P. wrote the manuscript, with comments from all authors.

Funding

This work was funded by National Geographic Grants (NGS-67216C-20 and NGS-97833C-22). XAH and KJM acknowledge additional funding by the Leverhulme Trust (RPG-2020-320) that supported this work.

Ethics Statement

This research was approved by the University of Costa Rica (UCR) and the Sistema de Areas de Conservación de Costa Rica (SINAC). Permit resolutions CIBET-085-2022 and CBio-21-2022 were granted by UCR,

and permits ACG-121-2021 and ACG-068-2022 were issued by the Área de Conservación Guanacaste. These permits comprehensively covered all aspects of the research, including ethical review, fieldwork, and molecular analyses for the entire duration of the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All raw sequencing data are hosted on the SRA under Project ID PRJNA1298236. All code and data needed to reproduce these analyses are hosted on GitHub at <https://github.com/xavharrison/RanoidesCostaRica24>. The database with strict curation of *Bd* inhibitory bacteria isolates used in this study is publicly available at <https://github.com/AmphiBac/AmphiBac-Database>.

References

- Antwis, R. E., and X. A. Harrison. 2018. "Probiotic Consortia Are Not Uniformly Effective Against Different Amphibian Chytrid Pathogen Isolates." *Molecular Ecology* 27, no. 2: 577–589. <https://doi.org/10.1111/mec.14456>.
- Bates, K. A., F. C. Clare, S. O'Hanlon, et al. 2018. "Amphibian Chytridiomycosis Outbreak Dynamics Are Linked With Host Skin Bacterial Community Structure." *Nature Communications* 9, no. 1: 693. <https://doi.org/10.1038/s41467-018-02967-w>.
- Bates, K. A., J. M. Shelton, V. L. Mercier, et al. 2019. "Captivity and Infection by the Fungal Pathogen *Batrachochytrium salamandrivorans* Perturb the Amphibian Skin Microbiome." *Frontiers in Microbiology* 10: 1834.
- Bates, K. A., U. Sommer, K. P. Hopkins, et al. 2022. "Microbiome Function Predicts Amphibian Chytridiomycosis Disease Dynamics." *Microbiome* 10, no. 1: 44. <https://doi.org/10.1186/s40168-021-01215-6>.
- Bürkner, P.-C. 2021. "Bayesian Item Response Modeling in R With Brms and Stan." *Journal of Statistical Software* 100, no. 5: 1–54. <https://doi.org/10.18637/jss.v100.i05>.
- Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13, no. 7: 581–583.
- Campbell, L. J., T. W. Garner, K. Hopkins, A. G. Griffiths, and X. A. Harrison. 2019. "Outbreaks of an Emerging Viral Disease Covary With Differences in the Composition of the Skin Microbiome of a Wild United Kingdom Amphibian." *Frontiers in Microbiology* 10: 1245.
- Campos, F. A., U. Kalbitzer, A. D. Melin, et al. 2020. "Differential Impact of Severe Drought on Infant Mortality in Two Sympatric Neotropical Primates." *Royal Society Open Science* 7, no. 4: 200302.
- Castro, S. M., G. A. Sanchez-Azofeifa, and H. Sato. 2018. "Effect of Drought on Productivity in a Costa Rican Tropical Dry Forest." *Environmental Research Letters* 13, no. 4: 045001.
- Chong, R., C. E. Grueber, S. Fox, et al. 2019. "Looking Like the Locals—Gut Microbiome Changes Post-Release in an Endangered Species." *Animal Microbiome* 1, no. 1: 8. <https://doi.org/10.1186/s42523-019-0012-4>.
- Couch, C. E., and C. W. Epps. 2022. "Host, Microbiome, and Complex Space: Applying Population and Landscape Genetic Approaches to Gut Microbiome Research in Wild Populations." *Journal of Heredity* 113, no. 3: 221–234. <https://doi.org/10.1093/jhered/esab078>.
- Dallas, J. W., and R. W. Warne. 2023. "Captivity and Animal Microbiomes: Potential Roles of Microbiota for Influencing Animal Conservation." *Microbial Ecology* 85, no. 3: 820–838.
- Davis, N. M., D. M. Proctor, S. P. Holmes, D. A. Relman, and B. J. Callahan. 2018. "Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data." *Microbiome* 6, no. 1: 226. <https://doi.org/10.1186/s40168-018-0605-2>.
- Estrada, A., M. C. Hughey, D. Medina, et al. 2019. "Skin Bacterial Communities of Neotropical Treefrogs Vary With Local Environmental Conditions at the Time of Sampling." *PeerJ* 7: e7044. <https://doi.org/10.7717/peerj.7044>.
- Estrada, A., D. Medina, B. Gratwicke, R. Ibáñez, and L. K. Belden. 2022. "Body Condition, Skin Bacterial Communities and Disease Status: Insights From the First Release Trial of the Limosa Harlequin Frog, *Atelopus limosus*." *Proceedings of the Royal Society B: Biological Sciences* 289, no. 1978: 20220586. <https://doi.org/10.1098/rspb.2022.0586>.
- García-Rodríguez, A., G. Chaves, C. Benavides-Varela, and R. Puschendorf. 2012. "Where Are the Survivors? Tracking Relictual Populations of Endangered Frogs in Costa Rica." *Diversity and Distributions* 18, no. 2: 204–212. <https://doi.org/10.1111/j.1472-4642.2011.00862.x>.
- Gloor, G. B., J. M. Macklaim, V. Pawlowsky-Glahn, and J. J. Egozcúe. 2017. "Microbiome Datasets Are Compositional: And This Is Not Optional." *Frontiers in Microbiology* 8: 2224. <https://doi.org/10.3389/fmicb.2017.02224>.
- Griffiths, S. M., X. A. Harrison, C. Weldon, et al. 2018. "Genetic Variability and Ontogeny Predict Microbiome Structure in a Disease-Challenged Montane Amphibian." *ISME Journal* 12, no. 10: 2506–2517.
- Harrison, X. A., E. Remotti, F. C. Clare, et al. 2025. "Environment Shapes the Association Between the Skin Microbiome and Infection Dynamics of the Dermocystid Pathogen *Amphibiothecum* in Palmate Newts (*Lissotriton helveticus*)." *bioRxiv: The Preprint Server for Biology*, 2025–11.
- Harrison, X. A., T. Sewell, M. Fisher, and R. E. Antwis. 2020. "Designing Probiotic Therapies With Broad-Spectrum Activity Against a Wildlife Pathogen." *Frontiers in Microbiology* 10: 3134. <https://doi.org/10.3389/fmicb.2019.03134>.
- Hernández-Gómez, O., and J. Hua. 2023. "From the Organismal to Biosphere Levels: Environmental Impacts on the Amphibian Microbiota." *FEMS Microbiology Reviews* 47, no. 1: fuad002. <https://doi.org/10.1093/femsre/fuad002>.
- Hijmans, R., A. Brown, and M. Barbosa. 2026. "terra: Spatial Data Analysis|CiNii Research." <https://cir.nii.ac.jp/crid/1360864422589423232>.
- Hossack, B. R., D. Hall, C. L. Crawford, et al. 2023. "Successful Eradication of Invasive American Bullfrogs Leads to Coextirpation of Emerging Pathogens." *Conservation Letters* 16, no. 5: e12970. <https://doi.org/10.1111/conl.12970>.
- Karger, D. N., O. Conrad, J. Böhner, et al. 2017. "Climatologies at High Resolution for the Earth's Land Surface Areas." *Scientific Data* 4, no. 1: 170122. <https://doi.org/10.1038/sdata.2017.122>.
- Kelleher, S. R., A. J. Silla, N. J. Dingemanse, and P. G. Byrne. 2017. "Body Size Predicts Between-Individual Differences in Exploration Behaviour in the Southern Corroboree Frog." *Animal Behaviour* 129: 161–170. <https://doi.org/10.1016/j.anbehav.2017.05.013>.
- Klocke, B., A. Estrada, M. Mataya, et al. 2023. "Movement and Survival of Captive-Bred Limosa Harlequin Frogs (*Atelopus limosus*) Released Into the Wild." *Frontiers in Amphibian and Reptile Science* 1: 1205938.
- Klocke, B., O. Garcés, E. Lassiter, et al. 2024. "Release Trial of Captive-Bred Variable Harlequin Frogs *Atelopus varius* Shows That Frogs Disperse Rapidly, Are Difficult to Recapture and Do Not Readily Regain Skin Toxicity." *Oryx* 58, no. 3: 323–335.
- Knapp, R. A., M. Q. Wilber, M. B. Joseph, T. C. Smith, and R. L. Grasso. 2024. "Reintroduction of Resistant Frogs Facilitates Landscape-Scale

- Recovery in the Presence of a Lethal Fungal Disease." *Nature Communications* 15, no. 1: 9436.
- Kolde, R. 2025. "pheatmap: Pretty Heatmaps." <https://doi.org/10.32614/CRAN.package.pheatmap>.
- Korpita, T. M., E. L. Muths, M. K. Watry, and V. J. McKenzie. 2023. "Captivity, Reintroductions, and the Rewilding of Amphibian-Associated Bacterial Communities." *Microbial Ecology* 86, no. 4: 2271–2281. <https://doi.org/10.1007/s00248-023-02229-3>.
- Krassowski, M. 2020. "ComplexUpset." <https://doi.org/10.5281/zenodo.3700590>.
- Kueneman, J. G., M. C. Bletz, M. Becker, et al. 2022. "Effects of Captivity and Rewilding on Amphibian Skin Microbiomes." *Biological Conservation* 271: 109576.
- Kueneman, J. G., L. W. Parfrey, D. C. Woodhams, H. M. Archer, R. Knight, and V. J. McKenzie. 2014. "The Amphibian Skin-Associated Microbiome Across Species, Space and Life History Stages." *Molecular Ecology* 23, no. 6: 1238–1250. <https://doi.org/10.1111/mec.12510>.
- Kurtz, Z., C. Mueller, E. Miraldi, and R. Bonneau. 2026. "SpiecEasi: Sparse Inverse Covariance for Ecological Statistical Inference." <https://doi.org/10.18129/B9.bioc.SpiecEasi>.
- Lex, A., N. Gehlenborg, H. Strobel, R. Vuilleumot, and H. Pfister. 2014. "UpSet: Visualization of Intersecting Sets." *IEEE Transactions on Visualization and Computer Graphics* 20, no. 12: 1983–1992. <https://doi.org/10.1109/TVCG.2014.2346248>.
- Lips, K. R., F. Brem, R. Brenes, et al. 2006. "Emerging Infectious Disease and the Loss of Biodiversity in a Neotropical Amphibian Community." *Proceedings of the National Academy of Sciences of the United States of America* 103, no. 9: 3165–3170. <https://doi.org/10.1073/pnas.0506889103>.
- Liukkonen, M., J. Muriel, J. Martínez-Padilla, et al. 2024. "Seasonal and Environmental Factors Contribute to the Variation in the Gut Microbiome: A Large-Scale Study of a Small Bird." *Journal of Animal Ecology* 93, no. 10: 1475–1492. <https://doi.org/10.1111/1365-2656.14153>.
- Longo, A. V., A. E. Savage, I. Hewson, and K. R. Zamudio. 2015. "Seasonal and Ontogenetic Variation of Skin Microbial Communities and Relationships to Natural Disease Dynamics in Declining Amphibians." *Royal Society Open Science* 2, no. 7: 140377.
- Loudon, A. H., D. C. Woodhams, L. W. Parfrey, et al. 2014. "Microbial Community Dynamics and Effect of Environmental Microbial Reservoirs on Red-Backed Salamanders (*Plethodon cinereus*)." *ISME Journal* 8, no. 4: 830–840.
- Marsh, K. J., S. Bearhop, and X. A. Harrison. 2024. "Linking Microbiome Temporal Dynamics to Host Ecology in the Wild." *Trends in Microbiology* 32: 1060–1071. [https://www.cell.com/trends/microbiology/fulltext/S0966-842X\(24\)00132-X](https://www.cell.com/trends/microbiology/fulltext/S0966-842X(24)00132-X).
- McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8, no. 4: e61217.
- Mongad, D. S., N. S. Chavan, N. P. Narwade, K. Dixit, Y. S. Shouche, and D. P. Dhotre. 2021. "MicFunPred: A Conserved Approach to Predict Functional Profiles From 16S rRNA Gene Sequence Data." *Genomics* 113, no. 6: 3635–3643.
- Monteiro, F. A. C., S. G. d. S. Bezerra, L. G. Z. de Castro, et al. 2023. "Neotropical Frog Foam Nest's Microbiomes." *Microorganisms* 11, no. 4: 900. <https://doi.org/10.3390/microorganisms11040900>.
- Muletz Wolz, C. R., S. A. Yarwood, E. H. Campbell Grant, R. C. Fleischer, and K. R. Lips. 2018. "Effects of Host Species and Environment on the Skin Microbiome of Plethodontid Salamanders." *Journal of Animal Ecology* 87, no. 2: 341–353. <https://doi.org/10.1111/1365-2656.12726>.
- Muths, E. 2003. "A Radio Transmitter Belt for Small Ranid Frogs." <https://pubs.usgs.gov/publication/1015077>.
- Neely, W. J., R. A. Martins, C. M. Mendonça da Silva, et al. 2023. "Linking Microbiome and Stress Hormone Responses in Wild Tropical Treefrogs Across Continuous and Fragmented Forests." *Communications Biology* 6, no. 1: 1261. <https://doi.org/10.1038/s42003-023-05600-9>.
- Niederle, M. V., J. Bosch, C. E. Ale, et al. 2019. "Skin-Associated Lactic Acid Bacteria From North American Bullfrogs as Potential Control Agents of *Batrachochytrium dendrobatidis*." *PLoS One* 14, no. 9: e0223020. <https://doi.org/10.1371/journal.pone.0223020>.
- Niku, J., W. Brooks, R. Herliansyah, et al. 2024. "gllvm: Generalized Linear Latent Variable Models."
- Niku, J., F. K. C. Hui, S. Taskinen, and D. I. Warton. 2019. "Gllvm—Fast Analysis of Multivariate Abundance Data With Generalized Linear Latent Variable Models in R." *Methods in Ecology and Evolution* 10: 2173–2182.
- Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2025. "vegan: Community Ecology Package." <https://CRAN.R-project.org/package=vegan>.
- Peschel, S. 2025. "NetCoMi: Network Construction and Comparison for Microbiome Data." <https://netcomi.de>.
- Powers, J. S., G. Vargas, T. J. Brodribb, et al. 2020. "A Catastrophic Tropical Drought Kills Hydraulically Vulnerable Tree Species." *Global Change Biology* 26, no. 5: 3122–3133. <https://doi.org/10.1111/gcb.15037>.
- Puschendorf, R., F. Bolaños, and G. Chaves. 2006. "The Amphibian Chytrid Fungus Along an Altitudinal Transect Before the First Reported Declines in Costa Rica." *Biological Conservation* 132, no. 1: 136–142.
- Puschendorf, R., A. C. Carnaval, J. VanDerWal, et al. 2009. "Distribution Models for the Amphibian Chytrid *Batrachochytrium dendrobatidis* in Costa Rica: Proposing Climatic Refuges as a Conservation Tool." *Diversity and Distributions* 15, no. 3: 401–408. <https://doi.org/10.1111/j.1472-4642.2008.00548.x>.
- Puschendorf, R., G. A. Chaves-Cordero, A. J. Crawford, and D. R. Brooks. 2005. "Eleutherodactylus ranoides (NCN). Dry Forest Population, Refuge From Decline? *Eleutherodactylus ranoides* (NCN). Población del bosque seco, refugio en disminución?" *Herpetological Review* 36: 53.
- Puschendorf, R., M. Wallace, M. M. Chavarria, et al. 2019. "Cryptic Diversity and Ranavirus Infection of a Critically Endangered Neotropical Frog Before and After Population Collapse." *Animal Conservation* 22, no. 5: 515–524. <https://doi.org/10.1111/acv.12498>.
- Quast, C., E. Pruesse, P. Yilmaz, et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41, no. Database issue: D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- Quensen, J., G. Simpson, and J. Oksanen. 2026. "ggordiplots: Make 'ggplot2' Versions of Vegan's Ordplots." <https://github.com/jfq3/ggordiplots>.
- Rebollar, E. A., T. Bridges, M. C. Hughey, D. Medina, L. K. Belden, and R. N. Harris. 2019. "Integrating the Role of Antifungal Bacteria Into Skin Symbiotic Communities of Three Neotropical Frog Species." *ISME Journal* 13, no. 7: 1763–1775. <https://doi.org/10.1038/s41396-019-0388-x>.
- Rebollar, E. A., A. Gutierrez-Preciado, C. Noecker, et al. 2018. "The Skin Microbiome of the Neotropical Frog *Craugastor fitzingeri*: Inferring Potential Bacterial-Host-Pathogen Interactions From Metagenomic Data." *Frontiers in Microbiology* 9: 466.
- Risely, A., P. G. Byrne, D. A. Hunter, A. S. Carranco, B. J. Hoye, and A. J. Silla. 2024. "Skin Bacterial and Fungal Microbiome Responses to Diet Supplementation and Rewilding in the Critically Endangered Southern Corroboree Frog." *Molecular Ecology* 33, no. 23: e17562. <https://doi.org/10.1111/mec.17562>.
- Ross, A. A., A. Rodrigues Hoffmann, and J. D. Neufeld. 2019. "The Skin Microbiome of Vertebrates." *Microbiome* 7, no. 1: 79. <https://doi.org/10.1186/s40168-019-0694-6>.

- Roth, T., J. Foley, J. Worth, J. Piovita-Scott, K. Pope, and S. Lawler. 2013. "Bacterial Flora on Cascades Frogs in the Klamath Mountains of California." *Comparative Immunology, Microbiology and Infectious Diseases* 36, no. 6: 591–598. <https://doi.org/10.1016/j.cimid.2013.07.002>.
- Sánchez-Murillo, R., E. Gazel, E. M. Schwarzenbach, et al. 2014. "Geochemical Evidence for Active Tropical Serpentinization in the Santa Elena Ophiolite, Costa Rica: An Analog of a Humid Early Earth?" *Geochemistry, Geophysics, Geosystems* 15, no. 5: 1783–1800. <https://doi.org/10.1002/2013GC005213>.
- Scheele, B. C., M. Hollanders, E. P. Hoffmann, et al. 2021. "Conservation Translocations for Amphibian Species Threatened by Chytrid Fungus: A Review, Conceptual Framework, and Recommendations." *Conservation Science and Practice* 3, no. 11: e524. <https://doi.org/10.1111/csp2.524>.
- Scheele, B. C., F. Pasmans, L. F. Skerratt, et al. 2019. "Amphibian Fungal Panzootic Causes Catastrophic and Ongoing Loss of Biodiversity." *Science* 363, no. 6434: 1459–1463. <https://doi.org/10.1126/science.aav0379>.
- Scheele, B. C., L. F. Skerratt, L. F. Grogan, et al. 2017. "After the Epidemic: Ongoing Declines, Stabilizations and Recoveries in Amphibians Afflicted by Chytridiomycosis." *Biological Conservation* 206: 37–46.
- Seddon, P., and K. Redford. 2025. "Current and Future Challenges to Conservation Translocations." *Nature Reviews Biodiversity* 1: 196–208. <https://doi.org/10.1038/s44358-025-00020-5>.
- Smith, K. G., K. R. Lips, and J. M. Chase. 2009. "Selecting for Extinction: Nonrandom Disease-Associated Extinction Homogenizes Amphibian Biotas." *Ecology Letters* 12, no. 10: 1069–1078. <https://doi.org/10.1111/j.1461-0248.2009.01363.x>.
- Stuart, S. N., J. S. Chanson, N. A. Cox, et al. 2004. "Status and Trends of Amphibian Declines and Extinctions Worldwide." *Science* 306, no. 5702: 1783–1786. <https://doi.org/10.1126/science.1103538>.
- Trevelline, B. K., S. S. Fontaine, B. K. Hartup, and K. D. Kohl. 2019. "Conservation Biology Needs a Microbial Renaissance: A Call for the Consideration of Host-Associated Microbiota in Wildlife Management Practices." *Proceedings of the Royal Society B: Biological Sciences* 286, no. 1895: 20182448. <https://doi.org/10.1098/rspb.2018.2448>.
- Varela, B. J., D. Lesbarrères, R. Ibáñez, and D. M. Green. 2018. "Environmental and Host Effects on Skin Bacterial Community Composition in Panamanian Frogs." *Frontiers in Microbiology* 9: 298. <https://doi.org/10.3389/fmicb.2018.00298>.
- West, A. G., D. W. Waite, P. Deines, et al. 2019. "The Microbiome in Threatened Species Conservation." *Biological Conservation* 229: 85–98.
- Wickham, H. 2011. "Ggplot2." *WIREs Computational Statistics* 3, no. 2: 180–185. <https://doi.org/10.1002/wics.147>.
- Wilke, C. O. 2025. "cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'." <https://doi.org/10.32614/CRAN.package.cowplot>.
- Woodhams, D. C., R. A. Alford, R. E. Antwis, et al. 2015. "Antifungal Isolates Database of Amphibian Skin-Associated Bacteria and Function Against Emerging Fungal Pathogens." *Ecology* 96, no. 2: 595. <https://doi.org/10.1890/14-1837.1>.
- Worsley, S. F., Z. Crighton, C. Z. Lee, et al. 2026. "Gut Microbiome Communities Vary Across Translocated Populations of the Seychelles Warbler." *Ecology and Evolution* 16, no. 6: e73750. <https://doi.org/10.1002/ece3.73750>.
- Worsley, S. F., E. Videvall, X. A. Harrison, J. R. Björk, F. Mazel, and K. M. Wanelik. 2024. "Probing the Functional Significance of Wild Animal Microbiomes Using Omics Data." *Functional Ecology* 38, no. 11: 2329–2349. <https://doi.org/10.1111/1365-2435.14650>.
- Xu, L., M. Xiang, W. Zhu, et al. 2020. "The Behavior of Amphibians Shapes Their Symbiotic Microbiomes." *mSystems* 5, no. 4: e00626-20. <https://doi.org/10.1128/mSystems.00626-20>.
- Zumbado-Ulate, H., F. Bolaños, G. Gutiérrez-Espeleta, and R. Puschendorf. 2014. "Extremely Low Prevalence of *Batrachochytrium dendrobatidis* in Frog Populations From Neotropical Dry Forest of Costa Rica Supports the Existence of a Climatic Refuge From Disease." *EcoHealth* 11, no. 4: 593–602. <https://doi.org/10.1007/s10393-014-0967-2>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Number of *Craugastor ranoides* individuals sampled per site and sampling period in the multi-population skin microbiome survey (48 individuals total), Santa Elena Peninsula, Área de Conservación Guanacaste, Costa Rica. All three populations were sampled in January 2022; Pedregal and Brazo del Potrero Grande were sampled again in April 2022. All sampling was conducted during the dry season. **Figure S1:** Rarefaction curves for samples used in the study. **Figure S2:** Bacterial richness by pH of the sampling location. Three main sampling sites (BdPG, M & P) are split by location of sampling within each catchment (A & B). **Figure S3:** Effect of Snout-Vent length (SVL) on bacterial alpha diversity (richness) by site. Though there was no statistical support for an effect of SVL or an SVL:site interaction, there was a positive trend at two sites and the credible intervals for their effects only just crossed zero.