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HOST MICROBE INTERACTIONS

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Habitat and Host Species Drive the Structure of Bacterial Communities of Two Neotropical Trap-Jaw *Odontomachus* Ants

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Abstract
Ants have long been known for their associations with other taxa, including macroscopic fungi and symbiotic bacteria. Recently, many ant species have had the composition and function of their bacterial communities investigated. Due to its behavioral and ecological diversity, the subfamily Ponerinae deserves more attention regarding its associated microbiota. Here, we used the V4 region of the 16S rRNA gene to characterize the bacterial communities of *Odontomachus chelifer* (ground-nesting) and *Odontomachus hastatus* (arboreal), two ponerine trap-jaw species commonly found in the Brazilian savanna ("Cerrado") and Atlantic rainforest. We investigated habitat effects (*O. chelifer* in the Cerrado and the Atlantic rainforest) and species-specific effects (both species in the Atlantic rainforest) on the bacterial communities' structure (composition and abundance) in two different body parts: cuticle and gaster. Bacterial communities differed in all populations studied. Cuticular communities were more diverse, while gaster communities presented variants common to other ants, including *Wolbachia* and *Candidatus Tokpelaia hoelldoblerii*. *Odontomachus chelifer* populations presented different communities in both body parts, highlighting the influence of habitat type. In the Atlantic rainforest, the outcome depended on the body part targeted. Cuticular communities were similar between species, reinforcing the habitat effect on bacterial communities, which are mainly composed of environmentally acquired taxa. Gaster communities, however, differed between the two *Odontomachus* species, suggesting species-specific effects and selective filters. Unclassified Firmicutes and uncultured Rhizobiales variants are the main components accounting for the observed differences. Our study indicates that both host species and habitat act synergistically, but to different degrees, to shape the bacterial communities in these *Odontomachus* species.

Keywords *Odontomachus* · Ponerinae · Bacterial communities · 16S rRNA gene · Cerrado · Atlantic Rainforest

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Introduction

Microorganisms have been associated with animals' evolutionary history since their origin, in a diversity of interactions [1]. Nevertheless, with more studies on host-associated microbial communities, it is clear now that through evolutionary history, not all animals maintain a stable symbiotic microbiota [2]. Both hosts and microorganisms can be selected to strengthen or relax their associations through deterministic or stochastic eco-evolutionary processes [3].

Disentangling the effects of host species (phylogenetic component) and the environment on the assembly process of microbial communities is important given that both factors can shape the structure (i.e., the composition of a microbial community and the abundance of its members; [4]) of associated communities in space and time [5]. Additionally, the microbial communities of the host's distinct body parts can be differentially affected by each of these factors due to their compartmentalization [6]. Phylogenetic history and environmental factors have been thoroughly investigated in animals [7–10]. Generally, outer surface microbial communities are less affected by host selection, thus harboring more diverse, environmentally related communities ([9, 11], but see [10]). Internal communities, however, are subject to constant selection by abiotic conditions and biotic interactions within the host, making them narrower and host-related, or even host-restricted ([5], but see [2]).

Studies on microbial communities associated with ants have increased markedly in the past decade [12]. The 'omics' revolution allowed myrmecologists to deepen their view of ants' eco-evolutionary relationships with microorganisms, in particular bacteria [13]. For instance, research on gut bacterial communities improved our understanding of the convergent occupation of forest canopies by distinct functional herbivore ant lineages and the role of bacteria in this transition [14–16]. Ants with nitrogen-rich diets have been shown to harbor abundant bacterial communities too [17, 18], although not as abundant as herbivorous ant species [13, 19].

To date, less attention has been given to bacterial communities associated with members of the ant subfamily Ponerinae, which is globally distributed and contains over 1200 species [20]. Ponerines are predominantly predatory ants, feeding mostly on arthropods [21], and may have specific bacterial communities related to their diet, as reported for species of the predatory ant subfamily Dorylinae [18]. *Odontomachus* is a pantropical ponerine genus containing 67 species distributed from semi-arid to rainforest habitats [20, 22]. *Odontomachus* trap-jaw ants are visually oriented hunters that nest on the ground or on plants [23, 24], feeding predominantly on litter-dwelling and arboreal arthropods [25–27]. Two species of *Odontomachus*, *O. chelifer* (Latreille, 1802) and *O. hastatus* (Fabricius, 1804) (Fig. 1), have previously been studied for their social organization and nesting habits [28, 29]. *Odontomachus chelifer* nests on the ground in Brazilian savanna ("Cerrado") and Atlantic rainforest and may complement its diet with nutritious fleshy fruits and seeds, acting as important secondary seed dispersers in their habitat [30–32]. *Odontomachus hastatus* nests among the roots of epiphytic bromeliads in the canopy of the Atlantic rainforest, where it is an important predator and provides nitrogen that enhances the growth of nest bromeliads [26, 33, 34]. Both *O. chelifer* and *O. hastatus* have facultatively polygynous colonies [28, 29], which could potentially account for intra- and inter-colonial variation in associated bacterial communities.

Studies on the microbiota associated with *Odontomachus* ants are scarce [16, 35–37]. Considering the importance of *Odontomachus chelifer* and *O. hastatus* for ecosystem functioning, expanding our knowledge about their associated bacterial communities should open new venues of exploration integrating field and molecular ecology. Here, we use 16S rRNA gene amplicon sequencing to characterize the bacterial communities associated with *O. chelifer* and *O.*

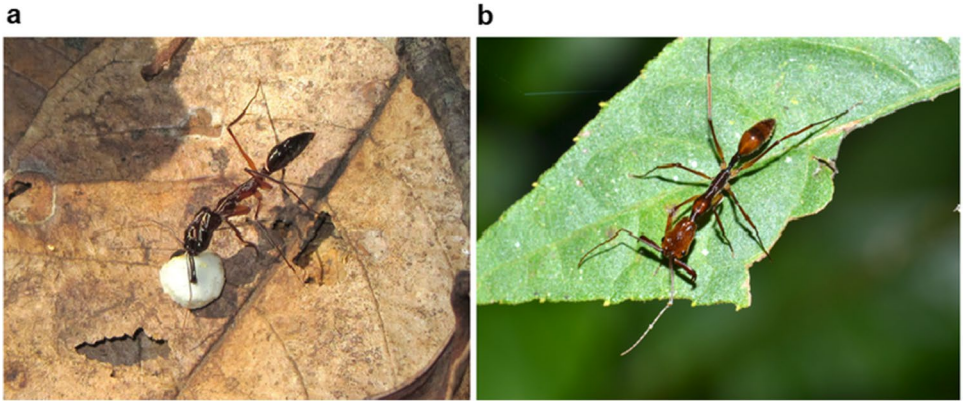

Fig. 1 Trap-jaw *Odontomachus* ants used as study organisms to investigate their bacterial communities in two Brazilian ecosystems: the Cerrado savanna (CS) and the Atlantic rainforest (AF). **a** Ground-nesting *O. chelifer* (CS and AF) carrying a nutritious arilated seed (photo by Verônica Magalhães) and **b** arboreal-nesting *O. hastatus* (AF) (photo by Luísa Mota)

hastatus in two main Neotropical environments: the Brazilian Cerrado and the Atlantic rainforest.

Beyond bacterial community characterization, we investigate the relative importance of host species and habitat in the assembling of the bacterial communities in these ant species, focusing on the communities found in the cuticle of their head and mesosoma, and in their gaster. The gaster is the posterior part of the ants' metasoma, which contains its digestive tract and reproductive system. These two body parts are important compartments since they are the main contact surfaces of the ant with the external world, with functions that involve protection against pathogens, digestion, nutrient provisioning, and communication, all of which are essential in social insects [38–42]. Despite its potential importance for protection and communication, the cuticular microbiota remains understudied in ants, partly because of their low yields of DNA [43]. This is delaying our understanding of the mechanisms shaping its structure and function when compared to the gut [11].

We addressed the following questions for each body part: (1) Do bacterial communities vary geographically (Cerrado vs. Atlantic rainforest) within the same host species (i.e., *O. chelifer*), and are cuticular communities more affected by habitat than gaster-associated communities? (2) Are bacterial communities species-specific in the Atlantic rainforest, where both species co-occur? If so, are internal bacterial communities more affected by host identity than cuticular communities?

This is the first study to investigate the richness and diversity of bacteria associated with trap-jaw *Odontomachus* ants, and potential sources of variation, in two major Neotropical biodiversity hotspots. We show that cuticular and gaster bacterial communities differ in composition and structure in all populations. *Odontomachus chelifer* presented inter-habitat variation in both body parts, highlighting the environmental influence on bacterial communities. Despite microhabitat differentiation between species (ground vs. arboreal nest), cuticular communities in the Atlantic rainforest had similar diversity, whereas gaster communities differed between *O. chelifer* and *O. hastatus*. Our study links ant-associated bacteria and ant habits and is a step forward in understanding the functionality of these microorganisms in the behavior and ecology of trap-jaw ants.

Materials and Methods

Ant Sampling

Odontomachus chelifer was collected in two localities: (1) a reserve of Cerrado at the "Reserva Biológica de Mogi-Guaçu," near Mogi-Guaçu, São Paulo State (22°15′S, 47°09′W), southeast Brazil. The vegetation physiognomy is called "Cerradão," a forest-like woodland with 50–90% of trees up to 10–12 m tall [44]. The annual temperature ranges from 16.3 to 23.5 °C, with accumulated rainfall varying from 250–300 mm in winter to 1100–1200 mm in summer (Reserve Management Plan: www.infraestruturameioambiente.sp.gov.br/institutodebotanica/mogi-guacu/); (2) an Atlantic rainforest reserve in the "Parque Estadual Serra do Mar-Núcleo Picinguaba," Ubatuba, São Paulo State (23°21′S, 44°50′W), southeast Brazil. *Odontomachus hastatus* was also collected at this Atlantic rainforest site, where it co-occurs with *O. chelifer*. The vegetation in this study area is a "restinga" forest growing on sandy soil, with trees up to 20 m tall [45]. The mean annual rainfall is 2624 mm, and the mean annual temperature is 21.2 °C. The relative humidity is greater than 80%, with rains throughout the year [46]. For a map of sampling, localities see Online Resource 1 (Figs. S1 and S2). Samplings were carried out in September 2018 in the Cerrado and in December 2018 in the Atlantic rainforest. All metadata are found in Online Resource 2.

Four *O. chelifer* colonies were sampled in the Cerrado (CS) and Atlantic rainforest sites (AF) and five *O. hastatus* colonies in the Atlantic rainforest site. The nests were at least 10 m apart to assure they were from different colonies [25]. Throughout the text, we refer to *O. chelifer* CS and AF together as "*O. chelifer* populations," whereas *O. chelifer* AF and *O. hastatus* are referred to as "co-occurring species."

Foragers were collected while leaving the nest and were individually stored in 99% alcohol at − 20 °C until DNA extraction, since this method apparently has little effect on bacterial community estimates [47]. Tweezers were rinsed in 2.5% bleach between each collection. As environmental controls, we collected soil from the entrances of *O. chelifer* nests (8 samples) and soil from epiphytic root clusters with and without *O. hastatus* nests (10 samples). Specimens were collected under scientific permits (SisBio #62,347–1 and #62,347–2, Instituto Florestal #396/2018, and Instituto de Botânica #2/2018) and registered on the SisGen platform (#AE5E54B).

Sample Preparation and DNA Extraction

We randomly selected 10 individuals from each colony for DNA extraction, totaling 130 individuals. The extraction was performed with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol but increasing the incubation time in the lysis stage to overnight at 56 °C. Firstly, the whole ants and environmental samples were washed to remove transient bacteria [43]. This was done by vortexing them in pure water for 30 s. To assess the differences between the cuticle and gaster bacterial communities, the ants were then sectioned in the constriction between the mesosoma and petiole. Tweezers were switched and cleaned in 5% bleach and pure distilled

water after each sectioning. Bacterial communities from both body parts totaled 260 samples.

Cuticle and environmental samples were added to lysis buffer and extraction proceeded with the wash (no maceration). Gaster samples were also added to lysis buffer, but they were further macerated in it to assess the internal bacterial communities, which are usually more abundant than external ones [43]. Four extraction controls were included by doing the same procedures on laboratory pure water.

PCR Amplification and Sequencing

The bacterial community of each sample was assessed by amplifying the V4 region of the 16S rRNA gene using a dual index approach [48], with primers 515F and 806R, following the Earth Microbiome Protocol [49]. PCR blank controls were included for each reaction, totaling 4 controls. Samples and controls were pooled and purified using a DNA Purification Kit (Macherey–Nagel, Germany). Samples were sequenced with paired-end 2 × 250 v2 chemistry on an Illumina MiSeq sequencer at the Tufts University Core Facility (TUCF Genomics, Boston, USA). Details on the PCR and library preparation can be found in Online Resource 1. Raw sequence reads were deposited on NCBI's SRA database (BioProject ID: PRJNA701315).

To account for possible cryptic diversity, a fragment of the mitochondrial gene Cytochrome c oxidase I (COI) from two individuals per colony was amplified using primers HCO1490 and LCO2190 [50], following a standard PCR procedure. Amplicons were purified and sent to Macrogen (South Korea) for sequencing. Sequences were quality trimmed using Geneious R11 (Biomatters) and sent to GenBank (accession numbers: MW587097-MW587122). We found no genetic variation within colonies of *O. chelifer* AF and *O. hastatus* (one haplotype each). Colonies of *O. chelifer* CS presented 3 different haplotypes differing from each other by 1–2 base pairs. The genetic uncorrected p-distances between *O. chelifer* CS and AF were, on average, 4.4%.

Sequence Processing and Quality-Filtering

Sequences were processed in QIIME 2 v2019.4 [51] on an Oracle Virtual Machine. Demultiplexed data were imported to QIIME 2, and sequences were validated, quality-filtered, and joined (forward and reverse reads) with default parameters using VSEARCH join-pairs with 251-nucleotide sequence length [52]. Deblur was applied to denoise the reads [53]. The resulting amplicon sequence variants (ASVs) were classified taxonomically with Silva 132 99% database [54]. The feature table and representative sequences were filtered to eliminate mitochondria and chloroplast sequences. The sequences were aligned with MAFFT [55], and the phylogenetic tree was built with FastTree 2 [56].

After initial processing, the feature table was submitted to different filtering steps to avoid contaminants and spurious sequences in the downstream analyses [57]. *Decontam* was the first used to remove contaminants based on their prevalence in samples and controls (threshold = 0.5) [58]. Variants that were present in only one sample were also excluded.

Because reagents and laboratory procedures can contaminate samples, common contaminants present in laboratory reagents were searched [59]. *Escherichia-Shigella* and *Acinetobacter lwoffi* were the most abundant variants in PCR and extraction controls, and were also present in 90.4 and 89.6% of the ant samples, respectively. Thus, both variants were removed from the data analysis table.

After filtering, samples were rarefied to 1000 reads, a normalization step in data analysis. Rarefaction was done after checking the best rarefaction depth for observed ASVs (Online Resource 1, Fig. S3; Online Resource 3) [60]. After rarefaction, four controls remained (CN1—DNA extraction control, PCR-CN1/2—PCR controls, and HC5—*O. hastatus* soil control), which were later removed from statistical analyses because of their small counts. Their compositions are shown in Fig. 2.

Diversity and Statistical Analyses

Statistical analyses were done using R v3.6.3 (www.r-project.org) and the packages *phyloseq* version 1.28.0 and *vegan* version 2.5–6 [61, 62]. Order level was chosen to represent the taxonomic diversity. Orders with less than 1% relative frequency were grouped under the name "Others." A barplot for each body part was built with *ggplot2* [63].

We assessed alpha diversity with two measures: the observed number of ASVs and the Shannon Diversity Index. The Kruskal–Wallis test was used to evaluate if there were differences in diversity between all body parts. Post hoc pairwise comparisons were performed then with the Wilcoxon rank sum test to evaluate the difference between habitats and host species. Benjamini–Hochberg *p*-value corrections for multiple testing were applied. Bacterial community differentiation between body parts and among colonies in each host population is presented in Online Resource 1 (Tables S3–S7).

To investigate the effect of habitat and host species on these communities' assembly, bacterial communities from both body parts were compared between populations of *O. chelifer* in different habitats (AF vs. CS) and between the co-occurring species in the Atlantic rainforest (*O. chelifer vs O. hastatus*). The feature table was filtered accordingly in QIIME 2 for each pairwise comparison. Species or habitats were used as explanatory variables on the PERMANOVA tests [64] and principal coordinates analyses were performed

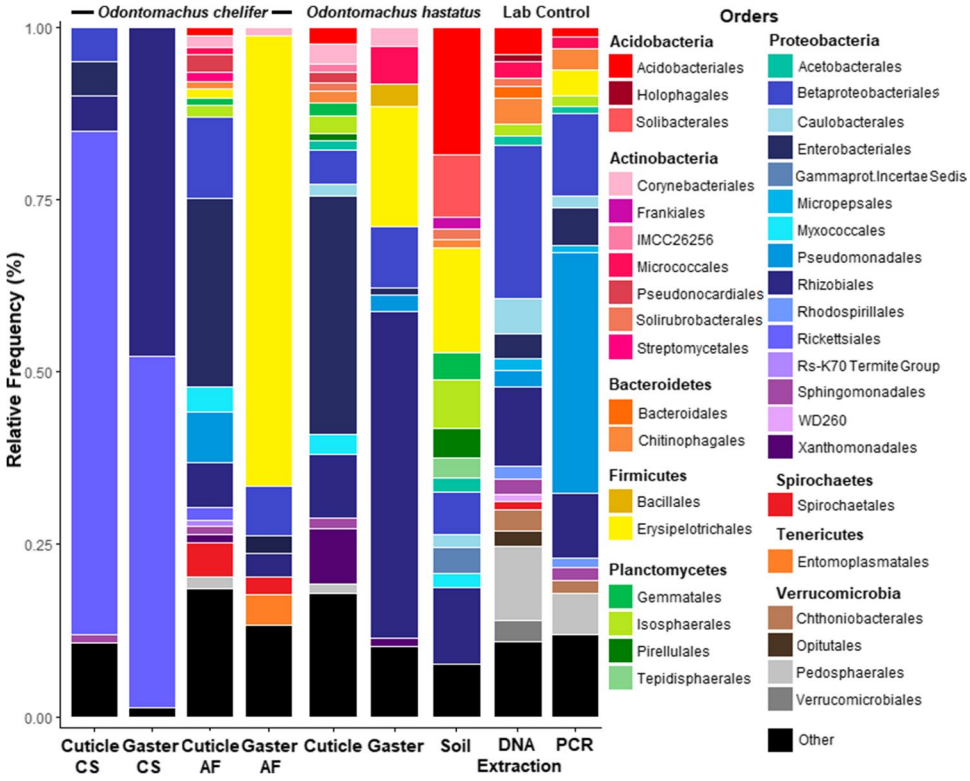

Fig. 2 Relative frequency of ASV orders in the cuticle and gaster bacterial communities of *Odontomachus chelifer* and *O. hastatus* in Cerrado savanna (CS) and Atlantic rainforest (AF), and also from the environmental control (soil with *O. hastatus* nest) and laboratory controls (DNA extraction and PCR). Bacterial orders with less than 1% of relative frequency are grouped under "Others." All samples were rarefied at 1000 sequences per sample. Sample number in each bar: *O. chelifer* CS cuticle = 29; *O. chelifer* CS gaster = 40; *O. chelifer* AF cuticle = 15; *O. chelifer* AF gaster = 39; *O. hastatus* AF cuticle = 14; *O. hastatus* AF gaster = 47; *O. hastatus* Soil Control = 1; DNA extraction control = 1; PCR Control = 2

on samples. Similarity percentage (SIMPER; Bray–Curtis dissimilarity) analyses were performed to investigate which ASVs were the main ones responsible for the differences found in the comparisons described above.

The importance of habitat and host species effects as drivers of the divergence of the bacterial communities seen in each body part was further tested, taking the bacterial communities of *O. chelifer* AF as a reference. For this, Monte Carlo exact permutation tests with 9999 permutations were done. We tested whether the distributions of observed beta dissimilarities between bacterial communities were different from null distributions generated [7]. One-tailed *p* values were used because there was an a priori expectation that cuticle bacterial communities in the same habitat (co-occurring *Odontomachus* species) and gaster bacterial communities in the same species (*O. chelifer* populations) would be more similar to each other (smaller beta diversity values) than those from different habitats or host species [8].

Since *Wolbachia* dominated *O. chelifer* CS samples compared with *O. chelifer* AF and *O. hastatus* (see "Results"), we chose to use mainly presence-absence metrics (Unweighted UniFrac and Jaccard) for the beta diversity comparisons presented [65], unless otherwise stated, to avoid misinterpreting diversity patterns (i.e., the abundance of *Wolbachia* would bias the observed distribution of beta dissimilarities of the *O. chelifer* populations, hampering our Monte Carlo exact permutation analysis). The presence-absence metrics are used to differentiate samples by the amount of unique ASVs in each sample. The difference is that UniFrac also considers the unique evolutionary history of each sample. Thus, unweighted UniFrac is a good complement to Jaccard because it helps to distinguish patterns in the samples not captured by the latter. Abundance metrics, like Bray–Curtis and weighted UniFrac, were avoided because the abundance of *Wolbachia* alone could create false-positive results in the comparison between *O. chelifer* CS and AF. In Online Resource 1, we present analyses without *Wolbachia* ASVs on the cuticle samples, as these bacteria might have been found there because of cross-contamination due to samples' low titers (see "Discussion").

Results

Sequencing of the V4 16S rRNA gene fragment for the 260 ant samples yielded 5,628,350 high-quality sequences encompassing a total of 2979 ASVs. After rarefaction to 1000 reads per sample, the analyses were carried out with 184 samples (*O. chelifer* CS cuticle = 29; *O. chelifer* CS gaster = 40; *O. chelifer* AF cuticle = 15; *O. chelifer* AF gaster = 39; *O. hastatus* cuticle = 14; *O. hastatus* gaster = 47; 70.77% of the samples) and 2738 ASVs (Online Resource 1, Table S1).

Diversity of the Bacterial Communities of the Cuticle and Gaster

In general, the bacterial communities of the cuticle and gaster of the *Odontomachus* ants analyzed were dominated by members of the phyla Proteobacteria, Firmicutes, and Actinobacteria (Fig. 2; Table S2). The cuticular bacterial composition of *O. chelifer* CS consisted mainly of Rickettsiales (73.04%; *Wolbachia* sp.), whereas the gaster bacterial community presented a balance of Rickettsiales (50.94%; *Wolbachia* sp.) and Rhizobiales (47.74%; uncultured *Bartonella* sp.). On the other hand, in the Atlantic rainforest, the cuticular bacterial composition in *O. chelifer* AF and *O. hastatus* consisted mainly of Enterobacteriales (27.47% and 34.62%, respectively; different unidentified Enterobacteriaceae). The gaster community in *O. chelifer* AF contained mostly Erysipelotrichales (65.43%; unclassified Firmicutes 2 and 3), whereas in *O. hastatus*, it consisted mainly of Rhizobiales (47.43%; uncultured Rhizobiaceae and *Candidatus Tokpelaia hoelldoblerii*).

Wolbachia was present in 74.5% of the samples, being found in both species and all colonies. The most common variant (*Wolbachia* ASV #01) represented 99.92% of the *Wolbachia* sequences and is one of 6 *Wolbachia* variants found in total. The others were rare and locally distributed (Online Resource 1, Fig. S4; Table S2).

The alpha diversity for each body part, within and between species, was significantly different (Kruskal–Wallis: Observed Richness: $x^2 = 64.176$, $df = 5$, $p = 1.661e-12$; Shannon: $x^2 = 49.56$, $df = 5$, $p = 1.705e-09$) (Fig. S5). Overall, cuticular bacterial diversity was greater in *O. chelifer* AF compared to the Cerrado in both metrics (Wilcoxon Rank Sum: Observed Richness: $p = 0.006$; Shannon: $p = 0.002$; Table 1). We found no difference, however, between their gaster bacterial communities (Wilcoxon rank sum: observed richness: $p = 0.559$; Shannon: $p = 0.366$; Table 1). Gaster samples in both habitats presented low median ASV richness and Shannon diversity compared to their respective cuticles.

Comparing co-occurring *Odontomachus* species, gaster bacterial alpha diversity was different between species in both metrics (Wilcoxon rank sum: obs. richness: $p = 0.026$; Shannon: $p = 0.003$; Table 1). The median values suggest that gaster diversity is slightly higher in *O. hastatus*; however, the variance found in *O. chelifer* was higher, as was also the average value obtained for richness. Variants of cuticular origin were possibly amplified in gaster samples, thus increasing the richness of the variance found. However, this effect should be consistent throughout the samples. Cuticle-associated bacterial communities, nevertheless, presented no difference between species (Wilcoxon rank sum: observed richness: $p = 0.913$; Shannon: $p = 0.983$; Table 1).

Habitat and Host Species Effects on Bacterial Communities of the Cuticle and Gaster

Cuticular Comparisons

Odontomachus chelifer CS and AF populations differed in their cuticular bacterial communities, as indicated by unweighted UniFrac and Jaccard metrics (see the Unweighted UniFrac PCoA in Fig. 3a; see Table 2 for PERMANOVA details). No difference was found, however, between the two co-occurring *Odontomachus* species in the Atlantic rainforest site for the unweighted (Fig. 3b; Table 2) and weighted UniFrac. Jaccard was significant, though, indicating that bacterial phylogenetic information was important for the similarity between their communities (Table 2). Cuticular comparisons were differentiated by Enterobacteriaceae variants and *Serratia* sp., whereas uncultured *Bartonella* sp. also contributes to the dissimilarity between populations of *O. chelifer* (Table S10, Online Resource 1).

The mean dissimilarity of the cuticular bacterial communities of the *O. chelifer* populations (between-habitat comparison) matched the mean dissimilarity distribution of the communities in co-occurring *Odontomachus* species (between-species comparison), against our a priori expectations. No difference was observed in the distribution for both metrics (Fig. 4a; Monte Carlo exact permutation test—One-tailed: Unweighted UniFrac—$p = 0.1648$ (99% confidence interval of the p value: 0.1553–0.1745); Jaccard—$p = 0.9982$ (0.9968–0.9991)).

Table 1 Bacterial alpha diversity of each body part (cuticle and gaster) of *Odontomachus chelifer* and *O. hastatus* sampled in Cerrado (CS) and Atlantic rainforest (AF); number of samples after rarefaction (*n*)

(a) Body part (*n*)	Observed ASV (median)	Shannon (median)
O. chelifer CS cuticle (29)	46.24 ± 59.41 (22)	1.67 ± 2.014 (0.731)
O. chelifer CS gaster (40)	6 ± 2.313 (6.5)	0.541 ± 0.487 (0.31)
O. chelifer AF cuticle (15)	130.9 ± 95.16 (96)	4.519 ± 2.461 (4.901)
O. chelifer AF gaster (39)	38.36 ± 76.41 (5)	1.736 ± 2.492 (0.064)
O. hastatus AF cuticle (14)	130.4 ± 110.6 (109)	4.247 ± 2.77 (5.659)
O. hastatus AF gaster (47)	21.89 ± 25.66 (7)	2.083 ± 1.665 (1.093)

Mean ± SD are presented for observed ASV richness and Shannon; medians are given between parentheses

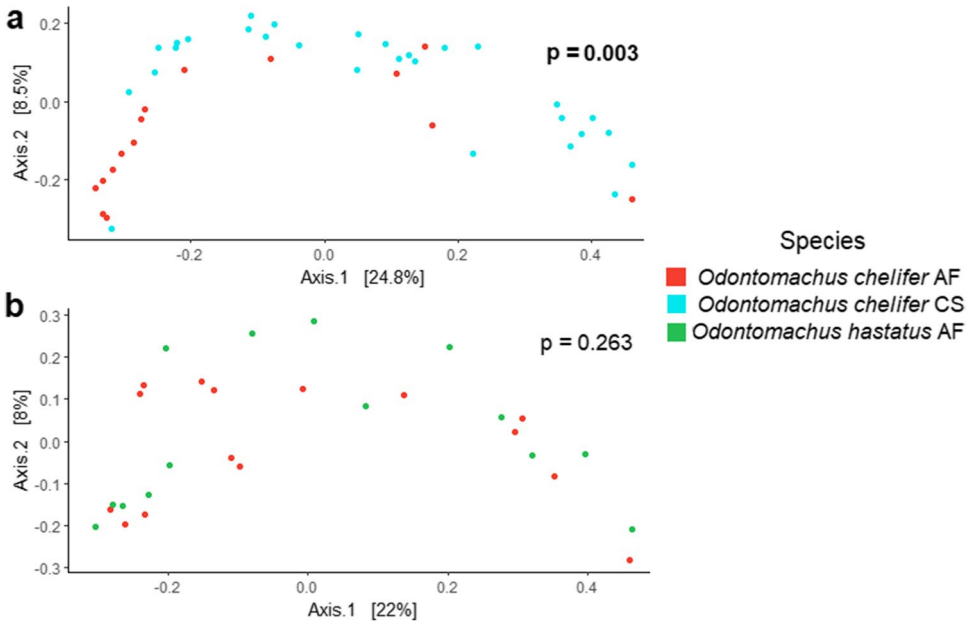

Fig. 3 Variation in bacterial communities from the cuticle in populations of *Odontomachus chelifer* in Cerrado savanna (CS) and Atlantic rainforest (AF), as well as in co-occurring *O. chelifer* and *O. hastatus* in the Atlantic rainforest. Two-dimensional plots of the principal coordinate analysis (unweighted UniFrac) of the cuticular bacterial communities for pairwise comparisons: **a** *O. chelifer* CS x *O. chelifer* AF and **b** *O. chelifer* AF×*O. hastatus* AF. The population of *O. chelifer* in the Atlantic rainforest is the same in both pairwise comparisons and appears in the same color. PERMANOVA *p*-values are shown; values in bold are significant. Cuticular Comparisons - between habitats (a) and between species (b)

Table 2 Permutational analysis of variance (PERMANOVA) values for the comparisons of cuticle/gaster pairs in *Odontomachus chelifer* and *O. hastatus* sampled in Cerrado savanna (CS) and Atlantic rainforest (AF) in southeast Brazil

	Jaccard			Unweighted UniFrac			Weighted UniFrac		
	Pseudo-*F*	Adj. R^2	*p* value	Pseudo-*F*	Adj. R^2	*p* value	Pseudo-*F*	Adj. R^2	*p* value
a. Cuticle									
Odontomachus chelifer CS and AF	1.678	0.038	**0.001**	3.252	0.072	**0.003**	–	–	–
Odontomachus chelifer AF and *O. hastatus* AF	1.256	0.044	**0.025**	1.134	0.040	0.263	0.888	0.032	0.450
b. Gaster									
Odontomachus chelifer CS and AF	7.886	0.093	**0.001**	9.999	0.115	**0.001**	–	–	–
Odontomachus chelifer AF and *O. hastatus* AF	9.234	0.099	**0.001**	5.585	0.062	**0.002**	24.569	0.226	**0.001**

Bacterial community data on Jaccard, unweighted UniFrac, and weighted UniFrac measures of dissimilarity for the comparisons of (a) cuticle and (b) gaster samples. Significant values are in bold ($p < 0.05$)

Gaster Comparisons

Bacterial communities from the gaster of *O. chelifer* differed between the AF and CS populations, as indicated by unweighted UniFrac and Jaccard metrics (Table 2; Fig. 5a), and also between the co-occurring species in the Atlantic rainforest for all metrics (Table 2; Fig. 5b). Three ASVs (*Wolbachia* sp., unclassified Firmicutes 3, and uncultured *Bartonella* sp.) were equally important in characterizing distinct *O. chelifer* populations (Table S10, Online Resource 1). Some of the same or closely related ASVs were also of significant contribution in differentiating communities of co-occurring *Odontomachus* (unclassified Firmicutes 1, 2, and 3; *Candidatus Tokpelaia hoelldoblerii*) (Table S10, Online Resource 1).

There were differences in the beta dissimilarities' distribution of the gaster bacterial communities of *Odontomachus* populations. Bacterial communities of co-occurring *Odontomachus* (between-species comparison) were more dissimilar than in the *O. chelifer* populations (between-habitat comparison) for both metrics, fitting our a priori expectation (Fig. 4b; Monte Carlo exact permutation test—One-tailed: unweighted UniFrac—$p = 1e-04$ (99%

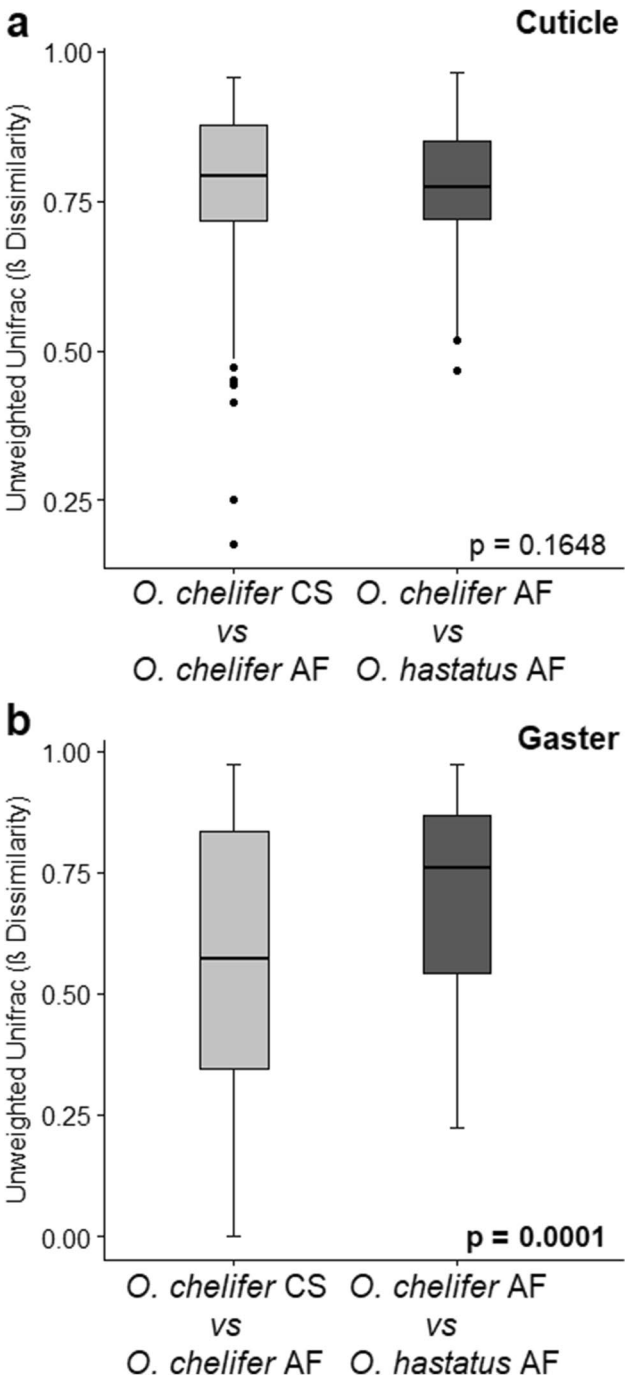

Fig. 4 Distribution of beta dissimilarity values (unweighted UniFrac) for pairwise comparisons between *Odontomachus chelifer* Cerrado savanna (CS) × *O. chelifer* Atlantic rainforest (AF) and between *O. chelifer* AF × *O. hastatus* AF for the **a** cuticular bacterial communities and **b** gaster bacterial communities. Monte Carlo exact permutation test *p* values are shown; values in bold are significant

confidence interval of the *p* value: 0.0000–0.0005); Jaccard—$p = 1e-04$ (0.0000–0.0005)). The main results are summarized in Fig. 6.

Discussion

Our study highlights the importance of host species and habitat effects on the structure of bacterial communities associated with *Odontomachus chelifer* and *O. hastatus*, showing that the outcomes may depend on the body part targeted. Internal communities in the gaster are mainly shaped by host species, as we see in the Atlantic rainforest for the co-occurring species. Our data for *O. chelifer* populations, however, shows that environmental variation also has a role in mediating bacterial community structure in the gaster within the same species in Cerrado vs. Atlantic rainforest. On the other hand, cuticular bacterial communities were highly influenced by habitat, regardless of the ant species. In such cases, the characteristic microbiota in a given habitat is probably acquired horizontally, stressing the importance of the environment's microbial composition for community assembling by the host (even with *Wolbachia* ASVs excluded, results were the same; Online Resource 1, Figs. S6 and S7; Tables S8 and S9).

Community Composition and Diversity

Bacterial communities found in the gaster are predominantly in the orders Erysipelotrichales (*O. chelifer* AF) and Rhizobiales (*O. chelifer* CS and *O. hastatus*), which belong to the phyla Firmicutes and Proteobacteria, respectively, and are commonly associated with the gastrointestinal tract of animals [66, 67], including ponerine ants (*Leptogenys* sp. and *Harpegnathos saltator*; Online Resource 1, Table S2) [16, 18]. The role of Rhizobiales in the nitrogen cycle of ants was first raised for the predatory ant *Harpegnathos saltator* [68], and since then this order has been found in many different subfamilies, especially the ASV *Candidatus Tokpelaia hoelldoblerii*. It is usually linked with nitrogen provision or recycling, depending on the ant diet [16, 39, 69, 70]. Considering their recurrent association with predatory ants in the Ponerinae and Dorylinae subfamilies [12, 18], nitrogen recycling or defense against entomopathogenic microorganisms are expected roles of these bacteria in the *Odontomachus* studied here [38]. In *O. chelifer* and *O. hastatus*, Erysipelotrichales and Rhizobiales ASVs might be important in recycling and providing usable nitrogen to the plants, a key ecosystem function of these ants [30, 33].

The cuticle of the Atlantic rainforest populations had a great abundance of the order Enterobacteriales, a gram-negative order of bacteria commonly found in the environment and in other ants [71]. Environmental acquisition is a probable route for this assembling (Online Resource 1; Table S2). Moreover, by attending to vertebrate feces to collect nutritious material [25, 72], *O. chelifer* may acquire microorganisms from other animals' guts. Recent studies

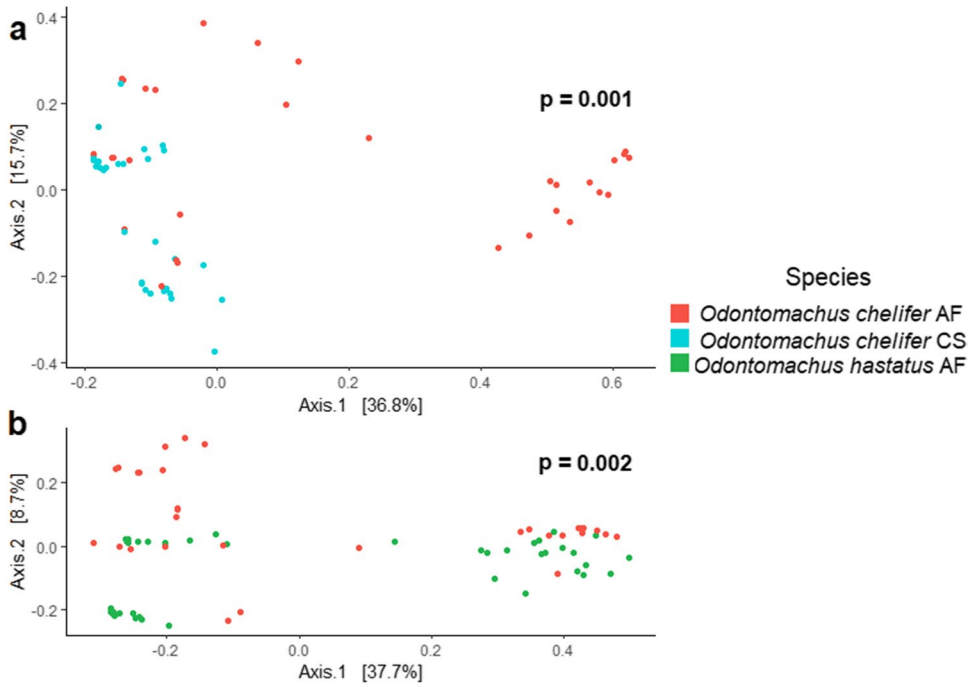

Fig. 5 Variation in bacterial communities from the gaster in populations of *Odontomachus chelifer* in Cerrado savanna (CS) and Atlantic rainforest (AF), as well as in co-occurring *O. chelifer* and *O. hastatus* in the Atlantic rainforest. Two-dimensional plots of the principal coordinate analysis (unweighted UniFrac) of the gaster bacterial communities for pairwise comparisons: **a** *O. chelifer* CS × *O. chelifer* AF and **b** *O. chelifer* AF × *O. hastatus* AF. The population of *O. chelifer* in the Atlantic rainforest is the same in both pairwise comparisons and appears in the same color. PERMANOVA *p* values are shown; values in bold are significant. Gaster Comparisons - between habitats (a) and between species (b)

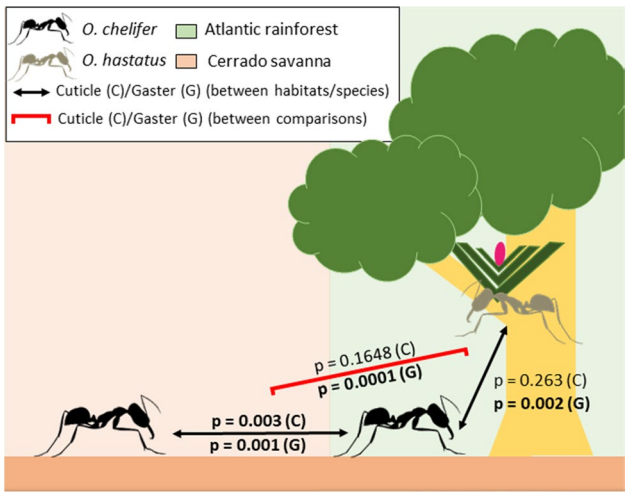

Fig. 6 Diagram of the main findings in our comparisons of the bacterial communities associated with *Odontomachus chelifer* and *O. hastatus* sampled in Cerrado savanna (CS) and Atlantic rainforest (AF) in southeast Brazil. Comparisons of the bacterial communities between habitats and species, within body parts, were performed using PERMANOVA (unweighted UniFrac). Black arrows show the PERMANOVA results for *O. chelifer* CS and *O. chelifer* AF and for *O. chelifer* AF and *O. hastatus* AF (see also Figs. 3 and 5). The red bracket shows the result of the Monte Carlo exact permutation test for the distribution of the mean beta dissimilarities (see also Fig. 4). Upper *p* values refer to comparisons of cuticular bacterial communities; lower *p* values refer to comparisons of gaster bacterial communities; values in bold are significant

show that some ant species have cuticle communities similar to the microbiota found in the nest material ([73], but see [11]). Cuticular bacterial communities are constantly exposed to the environment and subjected to regular interactions with environmental molecules and microorganisms of the ants' surroundings and could be more variable [74].

The bacterial communities of *O. chelifer* in the Cerrado savanna were dominated by *Wolbachia* (Rickettsiales). Polygynous species have greater chances of presenting these heritable bacteria because egg-laying queens come from diverse backgrounds [75]. When dealing with endosymbionts, however, caution is important since their abundance can mask or distort the diversity assessment of the community [76]. Low-abundance samples, like the ones from the cuticle, could easily be cross-contaminated, even though *Wolbachia* is found in many different insect tissues and is present in the late stages of egg development in ants [77, 78]. Nevertheless, our analyses showed that both body parts still carried distinct communities in datasets with and without *Wolbachia* (Online Resource 1, pp 18–23).

All ant populations in this study presented differences between cuticle and gaster bacterial communities. Other studies also report greater diversity in the bacterial community of the head and mesosoma compared to the gaster [76]. Army ants (*Dorylus* sp. and *Aenictus* sp.) present 1 or 2 OTUs (97% sequence similarity cutoff) in their gut, with some rare OTUs there as well [18]. That is similar to the *Odontomachus* gaster communities reported here, where most harbored less than 8 variants considering the entire gaster. A pattern of low diversity was found in these *Odontomachus* and other carnivorous ants, as the diversity components of gaster bacterial communities depend on gut length, presence of specialized structures, diet, presence of the heritable bacterium, or other host-related traits [79].

Gaster bacterial communities were colony-specific for both *O. chelifer* and *O. hastatus.* Colony differences are discussed in detail in Online Resource 1.

The diversity in some of our gaster samples from the Atlantic rainforest may be overestimated, possibly due to the presence of some variants from the diverse cuticular samples during DNA extraction, increasing standard deviations. Although our extraction method mixed to some degree internal and external communities, we preferred to be conservative in filtering body parts, thus preventing the exclusion of real ASVs.

Habitat Effects on the Bacterial Communities of Odontomachus chelifer and O. hastatus

Our data suggest that bacterial communities, internal and external, vary geographically within the same species. Further, the external cuticular communities are more affected by the surrounding environment (habitat and microhabitat) than the gaster internal communities. Although habitat had a stronger effect on gaster communities than expected in *O. chelifer*, our findings agree with the hypothesis that external communities are primarily influenced by the surrounding environment.

We found a significant difference in alpha (richness and Shannon index) and beta diversity (Jaccard and unweighted UniFrac) between the cuticular bacterial communities of the Cerrado savanna and the Atlantic rainforest samples. Spatial distance is an important factor in structuring biotic communities because it is tied to environmental variation and the dispersal abilities of different species [80]. Furthermore, habitat variation between the Cerrado savanna and Atlantic rainforest includes contrasting climatic regimes and markedly distinct vegetation physiognomies [44, 45], both of which should affect the bacterial communities found in these habitats [81]. The denser vegetation structure of the Atlantic rainforest compared to the Cerrado guarantees constant temperature and humidity and a thicker layer of leaf litter throughout the year. This provides structural and resource complexity to this microhabitat [82], sustaining a greater diversity of ASVs on the ground of the Atlantic rainforest. Indeed, leaf litter dynamics are complex and present contrasting patterns in these biomes [83, 84], with the Cerrado savanna presenting variation according to seasonality, which affects nutrient levels and microbial diversity throughout the year [85]. Thus, seasonality differences can mediate the observed disparity in *O. chelifer* cuticle-associated communities, and this should be investigated. The disparity is seen in the cuticular bacterial communities of *O. chelifer* from the Cerrado and the Atlantic rainforests, likely resulting from different sets of environmentally acquired bacteria. Divergence of the associated community occurring in allopatry, or the compositional nature of the data itself, could also play a role here. Studies with ants (*Camponotus* and *Pheidole*) and with salamanders also point out the role of the environment (as different sites or regions) in community structure differentiation [76, 86, 87].

As reported for the cuticular communities, gaster bacterial communities in *O. chelifer* populations also presented significant variation for Jaccard and unweighted UniFrac metrics (but not in alpha diversity, see Table 1). Therefore, habitat type can also interfere with the internal communities, such as differences in habitat structure and climate, as discussed above. Additionally, colony-level differences in age, polygyny, and diet can also affect associated bacterial communities [5]. Indeed, ant colonies did represent a source of variation in this comparison (check Online Resource 1, Tables S4 to S7). Previous studies with *O. chelifer* in the Cerrado showed that its diet depends more on seasonal plant resources (nutritious fleshy fruits and seeds) since litter-dwelling arthropods are less abundant in the savanna's dry season [25, 31, 32]. Dietary seasonal changes can impact the bacterial community structure by changing the nutrient balances and the bacteria associated with the food sources.

Apart from habitat variation, results on the cuticular bacterial communities of co-occurring *O. chelifer and O. hastatus* from the Atlantic rainforest corroborated the importance of the environment as the source of bacteria to the external communities. This was supported by the absence of significant variation in their communities, as well as by the similar richness and Shannon diversity levels in the cuticular communities of both species. Regardless of microhabitat occupation (ground-nesting *O. chelifer* vs. arboreal *O. hastatus*), which defines the pool of bacteria that these species get in contact with [88, 89], the shared environment or possibly similar host selective traits had more importance in structuring their cuticular bacterial communities (as in salamanders) [87].

Host Species Effects on the Internal Bacterial Communities of Odontomachus chelifer and O. hastatus

Internal communities are, generally, in constant selection imposed by the host and its microbiome, experiencing a more stable environment but with stronger filters than the ones experienced by communities on the cuticle [3, 76, 89, 90]. Internal communities should then be species-specific, or at least present less variability within species. Our data support this hypothesis. Indeed, we found species-specificity in the gaster bacterial communities of the two co-occurring *Odontomachus* species and lower beta diversity values in the communities of *O. chelifer* populations.

Due to the location of internal bacterial communities in the ants' bodies, with good conditions to proliferate, different mechanisms to keep the microbiome in a neutral

state (or beneficial to the ant) have been selected [3]; an example is a filter found in the *Cephalotes* (Myrmicinae) proventriculus [90]. Interestingly, although ponerine ants do not have a developed proventriculus as seen in *Cephalotes* [21], a study with *Odontomachus monticola* found differences between the bacterial communities of the infrabuccal pocket (external) and gut sections (internal) [37]. Most of the bacteria found in the gaster of *O. chelifer* and *O. hastatus* presented sequence similarities to bacteria found in other ants too (Online Resource 1, Table S2). This provides compelling evidence that host filtering from the local pool of species, vertical transmission, and possibly host restriction, are taking part in the assembly of these internal communities [3, 5].

Co-occurring *Odontomachus* species had different gaster communities in terms of alpha and beta diversities. This result highlights the importance of host species factors in their structuring [89], as these hosts are found in the same habitat and have similar dietary compositions [25, 26] and similar external bacterial communities as reported here. Despite the differentiation between *O. chelifer* populations regarding their gaster bacterial communities (Jaccard and unweighted UniFrac), they have lower mean values of beta dissimilarity than the co-occurring *Odontomachus* in the Atlantic rainforest, reinforcing the importance of host species on the assembly and structure of bacterial communities. Species-specific traits (e.g., diet, behavior, physiology, and microhabitat) may facilitate the acquisition of similar bacterial communities, reducing interindividual variability within each species [7, 8, 18].

In ants, bacteria are found in the gut and non-gut tissues in the gaster [13]. In some *Cephalotes* species, gaster communities were found to be mainly from the gut, especially because they lacked abundant heritable bacteria (i.e., *Wolbachia*) in non-gut tissues [91]. The route to gut colonization for most bacteria is through diet ingestion or social interactions in the early life of the callow worker [89]. *Cephalotes'* gut bacterial communities are phylogenetically conserved regardless of environmental conditions or location [7]. Differently, *Linepithema humile* (Dolichoderinae) and *Paraponera clavata* (Paraponerinae) have less conserved bacterial communities than the former example, probably harboring commensals [92, 93]. This is the case of the *Odontomachus* species studied here. The gaster harbors phylogenetically diverse bacterial communities (Fig. 2) housed in the gut and non-gut tissues [77], with few conserved variants between colonies or populations within each species. These ASVs are potentially acquired from their social environment or vertically transmitted, highlighting the importance of understanding the multiple acquisition sources and structuring forces.

Conclusions

In conclusion, *Odontomachus chelifer* and *O. hastatus* harbor distinct communities in their cuticle and gaster, which are distinctively affected by habitat and host species, corroborating what has been found in other ant species [76]. All populations presented variable cuticle-associated communities, likely acquired through horizontal transmission, and less differentiated in the co-occurring species. On the other hand, gaster bacterial communities presented greater species-specificity, suggesting that intrinsic behavioral, physiological, and genetic factors of each species may shape internal microbiotas.

Our study reveals the structure of the bacterial communities associated with these common trap-jaw ant species of the Cerrado savanna and Atlantic rainforest, two Neotropical biodiversity hotspots. We hope that our study will stimulate future investigations into the microbiota associated with ants, especially in Neotropical environments where ecological data are lacking for most of the ant fauna [94]. We add knowledge to the natural history of *Odontomachus chelifer* and *O. hastatus* and their ecological roles in their communities, unraveling associated bacteria in distinct body compartments and habitats and indicating possible sources of bacterial community variation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-022-02064-y.

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Author Contribution All authors contributed to the design of the study. F.P.R. and M.U.V.R. collected the samples. F.P.R., M.U.V.R., and M.L.L. carried out the molecular extraction and amplification. M.B. contributed with laboratory reagents. F.P.R., M.U.V.R., and M.L.L. performed the bioinformatics and the statistical analyses. F.P.R., M.U.V.R., M.L.L., M.B., and P.S.O. contributed to the writing of the manuscript.

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Data Availability Voucher specimens of both species and locations were deposited in the Hymenoptera section of the Museum of Zoology at the State University of Campinas (Accession Numbers: ZUEC-HYM-7675 to ZUEC-HYM-7684). DNA sequences were deposited in GenBank (COI: Accession numbers MW587097 to MW587122; Bacterial sequences: SRA Accession numbers SAMN17861685 to SAMN17861972).

Code Availability For custom code, contact the first author by e-mail at feprocha93@gmail.com.

Disclosures

Ethics Approval Not applicable.

Conflict of Interest The authors declare co conflict of interest.

References

1. McFall-Ngai M et al (2013) Animals in a bacterial world, a new imperative for the life sciences. PNAS 110(9):3229–3236. https://doi.org/10.1073/pnas.1218525110
2. Hammer TJ, Sanders JG, Fierer N (2019) Not all animals need a microbiome. FEMS Microbiol Lett 366(10):1–11. https://doi.org/10.1093/femsle/fnz117
3. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S (2017) The evolution of the host microbiome as an ecosystem on a leash. Nature 548(7665):43–51. https://doi.org/10.1038/nature23292
4. Friedrich MW (2011) Microbial communities, structure, and function. In: Reitner J, Thiel V (eds.) Encyclopedia of geobiology. Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-9212-1_144
5. Kohl KD (2020) Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities. Phil Trans R Soc B 375:20190251. https://doi.org/10.1098/rstb.2019.0251
6. Chomicki G, Werner GDA, West SA, Kiers ET (2020) Compartmentalization drives the evolution of symbiotic cooperation. Phil Trans R Soc B 375:20190602. https://doi.org/10.1098/rstb.2019.0602
7. Sanders JG, Powell S, Kronauer DJC, Vasconcelos HL, Frederickson ME, Pierce NE (2014) Stability and phylogenetic correlation in gut microbiota: Lessons from ants and apes. Mol Ecol 23(6):1268–1283. https://doi.org/10.1111/mec.12611
8. Knowles SCL, Eccles RM, Baltrūnaitė L (2019) Species identity dominates over environment in shaping the microbiota of small mammals. Ecol Lett 22(5):826–837. https://doi.org/10.1111/ele.13240
9. Bik EM, Costello EK, Switzer AD, Callahan BJ, Holmes SP, Wells RS, Carlin KP, Jensen ED, Venn-Watson S, Relman DA (2016) Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. Nat Commun 7:10516. https://doi.org/10.1038/ncomms10516
10. Varela BJ, Lesbarrères D, Ibánez R, Green DM (2018) Environmental and host effects on skin bacterial community composition in panamanian frogs. Front Microbiol 9:298. https://doi.org/10.3389/fmicb.2018.00298
11. Birer C, Moreau CS, Tysklind N, Zinger L, Duplais C (2020) Disentangling the assembly mechanisms of ant cuticular bacterial communities of two Amazonian ant species sharing a common arboreal nest. Mol Ecol 29(7):1372–1385. https://doi.org/10.1111/mec.15400
12. Pringle EG (2019) Convergence, constraint and the potential for mutualism between ants and gut microbes. Mol Ecol 28(4):699–702. https://doi.org/10.1111/mec.14998
13. Moreau CS (2020) Symbioses among ants and microbes. Current Opinion in Insect Science 39:1–5. https://doi.org/10.1016/j.cois.2020.01.002
14. Anderson KE et al (2012) Highly similar microbial communities are shared among related and trophically similar ant species. Mol Ecol 21(9):2282–2296. https://doi.org/10.1111/j.1365-294X.2011.05464.x
15. Blüthgen N, Gebauer G, Fiedler K (2003) Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia 137(3):426–435. https://doi.org/10.1007/s00442-003-1347-8
16. Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, Pierce NE (2009) Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. PNAS 106(50):21236–21241. https://doi.org/10.1073/pnas.0907926106
17. Funaro CF, Kronauer DJC, Moreau CS, Goldman-Huertas B, Pierce NE, Russell JA (2011) Army ants harbor a host-specific clade of Entomoplasmatales bacteria. Appl Environ Microbiol 77(1):346–350. https://doi.org/10.1128/AEM.01896-10
18. Łukasik P, Newton JA, Sanders JG, Hu Y, Moreau CS, Kronauer DJC, O'Donnell S, Koga R, Russell JA (2017) The structured diversity of specialized gut symbionts of the New World army ants. Mol Ecol 26(14):3808–3825. https://doi.org/10.1111/mec.14140
19. Sanders JG, Lukasik P, Frederickson ME, Russell JA, Koga R, Knight R, Pierce NE (2017) Dramatic differences in gut bacterial densities correlate with diet and habitat in rainforest ants. Integr Comp Biol 57:705–722. https://doi.org/10.1093/icb/icx088
20. Bolton B (2020) An online catalog of the ants of the world. Retrieved on August 31, 2020, from https://antcat.org
21. Hölldobler B, Wilson EO (1990) The Ants. Harvard University Press, Cambridge
22. Brown WL Jr (1976) Contributions toward a reclassification of the Formicidae. Part VI. Ponerinae, Tribe Ponerini, Subtribe Odontomachiti. Section A. Introduction, subtribal characters. Genus Odontomachus. Studia Entomologica 19(1–4):67–171
23. Oliveira PS, Hölldobler B (1989) Orientation and communication in the Neotropical ant *Odontomachus bauri* Emery (Hymenoptera, Formicidae, Ponerinae). Ethology 83:154–166
24. Rodrigues PAP, Oliveira PS (2014) Visual navigation in the Neotropical ant *Odontomachus hastatus* (Formicidae, Ponerinae), a predominantly nocturnal, canopy-dwelling predator of the Atlantic rainforest. Behav Proc 109:48–57. https://doi.org/10.1016/j.beproc.2014.06.007
25. Raimundo RLG, Freitas AVL, Oliveira PS (2009) Seasonal patterns in activity rhythm and foraging ecology in the neotropical forest-dwelling ant, *Odontomachus chelifer* (Formicidae: Ponerinae). Ann Entomol Soc Am 102(6):1151–1157. https://doi.org/10.1603/008.102.0625
26. Camargo RX, Oliveira PS (2012) Natural history of the neotropical arboreal ant, *Odontomachus hastatus*: Nest sites, foraging schedule, and diet. J Insect Sci 12(48):1–9. https://doi.org/10.1673/031.012.4801
27. Larabee FJ, Suarez AV (2014) The evolution and functional morphology of trap-jaw ants. Myrmecological News 20:25–36
28. Medeiros FNS, Lopes LE, Moutinho PRS, Oliveira PS, Hölldobler B (1992) Functional polygyny, agonistic interactions and reproductive dominance in the neotropical ant *Odontomachus chelifer* (Hymenoptera, Formicidae, Ponerinae). Ethology 91:134–146. https://doi.org/10.1111/j.1439-0310.1992.tb00857.x
29. Oliveira PS, Camargo RX, Fourcassié V (2011) Nesting patterns, ecological correlates of polygyny and social organization in the neotropical arboreal ant *Odontomachus hastatus*

(Formicidae, Ponerinae). Insectes Soc 58(2):207–217. https://doi.org/10.1007/s00040-010-0138-6
30. Passos L, Oliveira PS (2002) Ants affect the distribution and performance of seedlings of *Clusia criuva*, a primarily bird-dispersed rain forest tree. J Ecol 90(3):517–528. https://doi.org/10.1046/j.1365-2745.2002.00687.x
31. Oliveira PS, Christianini AV, Bieber AGD, Pizo MA (2017) Anthropogenic disturbances affect the interactions between ants and fleshy fruits in two neotropical biodiversity hotspots. In: Oliveira PS, Koptur S (eds) Ant-plant interactions: impacts of humans on terrestrial ecosystems. Cambridge University Press, Cambridge, UK, pp 133–156
32. Magalhães VB, Espírito Santo NB, Salles LFP, Soares H, Oliveira PS (2018) Secondary seed dispersal by ants in Neotropical cerrado savanna: Species-specific effects on seeds and seedlings of Siparuna guianensis (Siparunaceae). Ecol Entomol 43(5):665–674. https://doi.org/10.1111/een.12640
33. Gonçalves AZ, Oliveira RS, Oliveira PS, Romero GQ (2016) Species-specific effects of ant inhabitants on bromeliad nutrition. PLoS ONE 11(3):1–12. https://doi.org/10.1371/journal.pone.0152113
34. Gonçalves AZ, Srivastava DS, Oliveira PS, Romero GQ (2017) Effects of predatory ants within and across ecosystems in bromeliad food webs. J Anim Ecol 86(4):790–799. https://doi.org/10.1111/1365-2656.12671
35 De Oliveira TB, Ferro M, Bacci M, De Souza DJ, Fontana R, Delabie JHC, Silva A (2016) Bacterial communities in the midgut of Ponerine ants (Hymenoptera: Formicidae: Ponerinae). Sociobiology 63(1):637–644. https://doi.org/10.13102/sociobiology.v63i1.882
36. Kautz S, Rubin BER, Russell JA, Moreau CS (2013) Surveying the microbiome of ants: Comparing 454 pyrosequencing with traditional methods to uncover bacterial diversity. Appl Environ Microbiol 79(2):525–534. https://doi.org/10.1128/AEM.03107-12
37. Zheng Z, Hu X, Xu Y, Wei C, He H (2021) Bacterial composition and diversity of the digestive tract of *Odontomachus monticola* Emery and *Ectomomyrmex javanus* Mayr. Insects 12(2):176. https://doi.org/10.3390/insects12020176
38. Kaltenpoth M, Engl T (2014) Defensive microbial symbionts in Hymenoptera. Funct Ecol 28(2):315–327. https://doi.org/10.1111/1365-2435.12089
39. Hu Y et al (2018) Herbivorous turtle ants obtain essential nutrients from a conserved nitrogen-recycling gut microbiome. Nat Commun 9:964. https://doi.org/10.1038/s41467-018-03357-y
40. Salem H et al (2020) Symbiont digestive range reflects host plant breadth in herbivorous beetles. Curr Biol 30(15):2875–2886. https://doi.org/10.1016/j.cub.2020.05.043
41. Archie EA, Tung J (2015) Social behavior and the microbiome. Curr Opin Behav Sci 6:28–34. https://doi.org/10.1016/j.cobeha.2015.07.008
42. Dosmann A, Bahet N (2016) Gordon DM (2016) Experimental modulation of external microbiome affects nestmate recognition in harvester ants (*Pogonomyrmex barbatus*). PeerJ 1:1–8. https://doi.org/10.7717/peerj.1566
43. Birer C, Tysklind N, Zinger L, Duplais C (2017) Comparative analysis of DNA extraction methods to study the body surface microbiota of insects: A case study with ant cuticular bacteria. Mol Ecol Resour 17(6):e34–e45. https://doi.org/10.1111/1755-0998.12688
44. Oliveira-Filho AT, Ratter JA (2002) Vegetation physiognomies and the woody flora of the Cerrado biome. In: Oliveira PS, Marquis RJ (eds) The Cerrados of Brazil: ecology and natural history of a neotropical savanna. Columbia University Press, New York, pp 91–120
45. Joly CA et al (1999) Evolution of the Brazilian phytogeography classification systems: implications for biodiversity conservation. Ciência e Cultura 51(5/6):331–348
46. San Martin-Gajardo I, Morellato LP (2003) Fenologia de Rubiaceae de sub-bosque em floresta Atlântica no sudeste do Brasil. Bra J Bot 26(3):299–309. https://doi.org/10.1590/S0100-84042003000300003
47. Binetruy F, Dupraz M, Buysse M, Duron O (2019) Surface sterilization methods impact measures of internal microbial diversity in ticks. Parasit Vectors 12:268. https://doi.org/10.1186/s13071-019-3517-5
48. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79(17):5112–5120. https://doi.org/10.1128/AEM.01043-13
49. Caporaso JG et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2018.8
50. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3(5):294–299
51. Bolyen E et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37(8):852–857. https://doi.org/10.1038/s41587-019-0209-9
52. Rognes T, Flouri T, Nichols B, Quince C (2016) Mahé F (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 10:1–22. https://doi.org/10.7717/peerj.2584
53 Amir A et al (2017) Deblur rapidly resolves single-nucleotide community sequence patterns. MSystems 2(2):1–7. https://doi.org/10.1186/gb-2012-13-9-r79
54. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35(21):7188–7196. https://doi.org/10.1093/nar/gkm864
55. Katoh K, Misawa K, Kuma K-I, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30(14):3059–3066. https://doi.org/10.1093/nar/gkf436
56. Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS ONE 5(3):e9490. https://doi.org/10.1371/journal.pone.0009490
57. Bokulich NA et al (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 10(1):57–59. https://doi.org/10.1038/nmeth.2276
58. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6(1):1–14. https://doi.org/10.1186/s40168-018-0605-2
59. Salter SJ et al (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 12(1):1–12. https://doi.org/10.1186/s12915-014-0087-z
60. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, … Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome, 5(1), 1–18https://doi.org/10.1186/s40168-017-0237-y
61. McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4):e61217. https://doi.org/10.1371/journal.pone.0061217
62. Oksanen J et al. (2019) Vegan: Community Ecology Package, R Package Version 2.5–6. Retrieved from https://cran.r-project.org/package=vegan

63. Wickham H (2016) ggplot2: Elegant graphics for data analysis. Springer-Verlag New York, New York, pp 213. Retrieved from https://ggplot2.tidyverse.org
64. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26(1):32–46. https://doi.org/10.1046/j.1442-9993.2001.01070.x
65. Lozupone C, Knight R (2005) UniFrac: A new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71(12):8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005
66. Bletz MC et al (2016) Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. Nat Commun 7:1–12. https://doi.org/10.1038/ncomms13699
67. Delsuc F, Metcalf JL, Wegener Parfrey L, Song SJ, González A, Knight R (2014) Convergence of gut microbiomes in myrmecophagous mammals. Mol Ecol 23(6):1301–1317. https://doi.org/10.1111/mec.12501
68. Neuvonen MM et al (2016) The genome of Rhizobiales bacteria in predatory ants reveals urease gene functions but no genes for nitrogen fixation. Sci Rep 6(November):1–11. https://doi.org/10.1038/srep39197
69. Ramalho MO, Duplais C, Orivel J, Dejean A, Gibson JC, Suarez AV, Moreau CS (2020) Development but not diet alters microbial communities in the Neotropical arboreal trap jaw ant *Daceton armigerum*: an exploratory study. Sci Rep 10:7350. https://doi.org/10.1038/s41598-020-64393-7
70. Rubin BER, Kautz S, Wray BD, Moreau CS (2018) Dietary specialization in mutualistic acacia-ants affects relative abundance but not identity of host-associated bacteria. Mol Ecol 28(4):900–916. https://doi.org/10.1111/mec.14834
71. Bitar MR, Pinto VD, Moreira LM, Ribeiro SP (2021) Gram-negative bacteria associated with a dominant arboreal ant species outcompete phyllosphere-associated bacteria species in a tropical canopy. Oecologia 195:959–970. https://doi.org/10.1007/s00442-021-04878-y
72. Pizo MA, Guimarães PR, Oliveira PS (2005) Seed removal by ants from faeces produced by different vertebrate species. Écoscience 12(1):136–140
73. Lucas J, Bill B, Stevenson B, Kaspari M (2017) The microbiome of the ant-built home: The microbial communities of a tropical arboreal ant and its nest. Ecosphere 8(2):e03619. https://doi.org/10.1002/ecs2.1639
74. Douglas AE (2015) Multiorganismal insects: Diversity and function of resident microorganisms. Annu Rev Entomol 60:17–34. https://doi.org/10.1146/annurev-ento-010814-020822
75. Russell JA (2012) The ants (Hymenoptera: Formicidae) are unique and enigmatic hosts of prevalent *Wolbachia* (Alphaproteobacteria) symbionts. Myrmecological News 16(January, 2011):7–23
76. Ramalho MO, Moreau CS, Bueno OC (2019) The potential role of environment in structuring the microbiota of *Camponotus* across parts of the body. Adv Entomol 7(3):47–70. https://doi.org/10.4236/ae.2019.73005
77. Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, O'Neill SL (1999) *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochem Mol Biol 29(2):153–160. https://doi.org/10.1016/S0965-1748(98)00119-2
78. Ramalho MO, Vieira AS, Pereira MC, Moreau CS, Bueno OC (2019) Transovarian transmission of *Blochmannia* and *Wolbachia* endosymbionts in the Neotropical weaver ant *Camponotus textor* (Hymenoptera, Formicidae). Curr Microbiol 75:855–873. https://doi.org/10.1007/s00284-018-1459-3
79. Engel P, Moran NA (2013) The gut microbiota of insects - diversity in structure and function. FEMS Microbiol Rev 37(5):699–735. https://doi.org/10.1111/1574-6976.12025
80. Hanson CA, Fuhrman JA, Horner-Devine C, Martiny JBH (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. Nat Rev Microbiol 10:497–506. https://doi.org/10.1038/nrmicro2795
81. Ramette A, Tiedje JM (2007) Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. PNAS 104(8):2761–2766. https://doi.org/10.1073/pnas.0610671104
82. Giesselman UC, Martins KG, Brändle M, Schädler M, Marques R, Brandl R (2010) Diversity and ecosystem functioning: Litter decomposition dynamics in the Atlantic Rainforest. Appl Soil Ecol 46:283–290. https://doi.org/10.1016/j.apsoil.2010.07.006
83. Purahong W et al (2016) Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Mol Ecol 25:4059–4074. https://doi.org/10.1111/mec.13739
84. Tonin AM et al (2017) Plant litter dynamics in the forest-stream interface: precipitation is a major control across tropical biomes. Sci Rep 7:10799. https://doi.org/10.1038/s41598-017-10576-8
85. Valenti MW, Cianciaruso MV, Batalha MA (2008) Seasonality of litterfall and leaf decomposition in a cerrado site. Braz J Biol 68(3):459–465. https://doi.org/10.1590/S1519-69842008000300002
86. Martins C, Moreau CS (2020) Influence of host phylogeny, geographical location and seed harvesting diet on the bacterial community of globally distributed *Pheidole* ants. PeerJ 8:e8492. https://doi.org/10.7717/peerj.8492
87. Wolz CRM, Yarwood SA, Grant EHC, Fleischer RC, Lips KR (2017) Effects of host species and environment on the skin microbiome of Plethodontid salamanders. J Anim Ecol 87(2):341–353. https://doi.org/10.1111/1365-2656.12726
88. Paoletti MG, Taylor RAJ, Stinner BR, Stinner DH, Benzing DH (1991) Diversity of soil fauna in the canopy and forest floor of a venezuelan cloud forest. Journal of Tropical Ecology 7(3): 373–383. http://www.jstor.org/stable/2559636
89. Moran NA, Ochman H, Hammer TJ (2019) Evolutionary and ecological consequences of gut microbial communities. Annu Rev Ecol Evol Syst 50:451–475. https://doi.org/10.1146/annurev-ecolsys-110617-062453
90. Lanan MC, Rodrigues PAP, Agellon A, Jansma P, Wheeler DE (2016) A bacterial filter protects and structures the gut microbiome of an insect. ISME J 10(8):1866–1876. https://doi.org/10.1038/ismej.2015.264
91. Flynn PJ, D'Amelio CL, Sanders JG, Russell JA, Moreau CS (2021) Localization of bacterial communities within gut compartments across *Cephalotes* turtle ants. Appl Environ Microbiol 87:e02803-e2820. https://doi.org/10.1128/AEM02803-20
92. Hu Y et al (2017) By their own devices: invasive Argentine ants have shifted diet without clear aid from symbiotic microbiota. Mol Ecol 26:1608–1630. https://doi.org/10.1111/mec.13991
93. Moreau CS, Rubin BER (2017) Diversity and persistence of the gut microbiome of the giant neotropical bullet ant. Integr Comp Biol 57(4):682–689. https://doi.org/10.1093/icb/icx037
94. Wilson EO (2017) Biodiversity research requires more boots on the ground. Nature Ecology and Evolution 1:1590–1591