



# Extinction of anciently associated gut bacterial symbionts in a clade of stingless bees

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## Abstract

Animal-microbe symbioses are often stable for millions of years. An example is the clade consisting of social corbiculate bees—honeybees, bumblebees, and stingless bees—in which a shared ancestor acquired specialized gut bacteria that subsequently diversified with hosts. This model may be incomplete, however, as few microbiomes have been characterized for stingless bees, which are diverse and ecologically dominant pollinators in the tropics. We surveyed gut microbiomes of Brazilian stingless bees, focusing on the genus *Melipona*, for which we sampled multiple species and biomes. Strikingly, *Melipona* lacks *Snodgrassella* and *Gilliamella*, bacterial symbionts ubiquitous in other social corbiculate bees. Instead, *Melipona* species harbor more environmental bacteria and bee-specific *Starmerella* yeasts. Loss of *Snodgrassella* and *Gilliamella* may stem from ecological shifts in *Melipona* or the acquisition of new symbionts as functional replacements. Our findings demonstrate the value of broadly sampling microbiome biodiversity and show that even ancient symbioses can be lost.

## Introduction

Microbial symbionts have contributed to the diversification of many animal lineages. Host-microbe associations are often stable [1, 2]; however, ancient symbionts can be lost, and sometimes replaced by novel symbionts [3, 4]. These transitions may be driven by erosion of symbiont functionality [5] or shifts in host ecology [6]. Although symbiont turnover has been documented for intracellular symbionts, its prevalence among gut symbionts is unclear. Here we report the dissolution of a long-standing symbiosis between social bees and specialized gut bacteria.

The eusocial corbiculate bees (Apidae) contain distinctive gut bacterial symbionts that are restricted to bees and that have co-diversified with their hosts [7–9]. In *Apis mellifera* (honeybees) and *Bombus impatiens* and *B. terrestris* (bumblebees), *Snodgrassella*, *Gilliamella* and *Lactobacillus* have been observed to contribute to host health [10–13]. However, gut microbiomes of stingless bees (Meliponini), the most diverse clade of highly social bees and major pollinators in the tropics [14], are understudied, and seem to vary more than microbiomes of other social bees [8, 15–17]. Here, we characterize gut bacteria and fungi associated with the genus *Melipona* from multiple Brazilian biomes.

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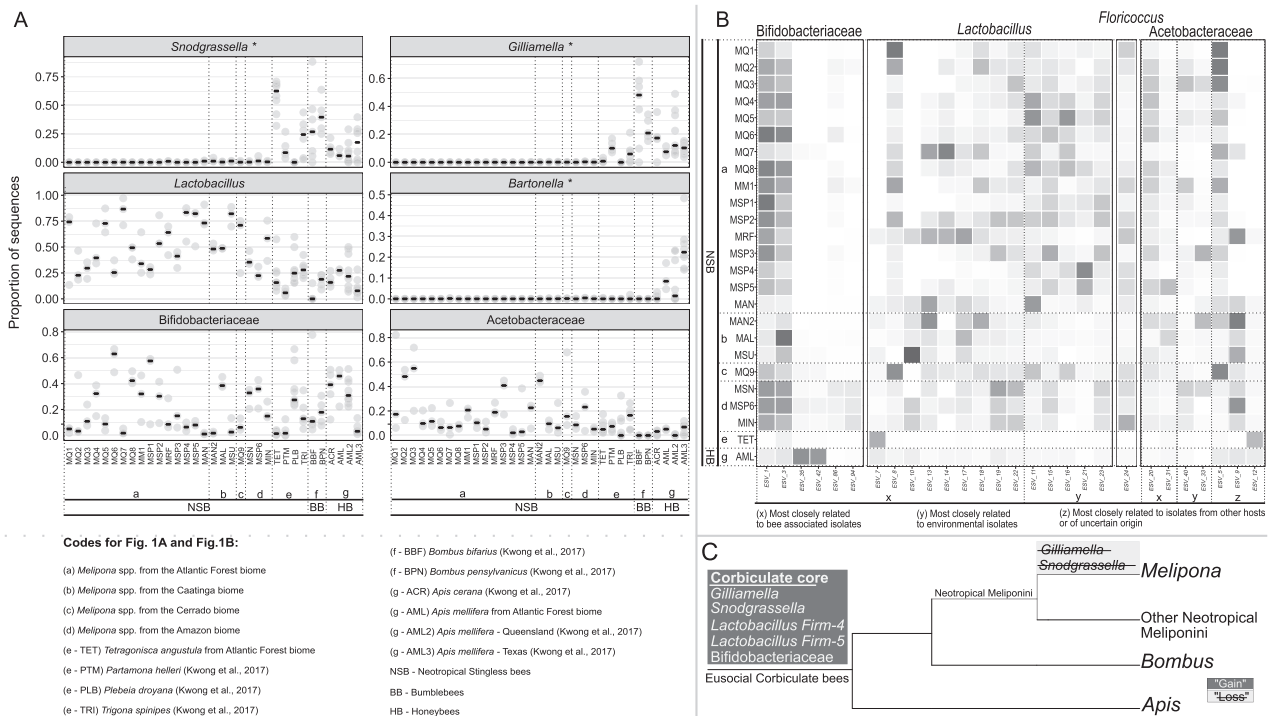
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## Methods and results

We sampled 3 colonies from each of 23 *Melipona* populations representing 4 Brazilian biomes (SISBIO/ICMBio authorization #69500-1). Three colonies of *Apis mellifera* and 8 of the stingless bee *Tetragonisca angustula* were also collected from one site. A pool of guts from five individuals/colony had regions of bacterial 16S rRNA and fungal ITS1 genes sequenced to characterize gut microbial community composition (Supplementary Table S1), and we analyzed these data (SRA accession #PRJNA678404) together with previously



**Fig. 1 Gut bacterial community composition and a model of symbiont gain and loss in eusocial corbiculate bees.** **A** Distribution of six bee-specific gut bacterial symbionts across Neotropical stingless bees (NSB), bumblebees (BB) and honeybees (HB). Bee populations are on the x-axis (more detail in Supplementary Table S1). Previously published data [8] are included for comparison. The y-axis shows the relative abundance of each bacterial group as the proportion of sequences. Circles represent replicate bee samples, and black bars represent the median proportion for each bee population. Taxa with significantly higher relative abundances in *Apis mellifera* than *Melipona* are denoted with an asterisk. **B** Heatmap comprising the most

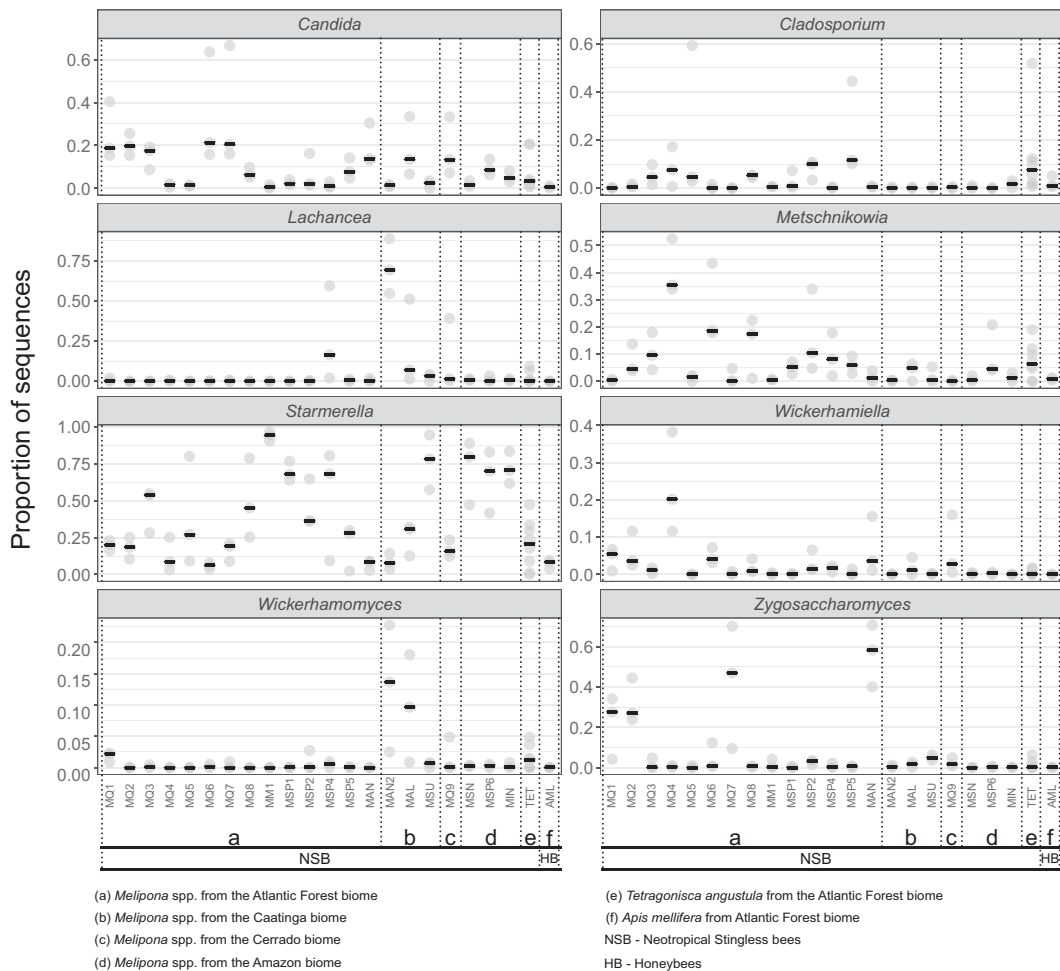
abundant ESVs belonging to *Melipona* core bacterial taxa (i.e., taxa present in all samples of all *Melipona* populations). The x-axis represents ESVs labeled by the source from which close relatives were isolated (Supplementary Figs. S1–S4), and the y-axis represents each bee population sequenced. Darker squares correspond to higher log-transformed mean relative abundances for a given ESV in each bee population. **C** Phylogeny of eusocial corbiculate bees (based on [20]) showing the gains [7–9] and our hypothesized losses of core symbionts. A color version of this figure is available in the Supplementary Material.

published data [8] (see Supplementary Methods). Our results show that *Melipona* lack *Snodgrassella* and *Gilliamella*, core gut symbionts of honeybees, bumblebees, and some other stingless bee clades (Fig. 1A). As expected, honeybee-specific *Bartonella apis* was also absent. These taxa were significantly lower in relative abundance in *Melipona* as compared with *A. mellifera* (Kruskal–Wallis  $p < 0.05$ ) (Fig. 1A). The most abundant and consistent bacteria in *Melipona* belong to *Lactobacillus*, Bifidobacteriaceae, Acetobacteraceae, and *Floricoccus* (Fig. 1B). For the core *Melipona* bacterial taxa (i.e., taxa containing exact sequence variants [ESVs] in all *Melipona* samples), we constructed phylogenies using the most abundant ESVs and related sequences from GenBank to differentiate widespread environmental taxa from specialized associates of social bees. *Melipona*-associated *Lactobacillus* and Acetobacteraceae include putatively environmental ESVs as well as bee-specific ESVs (Fig. 1B, Supplementary Figs. S1, S2, and Table S2). Bifidobacteriaceae ESVs in *Melipona* are closely related to bee isolates (Fig. 1B, Supplementary Fig. S3 and Table S2). In contrast, the core

*Floricoccus* ESV is closely related to environmental isolates (Fig. 1B, Supplementary Fig. S4 and Table S2). The fungal gut microbiome of *Melipona* is mainly composed of *Starmerella*, *Lachancea*, *Zygosaccharomyces*, and a few other yeasts (Fig. 2). Of these, *Starmerella* are noteworthy in that they form bee/bee-hive-specific clades (Supplementary Fig. S5), were present in all *Melipona* samples and comprised a higher proportion of gut microbiomes in *Melipona* than in other social bee species (Kruskal–Wallis  $p < 0.05$ ) (Fig. 2). Apparent symbiont loss from *Melipona* (Fig. 1C) is not due to incomplete sampling, as rarefaction curves confirm sufficient characterization of bacterial communities (Supplementary Table S3 and Fig. S6).

## Discussion

Although present in our *A. mellifera* and *T. angustula* samples, *Snodgrassella* and *Gilliamella* were extremely rare or absent in samples of 23 *Melipona* populations from four



**Fig. 2** Distribution of eight dominant fungi across *Melipona*, *Tetragonisca angustula* and *Apis mellifera*. The x-axis represents sequenced bee populations (Supplementary Table S1). The y-axis shows the relative abundance of each fungal genus as the proportion of

sequences for each bee population. Circles represent replicate bee samples, and black bars represent the median proportion for each bee population. A color version of this figure is available in the Supplementary Material.

biomes in Brazil (Fig. 1A). When present, the low abundance likely reflects artefacts such as cross-contamination. These findings, together with previous work [15, 17], suggest that *Snodgrassella* and *Gilliamella* have been lost entirely, or almost entirely, from the sampled species of *Melipona*. This shift is unlikely to have occurred in the common ancestor of stingless bees, because several other Neotropical stingless bees, including *T. angustula* and other species occurring in Brazil, do maintain these associations [8] (Fig. 1A and C). Furthermore, such losses are not geographically restricted as they were observed in *Melipona* from four Brazilian biomes (Fig. 1A) and from Central America [15]. While a few other stingless bee genera have been reported to lack *Snodgrassella* and *Gilliamella*, based on limited sampling [8, 15, 16], our finding of consistent absence from *Melipona* provides the strongest evidence of symbiont loss in a major social bee clade. Since *Snodgrassella* and *Gilliamella* can contribute to the health of honeybees and bumblebees [10–13], it is possible that

*Melipona* has undergone ecological shifts that release them from dependence on symbiont-based nutrition or defense. Alternatively, either persisting members of the ancestral microbiome, or newly acquired symbionts, may compensate for their absence. *Melipona* retains bee-specialized strains of *Lactobacillus* (Supplementary Fig. S1), Acetobacteraceae (Supplementary Fig. S2), and Bifidobacteriaceae (Supplementary Fig. S3), which in theory could have gained new functions, such as metabolic and protective capabilities. *Melipona* has also gained new microbial associations, such as environmental strains of *Lactobacillus*, Acetobacteraceae, and *Floriccoccus*. These bacteria are widespread in *Melipona* but only occasionally found in *T. angustula* and *A. mellifera* (Fig. 1B). Although phylogenetic evidence suggests they are acquired from the environment, the prevalence of these taxa points toward a stable, and possibly functional association with *Melipona*. Furthermore, certain fungi are also associated with *Melipona* (Fig. 2). *Starmerella*, the most abundant and widespread of

these, has been found in stingless bee adults, pollen provisions and honey [18], suggesting specialization for the bee niche (Supplementary Fig. S5 and Table S2). In other insects, fungi have occasionally replaced degenerating bacterial symbionts [3], and dependence on a fungal symbiont is documented for another stingless bee, *Scaptotrigona depilis* [19]. Experimental work on *Melipona* and other stingless bees could give insights into the functional basis and dynamic nature of bee microbiomes.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

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