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Home Sweet Home: Yeasts Living in Substrates Associated with *Melipona scutellaris* in the Brazilian Atlantic Forest

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Abstract

The interaction between stingless bees and fungi has garnered recent interest due to potential mutual benefits. In Brazil, *Melipona scutellaris* stands out ecologically and economically. We investigated its microbiota, focusing on yeast species in pot-honey, pot-pollen, and nest surfaces. Samples from the Atlantic Forest (Pernambuco state, Brazil) were analyzed through morphological and physiological methods and D1/D2 LSU rDNA sequence analysis. We identified 20 yeast species, comprising 15 ascomycetes and five basidiomycetes, representing 11 and five genera, respectively. All yeast species, except *Blastobotrys meliponae*, were reported for the first time in association with *M. scutellaris*. Honey exhibited the highest species richness. In this study, the species *Blastobotrys meliponae*, *Meyerozyma caribbica*, *Wickerhamomyces anomalus*, and *Moniliella carnis* were exclusively isolated from honey. The understanding of the ecological association between stingless bees and yeasts, as revealed in our research, can significantly aid in insect conservation programs. We present the first report of yeasts associated with *M. scutellaris* substrates living in the Brazilian Atlantic Forest.

Introduction

The Brazilian Atlantic Forest is one of the world's most diverse tropical forests, housing numerous species of plants, animals, and microorganisms (Myers et al., 2000; Marques et al., 2021; Barbosa et al., 2022). Stingless bees play a crucial role in this ecosystem by providing essential pollination ecological services to plant species (Lorenzon & Morgado, 2008). Among the native stingless bee species, *Melipona scutellaris* Latreille, 1811 stands out as a remarkable species for the environmental ecology and economy of the Atlantic Forest regions in the Northeast of Brazil (Alves et al., 2012).

Stingless bees have been reported to live in association with several fungal species, which are vital for the bees' survival. Conversely, the bees facilitate the dispersion of fungal propagules (Cano et al., 1994; Gilliam, 1997; Rosa et al., 2003; Pagnocca et al., 2008; Menezes et al., 2015; Barbosa et al. 2016, 2017, 2018). Yeasts appear to play an essential role in bee nutrition and protection against harmful organisms that could jeopardize the colony's survival (Gilliam et al., 1990; Gilliam, 1997). Over the years, studies have explored the diversity of yeasts associated with various hosts/substrates, including bee species (Pimentel et al., 2005; Ganter, 2006; Meireles et al., 2022). Efforts to increase knowledge about the richness and diversity of yeasts associated with different substrates of stingless bee's nests in tropical environments are crucial due to the bees' role in the ecosystem.

In Brazil, few studies have been conducted to investigate the mycobiota associated with stingless bees. For instance, Camargo et al. (1992) demonstrated *Ptilotrigona lurida* employs yeasts of the genus *Candida* to dehydrate stored pollen, a crucial process preventing deterioration and protecting against

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organisms like phorid (*Phoridae*), ensuring colony well-being. Rosa et al. (2003) explored yeast communities linked to stingless bees and o demonstrated a strong connection with *Tetragonisca angustula* and *Frieseomelitta varia* with the yeast *Starmerella meliponinorum* and *Melipona quadrifasciata* with *Candida apicola*. Meireles et al. (2022) reported several yeasts from the nests of the bees *Melipona interrupta* and *Cephalotrigona femorata*, native to Central Amazonia. Beux et al. (2022) found *S. meliponinorum*, *Candida magnoliae*, and *Zygosaccharomyces bailli* in samples of honey and pollen pots from *T. angustula*. Most recently, de Paula et al. (2023) examined brood cell samples from 19 Brazilian stingless bee species, isolating the osmophilic yeast *Zygosaccharomyces* from eight bee species. The yeast exhibited unique characteristics, forming pseudohyphae and accumulating ergosterol in lipid droplets, similar to the pattern previously observed for *Scaptotrigona depilis*.

In the Northeast region of Brazil, Barbosa et al. (2016) examined the richness of yeasts associated with honey obtained from stingless bees living in the Brazilian tropical dry forest (Caatinga). They observed 12 yeast species primarily belonging to the genera *Candida*, *Debaryomyces*, *Dekkera*, *Pichia*, and *Kloeckera*, most of which were classified under the phylum Ascomycota. As part of a project initiated in 2014 to study the mycobiota associated with pot-honey, pot-pollen, and the interior (surface) of *Melipona scutellaris* bee nests in Atlantic Forest areas of Northeastern Brazil, Barbosa et al. (2017) examined the occurrence of *Monascus* species. They described three new species isolated from bee-related substrates. Barbosa et al. (2018) identified twenty-one *Penicillium* species and six *Talaromyces* species, including seven new species. The species *Blastobotrys meliponae* (Crous et al., 2016), *Castanediella meliponae* (Hyde et al., 2019), and *Penicillium nordestinense* (Barbosa et al., 2022) were also new species described during this project. The published results thus far suggest that the mycobiota associated with native bees is an area that deserves attention, as it holds the potential for taxonomic novelties, ecological interactions, and biotechnological developments.

The association between fungi and bees in the tropical rainforest of Brazil remains a knowledge gap that we are actively working on to comprehend their relationship and contribution to the Atlantic Forest biome in the Northeast region of Brazil (Barbosa et al., 2017, 2018). Building upon this existing knowledge and aiming to enhance our understanding of this relationship, this paper focuses on the diversity of yeasts associated with pot-honey, pot-pollen, and the inner surface of *Melipona scutellaris* nests in the Atlantic Rainforest of Brazil.

Material and Methods

Study area

The study was conducted in the Parque Estadual Dois Irmãos, Recife, Brazil. This Park, a state conservation unit, is one fragment of the Atlantic Forest located in a densely populated area of the northwest of the city of Recife, Pernambuco state (8°7′30″S, 34°52′30″W and 8°40 3600S, 34°570 3400W). The Park has an area of about 1,161 ha and is one of the most representative fragments of the Atlantic Forest in Recife. This Atlantic Forest conservation unit also harbors the municipal zoo (SEMAS et al., 2021). The Parque Estadual Dois Irmãos administration authorized the collections and under the supervision of the Associação de Apicultores e Meliponicultores de Pernambuco (APIME).

Sampling of substrates

Samples of pot-honey, pot-pollen, and surface of storage pot were collected from nests of *Melipona scutellaris* (CEPANN 7714) in the Parque Estadual Dois Irmãos, Pernambuco state, Brazil, between January and June 2014 (totaling six collections). In each collection, three nests were used, and in each four samples of each substrate were collected (pot-honey, pot-pollen, surface of storage pot). See Figure 1.

Yeast Isolation

The obtention of yeasts (isolation) from each substrate was performed as Barbosa et al. (2017) described. Briefly,

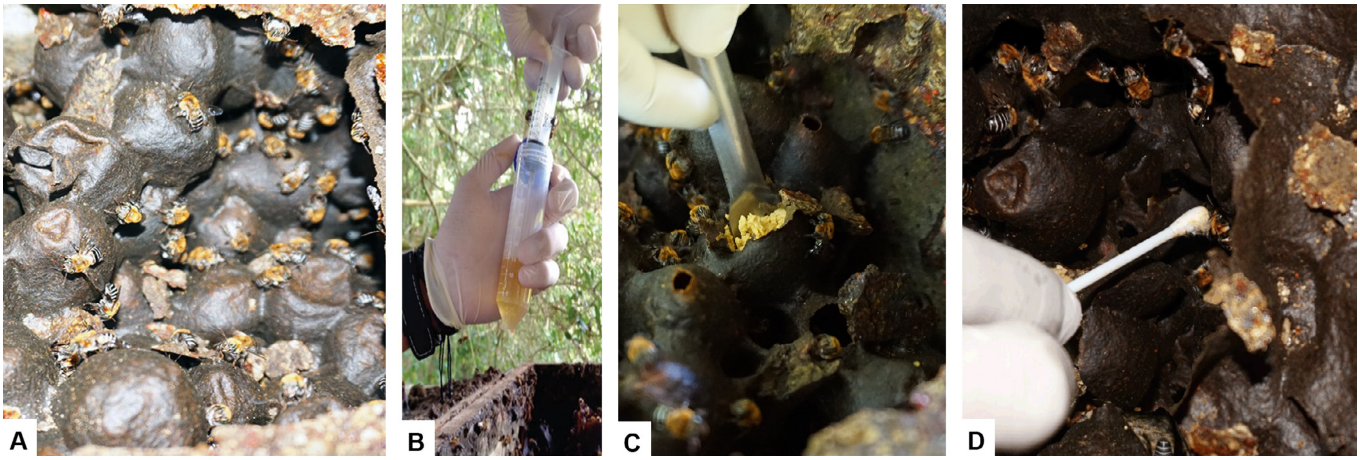


Fig 1. Sampling of diverse substrates from a *Melipona scutellaris* nest. (A) Pot-honey, (B) Honey samples collected by suction with syringes, (C) Pot-pollen, and pollen samples collected, (D) collection in surface of pots with swab.

in each collection, for the pot-honey and pot-pollen samples, 25 g (obtained in each collection) of each was suspended in 225 mL peptone water (0.1 %), and decimal dilutions were made up to 10^{-3}. For the collection of the samples' inner nest surface, a sterile cotton swab (1 for each nest, in each collection) was used to sample the surface of the pollen and honey pots, and brood cells. The swab was soaked in 3 mL peptone water (0.1 %) and vortexed vigorously. Subsequently, 1 mL of each dilution was spread on the surface of Petri dishes with dichloran 18% glycerol agar (DG18) (Himedia) and malt extract agar (Himedia) supplemented with chloramphenicol (0,05g/L). The same nests were sampled throughout the study. All culture media were prepared according to their respective manufacturer.

Yeast identification

Initially, yeast isolates were classified into morphological groups based on macroscopic and microscopic characteristics, including cell type, colony color, colony shape, margins, and elevation (Lacaz, 2000; Kurtzman et al. 2011a). Additionally, physiological properties were examined using standard yeast identification methods outlined in Kurtzman et al. (2011a). Representative isolates with uncertain morphophysiological identification underwent molecular identification. According to the manufacturer's protocol, DNA extraction was performed from 7-day-old cultures grown on malt extract agar (MEA, Oxoid) using the UltraClean Microbial DNA kit (MoBio Laboratories, Solana Beach, CA, USA). To amplify a portion of the nuclear ribosomal large subunit (LSU) rDNA, a polymerase chain reaction (PCR) was carried out with the NL1 and NL4 primers (O'Donnell, 1993). The PCR products were then purified using the PureLink - PCR Purification Kit (Invitrogen®) and sequenced bidirectionally with the same primers. Assembled contigs were generated by aligning forward and reverse sequences with the same protocols used in de Barbosa et al. (2017). We used the BLASTn tool at NCBI to assess the similarity of strains' D1/D2 domain within yeast species. Strains were categorized using a 99% LSU region identity threshold, following Kurtzman et al. (2011b), which indicates the same species. Newly generated sequences were deposited in GenBank and assigned accession numbers (Table 1).

Table 1. List of identified yeast taxa isolated from pot-honey, pot-pollen, and storage pot surface inside the nest of *Melipona scutellaris* living in a Brazilian Atlantic Forest, Pernambuco state, Brazil.

Yeasts	Pot-honey	Pot-pollen	Storage pot surface	LSU GenBank
Ascomycota				
Aureobasidium pullulans	A	P	P	OR568054,OR568055
Blastobotrys chiropterorum	P	P	P	OR568056,OR568057
Blastobotrys terrestris	P	P	P	OR568058,OR568059,OR568060, OR568061
Blastobotrys meliponae	P	A	A	KR779215,KR779216,KR779217
Candida cf. *pseudointermedia*	P	P	P	OR568062,OR568063
Debaryomyces cf. *nepalensis*	P	P	P	OR568064, OR568065
Kodamaea ohmeri	P	A	P	OR568066,OR568067,OR568068
Metschnikowia koreensis	P	P	P	OR568069,OR568070,OR568071
Meyerozyma cf. *guilliermondii*	P	P	P	OR568072,OR568073
Meyerozyma caribbica	P	A	A	OR568958,OR568959,OR568960
Priceomyces melissophilus	P	P	P	OR568074,OR568075,OR568076
Starmerella bombicola	P	P	P	OR568077,OR568078,OR568079
Starmerella etchellsii	P	P	P	OR568961, OR568962,OR568963,OR568964
Torulaspora delbrueckii	P	P	P	OR568080,OR568081,OR568082
Wickerhamomyces anomalus	P	A	A	OR568083,OR568084,OR568085
Basidiomycota				
Pseudozyma hubeiensis	P	P	P	OR568086, OR568087, OR568088, OR568089
Cutaneotrichosporon jirovecii	A	P	P	OR568090, OR568091
Moniliella carnis	P	A	A	OR568092, OR568093, OR568094
Moniliella suaveolens	P	P	A	OR568095, OR568096, OR568097
Sakaguchia sp .	P	P	A	OR568098
Richness	18	15	14	

P = fungal taxon present (observed). A = fungal taxon absent (not observed).

Ecological and statistical analyses

Yeast abundance was assessed through the total number of colonies (colony-forming units, CFU), and the data underwent ANOVA using the F test with a significance level of 1%. Significant results were tested using post hoc Tukey's test at a significance level of 5%. The following ecological analyses were performed by selecting only species identified, at least, at the genus level based on presence and abundance data. The species richness (S) (Shannon & Weaver, 1971) for the yeasts collected across the substrates (bee pollen, nest surfaces, and honey) was estimated. All analyses were performed using the software R v. 4.1.0 (R Core Team, 2021), along with packages "vegan", "agricolae", "lsmeans", "emmeans", and "ggplot2" (Graves et al., 2015; Lenth, 2016; Oksanen et al., 2020; Mendiburu, 2021). A Venn diagram was constructed using the online tool "InteractiVenn" (Wickham, 2016) to visually represent identified species' logical distribution and relationships among observed samples.

Results

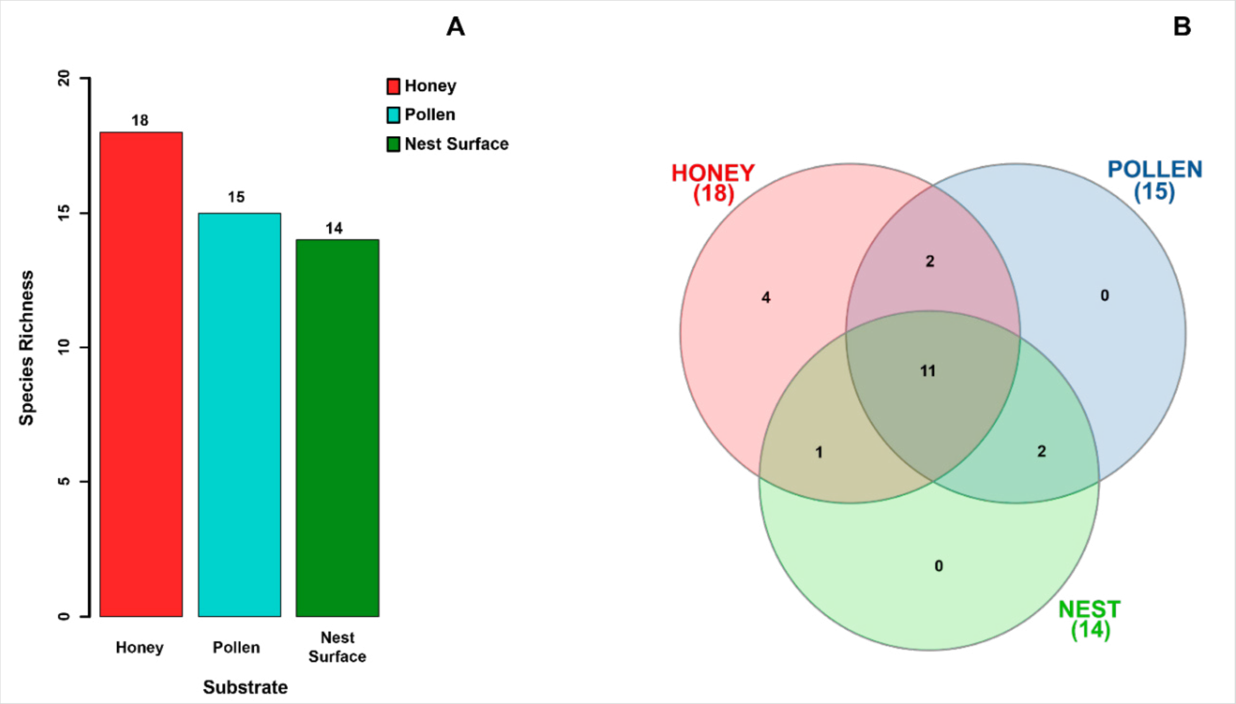
Yeasts identification

The fungal isolates were identified through DNA sequence analysis of 65 representatives selected based on morphological and physiological features. Using sequences of the D1/D2 domain of the LSU rDNA region, we identified 20 yeast species representing 15 genera, of which 11 were ascomycetes and four were basidiomycetes (Table 1). The ascomycetous genera were represented by 15 taxa, while basidiomycetes by five taxa. All yeast species are being reported for the first time in association with *Melipona scutellaris*, except for *Blastobotrys meliponae,* a new species previously described (Crous et al., 2016).

Species distribution and diversity

A total of 1,375 colony-forming unit (CFU) yeast isolates were obtained from substrates related to *M. scutellaris*. Among these, the fungal abundance varied, with 709 CFU/mL detected in pot-honey, 232 CFU/g in pot-pollen, and 434 CFU on the nest surface (Table 1). Regarding species richness, honey exhibited the highest diversity (18 species) compared to pot-pollen (15 species) and surfaces of storage pots (14 species). Notably, *B. meliponae*, *M. caribbica,* and *W. anomalus*, along with *M. carnis*, were exclusively isolated from honey. Furthermore, specific species were solely found in combination with pot-honey and pot-pollen (*Moniliella suaveolens* and *Sakaguchia* sp.), honey and the nest surface (*Kodamaea ohmeri*), and pot-pollen and the nest surface (*Aureobasidium pullulans* and *Cutaneotrichosporon jirovecii*).

The Venn diagram (Figure 2) illustrates yeast species across the analyzed samples, demonstrating that eleven species are commonly shared among the three substrates. These species include *Blastobotrys chiropterorum*, *Blastobotrys terrestris*, *Candida* cf. *pseudointermedia*, *Debaryomyces* cf. *nepalensis*, *Metschnikowia koreensis*, *Meyerozyma* cf. *guilliermondii*, *Priceomyces melissophilus*, *Starmerella bombicola*, *Starmerella etchellsii*, *Torulaspora delbrueckii*, and *Pseudozyma hubeiensis*.



Fige 2. (A) Bar chart based on species richness and (B) Venn diagram showing yeast species among the studied samples. Nest surface and pollen had no exclusive species.

Discussion

In Brazil, few mycological studies have explored fungi in substrates related to bees (Barbosa et al., 2016, 2017, 2018; Souza et al., 2018; Meireles et al., 2022; Santos et al., 2023). Most focus on the microbiological quality of bee-associated substrates like honey (e.g., Souza et al., 2009; Gois et al., 2010). A significant knowledge gap exists regarding the association between tropical bees and fungal species, especially yeast diversity. Blackwell (2011) emphasized the largely unexplored diversity of fungi associated with insects, highlighting our limited understanding of this aspect. This observation still holds today, as the fungal diversity associated with insects remains largely undocumented, making insects a cryptic biome in terms of their fungal associations.

The yeast community structure differed among the studied substrates (pot-honey, pot-pollen, and nest surface), likely due to substrate chemical composition and interactions with other microorganisms, especially bacteria, which could act as limiting factors (Olaitan et al., 2007). Notably, only a few yeast species were common across substrates, suggesting that their locations might not be their active growth sites but the survival points during transitions between habitats where they thrive (Starmer & Lachance, 2011). It is essential to mention that some yeast colonies didn’t remain viable on artificial media during isolation and identification. Additionally, some colonies were overtaken by fast-growing filamentous fungi, obstructing identification. Therefore, future research should consider using culture-independent methods to understand yeast diversity better and surpass limitations linked to culture-based approaches.

The prevalent yeasts isolated in this study belong to the Ascomycota phylum. *Meyerozyma guilliermondii* (= *Pichia guilliermondii*), one of the most abundant yeasts in our study, is widespread and not tied to a specific host insect (Zacchi & Vaughan-Martini, 2002). Another instance is *Wickerhamomyces anomalus* (= *Hansenula anomala* and *Pichia anomala*). This species is a mutualistic symbiont in various insect species, including dipteran disease vectors. It has been discovered in numerous samples of foraging bees and is also prevalent in many substrates, including flowers, leaves, food, and aquatic habitats (Cappelli et al., 2021). The genus *Debarromyces* is closely related to *Pichia* and is also linked to insects (e.g., *D. robertsiae* = *Pichia robertsiae*), appearing to be associated with carpenter bees (van der Walt, 1959; Batra et al., 1973). The species *D. hansenii* has been isolated from adults and garbage pellets of *Melipona quadrifasciara* and propolis of *Tetragonista angustula* (Rosa et al., 2003).

Metschnikowia species have been documented in association with insects from orders Diptera, Coleoptera, and Hymenoptera (Lachance et al., 2003; Lachance, 2011) and fruits, flowers, insects, and nectar (Nguyen et al., 2006; Vega et al., 2012; Kaewwichian et al., 2012). A study conducted by Good et al. (2014) discovered that microbial growth in nectar can significantly affect the foraging choices of honeybees (*Apis mellifera*). Bees tend to avoid nectar containing bacterial species, whereas the presence of yeast species like *M. reukaufii* does not alter their feeding preferences. Furthermore, research into the co-speciation hypothesis between the yeast *M. gruessii* and bumblebees (*Bombus*) revealed a remarkable morphological adaptation in this yeast species, allowing it to adhere to the trichomes located in the buccal region of bees (Brysch-Herzberg, 2004). While our study did not identify these specific taxa, it underscores the intriguing ecological dynamics within this genus.

Previous investigations on native bee species in Brazil consistently identified *Starmerella* species as the most frequently isolated yeasts (Detry et al., 2020). This genus encompasses about 50 species, most associated with bees and their floral habitats (Santos et al., 2018; Gonçalves et al., 2020). The *Starmerella* clade includes two teleomorphic species and several asexual *Candida* species, found in honey, provisional pollen, nectar, and waste deposits in hives and bee nests (Golonka & Vilgalys, 2013). For example, *S. bombicola*, isolated in our study, is linked to *Apis florea* and other insect pollinators (Rosa & Lachance, 1998; Rosa et al., 2003; Brysch-Herzberg, 2004; de Paula et al., 2021). *Starmerella etchellsii*, associated mainly with stingless bees, has been isolated from nest debris and adult bees of *Tetragonisca angustula* (Rosa et al., 2003). The precise nature of the potential symbiotic relationship is not fully understood, but it’s believed to impact pollen maturation (Starmer & Lachance, 2011). *Starmerella* species display nutritional specialization, utilizing a limited range of carbohydrate and nitrogen sources (Rosa & Lachance, 1998). Furthermore, numerous species produce significant amounts of sophorolipids, which might function as extracellular storage materials, aiding adaptation to high osmotic pressure due to elevated sugar concentrations (Hommel et al., 1994). The repeated isolation of numerous *Starmerella* species from bees strongly suggests their metabolic activity holds importance in this ecosystem and within host organisms (Detry et al., 2020).

Another commonly associated genus with bees, *Blastobotrys*, was the yeast most frequently isolated in this study, encompassing species previously categorized under *Arxula* and *Sympodiomyces*. Certain species were reported from insects, such as *B. attinorum* (= *Sympodiomyces attinorum*) from ants of the *Attini* tribe (Carreiro et al., 2004). In 2016, we introduced *B. meliponae* as a new species isolated from the bee *M. scutellaris* honey collected in the Brazilian Atlantic Forest (Crous et al., 2016). Given our study’s substantial number of isolates, this genus might interact ecologically with this bee, warranting investigation into their ecological relationship to understand their ecological role better. Our study also encountered a significant cluster of yeasts belonging to the genus *Moniliella*. Species within this genus possess xerophilic and fermentative abilities, commonly associated with honey, pollen, tobacco, human sputum, and spoiled foods (de Hoog et al., 1998). For instance, *M. pollinis* was first isolated from fresh pollen within a honeycomb (Dooms et al., 1971), *M. byzovi* from flowers (Thanh et al., 2013), and *M. mellis* from honey (Rao & De Hoog, 1975).

Moreover, we identified *Kodamaea ohmeri* in our study. This yeast species can be found in various substrates and is frequently carried by larvae of the small hive beetle (Amos et al., 2018). Previous research has indicated that when *K. ohmeri* grows on pollen collected (bee-bread) by bees, it emits volatile compounds similar to honey bee alarm pheromones (Torto et al., 2007; Benda et al., 2008). These same compounds attract small beetles to the colony, rendering the hive uninhabitable for bees (Torto et al., 2007b; Benda et al., 2008). It is noteworthy that during our study, no hive damage was observed.

Other researchers have also identified *Candida*, *Wickerhamomyces*, *Priceomyces*, and *Torulaspora* yeasts when studying various insects (Rosa et al., 2003; Fleet, 2011). *Candida* species are commonly found in insect substrates (Fleet, 2011; Santos et al., 2020), including Brazilian stingless bees (Rosa et al., 2003; Barbosa et al., 2016; Costa Neto & Morais, 2020). Barbosa et al. (2016) reported many *Candida* isolates among their yeast findings in a study of honey s amples from stingless bees living Brazilian Caatinga forest. Our study also noted a high occurrence of *C. pseudointermedia* in honey samples. Isolates from the *C. intermedia* clade are commonly associated with insects (Nguyen et al., 2007; Nakase et al., 2011), although this yeast hadn't been previously reported in honey samples from stingless bees. Another noteworthy observation was the presence of *Wickerhamomyces anomalus* in honey samples. Various *Wickerhamomyces* species have been associated with stingless bees, including *W. anomalus* and *W. pijperi* (Costa Neto & Morais, 2020) and *W. sydowiorum* in Brazil (Echeverrigaray et al., 2021). Species from the *Priceomyces* genus are also commonly linked with insects (Kurtzman et al., 2011a; Crous et al., 2016). For example, *P. melissophilus* (= *Pichia melissophila*) was initially identified in honey samples from South Africa (van der Walt, van der Klift, 1972). Our study obtained similar outcomes, reporting *P. melissophilus* in honey samples. *Torulaspora delbrueckii* was another species observed in our study. These yeasts are often associated with stingless bees (Costa et al., 2020) and honey samples (Barry et al., 2018; Echeverrigaray et al., 2021). The frequency of these isolates indicates that these yeasts are widespread among insects, primarily because they are found in food sources like nectar and pollen for these insects (Pimentel et al., 2005; Canto et al., 2017; Santos et al., 2020).

Our study obtained few basidiomycetous yeasts in honey. This observation might stem from the fact that basidiomycetes yeasts generally struggle to tolerate high sugar concentrations compared to ascomycetous yeasts (Buzzini et al., 2018). For instance, Brysch-Hetzberg (2004) noted the prevalence of ascomycetous yeasts among seven *Bombus* bee species and floral resources visited by bees. In our investigation, the distribution of yeast species across substrates (honey, pollen, and surface) implies that their presence could be occasional rather than consistent settlers. The relatively limited number of yeast species identified, particularly within the Basidiomycota, might indicate restricted diversity or reflect challenges in cultivating many of these species outside their nest environments.

While a few studies, including next-generation sequencing of fungal bee microbiota, have been conducted (Cornman et al., 2012; Yun et al., 2018), a knowledge gap remains in understanding molds and yeasts in bees substrates and nests, as well as their significance in the ecosystem. This study revealed that specific yeast genera were present in pot-pollen and pot-honey. Stingless bees prefer fermented pollen as food, but the specific role of yeasts in the metabolic conversion of nutrients in pollen and honey remains unresolved (Vollet-Neto et al., 2016). The mutual benefits of the fungi-insect association have piqued continuous interest, implying a connection involving cellular protection and dispersion (Douglas & Smith, 1989; Nicoletti & Becchimanzi, 2022). Other potential yeast functions include detoxification of harmful substances, defense against biotic stresses, chemical communication, and nutritional contributions, as yeasts are rich in B vitamins (B3 and B5), proteins, metals, amino acids, and other nutrients (Gibson & Hunter, 2010; Witzgall et al., 2012; Engel & Moran, 2013).

Although initial studies exist, the microbiota linked to microenvironments within nests of stingless bees living in the Atlantic Forest remains incompletely understood (Barbosa et al., 2017, 2018). Information about yeasts' involvement in food fermentation of *Melipona scutellaris* and their impact on immune health is lacking. The architectural design of the stingless bee's nests, featuring storage pots combined with elevated humidity and temperature, establishes an optimal setting for a natural bioreactor, creating a favorable environment for the growth of yeasts and other microorganisms with potential biotechnological applications in the future (Barbosa, personal observation).

The distinct yeast communities observed across the three studied substrates emphasize the importance of conducting bee-associated surveys to uncover yeast species diversity linked to various hosts and substrates within the Atlantic Forest in Brazil. Similar studies are urgent and crucial for comprehending the broader significance of the ecology of Brazilian forests and bees, thereby contributing to local and global yeast diversity estimations (Boekhout et al., 2022). Given the global decline of bee populations, it is essential to understand the potential impact of yeasts on their well-being and lifestyles, especially within key species like those found in tropical rainforests. This knowledge can provide insights into whether and how yeasts play a role in supporting these essential pollinators and contribute to the overall health of these ecosystems.

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Authors' Contributions

RNB: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing-original Draft, Writing-review & Editing, Visualization.
JDPB: Data Curation, Writing-original Draft, Writing-review.
JCM: Data Curation, Writing-original Draft, Writing-review.
JEFS: Investigation, Data Curation, Writing-original Draft, Writing-review & Editing, Visualization
IOJ: Data Curation, Writing-original Draft, Writing-review.
CMSM: Funding acquisition, Supervision, Writing-original Draft, Writing-review.
NTO: Project administration, Supervision, Writing-original Draft, Writing-review.

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