

Chapter 11

Microorganisms Associated with Stingless Bees

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11.1 Introduction

Evidence for the great biodiversity associated with stingless bees is obtained from the variety of materials and structures used to build their nests. Inside the nest, there are different shapes and arrangements of brood cells and food storage containers. Wax secreted by stingless bees is mixed with plant resins to produce cerumen (Wille and Michener 1973; Michener 1974; Roubik 1983). Honey and pollen are stored in separate cerumen pots (Fig. 11.1). The size and shape of these pots vary among bee species. Stored nectar or ripened honey is found in the extremes of the nest cavity (for storage during heavy flowering periods), while pollen and some honey surround the brood area (Roubik 2006).

Diverse ethnomedicinal properties attributed to stingless bee honeys are known in Brazil, Ecuador, Guatemala, Mexico, and Venezuela (Vit et al. 2004; Mendes and Antonini 2008; Guerrini et al. 2009), where pot-honey is worth up to 20 times more than *Apis mellifera* honey (Nogueira-Neto 1997; Vit et al. 1998).

Most of the studies of the microorganisms associated with stingless bees were carried out with the objective of describing the bacterial and fungal communities associated with these bees. However, data on the functional relationship between

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Fig. 11.1 Honey and pollen of *Melipona quinquefasciata* stored in separate cerumen pots. Photo: P.S. São Thiago Calaça

microorganisms and stingless bees are scarce. Although honey has some distinct properties that inhibit the growth of microorganisms, such as high sugar concentrations and high acidity (Snowdon and Cliver 1996), microbial fermentation has been suspected to contribute to the transformation of pollen into bee bread and in the formation of the honey itself. Microorganisms may also have a role in honey maturation and in the biochemical modification of stored pot pollen. After its collection by bees from flowers, the pollen stored inside meliponine nests becomes biochemically distinct due to fermentation processes, but it is not clear if yeasts or bacteria (or both) are responsible for these processes (Ganter 2006).

The association of microorganisms with honey, pollen, immature, and adult bees is indicative of a functional relationship with these insects. In this chapter, we will discuss the presence of different species of bacteria, molds, and yeasts associated with stingless bees and the possibility of the existence of a symbiotic relationship between these organisms.

11.2 Bees and Microbes

Insects engage in a vast array of symbiotic relationships with a wide diversity of microorganisms, in which some of them benefit the host nutritionally and provide protection from natural enemies (Klepzig et al. 2009). Yeasts, for example, are a food source for insects and are known to be the main source of sterols, vitamins, and protein for adult and larval stages of *Drosophila* (Morais et al. 1995b). The number of symbionts of the ground-dwelling ants and termites is large compared to that of social wasps and bees (Wilson 1971; Kistner 1982). According to Peruquetti (2000), the highly social stingless bees (Apidae, Meliponini) seem to be an exception to this rule. Their nests have many guests, including mites, moths, cockroaches, flies, beetles,

fungi, and bacteria, some of which are obligate symbionts (Wasmann 1904; Salt 1929; Nogueira-Neto 1970; Machado 1971; Flechtmann and Camargo 1974; Aponte 1996; Kerr et al. 1996).

Insect species are important vectors of microorganisms, including bacteria, fungi, and protozoans (Starmer and Lachance 2011; Redak et al. 2004; Purcell 1982). For example, the distribution and habitat specificity of yeasts depend primarily on the insect vectors but are also dependent on the substrate composition and the presence of inhibitory compounds (Morais and Rosa 2000; Morais et al. 1995a; Starmer et al. 1976).

Various studies have aimed to characterize the microbial community associated with bees (Gilliam et al. 1984; Gilliam 1997; Inglis et al. 1993; Rosa et al. 1999, 2003; Teixeira et al. 2003). The microbiota of the European honey bee (*Apis mellifera*) has been isolated and identified (Gilliam 1997; Gilliam and Morton 1978; Piccini et al. 2004; Rada et al. 1997). These microbes are believed to help chemical conversion in the intestinal tract, preservation of pollen stored in comb cells, and production of antimycotic substances against the chalkbrood pathogen (Gilliam 1997).

Most of the bacteria isolated from brood combs and hive floors of the honey bee belong to the genera *Bacillus* and *Corynebacterium* (Piccini et al. 2004). Studies on the microbiota of the alfalfa leafcutting bee showed a dominance of fungi (e.g., *Aspergillus niger*, *Penicillium* sp., and *Saccharomyces* sp.) and bacteria (e.g., *Bacillus circulans*, *B. mycoides*, *Enterobacter agglomerans*, and *Pseudomonas* sp.) (Goerzen 1991). Other spore-forming bacteria belonging to the genus *Bacillus* were found to be prevalent in larval populations of two solitary bees (*Centris pallida* and *Anthophora* sp.) (Gilliam et al. 1984, 1990a).

Bacteria of the genus *Lactobacillus* were identified in *A. mellifera* and *A. mellifera scutellata* (Mohr and Tabbe 2006; Jeyaprakash et al. 2003). Recently, a novel bacterial flora composed of lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* was found in the stomach of *A. mellifera* (Olofsson and Vásquez 2008). In contrast, Evans and Armstrong (2006) failed to find *Lactobacillus* species in *A. mellifera*, suggesting that the gut microbial population is not constant even within the same species. Yoshiyama and Kimura (2009) did not find *Lactobacillus* species in the gut of *A. cerana japonica*, but they detected the following gut bacterial groups that had not been found in other *Apis* species: *Staphylococcus saprophyticus* (Firmicutes), *Kocuria* sp., *Tsukamurella tyrosinosolvens*, *Microbacterium* sp. (Actinobacteria), *Sphingomonas melonis*, *Mesorhizobium* sp. (Alphaproteobacteria), *Janthinobacterium* sp. (Betaproteobacteria), *Escherichia coli*, *Pseudomonas* sp., *Providencia alcalifaciens*, *Erwinia tasmaniensis*, and *Moraxella* sp. (Gammaproteobacteria). Honey bees visit flowers of many types, which vary geographically and seasonally. Furthermore, honey bees of different species tend to visit flowers of a particular species. Thus, Yoshiyama and Kimura (2009) suggest that variation of a characteristic gut bacterial flora in *Apis* species is likely to be related to variation in the food source, and this may be also true for other plant pollinators such as meliponines.

The bacteria *Streptomyces* sp. have also frequently been found in pollen, provisions, and alimentary canals of alfalfa leafcutter bees (*Megachile rotundata*), and these bacteria are considered to be part of the resident microbiota of the bee

(Inglis et al. 1993). *Streptomyces fradiae* was isolated from the hive materials of *A. florea*, and *S. drozdrwiczii*, *S. albidoflavus*, and *S. badius* were isolated from *A. cerana* in Thailand.

According to Promnuan et al. (2009), *Streptomyces* species show a symbiotic relationship with some insects. A unique association between a new *Streptomyces* species and the European beewolf (*Philanthus triangulum*), a solitary hunting wasp, was reported. The beewolf females harbor the *Streptomyces* bacteria in specialized antennal glands and apply them to the brood cell prior to oviposition. The bacteria are taken up by the larva and are also found on the walls of the cocoon. Bioassays indicated that the streptomycetes protect the cocoon from fungal infestation and significantly enhance the survival probability of the larva, possibly by producing antibiotics (Kaltenpoth et al. 2005).

Rosa et al. (1999) found that a killer toxin-producing *Mucor* species was a dominant fungus, together with the yeast *C. batistae*, in nearly 100 nests of the solitary bee, *Diadasina distincta*. This fungus may play a role in pollen maturation because it presents proteolytic and pectinolytic ability that could be combined with the yeast fermentative and lipolytic function for pollen transformation (Rosa et al. 1999).

Inglis et al. (1993) showed that *Candida bombicola* (*Starmerella bombicola*) is frequently found in nectar, pollen, and provisions of the solitary bee *Megachile rotundata*. Rosa et al. (1999) isolated *Candida batistae* from the solitary bees *D. distincta* and *Ptilothrix plumata* in Brazil, and the authors suggested a possible mutualistic interaction between this yeast species and the bees. Pimentel et al. (2005) described two new species of yeasts, *Candida riocensis* and *Candida cellae*, associated with two solitary bees, *Megachile* sp. and *Centris tarsata*, in the Atlantic rain forest of Brazil.

At this time, the *Starmerella* clade contains more than 40 yeast species, most of which were isolated from bees (Table 11.1). This clade is defined as a single branch in the Ascomycetes that present the common ecological traits of the association with insects and ephemeral flowers. Species belonging to this clade, such as *C. magnoliae*, *C. batistae*, *S. bombicola*, and *S. meliponinorum*, are thought to be involved in a mutualistic relationship with bees (Gilliam 1979a; Inglis et al. 1993; Rosa et al. 1999). In addition to the two *Starmerella* species, *Candida bombi* is common in European bumble bees (Brysch-Heberg 2004). *Candida davenportii*, *C. apicola*, *C. bombi*, *C. powellii*, *C. floricola*, *C. tilneyi*, *C. vaccinii*, *C. sorbosivorans*, *C. magnoliae*, and *C. apis* have been isolated from bees, wasps, substrates that these insects visit and from other insects that visit the same substrates (Lachance et al. 2001a,b; Trindade et al. 2002).

11.3 Bacteria Associated with Stingless Bees and Their Ecological Roles

Bacteria maintain a symbiotic relationship with various groups of bees (Roubik 1989). Although the interior of the nests of stingless bees has a high relative humidity and contains mud and large quantities of feces and other detritus, relatively few

Table 11.1 Some yeast species in the *Starmerella* clade and their association with bees^a

Yeast species	Bee species or bee substrate of isolation	Locality
<i>Candida apicola</i>	Bee gut <i>Melipona quadrifasciata</i> , <i>M. rufiventris</i> , <i>Trigona</i> spp., and their hives and pollen	Croatia Brazil, Costa Rica, Malaysia
<i>C. apis</i>	Trachea of a bee	UK
<i>C. batistae</i>	Ground nesting solitary bee	Brazil
<i>C. bombi</i>	<i>Bombus terrestris</i> , <i>B. hortorum</i> , <i>B.</i> <i>cryptarum</i> , <i>Bombus</i> sp.	France, Germany
<i>C. cellae</i>	<i>Centris tarsata</i> (solitary bee)	Brazil
<i>C. davenportii</i>	Dead wasp	UK
<i>C. etchellsii</i>	<i>Trigona</i> Unknown bee in <i>Opuntia</i> flowers	Costa Rica USA
<i>C. floricola</i>	<i>Ipomoea</i> flowers visited by bees	Brazil
<i>C. floris</i>	<i>Trigona</i> spp.	Costa Rica
<i>C. geochares</i>	Honey of <i>T. angustula</i> and <i>M.</i> <i>quinqüefasciata</i>	Brazil, South Africa
<i>C. magnolia</i>	Bee gut and pollen (<i>Apis mellifera</i>)	Croatia, USA
<i>C. powellii</i>	Unknown bee on <i>Ipomoea</i>	Costa Rica
<i>C. riococensis</i>	Pollen and nectar provision of <i>Megachile</i> sp.	Brazil
<i>C. tilneyi</i>	Halictid bee in <i>Ipomoea carnea</i>	Costa Rica
<i>Starmerella bombicola</i>	Honey and pollen of <i>T. angustula</i> , <i>M.</i> <i>quinqüefasciata</i> , <i>M. quadrifasciata</i> , and <i>F. varia</i> <i>Bombus</i> sp. <i>Trigona fulviventris</i>	Brazil Canada Costa Rica
<i>S. meliponinorum</i>	Honey and pollen of <i>T. angustula</i> , <i>M.</i> <i>quadrifasciata</i> , <i>M. rufiventris</i> , and <i>F.</i> <i>varia</i> <i>Trigona</i> sp.	Brazil Costa Rica

^aData from Lachance (2011)

bacteria are found in the nest, probably due to antibiotic substances in the nest materials and inhibitors produced by the bees themselves to suppress competitors (Roubik 1983). Bacteria present in the bee nests seem to have an important role in pot-honey maybe by inhibiting spoilage bacteria. In the intestinal tract of *M. quadrifasciata*, five different types of *Bacillus* spp. are found, although only one species may maintain a close relationship with the bee because it is found in bee's intestines and also in pot-honey (Cruz-Landim 1996). It is possible that *Bacillus meliponotrophicus* is responsible for a type of pre-digestion of honey and pollen produced by *M. quadrifasciata* (Nogueira-Neto 1997). Machado (1971) has shown that *B. meliponotrophicus* is associated with *Trigona* and *Melipona* but not with *Apis* and *Bombus*, which are phylogenetically related to the stingless bees. In the *M. quadrifasciata* colonies, bacteria are present in high concentrations in larval food and honey pots, where they take part in the fermentation process. The relationship between the bacterial species and the bee is obligatory because the use of antibiotics/streptomycin in the food led to the disappearance of the colony.

Spore-forming bacteria belonging to the genus *Bacillus* were found in some nests of stingless bees *Melipona panamica* (*B. alvei*, *B. circulans*, and *B. megaterium*) and *Trigona necrophaga* (*B. circulans*, *B. licheniformis*, *P. megaterium*, *B. pumilis*, and *B. subtilis*) in Panama (Gilliam et al. 1985, 1990b).

Lactic acid bacteria (probably *Lactobacillus* species) were isolated in high numbers from honey and pollen samples of *T. angustula* and *M. quadrifasciata* (C.A. Rosa, unpublished results). These bacteria likely have a role in the honey maturation of these bees by suppressing spoilage bacteria, as we speculate above.

Two stingless bees, *Tetragourla laeviceps* and *Tetragourla fuscobalteata*, commonly found in the northern region of Thailand, are known to construct nests inside forest trees. Bacterial communities of *T. laeviceps* included *Streptomyces pseudogriseolus*, *S. rochei*, *S. drozdowiczii*, *S. mutabilis*, *S. minutiscleroticus*, *S. albus*, *S. tosaensis*, and *S. malaysiensis*. In contrast, in the *T. fuscobalteata* hives, *S. ambofaciens*, *S. mutabilis*, *S. coalescens*, and *S. violaceoruber* were isolated from brood cells (Promnuan et al. 2009). The ecological role of the bacterial community still needs to be determined.

Although beneficial endosymbiosis has been described in many solitary and colonial insects that vary from obligate and intracellular to facultative and extracellular within the gut lumen (Kikuchi 2009). Anderson et al. (2011) point that virtually nothing is known about beneficial symbionts of bees. Mohr and Tabbe (2006) suggest the existence of cosmopolitan gut bacteria in bees, although Koch and Schmid-Hempel (2011) affirm that bumble bee gut presents a highly specific microflora largely different from bacteria associated with guts of honey bees. Killer et al (2009) described a new species *Bifidobacterium bombi* among gram-positive-staining, anaerobic, non-spore-forming, lactate- and acetate-producing bacteria isolated from the digestive tracts of different bumble bee species (*Bombus lucorum*, *Bombus pascuorum*, and *Bombus lapidarius*). Recent studies on the microbial flora of the honey bee gut have revealed an apparently highly specific community of resident bacteria that might play a role in immune defense and food preservation for their hosts. As pointed by Anderson et al. (2011), honey bees used in agriculture are stressed by a plethora of agricultural chemicals and their associated by products, and this may be a general situation for most bees including wild meliponing, and those antibacterial agents may kill bacterial symbionts resulting in the decline of bee populations as seen for honey bees in part of the world.

11.4 Molds Associated with Stingless Bees

There are few reports on molds associated with stingless bees. Roubik and Wheeler (1982) report the presence of *Stemphylium* (similar to those that decompose wood) in nests of *M. panamica*. Fungal identification was performed by observation of spores and hyphae found in the stomach of a beetle of the genus *Scotocryptus* that inhabits the nests of stingless bees. Gilliam et al. (1990b) reported the presence of a

green fungus in the honey of *M. Panamica*. Melo (1996) also reported a dark purple fungus in the cerumen of *M. capixaba*. However, the ecological roles of these fungi have not been determined.

Early mycological studies recognized that certain molds are common saprophytes both on and inside dead honey bees and brood combs and are probably unable to become established within the bee or the hive (Betts 1920). Fungus-associated spoilage of provisions and mortality of honey bees are rare (Batra et al. 1973). Gilliam et al. (1988) showed that only *Ascosphaera apis*, which causes chalkbrood disease, is of economic importance. Egorova (1971) isolated *Aspergillus flavus*, *A. versicolor*, *Mucor alboalter*, *Penicillium granulatum*, *P. solitum*, and *Sporotrichum olivecum* from bee bread. Two studies, Chevitchik (1950) and Pain and Maugnet (1966), did not mention molds in pollen or bee bread (the actual food consumed by bee larvae). However, Gilliam et al. (1989) isolated *Aureobasidium pullulans*, *P. corylophilum*, *P. crustosum*, and *Rhizopus nigricans* (*R. stonolifer*) in pollen and bee bread but not from floral pollen. These authors determined that these isolates may have been introduced by the bees. They noticed that the number of isolates decreased after storage by the bees, and *Mucor* sp., the dominant mold in floral pollen, was not found in corbicular pollen or bee bread. They concluded that, as with yeasts (Gilliam 1979a) and *Bacillus* spp. (Gilliam 1979b), the mold biota of corbicular pollen and bee bread may be the result of microbial inoculation by the bees and chemical changes in pollen that allow some species but not others to survive, as noted by Klungness and Peng (1983).

In the course of a study on pollen diets of three sympatric species of stingless bees *Heterotrigona collina*, *Tetragonmla melina*, and *T. melanocephala* in Sabah, Malaysia, Eltz et al. (2002) observed that large fractions of the foragers of three colonies of *H. collina* collected corbicular loads of fungal spores in lieu of pollen. Collection of spores continued for at least three consecutive days. The spores were brought to germination in the laboratory, and the culture was identified as mold of the genus *Rhizopus*. Their observations represent the first reported case of the collection of *Rhizopus* mold spores in lieu of pollen by bees and a rare case of the collection of fungal spores by bees other than honey bees (*Apis*) (Eltz et al. 2002).

Yeasts and molds are found naturally in honey, according to Gilliam (1997), who argues that microorganisms associated with bees are non-pathogenic and that most of these microorganisms are not yet known. Eltz et al. (2002) affirm that the fungi collection sometimes replaces pollen harvesting in *Apis*, *Trigona*, and *Partamona*. Ferraz et al. (2006) detected *Aspergillus* sp., *A. niger*, *Penicillium* sp., *A. terreus*, *Curvularia* sp., *Monilia* sp., *Nigrospora* sp., *Cladosporium* sp., and *Trichoderma* sp. in “jandaíra” *Melipona subnitida*, which inhabit the semiarid rocky areas of Brazilian Northeast. A species of *Curvularia* was reported as an inhabitant of *Trigona* sp. inhabiting the dry Caatinga ecosystem of Northeastern Brazil (Ferraz et al. 2006). However, the ecological role of these filamentous fungal species in the bee nests has not been determined. Indeed, Gibson and Hunter (2005) noted that the distinction between commensal and mutualistic interactions is often difficult to discern.

11.5 Yeasts Associated with Stingless Bees

Bee nests harbor a diversified yeast microbiota, and their role in biochemistry, nutrition, and physiology of bees has been investigated (Teixeira et al. 2003). According to Gilliam (1997), in social species, yeasts may have an important role in the conversion of pollen into available nutrients. Early studies showed that microbiota of pollen taken directly from flowers, corbicular pollen, and pollen stored in comb cells in the hive (bee bread) are similar. Foraging bees add microbes to pollen during collection and the same species of bacteria and yeasts are found in guts of worker bees and in corbicular pollen (Gilliam 1979a; Gilliam et al. 1984; Gilliam and Prest 1987). These microorganisms may be involved in the metabolic conversion, fermentation, and preservation of the stored food. The conversion of pollen to bee bread has often been postulated to be the result of microbial action, principally a lactic acid fermentation caused by bacteria and yeasts (Haydak 1958).

Yeasts have been isolated from honey bees, stingless bees, and solitary bees (Gilliam 1997; Rosa et al. 2003; Brysch-Heberg 2004). The Amazonian species *Ptilotrigona lurida* maintains mutualistic interactions with an unidentified yeast species that is believed to be responsible for dehydrating and retarding the deterioration of the pollen in the bee nest (Camargo et al. 1992). *Starmerella meliponinorum* was described in association with nests of the eusocial stingless bee, *T. angustula*, and could also be associated with food, both honey and pollen, propolis, detritus, and adult individuals of *M. quadrifasciata*, *M. rufiventris*, *T. angustula*, and *T. fulviventris* (Rosa et al. 2003; Teixeira et al. 2003). *Starmerella meliponinorum* and *C. apicola*, also part of the *Starmerella* clade, have been consistently isolated from *T. angustula* adults, honey, pollen provisions and refuse, *M. quadrifasciata* and *M. rufiventris* in Brazil, and *Heterotrigona Tetragonula* sp. in Malaysia. Therefore, they may have a mutualistic relationship with stingless bees. Most of the described species in the *Starmerella* clade are associated with bees or related habitats (Rosa et al. 2003). Some species in the clade are also found in other environments. In addition to the two *Starmerella* species, *S. bombycolina* and *S. meliponinorum*, *C. apicola* and closely related types are found in tropical meliponine bees worldwide (Lachance 2011).

Rosa et al. (2003) showed that the yeast community associated with *T. angustula*, *M. quadrifasciata*, and *Frieseomelitta varia* is specific to these bee species, although the ecological roles of the yeasts have not yet been defined. A large number of other yeast species were isolated from various adults of these three bee species, including *Aureobasidium pullulans*, *Pseudozyma antarctica*, and various species of *Cryptococcus* and *Rhodotorula* that may represent a transient mycota vectored by bees. *Debaryomyces hansenii* was isolated from adults and garbage pellets of *M. quadrifasciata* and from a propolis sample of *T. angustula*. This halotolerant and osmotolerant generalist is a frequent contaminant of human food and usually rare on the phylloplane (Fonseca and Inácio 2006; Kurtzman 2011a, 2011b). It was reported to cause spoilage of *A. mellifera* honey (Snowdon and Cliver 1996). Highly osmotolerant species of *Zygosaccharomyces* were isolated from the honey of *T. angustula*, from an adult *M. quadrifasciata* and from a garbage pellet of *F. varia*. *Zygosaccharomyces machadoi* was isolated from a garbage pellet of *T. angustula*



Fig. 11.2 Ripe honey of *Melipona quinquefasciata*

(Rosa and Lachance 2005). The new species *Zygosaccharomyces siamensis* was isolated from raw honey of *A. mellifera*, *A. dorsata*, and *Tetragonula pagdeni* in Thailand (Saksinchai et al. 2012). These yeasts might act as an agent of pot-honey spoilage for these bees, as argued by Rosa et al. (2003). Other yeasts already isolated from stingless bees are *Hyphopichia burtonii* (Kurtzman 2011a) and *Priceomyces mellissophilus* (Kurtzman 2011b), whereas *M. kunwiensis* and *M. reukaufii* are consistently isolated from *Bombus* bee species (Lachance 2011).

Calaçá (2011) reported that the number of yeast cells was higher in unripe pot-honey than in ripe honey of *M. quinquefasciata* (Fig. 11.2) collected in Brazil, which indicates that abundance and diversity of yeasts decreases during honey ripeness. *Candida* sp. MUCL 4571, a new undescribed species sister of *C. apicola*, was the prevalent species in the samples and could have a mutualistic association with this bee.

11.6 A Possible Mutualistic Interaction Between Yeasts and Bees?

High yeast counts in larval provisions suggest that these microorganisms are metabolically active, and that the enzymes they produce may be important for the improvement of the nutritional characteristics of pollen. Both social and solitary bees introduce yeasts into their nests (Gilliam 1997), which possibly bring nutritional benefits to larvae. Bees require nutrients, such as proteins, lipids, and vitamins, from pollen and carbohydrates from nectar (Standifer et al. 1980). Corbicular pollen is transformed into bee bread (comb pollen) through a fermentative process that is carried out primarily by yeasts (Pain and Maugeat 1966) and brings a higher nutritional value and availability of amino acids in the bee bread compared to corbicular pollen (Loper et al. 1980; Standifer et al. 1980).

Gibson and Hunter (2005) defined five stages in the pathway to obligate mutualism: (1) consistent and extended contact; (2) avoidance of lethal harm during contact; (3) coadaptation, leading to increased tolerance; (4) further coadaptation, leading to dependence and/or interdependence; and (5) permanent association. In studies of the association of yeasts and *Chrysoperla* lacewings, Gibson and Hunter (2005) argue that the ease with which the yeasts can be cultured suggests that these two organisms are not interdependent obligate mutualists, as in case of bacterial symbionts (Douglas 1998). Although they could not find evidence that resident yeasts bring nutritional benefits to the lacewings, they were not able to cultivate yeast-free lacewings and, therefore, could not reach a conclusion on the role of yeasts in the interaction. Our own studies on the yeasts associated with the bees *M. quinquefasciata* in Minas Gerais (Southeastern Brazil) and *M. compressipes*, *M. scutellaris*, *Plebeia* sp., *Scaptotrigona polysticta*, and *S. tubiba* in Cerrado ecosystems of Central North Brazil indicate that those yeast strains are very difficult to maintain in culture collections, and various strains die before a complete identification is reached, raising the possibility that association with the bees is important for survival of those yeasts. Further investigation is needed to reach any conclusions on the mutualistic interactions between stingless bees and yeasts.

Records of yeast-insect associations in which the role of the yeasts is not well understood include: green June beetles (Vishniac and Johnson 1990), nitidulid beetles (Lachance et al. 2003), clerid beetles (Lachance et al. 2001a), encyrtid parasitoids (Lebeck 1989), ichneumonid parasitoids (Middeldorf and Ruthmann 1984), fire ants (Ba and Phillips 1996), leafcutting bees (Teixeira et al. 2003), solitary digger bees (Rosa et al. 1999), vespid wasps and bumble bees (Stratford et al. 2002), honey bees (Spencer and Spencer 1997), and the green lacewings in the genus *Chrysoperla* (Hagen et al. 1970; Gibson and Hunter 2005). Although we cannot rule out the possibility that stingless bees are simply vectors for yeasts, Lachance et al. (2011) affirm that the insect vectors appear to be the primary agents responsible for the organization of the yeast communities, a role of great importance for the understanding of yeast ecology in all ecosystems.

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