Chemical Ecology in Insect-microbe Interactions in the Neotropics

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ABSTRACT

Small molecules frequently mediate symbiotic interactions between microorganisms and their hosts. Brazil harbors the highest diversity of insects in the world; however, just recently, efforts have been directed to deciphering the chemical signals involved in the symbioses of microorganisms and social insects. The current scenario of natural products research guided by chemical ecology is discussed in this review. Two groups of social insects have been prioritized in the studies, fungus-farming ants and stingless bees, leading to the identification of natural products involved in defensive and nutritional symbioses. Some of the compounds also present potential pharmaceutical applications as antimicrobials, and this is likely related to their ecological roles. Microbial symbioses in termites and wasps are suggested promising sources of biologically active small molecules. Aspects related to public policies for insect biodiversity preservation are also highlighted.

Introduction

Brazil is one of the world's 17 megadiverse countries that host between 15–20% of the entire world's biological diversity, including the greatest number of endemic species [1], offering innumerous research opportunities and sustainable technological development. Natural products research in Brazil has been historically focused on plant-derived compounds and evaluation of their biological activities [2], with only recent efforts directed to microbial-derived compounds and marine organisms [3–5].

Recent estimates based on DNA sequence information have shown that microorganisms consist in the highest number of living species, most of them yet to be described [6], but there are no records on the number of microbial species in Brazil. This untapped ecological niche represents a huge potential for natural products discovery, as suggested by sequencing of environmental bacterial DNA collected in different biomes [7].

Microorganisms are the most ancient form of life on earth and have established symbiotic interactions with several other organisms, from mammals to arthropods and plants [8]. Symbiosis involves any intimate species interaction, either positive or negative, including mutualism, commensalism, and parasitism, as defined by Bary [9]. Indeed, symbioses with microorganisms have contributed with innovations in eukaryotic evolution [10]. Much of the communication between microorganisms and between microorganisms and their environment is based on chemical interactions [11], the subject of research in the interdisciplinary field of chemical ecology [12]. Deciphering the chemical language between species is key to understanding how they interact and provides an opportunity for ecologically-guided natural products discovery for biotechnological purposes.

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Insects dominate the known diversity of living organisms. Class Insecta comprises about 1 million described species and makes up 83.5% of all species in the Phylum Arthropoda [13]. Brazil harbors the highest diversity of insects in the world [14], but natural products research on insects is mainly focused on pheromones in the country [15]. As with other organisms, insects have developed a plethora of interspecies interactions with microorganisms, and social insects are fascinating examples of multipartite symbiosis.

Eusociality is an evolutionarily advanced level of social organization nearly confined to insects, especially ants, bees, wasps, and termites. Eusocial adult insects in a colony belong to different overlapping generations, care cooperatively for the offspring, and are divided into reproductive and nonreproductive castes [16]. Some social insects have also evolved symbiotic mutualistic interactions with fungi in which nutrient exchange between species is the key behind this association. Insects provide food resources–usually plant-derived material difficult to digest–to the fungi and protect them against opportunistic pathogens. In return, fungi can supply nutrients and molecules important for insect's physiological functions [17, 18]. Social insects are particularly attractive for microbial disease agents, since they live in high populational densities in relatively homeostatic nest environments and store food resources [19]. To avoid pathogens, social insects have evolved several strategies, such as grooming, nest hygiene, and chemical defenses [19, 20]. Another defensive strategy of insects is the symbiotic association with bacteria that produce and secrete biologically active small molecules that are selective against pathogens [20].

The biosynthetic potential of insect-associated microbes worldwide is covered elsewhere [17, 20–22], and here we highlight some of the work done on the Brazilian biodiversity. The biological activity of some microbial strains isolated from Brazilian social insects has been investigated but not exhaustively studied. These microbial isolates comprise yeasts, proteobacteria, and actinobacteria, which produce a variety of biologically active small molecules.

Natural Products Mediating Microbial Symbiosis Fungus-Farming Ants

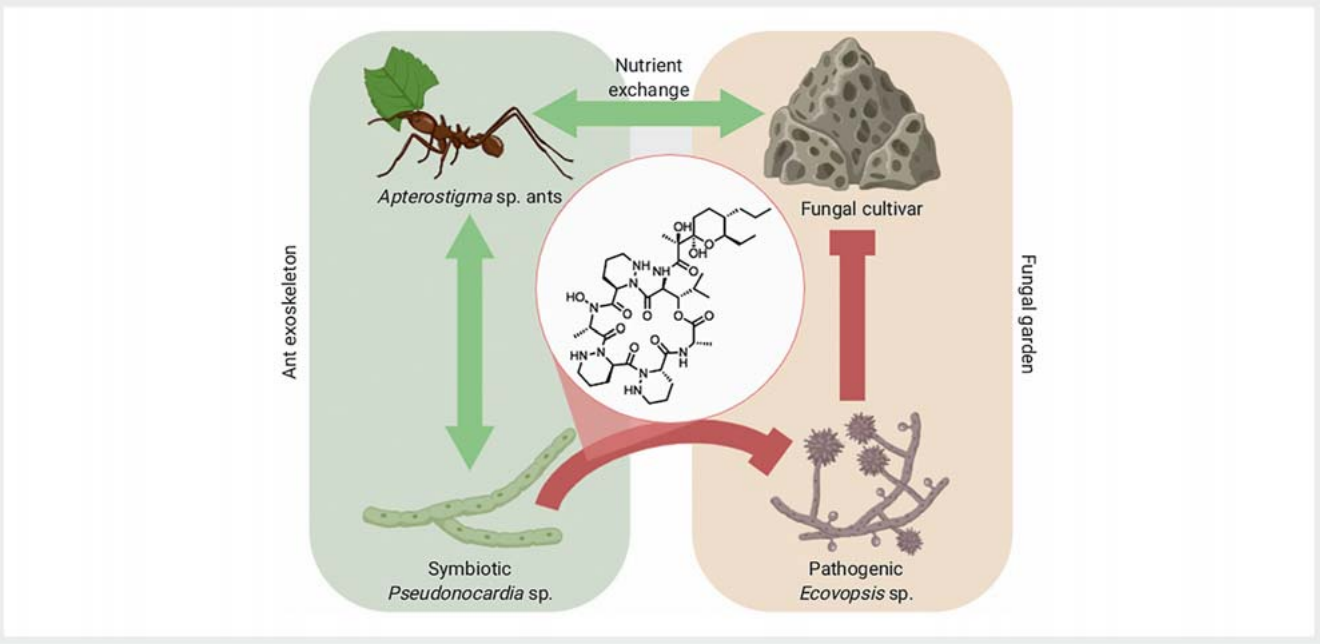
E. O. Wilson, a recognized American biologist, naturalist, and writer, stated in 1975: "*There are more species of ants in a square kilometer of Brazilian forest than all the species of primates in the world, more workers in a single colony of driver ants than all the lions and elephants in Africa*" [23].

Fungus-farming "attine" ants (Formicidae: Myrmicinae: Attini: Attina) originated in a single ancestral attine in Amazon around 45 million y ago [24]. Approximately 250 species of fungus-farming ants are found in the "New World", ranging from North America to South America [25]. Attine ants collect plant and other material they forage from the environment to nurture basidiomycete fungi they cultivate for food. During the evolution of these interactions, the foraged material has been diversified, giving rise to the so-called "basal" and "highly evolved" agricultural systems, based on the substrate that the fungal crop is fed [26]. Other microbes found in this multipartite symbiosis are a specialized pathogenic fungus from the genus *Escovopsis*, which can suppress the crop fungal cultivar and destroy the ant colony [27], and a symbiotic actinobacterium usually belonging to the genus *Pseudonocardia*, which produces small molecules that selectively inhibit the pathogenic fungus over the crop fungus [28, 29]. Dentigerumycin, produced by *Pseudonocardia* associated with *Apterostigma* ants collected in Central America, was the first selective antifungal characterized as mediator of the defensive symbiosis in attine ants (▶ **Fig. 1**) [30]. Even though there is a reasonable number of publications examining the chemistry involved in this symbiosis from attine colonies sampled in Central America [30–34], the potential of the microbial symbionts from Brazilian attine ants still remains to be explored.

Some attine ants have their exoskeletons covered by the actinobacteria, whereas others do not show the same obvious association. Ants of *Atta* genus, for instance, do not show specialized crypts–morphological structures that harbor actinobacteria–in their bodies. The bacterial symbionts are supposed to be internalized inside ants' bodies [35] or even in other places of the colony. The hypothesis that opportunistic pathogens were inhibited by microorganisms living in the colony was therefore tested. Different parts of *Atta sexdens* colonies (fungal garden, waste deposit, and surface of leaves collected by ants) were sampled and resulted in the isolation of 99 yeast strains. These strains had their inhibitory activities tested against 6 reporter yeast strains and also against each other. The results showed that 77 strains (78%) inhibited the growth of the competing strain. This high number of active strains pointed toward a role in maintaining the nest microbial community [36]. Investigations were also conducted into the role of bacterial strains associated with the fungal gardens of *Atta sexdens* ants collected in eucalyptus plantations in Rio de Janeiro state, Brazil. A high number of colonies was found to be associated with *Burkholderia* sp. strains, which inhibited different entomopathogenic fungi, such as *Metarhizium anisopliae, Beauveria bassiana*, the saprophytic fungus *Verticillium lecaniii,* and the specific ant pathogen *Escovopsis weberi*. Although these strains had antifungal activity against different fungi, they did not show any activity against the fungal cultivar. *Burkholderia* sp. strains were isolated from 32 out of 57 ant nests (56%), also suggesting an important ecological role [37].

As well as producing small molecules with defensive roles, Proteobacteria can also fulfill other ecological functions. *Serratia marcescens* isolated from *Atta sexdens rubropilosa* colonies produce volatile pyrazines, including 2,5-dimethylpyrazine (**1**) and 3-ethyl-2,5-dimethylpyrazine (**2**) (▶ **Fig. 2**), that are components of ants' trail pheromone [38]. However, the dependence of ants on the microbial biosynthesis of trail pheromones remains to be elucidated. The microbial involvement in the production of pheromones has been recognized for some insects, but more efforts are needed to experimentally validate connections between the presence of specific symbionts, changes in the host's chemistry, and behavioral effects [39].

Leaf-cutter ants start new colonies with queen ants fecundated during the nuptial flight; however, mortality is extremely high during the nuptial flight and immediately afterward [24]. *A. sexdens rubropilosa* queen ants were found to be infected by the entomopathogenic fungus *Aspergillus nomius* after nuptial



▶ **Fig. 1** Ecological relationships between microorganisms and attine ants. Mutualistic interactions are represented by green arrows and red T-bars represent harmful interactions. Investigations into this system led to the isolation of the antifungal dentigerumycin [30]. Source: Monica Tallarico Pupo

flight. The fungus produced aflatoxin B1 (**3**) and aflatoxin G1 (**4**) (▶ **Fig. 2**) both *in situ* and *in vitro*. These compounds may play a pivotal role in the fungal pathogenicity observed for the *Atta* queens [40].

Different from *Atta* ants, leaf-cutter ants of genus *Acromyrmex* usually carry the symbiotic actinobacteria in crypts in their exoskeletons. *Acromyrmex subterraneus brunneus* worker ants at USP-campus, Piracicaba-SP, Brazil were sampled for the presence of actinobacteria, leading to the isolation of 20 actinobacteria strains. Among these bacteria, 17 strains belong to the genus *Streptomyces*, and the remaining are *Pseudonocardia, Kitasatospora*, and *Propionicimonas*. The majority of *Streptomyces* isolates inhibited the growth of the nest pathogen *Escovopsis weberi* [41]. These studies suggested that the ant-associated microbes isolated from samples collected in Brazil–other than *Pseudonocardia*–produce secondary metabolites with biological activity; however, chemical compounds responsible for antimicrobial activities are still elusive.

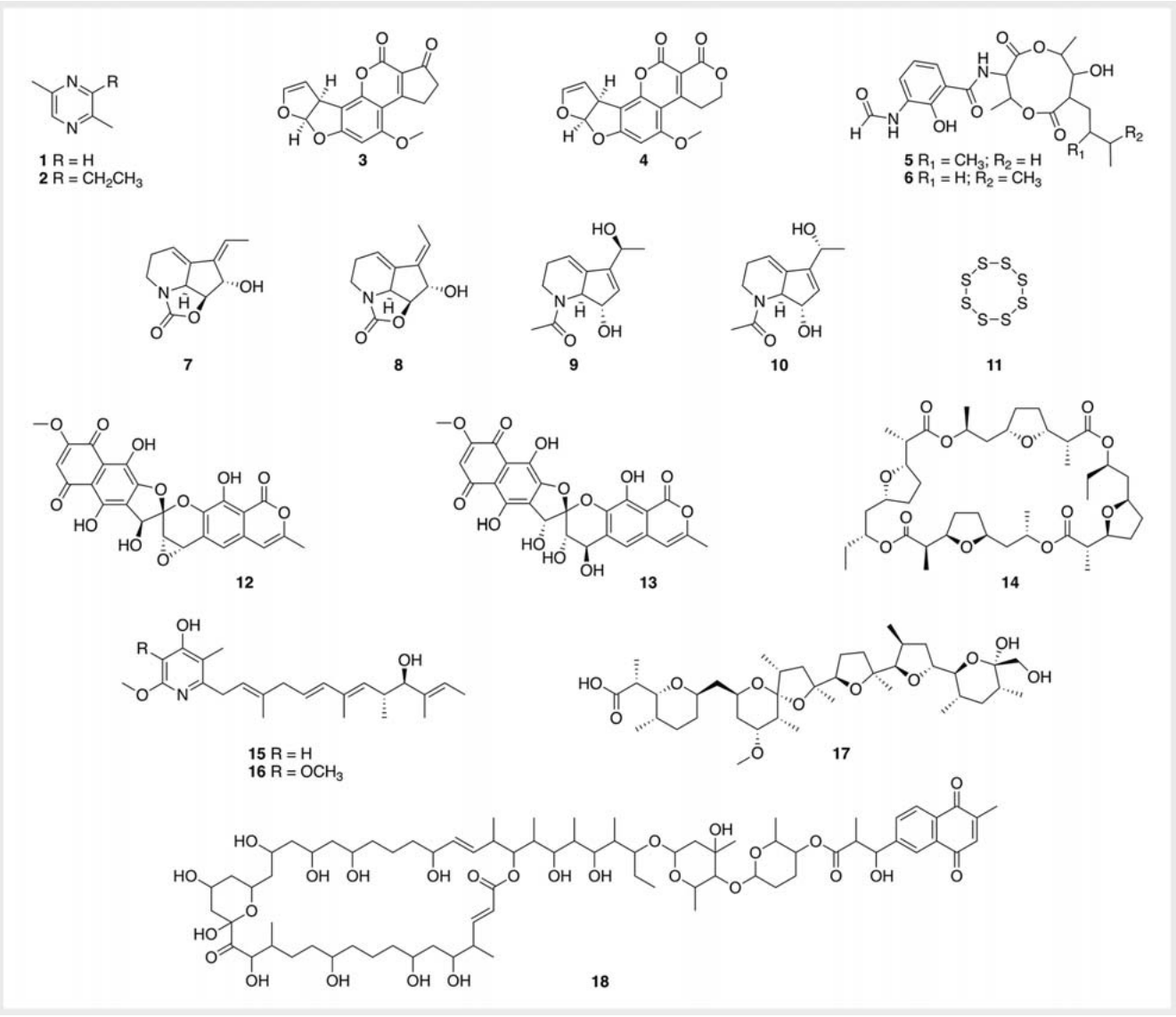
Actinobacteria can produce compounds showing great chemical diversity and a large variety of biological activities, and have evolved protective symbiotic interactions with different organisms [42]. Indeed, *Streptomyces* has been proven to be a good source of antimicrobial defenses in insects [22]. The first compounds from *Streptomyces* strains associated with fungus-farming ants in Brazil were the antimycins urauchimycin A (**5**) and urauchimycin B (**6**) (▶ **Fig. 2**) [43], with broad and potent antifungal activity against medically important *Candida* strains.

Recently, interdisciplinary research groups in Brazil have been mainly focused on the characterization of biologically active natural products from microbial symbionts of social insects [44]. Using an ecological-driven approach, the actinobacterial symbionts of attine ants have been systematically screened against the specialized pathogenic fungus *Escovopsis* and then selected for further screening against other bacterial, fungal, and protozoan human pathogens.

The search for symbionts of *Acromyrmex subterraneus brunneus* ants, collected at USP-campus, Ribeirão Preto-SP, led to the isolation of *Streptomyces chartreusis* ICBG377, recovered from the fungal garden. The actinobacterium produces the antibiotic streptazolin (**7**), its *E*-isomer (**8**), strepchazolin A (**9**), strepchazolin B (**10**), and the inorganic compound cyclooctasulfur (**11**), the active compound against *Escovopsis* (▶ **Fig. 2**) [45]. Compound **11** was also produced by *S. chartreusis* ICBG323, isolated from the exoskeleton of winged males of *Mycocepurus goeldii* [45].

The actinobacterium *S. puniceus* ICBG378, isolated from *Acromyrmex rugosus rugosus* ants, produces griseorhodin A (**12**) and griseorhodin C (**13**), natural products known by their cytotoxic activity against cancer cell lines [46], and dinactin (**14**), active against *Escovopsis* (▶ **Fig. 2**) [47]. Dinactin (**14**) was also active against *Leishmania donovani*, one of the etiological agents of leishmaniasis, a neglected tropical disease that causes thousands of deaths yearly in developing countries.

Similarly, *Cyphomyrmex*-associated *Streptomyces* sp. ICBG292 produced Mer-A2026B (**15**), piericidin-A1 (**16**), and nigericin (**17**) (▶ **Fig. 2**), all active against *Escovopsis* and against intracellular amastigotes of *L. donovani*. Compounds **15** and **16** were also isolated from *Atta*-associated *Streptomyces*, while **14** and **17** showed the most potent leishmanicidal activities, with good selectivity indexes [47]. The biological activity of these compounds highlights the importance of exploring different sources for prospecting compounds that can help treating human diseases [47].



▶ **Fig. 2** Compounds isolated from microbial symbionts of attine ants collected in Brazil.

The hypothesis that the ant microbiome is a good source for exploring new medically useful antimicrobial agents was further reiterated. The isolation of cyphomycin (**18**) (▶ **Fig. 2**), a new antifungal polyketide, proved that the Brazilian biodiversity should be explored in order to find new candidates for the treatment of fungal infections. Cyphomycin (**18**) was produced by a *Cyphomyrmex*-associated *Streptomyces* strain and showed potent activity against human fungal pathogens both *in vitro* and *in vivo* [22].

Natural Products Mediating Microbial Symbiosis in Stingless Bees

Although a wide diversity of microorganisms, such as bacteria, fungi, and yeasts are found to be associated with bees, little is known about their role as beneficial symbionts [48]. Indeed, honey bees across the world harbor a rich bacterial community [49–52]. Lactic acid bacteria (LAB) are frequently isolated from the guts of honey bees and bumble bees, and it is believed that this specific microbiota coevolved with their hosts. playing roles in nutrition and defense against pathogens [53–56].

More information is available regarding microbial pathogens of honey bees, which span several kingdoms, including the most damaging threats such as viruses, bacteria, and fungi [57]. *Paenibacillus larvae* and *Melissococcus plutonius*, the infective agents of American and European foulbrood diseases, respectively, are major bacterial threats to honey bees (*Apis* species) [57]. The American foulbrood disease (AFB) is widespread in honey bee larvae [58, 59] and drastically impacts the apiculture and the pollination of crops and wild plants [60]. The long-lived spores produced by the bacterium are infectious only for larvae, especially in early larval stages [61]. Burning the infected colonies is one usual treatment for AFB [62]. Antibiotics such as oxytetracycline are used in some countries for prevention and treatment of contami-

nated colonies still, but this approach is not effective against spores [63–67]. Other problems can be caused using antibiotics as their chemical residues accumulate in honey, reducing the longevity of the bees and selecting resistant *P. larvae* strains [61,68]. The European foulbrood disease is caused by the globally distributed Gram-positive, non-spore-forming bacterium *M. plutonius* [69]. Ingestion of larval food contaminated with *M. plutonius* causes infection in larvae. Bumble bees (*Bombus terrestris*), important pollinators of the northern hemisphere, are colonized by the virulent parasite trypanosomatid *Crithidia bombi* (Trypanosomatidae, Zoomastigophorea) [70]. These infections have a variety of consequences, such as the mortality of the colonies.

While microbial diseases are better understood for honey bees, little is known about microbial diseases affecting stingless bees. Stingless bees (Apidae: Meliponini) are a large group of bees with more than 500 species described, around 300 of them occur in Brazil [71]. Although *A. mellifera* is originally from Africa, this species is an important pollinator widespread around the world [72]. The global distribution of honey bees favors their microbial pathogens to spillover stingless bees native to tropical and subtropical regions [73]. Indeed, some honey bee pathogens already detected in stingless bees include the disease-causing bacterium *Lysinibacillus sphaericus* in Australia [74], the acute bee paralysis virus (ABPV) in Brazil [75], the bacterium *M. plutonius* in Brazil [76], and the fungus *Nosema ceranae* in laboratory colonies [73]. Therefore, an ecological approach to study bacterial symbionts of stingless bees involved in defensive responses can be based on microbial pathogens of honey bees and bumble bees.

Research has been directed toward the role of the associated microbiota in protecting bees against pathogens. LAB have a potential role in controlling the bacterial pathogens causing American and European foulbrood diseases [77]. Stingless bees from different geographical regions also carry LAB [78] that might possess similar functions. Bacteriocin-like compounds, active against *P. larvae* were also identified from LAB isolated from honey bees in Argentina [79]. Actinobacteria have also been isolated from stingless bees from other tropical and subtropical regions. Antibiotic-producing *Streptomyces* spp. were isolated from the stingless bee *Tetragonisca angustula* in Costa Rica, showing antimicrobial activities against a variety of human pathogens [80]. Actinobacteria strains active against *P. larvae* and *M. plutonius* were isolated from colonies of honey bees (*A. mellifera, A. cerana, A. florae*) and stingless bees (*Trigona laeviceps* and *T. fuscobalteata*) in Thailand [81]. However, the small molecules mediating these defensive symbioses have not been comprehensively studied.

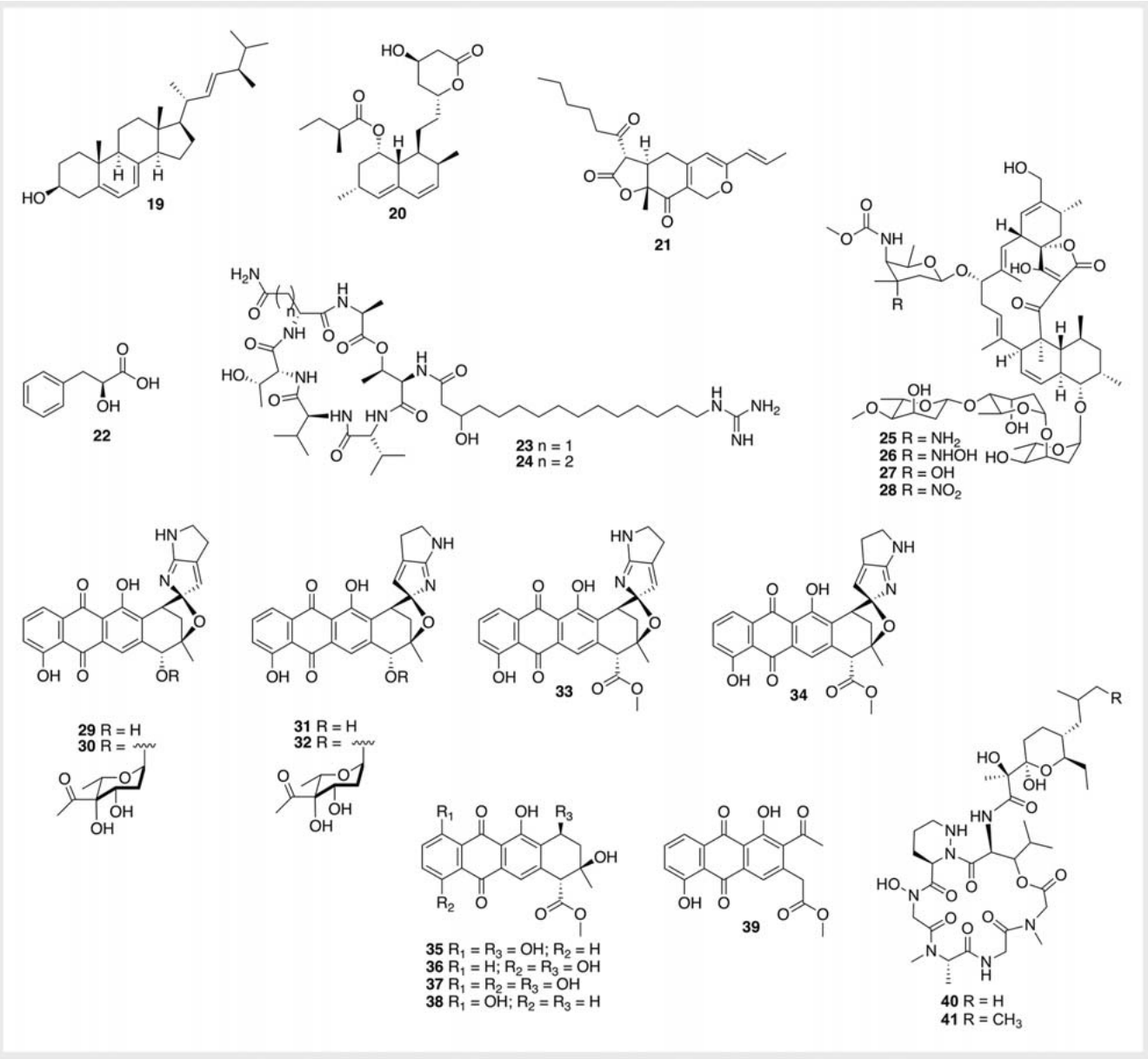
The first unprecedented example of nutritional symbiosis in stingless bees is the Brazilian bee *Scaptotrigona depilis*, which is surrounded by a complex microbial community. *S. depilis* cultivates a fungus of the genus *Zygosaccharomyces* in the brood cell, which provides ergosterol (**19**) (▶ **Fig. 3**) as a precursor for ecdysteroid biosynthesis and, consequently, for proper larval development and metamorphosis [82,83]. Two additional fungi are also active in the cerumen of brood cells, *Candida* sp. and *Monascus ruber*, which modulate *Zygosaccharomyces* sp. growth. *Candida* sp. produces volatile alcohols such as ethanol and isoamyl alcohol that stimulate the growth of *Zygosaccharomyces* sp., while *Monascus ruber* inhibits *Zygosaccharomyces* sp. and *Candida* sp. by the production of lovastatin (**20**) and monascin (**21**), respectively (▶ **Fig. 3**) [84]. Fungi of the genus *Monascus* were also found in association with other species of stingless bees in Brazil, but their chemical-ecological functions remain unknown [85]. The larvae of *S. depilis* also engage in associations with microbes. Genome analyses of the *Bacillus* sp. SDLI1 isolated from *S. depilis* larvae indicated the presence of biosynthetic gene clusters that encode the production of a variety of antibiotics, suggesting a bacterial defensive symbiosis [86]. *Bacillus* spp. have been commonly associated with honey bees' larvae and inhibit *P. larvae* [87,88]. The isolation of *Bacillus* DNA in fossils showed close phylogenetic relationships with strains typically isolated from stingless bees, which could provide information about the evolution of microbe-insect symbiosis [89].

The stingless bee *Melipona scutellaris* inhabits in Northeast Brazil and engages in a relationship with various bacteria. The ecological-driven approach of bioassays against entomopathogens led to the identification of some bacterial strains as possible defensive symbionts and their chemical signals. The bacterium *Paenibacillus polymyxa* was isolated from the larval food of *M. scutellaris* and produces (L)-(−)-phenyllactic acid (**22**) and a family of 9 lipodepsipeptides known as fusaricidins, including the major compounds fusaricidin A (**23**) and fusaricidin B (**24**) (▶ **Fig. 3**), active against the entomopathogenic fungus *B. bassiana* and bacterium *P. larvae*. Interestingly, fusaricidins **23** and **24** were also detected directly in the larval food of different sampled colonies, suggesting a beneficial defensive role against pathogens [90]. Adult *M. scutellaris* bees also carry several actinobacteria in their bodies. *Streptomyces* sp. ICBG1323 and *Micromonospora* sp. ICBG1321 were isolated from nurse and forager bees, respectively. Two families of structurally complex bioactive polyketides were isolated from the associated strains: lobophorins (**25**–**28**) from *Streptomyces* sp. ICBG1323 and anthracyclines from *Micromonospora* sp. ICBG132 (**29**–**39**), including the rare quinocyclines **29**–**34** and the novel compound **39** (▶ **Fig. 3**). The compounds presented variable levels of activities against *P. larvae*. Compounds **28** and **30** showed the higher antibacterial activity, better than the control antibiotics [91]. Finally, two new compounds were isolated from *Streptomyces* sp. ICBG1318 strain in association with *M. scutellaris* nurse bees. The novel cyclodepsipeptides named meliponamycin A (**40**) and meliponamycin B (**41**) (▶ **Fig. 3**) were strongly active against *P. larvae* and human pathogens, such as *Staphylococcus aureus* and *L. infantum* [92].

The examples highlight that more research on the stingless bees-associated microbiota is essential to enhance the current knowledge of the molecular signals involved in these symbiotic interactions. This knowledge might contribute to design policies for the preservation of these important pollinators of native flora and agricultural crops.

Microbial Symbiosis in Wasps

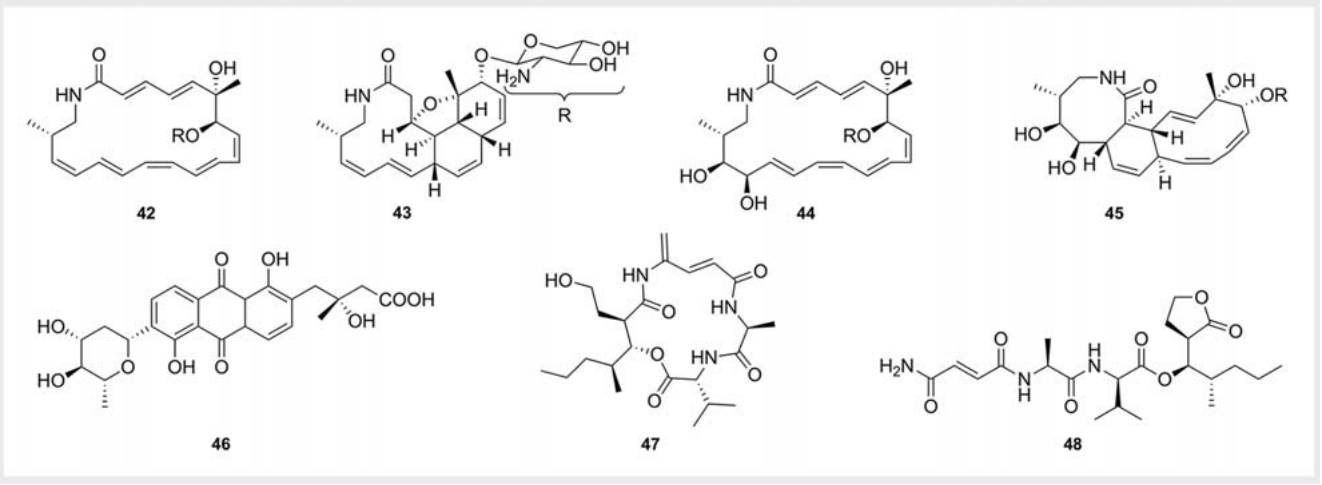
Previous research efforts on digger wasps of the genus *Philanthus* (beewolves; Hymenoptera, Crabronidae), which consists of more than 100 species widespread in Europe, Africa, Asia, and North America, showed association with the symbiotic actinobacteria *Candidatus Streptomyces philanthi* [93,94]. The symbionts are cul-



▶ **Fig. 3** Compounds isolated from microbial symbionts of Brazilian stingless bees.

tivated in specialized antennal gland reservoirs and transferred to the brood cells where they produce antibiotics such as streptochlorin and piericidin derivatives responsible for protecting the wasps' larvae against pathogens [95,96]. The Brazilian digger wasps *Trachypus boharti* also present bacteria in the antennal gland reservoirs. Gene sequences revealed that among all antennal symbionts described, the Brazilian wasps cultivate the most distantly related actinobacteria [97]. However, the chemistry behind this protective symbiosis remains to be uncovered.

Another example of wasp-microbe symbiosis is established between the parasitic wasp *Asobara tabida* with the bacterium *Wolbachia*. The bacterium is vertically transmitted via the eggs by wasps and plays a fundamental role in oogenesis completion [98]. The treatment with antibiotics to eliminate *Wolbachia* found that aposymbiotic females of *A. tabida* are reproductively sterile, being unable to produce viable offspring [99]. An intense apoptosis process is responsible for the absence of egg production, and there is evidence that *Wolbachia* inhibits the programmed cell death by the disruption of cellular physiology of the host [100]. The symbiosis with the bacterium *Wolbachia* was found for wasps of the genus *Encarsia*; meanwhile, another bacterium described as "*Encarsia* bacterium" was found to be associated with a population of *Encarsia* wasps, including *E. pergandiella* collected in Brazil. The bacterium is related to parthenogenesis [101]. The symbiont "*Candidatus Cardinium hertigii*" associated with *Encarsia* wasps from Brazil and USA is also linked to reproductive alterations in the host [102].



▶ **Fig. 4** Compounds isolated from microbial symbionts of termites.

Parasitoid wasps lay their eggs into other arthropods who are hosts for wasp larval development [103]. Braconid wasps engaged in an ancient relationship with polydnavirus that suppress host defense mechanisms and permit the larval development [104–106]. This symbiosis is so old (about 70 million y) that the genes involved in viral replication have been incorporated into the wasp genome [107].

Invasive wood wasps *Sirex noctilio* collected in USA are associated with *Streptomyces* strains with specific enzymatic activities responsible for degrading cellulose, which is used by the insect as source of energy [108]. Wood wasps still hold a close relationship with a fungal symbiont *Amylostereum chailletii* that feeds wasp larvae, providing them with digestive enzymes [109].

Very few efforts have been pursued on revealing the chemical signaling in interactions between microbes and wasps in Brazil. The country harbors the richest fauna of social wasps (Polistinae) in the world, with more than 300 species, 104 of them endemic from Brazil [110], offering several opportunities for chemical ecology based natural products discovery.

Microbial Symbiosis in Termites

Although fungus-farming ants and fungus-growing termites share behavioral similarities, and both seem to rely on the presence of symbiotic actinobacteria to chemically defend their nests against fungal pathogens [54, 111], they do not share a common ancestor with the same characteristics [112]. Moreover, these insects differ from each other in the geographical distribution. While attine ants originated and are found in the “New World”, fungus growing Macrotermitinae termites (Termitidae: Macrotermitinae) originated in Africa and comprise about 330 species distributed in the “Old World”, including Africa and Asia [113]. Macrotermitinae termites are subdivided into 11 genera [114].

Brazil houses around 300 species of termites belonging to the families Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae [115]. Termites contribute to structure and composition of soils by efficiently degrading biomass with the aid of resident gut microbiota, so most of the research in Brazil has focused on the enzymatic potential of termite-associated microbiota [116]. Termite microbiota might also have a contribution in defensive symbiosis by the production of secondary metabolites. Indeed, two *Streptomyces* strains from termite mounds collected in Bahia State showed percentages of inhibition above 98% against bovine viral diarrhea virus (BVDV), but the active compound has not been identified [117].

There are some examples of natural products produced by actinobacteria in association with African fungus-growing termites [118–122]. *Amycolatopsis* sp. produced macrotermycins A–D (**42–45**) (▶ **Fig. 4**). Besides the antifungal ecological role, these 4 macrolactams presented antibacterial activity against *S. aureus* [123]. The polyketide fridamycin A (**46**) (▶ **Fig. 4**) was isolated from the termite-associated *Actinomadura* sp.; it demonstrated glucose uptake stimulation and could be an option for type 2 diabetes therapeutics [124]. Microtermolides A (**47**) and B (**48**) (▶ **Fig. 4**) were isolated from a fungus-growing termite-associated *Streptomyces* sp. [125, 126]. Both compounds are depsipeptides; moreover, microtermolide B is a rare linear depsipeptide and seems to be the first one of this class produced by a *Streptomyces* strain. The lack of extensive work on termite-associated actinobacteria in Brazil, however, can be due to the absence of fungus-growing termites in this region of the world [127]. Brazilian termites, as wasps, are eusocial insects. Even though there is not much work on Brazilian termites, this system seems promising since their colonies are susceptible to parasitic pressure, and previous works have demonstrated the beneficial association between social insects and antibiotic-producing microbes.

Conclusion

Brazil harbors an impressive reservoir of genetic resources in different biomes, including the highest number of known insects in the world and an undescribed microbial diversity. The chemistry of microbial natural products and chemical ecology of microbial symbiosis are complementary research fields in their early days in

the country. Two decades have passed since the publication of the first natural product from a microbial source [128], and 7 y since the first report on natural products mediating microbial interactions [129] in Brazil.

The chemistry involved in interspecies interactions between insects and microbes remains largely to be unveiled. The examples showed here are just a glimpse of the chemodiversity involved in nutritional and defensive microbial symbiosis in some species of attine ants and stingless bees in Brazil. Several other species of these insects should be investigated, as well as other Brazilian social insects such as termites and wasps.

The understanding of how species interact in nature is instrumental to design sustainable approaches for their uses. Besides improving the knowledge about interspecies interactions, the chemical ecology approach to studying insect-microbe symbiosis might lead to the identification of biologically active compounds with privileged scaffolds for biotechnological development, mainly as agrochemicals and pharmaceuticals. The huge biodiversity remaining in Brazil potentially encodes useful products to be developed based on sustainable practices. Efforts of multidisciplinary research groups in chemistry, microbiology, molecular biology, entomology, and pharmacology are instrumental to achieve such results.

Not less important is the regular and prioritized governmental financial support for research in the field. The biodiversity of insects has declined worldwide; therefore, researchers and government in Brazil might act synergistically. According to Sánchez-Bayoa & Wyckhuys [130], the main drivers of insect decline are: i) habitat loss and conversion to intensive agriculture and urbanization; ii) pollution, mainly that by synthetic pesticides and fertilizers; iii) biological factors, including pathogens and introduced species; and iv) climate change. It is out of scope to discuss each one of those factors in this article but needless to explain that all of them occur in Brazil.

Strong public policies are urgently needed to protect Brazilian biodiversity. Specifically, it is important to consider the preservation of native insects and, consequently, the benefits they provide–in association with resident microbes–in the structure and functioning of the ecosystems.

Contributors' Statement

Conception and design of the work: M. T. Pupo; Data collection: C. Menegatti, T. T. H. Fukuda, M. T. Pupo; Analysis and interpretation of the data: C. Menegatti, T. T. H. Fukuda, M. T. Pupo; Drafting the manuscript: C. Menegatti, T. T. H. Fukuda, M. T. Pupo; Critical revision of the manuscript: M. T. Pupo.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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