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Versatile and Dynamic Symbioses Between Insects and *Burkholderia* Bacteria

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Burkholderia, symbiosis, mutualism, pathogenicity, lifestyle transition, vertical and horizontal transmission

Abstract

Symbiotic associations with microorganisms represent major sources of ecological and evolutionary innovations in insects. Multiple insect taxa engage in symbioses with bacteria of the genus *Burkholderia*, a diverse group that is widespread across different environments and whose members can be mutualistic or pathogenic to plants, fungi, and animals. *Burkholderia* symbionts provide nutritional benefits and resistance against insecticides to stinkbugs, defend *Lagria* beetle eggs against pathogenic fungi, and may be involved in nitrogen metabolism in ants. In contrast to many other insect symbioses, the known associations with *Burkholderia* are characterized by environmental symbiont acquisition or mixed-mode transmission, resulting in interesting ecological and evolutionary dynamics of symbiont strain composition. Insect–*Burkholderia* symbioses present valuable model systems from which to derive insights into general principles governing symbiotic interactions because they are often experimentally and genetically tractable and span a large fraction of the diversity of functions, localizations, and transmission routes represented in insect symbioses.

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INTRODUCTION

Insects constantly interact with a diverse array of microorganisms in their environments and can establish life-long associations with specific microbial partners. These symbiotic partnerships often have profound implications for the biology of the insect host and can be key drivers of niche colonization and ecological innovation due to their impact on the host's nutrition, digestion, toxin resistance, or defense against natural enemies (25, 36). Yet little is known about why insects associate with a particular microbial partner instead of other potential ones. Specific microbial taxa might be favored for the evolution of symbiosis, depending on factors such as the microbe's metabolic capabilities, virulence, stress resistance, and habitat overlap with the host. *Burkholderia* bacteria are a particularly interesting microbial group in this context, given their extraordinary ecological versatility and repeated appearance as associates of many different eukaryotic hosts, including several insect taxa (40, 73, 105, 164). In this review, we provide an overview of the ecology and phylogenetic affiliations of insect-associated *Burkholderia*, highlight features that are potentially favorable for transitioning to a symbiotic lifestyle, and propose promising approaches to study symbiosis using *Burkholderia*–insect associations as tractable model systems.

THE VERSATILE BURKHOLDERIA

First defined in the early 1990s, *Burkholderia*, a genus of β -Proteobacteria, originally comprised only seven species previously designated as *Pseudomonas* (172). The initial representatives of the genus were mostly known for their pathogenicity in plants and animals. Within the past three decades, however, the number of species rose to nearly 100, including strains from a remarkable diversity of environments and lifestyles (20, 28). Most recently, the expanding *Burkholderia* clade was taxonomically revised, and the reassignment of several strains to two major new genera, *Paraburkholderia* and *Caballeronia*, as well as the three smaller genera, *Robbsia*, *Mycetohabitans*, and *Trinickia*, was proposed (3, 22, 33).

Although the species remaining in the genus *Burkholderia* sensu stricto (s.str.) are those most commonly linked to pathogenicity, evidence for mutualistic partnerships with insects or occasional contributions to plant health suggest that virulence is variable among members of this clade, with the potential for dynamic lifestyle shifts. Several of these species, including *Burkholderia gladioli*, *Burkholderia glumae*, and *Burkholderia plantarii*, are recognized agents of plant disease and can cause wilt, rot, blight, or canker in a range of different monocotyledonous and dicotyledonous plants (17, 47). Other species of this clade can be primary pathogens of humans and animals (14) or opportunistic human pathogens causing life-threatening infections in immunocompromised patients (166). Interestingly, closely related strains are also able to promote plant growth and yield directly by altering ethylene levels or fixing nitrogen, or indirectly due to the production of antimicrobial compounds that repress soil-borne plant pathogens (166). Furthermore, some strains can act as bioremediation agents by degrading recalcitrant compounds in herbicides and pesticides (158). These observations have received special attention for agricultural purposes given that *Burkholderia* are among the dominant bacterial taxa in the rhizosphere of several important crops, including rice, maize, pea, cotton, and coffee (158, 166).

The *Paraburkholderia* lineage primarily contains nonpathogenic strains with beneficial effects on plants, such as nitrogen fixation in legume root nodules, degradation of aromatic compounds, phosphate solubilization, or induction of stress resistance (153). At least three species isolated from mosses are also capable of producing antifungal compounds that might be useful for biocontrol in agriculture, yet this usage entails risks due to potential virulence for other eukaryotes (28). Finally, *Caballeronia* corresponds to what was previously called the glathei group, which harbors environmental species common in soil or water (3), obligate leaf-nodulating symbionts of several

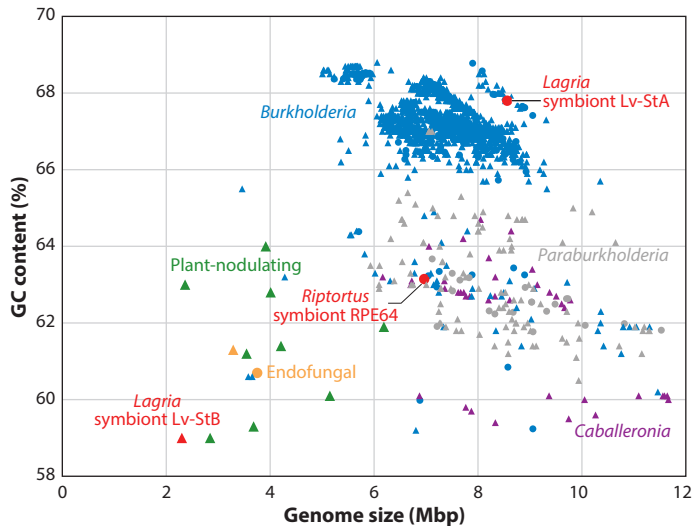


Figure 1

Size and GC content of *Burkholderia*, *Paraburkholderia*, and *Caballeronia* genomes. Data for all annotated genomes available in the NCBI database were included. Triangles represent draft and circles complete genomes. Plant-nodulating (green), endofungal (yellow), and the three available insect symbiont genomes (red) are highlighted as larger symbols.

plant species (126), and most symbiotic strains of true bugs discovered to date, as is described in the following sections.

Given the ongoing taxonomic rearrangements of the genus and for the sake of readability, throughout this review we use *Burkholderia* to refer to the entire clade containing *Burkholderia*, *Paraburkholderia*, *Caballeronia*, *Robbsia*, *Mycetohabitans*, and *Trinickia* and *Burkholderia* s.str. when referring specifically to the new and more confined genus designation.

GENOMIC HALLMARKS OF *BURKHOLDERIA*

Investigating the genomes of a considerable number of *Burkholderia* strains has provided insights into several common traits that are key to understanding the ecology of the group. *Burkholderia* typically have large genomes (on average 7–8 Mb; **Figure 1**) that usually include two or three chromosomes (4, 20). They range in size from 2.3 Mb estimated for a symbiotic strain of an insect (41) up to 11.5 Mb in the soil-living *Burkholderia terrae* BS001 (111). Although there are differences between the genera constituting *Burkholderia* sensu lato (s.l.), their genomes generally have a characteristically high GC content (3) (**Figure 1**). In addition, the high frequency of genomic islands and insertion sequences in many *Burkholderia* strains is thought to be associated with increased functional diversity (12, 41, 102). The multireplicon structure of their genomes is similarly likely to contribute to genomic variability. In bacteria with several replicons, the larger primary chromosome usually carries more essential genes and is evolutionarily conserved, while secondary chromosomes are more variable and can work as evolutionary test-beds (4). For example, the second and third chromosomes of *Burkholderia multivorans* are enriched in genes involved in defense mechanisms and secondary metabolism (122), highlighting the large potential for antibiotic biosynthesis across the group, including many antifungal, antibacterial, herbicidal, and insecticidal compounds (20, 125, 167). It has been estimated that, on average, 7% of the genome is devoted to secondary metabolite production in *Burkholderia* s.l., although there is significant variation in the

different subclades. In strains now corresponding to *Paraburkholderia* and *Caballeronia*, secondary metabolite gene clusters account for 5% of their genomes or less, whereas the percentage in members of *Burkholderia* s.str. can lie around 10% (20). The production of many of these antibiotics, as well as other traits relevant for host-associated lifestyles, including swarming motility, biofilm formation, and the expression of several virulence factors, relies on quorum sensing (27, 54, 112, 157). Although the importance of quorum sensing has been studied in depth mainly in pathogenic *Burkholderia* s.str., the mechanism seems to be widespread across *Burkholderia* s.l. (15, 167).

One of the most appealing characteristics of this bacterial group is the diversity of organisms that its members can associate with, as well as the contrasting roles that they can play, ranging from virulent pathogens to obligate mutualists. Insects have been studied to understand these different facets as naturally occurring hosts of beneficial *Burkholderia* (39, 40, 161), as well as through artificial infections to investigate *Burkholderia*-caused diseases in animals (132, 143). The following sections summarize available information on insect–*Burkholderia* relationships with varying levels of stability and different localizations in the insect body, highlighting established model systems that are especially amenable to manipulative experimentation.

BURKHOLDERIA IN STINKBUGS

Taxonomic Distribution and Evolution of the Stinkbug–*Burkholderia* Symbiosis

The most widespread and intensively studied associations of insects with *Burkholderia* are found within the herbivorous stinkbugs (Hemiptera, Pentatomomorpha) (73). Of the five extant stinkbug superfamilies, the Lygaeoidea (including Idiostoloidea), Coreoidea, and Pyrrhocoroidea contain taxa known to cultivate *Burkholderia* symbionts in specialized crypts or tubules attached to a particular region of the midgut, the so-called M4 region (**Figure 2a**) (73, 75, 154, 155, 161). In the lumen of these organs, the symbionts live extracellularly in dense aggregations (6, 49, 73, 75, 94, 116). Given its widespread occurrence in the midgut-associated crypts of diverse stinkbug families, the symbiosis with *Burkholderia* is assumed to have evolved in the last common ancestor of the Lygaeoidea, Coreoidea, and Pyrrhocoroidea (49, 155). Secondarily, multiple lineages have lost the *Burkholderia* symbionts and transitioned to interactions with other microbes, rendering Pentatomomorpha a particularly diverse insect taxon in terms of its symbiotic associations (**Figure 2b**) (154). One of these transitions occurred within the Pyrrhocoroidea, with the Pyrrhocoridae losing the midgut M4-associated *Burkholderia* in favor of a community of Actinobacteria, Firmicutes, and γ -Proteobacteria localized in the anoxic M3 region of the midgut (155, 156). Within Coreoidea, the family Rhopalidae appears to be devoid of *Burkholderia* (73), but to date, detailed studies on other possible microbial associates of this group are lacking. Finally, symbiotic associations are most dynamic in the Lygaeoidea, with multiple transitions from the *Burkholderia* M4 symbiosis to diverse bacteriome-localized γ -proteobacterial symbionts, notably within the families Artheneidae, Blissidae, Oxyarenidae, Geocoridae, and Lygaeidae (**Figure 2b**) (73, 95, 161). Thus, despite its widespread occurrence, the symbiosis with *Burkholderia* appears to be evolutionarily fragile, experiencing multiple transitions to other symbiotic associations.

Fitness Implications for the Host

The contribution of *Burkholderia* symbionts to their stinkbug hosts' metabolisms remains incompletely understood. Several studies across multiple bug species reported retarded development, impaired survival, and/or reduced adult size in insects that were deprived of their symbionts, indicating a nutritional benefit provided by *Burkholderia* (6, 46, 72, 99, 116, 171). Similar effects were observed for symbiont-depleted stinkbugs of species that are associated with taxonomically

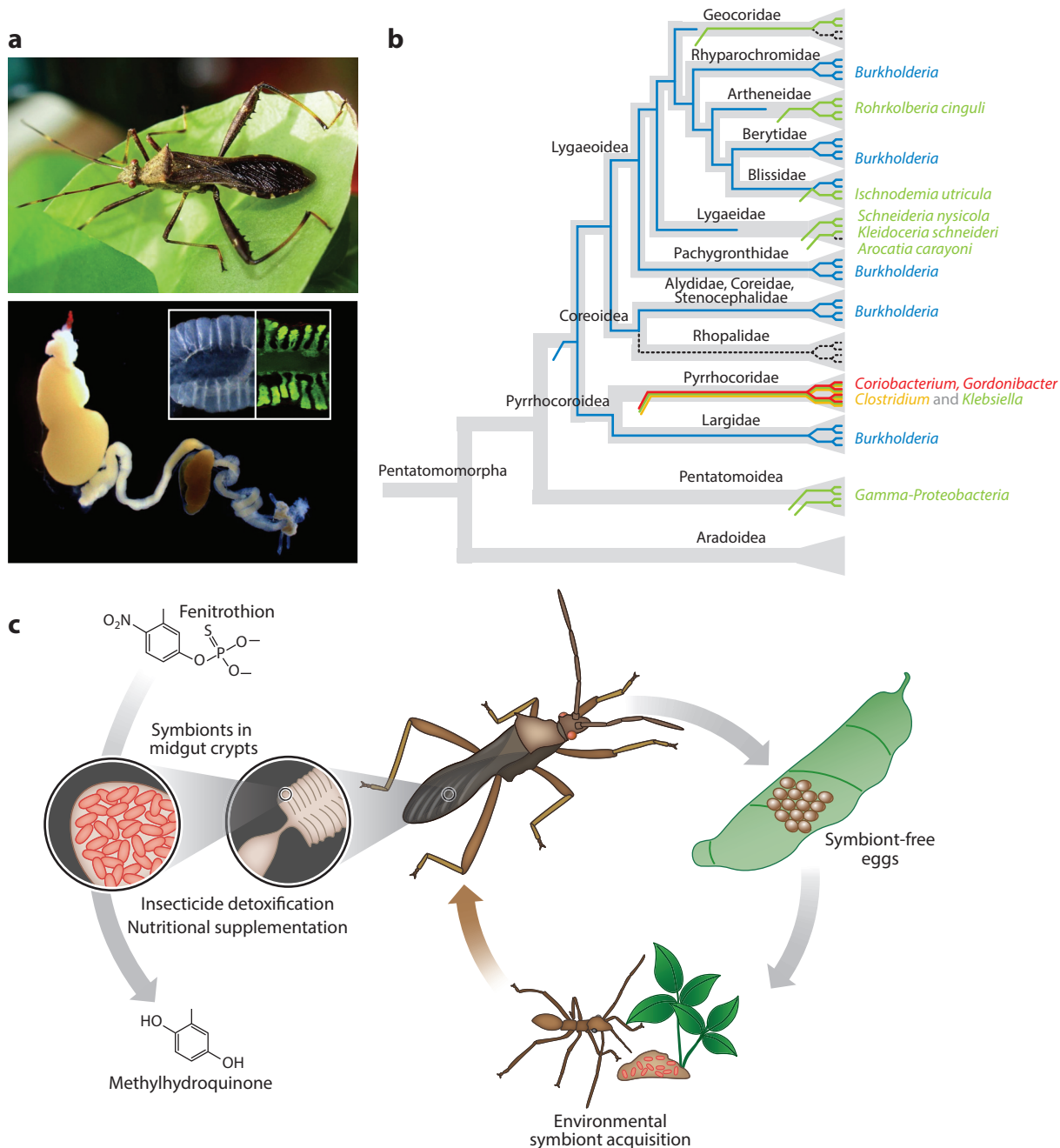


Figure 2

Overview of the symbiosis between *Riptortus* bugs and nutrient-supplementing and detoxifying *Burkholderia* symbionts. (a) Adult *Riptortus pedestris* (top), dissected gut of an adult bug (bottom), and symbiont-containing crypt-region of the M4 (inset) as bright-field and fluorescence micrographs (*Burkholderia* labeled in green). Copyright 2012, National Academy of Sciences (71). (b) Schematic phylogeny of Pentatomomorpha, highlighting the inferred origin and losses of *Burkholderia* symbionts in midgut crypts. Modified from Reference 154, with permission from Cell Press. (c) Life cycle of *R. pedestris*, with environmental acquisition of *Burkholderia* symbionts from the soil in early nymphal instars and nutritional and detoxifying benefits provided by the symbionts to developing and adult insects.

distinct bacterial symbionts (52, 138). Notably, genomic analyses of the γ -proteobacterial symbionts in plant sap-feeding Urostylididae (Pentatomoidea) revealed their potential for supplementing their host's diet with essential amino acids and vitamins (65), a symbiotic function that is widespread among sap-feeding Hemiptera (24). In seed-feeding Pyrrhocoridae (Pyrrhocoroidea), actinobacterial symbionts provision B-vitamins to their hosts that are scarce in the seed-based diet (136). Based on these observations, the *Burkholderia* symbionts of stinkbugs have been hypothesized to likewise contribute to their host's metabolism through the provisioning of essential amino acids and/or vitamins (73), but experimental evidence remains lacking.

While the primary function of *Burkholderia* symbionts in stinkbugs remains elusive, a secondary context-dependent benefit has been reported in the bean bug *Riptortus pedestris* (Hemiptera, Alydidae): These bugs can associate with fenitrothion-resistant *Burkholderia* strains and thereby acquire resistance to this insecticide (**Figure 2c**) (71). Applying fenitrothion to the soil resulted in a strong enrichment of the proportion of fenitrothion-resistant *Burkholderia*, which successfully established a symbiotic infection in exposed bean bugs (58, 71, 159). Importantly, bugs with insecticide-resistant symbionts exhibited significantly enhanced survival upon oral and percutaneous exposure to fenitrothion, presumably providing a strong fitness benefit to the bugs in environments with high pesticide application (71). Under field conditions, however, bugs with insecticide-resistant symbionts were only discovered in a population of *Cavelerius saccharivorus* (Blissidae) that was exposed to heavy fenitrothion spraying over five consecutive years (71). Nevertheless, the observation that over 99% of fenitrothion-degrading bacteria isolated from exposed field soils belong to the genus *Burkholderia* and cluster with the stinkbug symbionts highlights the potential for the rapid emergence of insecticide resistance in pest insects through the acquisition of insecticide-degrading *Burkholderia* symbionts (160).

Symbiont Transmission

An unusual aspect of the stinkbug–*Burkholderia* association is the predominance of horizontal symbiont transmission (**Figure 2c**) (72). This is surprising, as vertical transmission appears to be very common among beneficial insect–microbe symbioses described so far and in many Hemiptera in particular (137). The *Burkholderia* symbionts, however, are acquired de novo from the environment by every host generation (46, 72, 73, 116, 170), akin to the well-known *Aliivibrio* symbionts of marine *Euprymna* squids (114) or the nitrogen-fixing rhizobia of plants (165). The only case of vertical *Burkholderia* transmission in stinkbugs reported to date is the oriental chinch bug (*C. saccharivorus*, Blissidae), where approximately 30% of the hatching nymphs acquire the symbionts from the egg surface (57). In all other cases, the symbionts appear to be exclusively acquired from *Burkholderia*-contaminated soil or plant material (46, 72, 73, 116, 170). In the bean bug, *R. pedestris*, symbiont acquisition occurs predominantly during the second and—to a lesser extent—third nymphal instars (74), and only approximately 80 symbiont cells are enough for a 50% probability of successful infection (76). Thus, efficient screening and filtering mechanisms must exist in the host to selectively acquire and/or enrich the bacterial symbionts in its midgut-associated structures (see below).

The Bean Bug–*Burkholderia* Symbiosis as a Model for Molecular Host–Symbiont Interactions

Investigation of the molecular underpinnings of host–symbiont interactions is often hampered by the difficulties in experimentally and/or genetically manipulating the symbiotic partners. Because of its tractability, however, the *Riptortus*–*Burkholderia* symbiosis has recently emerged as a promising model system to characterize the molecular cross-talk between an insect host and its extracellular symbionts (161). Both host and symbiont are easily culturable in the laboratory

(73, 75), and different host–symbiont combinations can be established by exposing axenically reared bugs to water contaminated with bacterial cultures of interest (72). In addition, multiple symbiont genomes (149, 162) and host transcriptomes (44) have already been sequenced, the symbionts are amenable to standard reverse genetics approaches (70, 82, 83, 86, 161), and candidate host genes can be manipulated by RNA interference (43, 118). Finally, consequences of the infection with different symbionts can be studied in terms of the symbionts' infection success, as well as the host's development and reproduction (71, 79, 89, 99). Studies employing a combination of these techniques have yielded a wealth of information on the host's control over the symbiosis, as well as the symbionts' genetic determinants that affect the outcome of the interaction with the host (82, 83, 86, 161). In particular, targeted mutagenesis of the symbionts and screening of transposon mutant libraries revealed three factors that are important for successful symbiosis establishment and maintenance: motility, cell envelope biosynthesis, and stress response.

Initial experiments on symbiont mutants lacking an N-acetylmuramyl-L-alanine amidase (AmiC) required for peptidoglycan degradation suggested that a single-cell morphology and motility are important for successful colonization of the *Riptortus* midgut (98). Transposon mutagenesis and screening of symbiosis-deficient mutants corroborated this finding and revealed that mutants lacking a flagella are incapable of passing the narrow constricted region (CR) connecting the M3 midgut region to the M4B (115). Interestingly, food particles and nonsymbiotic *Escherichia coli* were also unable to pass the CR, indicating that this region ensures a selective passage of symbiotic microbes (115). Comparative analyses revealed that this symbiont sorting organ evolved in concert with the M4-associated crypts at the base of the clade comprising the Pentatomoidea, Pyrrhocoroidea, Coreoidea, and Lygaeoidea (115). A recent study uncovered a novel mode of motility that the symbionts employ to corkscrew their way through the constricted region by wrapping the left-handed flagella around their body and turning it in a clockwise fashion (90). Thus, this particular way of movement, which is also found in the bioluminescent *Aliivibrio fischeri* symbiont that selectively colonizes bobtail squids, may contribute to the selectivity of the CR and allow the host to distinguish friend from foe (90).

The bacterial cell envelope constitutes the interface that first comes into contact with a host, so cell wall components generally play an integral role in mediating host–microbe interactions (36, 128). In the *Riptortus* symbionts, genes involved in the biosynthesis of different cell wall components have been found to be important in regulating symbiosis establishment and maintenance, as well as interaction with the host's immune system (82, 83). *UppP*, the major phosphatase mediating the dephosphorylation of the lipid carrier undecaprenyl pyrophosphate to derive various cell wall components, provides resistance against lysozyme, as well as osmotic and physical stress in vitro, and is essential for proliferation and the establishment of a normal infection in vivo after reaching the *Riptortus* midgut (84). The core oligosaccharide and O-antigen of lipopolysaccharide (LPS) are also of central importance. In contrast to in vitro cultures, symbiont cells isolated from the *Riptortus* midgut lack the O-antigen and show increased susceptibility to the host's immune effectors (60, 88). Correspondingly, O-antigen-deficient mutants exhibited reduced infectivity in early stages but then proliferated equally well in the midgut and established a normal infection (87). By contrast, the LPS core oligosaccharide turned out to be essential for both successful colonization and proliferation of the symbionts in the bug's midgut (78).

As might be expected, natural and experimental modifications in the bacterial cell wall affect the symbiont's interaction with the host's immune system. Early transcriptomic analyses revealed that many immune genes are differentially expressed across the gut regions and between symbiotic and aposymbiotic insects, with a notable enrichment of known and putative antimicrobial peptide (AMP) genes (44). Some of these immune effectors are effective against the symbiotic *Burkholderia* and may thus play a role in symbiosis regulation during development (77) or

confinement to particular gut regions (60). The M1 region exhibits strong bioactivity against O-antigen-deficient symbiont mutants, which is at least partly due to a food plant-derived (soybean) Kunitz-type trypsin inhibitor (60). Thus, O-antigen is important for successful passage through the anterior midgut regions but is later lost upon infection of the M4 (60, 88). Interestingly, the M4B region also displays antibacterial activity against symbiotic but not against cultured *Burkholderia* cells, and this activity is upregulated upon symbiont presence (80). Based on this observation, a cathepsin-L-like protease was isolated from the M4B lysate and found to be toxic to the symbionts (10). Furthermore, the AMP rip-thanatoin is specifically upregulated in the M4 region of symbiotic bugs after an immune challenge, displays high antimicrobial activity (118), and may be involved in controlling symbiont titers in the M4 region (118).

The interaction of hosts and symbionts also has implications for the systemic immune response of the host: Aposymbiotic bugs displayed lower AMP expression and antimicrobial activity in the hemolymph, resulting in a higher susceptibility to pathogen challenge (85). Likewise, bugs infected with symbiont mutants deficient in the LPS core oligosaccharide were more susceptible to septic bacterial challenge (78). Similarly, symbiotic microbes have been found to be essential for proper development of the host's immune system in other insects, e.g., in tsetse flies (168). Thus, the molecular interplay between hosts and symbionts is necessary not only for successful establishment of the specific symbiotic interaction, but also for the defense of the host against pathogens.

The third aspect found to be important for successful symbiosis establishment and maintenance is related to the symbionts' adaptation to harsh environmental conditions. A comparative analysis of protein patterns between symbiotic *Burkholderia* living in the gut versus the same strain cultured in vitro revealed, under in vivo conditions, increased amounts of phasin (PhaP), a protein associating with polyhydroxyalkanoates (PHA) that serve as a carbon storage in many bacteria and confer resistance to nutritional depletion and other environmental stresses (89). Concordantly, targeted mutagenesis of genes involved in PHA biosynthesis (*phaB* and *phaC*) resulted in cells with reduced amounts of stored PHA, lower resistance to environmental stresses, and impaired colonization of the *Riptortus* midgut, which in turn resulted in reduced adult size of the host (89). Similar effects were observed when knocking out the PhaP biosynthesis negative regulator PhaR or the PhaP gene *phaP3*, but not when the genes encoding other PhaP proteins were impaired (61).

Another important bacterial adaptation for surviving challenging environmental conditions is the formation of biofilms, which is regulated by the level of cyclic di-GMP and, thus, affected by the intracellular purine pool (18). Concordantly, mutations in several purine biosynthesis genes resulted in impaired biofilm formation and low levels of colonization of the bug's midgut crypts (79, 81, 82). In particular, the biofilm-defective *purT* mutant showed lower levels of cyclic di-GMP and reduced population size upon infection in bugs, and the hosts experienced slower growth and reduced adult body weight (81). As both biofilm formation and PHA metabolism constitute adaptations to stressful conditions, the *Riptortus* gut likely provides a challenging environment for the symbionts (82).

SYMBIOTIC *BURKHOLDERIA* IN OTHER HEMIPTERA

While the association of *Burkholderia* with several true bugs (Heteroptera) is the most thoroughly characterized among Hemiptera, members of the bacterial genus have also been found in two other major hemipteran suborders, namely the Auchenorrhyncha and the Sternorrhyncha. A strain closest to *Burkholderia fungorum* was found at frequencies of 21% and 29%, respectively, in two out of four investigated populations of the leafhopper *Macrostelus strifrons* (56). *Macrostelus*

leafhoppers carry *Candidatus*¹ *Sulcia muelleri* and *Candidatus* *Nasuia deltocephalinicola* as obligate bacteriome-associated symbionts, yet five other bacteria including *Burkholderia* were identified in DNA extracts from the same tissues. The *Burkholderia* are also present in the ovaries and are absent in male individuals. Whether these bacteria are consistently vertically transmitted and/or have any significant effect on leafhopper fitness is not known. Among the Sternorrhyncha, two species of scale insects belonging to the Eriococcidae or felt scales harbor *Burkholderia* bacteria in the fat body and ovaries (105). Histological observations in *Gossyparia spuria* and *Acanthococcus aceris* show that the bacteria invade the neck of the ovarioles and then enter the oocyte cytoplasm, providing strong evidence for vertical transmission within the egg. The strains from the two different species are 98% similar to each other based on the 16S rRNA gene, and their closest relatives belong to the *Burkholderia* s.str. clade. While the infection frequency of *Burkholderia* in these or other scale insects has not yet been reported, their specialized localization in these species suggests a close interaction and merits further investigation of potential functional roles.

BURKHOLDERIA IN LAGRIINAE BEETLES

Symbiont Localization and Transmission

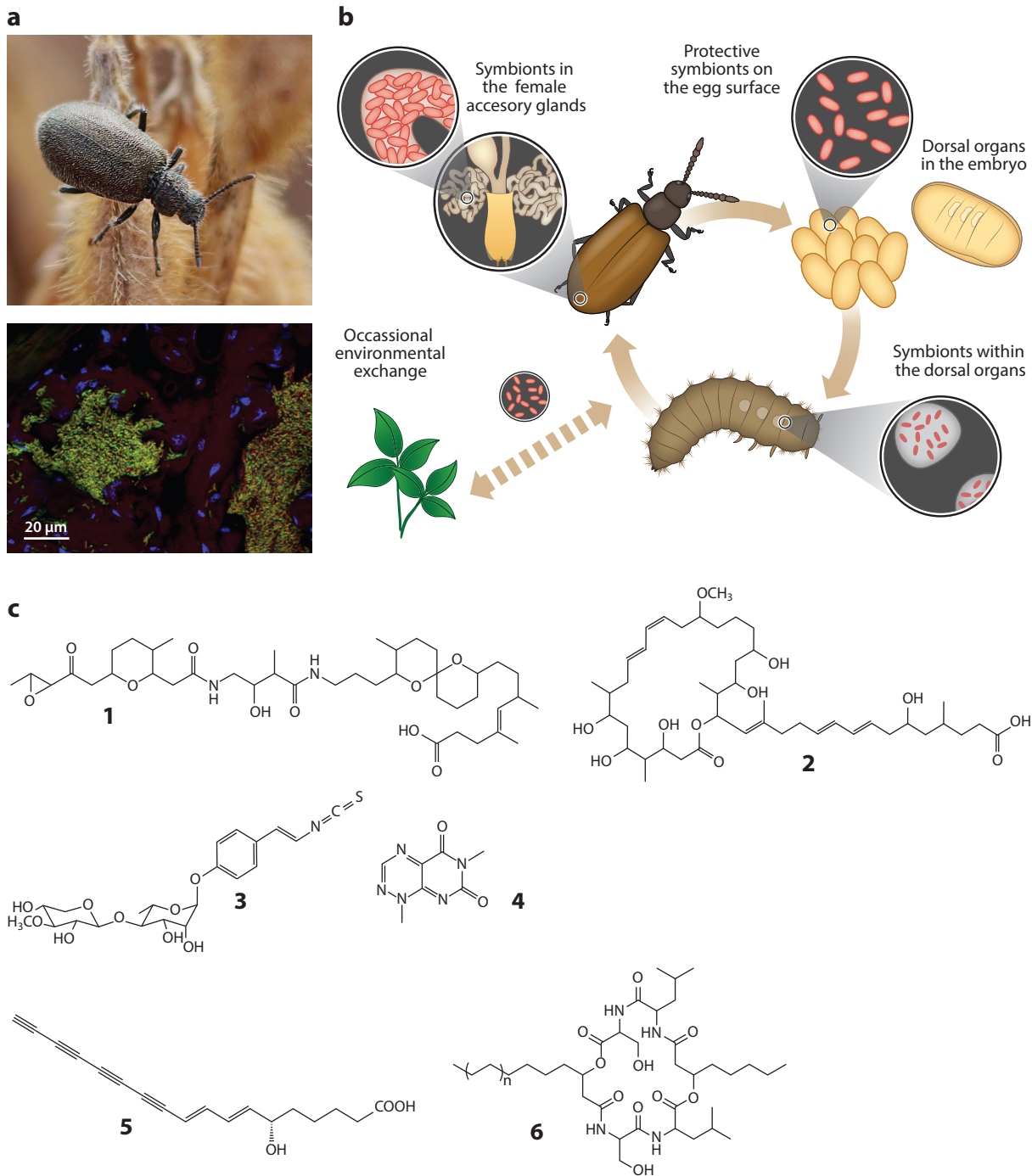
At least six species within the beetle subfamily Lagriinae (Tenebrionidae) host symbiotic *B. gladioli* (40). Adult females harbor the symbionts extracellularly in paired accessory glands of the reproductive system (**Figure 3a**), while the symbionts are absent in adult males. According to morphological observations reported almost a century ago, presumably homologous glands are present in females of at least 83 species of the Lagriinae, formerly Lagriidae (151). Hans Jürgen Stammer, the first to investigate this symbiosis, discovered a remarkable morphological diversity in the organs across different species, including tubular, branched, and lobed structures of varying size (151). In most species, including the European *Lagria hirta*, the symbionts are also found within elongated structures on each side of the oviduct and within the ovipositor. Whether these organs and the glands are populated with *Burkholderia* or other symbionts in all species, and whether they evolved in the context of the symbiosis or were co-opted for symbiont maintenance and transmission, remain to be investigated.

Observations in *L. hirta* and *Lagria villosa* indicate that there is a route for vertical transmission of the symbionts, i.e., from mother to offspring (39, 40, 151): During oviposition, the mother smears the eggs with a *Burkholderia*-containing secretion. The bacteria then enter the egg, presumably through the micropyle, and colonize three cuticular invaginations located dorsally in the embryo (**Figure 3b**), which persist during larval development (151). Structures similar to those in adult Lagriinae females are present in several cerambycid, anobiid, and curculionid beetles harboring yeast or bacterial symbionts. However, Stammer, and later Buchner, referred to the dorsal compartments of the *Lagria* larvae as especially unusual and potentially unique organs for bearing symbiotic bacteria in an insect (9, 151).

Functional Role of the Symbionts

In line with the high bioactive potential of *Burkholderia*, the symbionts of Lagriinae beetles play a key role for defense. Manipulative experiments using *L. villosa* demonstrated that antagonistic fungi readily grow on the beetle eggs if the symbionts are removed, with detrimental consequences for the survival of young larvae (40). *B. gladioli* can produce a range of antifungal and antibacterial secondary metabolites (**Figure 3c**), and the produced compounds differ between symbiont

¹*Candidatus* (Latin for candidate, abbreviated *Ca.*) is used as an interim status in prokaryote nomenclature for an organism that is described but cannot be maintained in a microbiological culture collection.



(Caption appears on following page)

Figure 3 (Figure appears on preceding page)

Overview of the protective *Lagria*–*Burkholderia* symbiosis. (a) *Lagria villosa* adult on a soybean pod (top) and fluorescence in situ hybridization (FISH) micrograph (bottom) on a cross-section of a female accessory gland of the same species, showing *Burkholderia* specifically labeled as an overlay of Burk16S_StB_2-Cy5 (green) and Burk_16S-Cy3 (red) probes, as well as host cell nuclei counterstained with DAPI (blue). Scale bar = 20 μ m. Image courtesy of Rebekka Janke. (b) Symbiont transmission route throughout the life cycle of the beetle. *Burkholderia gladioli* symbionts are represented as red-colored rods. (c) Bioactive compounds produced by strains associated with *L. villosa*. The dominant strain, *B. gladioli* Lv-StB, produces lagriamide (1), while the culturable strain *B. gladioli* Lv-StA produces lagriene (3), sinapigladioside (4), toxoflavin (5), caryoynencin (6), and icosalide (7) (see References 40, 41).

strains. The dominant symbiont strain in *L. villosa* field populations produces lagriamide (1), an antifungal polyketide that closely resembles symbiont-produced defensive compounds found in marine tunicates. The gene cluster involved in the biosynthesis of lagriamide lies on a genomic island and was therefore likely acquired through horizontal gene transfer (41). A second and readily culturable strain isolated from *L. villosa* is able to produce sinapigladioside (4), an antimicrobial substance with rare characteristics among bacterially synthesized chemical structures (40). It contains an isothiocyanate moiety, a chemical group common in plant secondary metabolites known as mustard oils yet unexpected in bacteria. The same *B. gladioli* strain can produce the antibacterial polyketide lagriene (3), the highly reactive caryoynencin (6), as well as toxoflavin (5, 40). Interestingly, the latter compound is associated with virulence of *B. gladioli* and *B. glumae* in plants (97). More recently, the antibiotic icosalide (7) was also identified among the metabolites produced by this strain (23). This lipocyclopeptide has surfactant properties and can inhibit bacterial swarming, which may play a role in host colonization (23). The symbiotic strain isolated from the beetles can also systemically infect soybean plants and cause a reduction in seed production, indicating that it can intimately interact with both insect and plant with opposing fitness effects on the two different hosts (40). This potential for dynamically transitioning between different hosts, as well as engaging in a free-living lifestyle, underscores the ecological versatility that is common among *Burkholderia*.

Diversity of *Burkholderia gladioli* Strains in *Lagnia* Beetles

In *Lagria* beetles, the coexistence of multiple closely related *B. gladioli* strains exhibiting different levels of host dependence is especially intriguing. While the culturable strain from *L. villosa* has a genome size (8.5 Mbp) that is comparable to most free-living *Burkholderia* reported to date, that of the unculturable and dominant strain in this beetle species is estimated to be only approximately one-fourth the size (2.3 Mbp) (Figure 1) (41). Additionally, other uncultured strains from *L. villosa* have genome sizes calculated around 3 and 4 Mbp (41). Genome reduction is common in vertically transmitted bacterial symbionts and has been extensively studied in insects, including intra- and extracellular symbionts (103, 109, 135). There are numerous examples of genome reduction reaching in some cases genomes as small as 112 kb (2). Thus, it is plausible that transmission modes differ between the *Lagria*-associated strains, resulting in gene loss and increased dependence in those that are vertically transmitted. Similarly, the genome of *Burkholderia rhizoxinica*, an obligate and vertically transmitted symbiont of the fungus *Rhizopus microsporus*, has a genome 3.75 Mb in size (96). In addition, several leaf nodule-associated *Burkholderia* symbionts of plant species in the Rubiaceae and Primulaceae families that are also vertically transmitted have genomes as small as 2.4 Mb, which is below that of any environmental *Burkholderia* (Figure 1) (126, 127). In cases of horizontal acquisition by *Lagria* beetles or mixed transmission mode of the strains, competition between the symbionts during host colonization or relocation should determine strain composition. Such a scenario might be comparable to symbiosis establishment by *Aliivibrio* bacteria in *Euprymna* squids, in which strain dominance in the symbiotic organs is defined by a combination

of colonization timing and compartmentalization (5), bacterial aggregation capacity (91), and direct interference between the strains (150). Differences in secondary metabolism might also play an important role in determining the symbiont community structure, since the bioactive potential is relevant for host defense. Thus, selective pressures on the insect host could favor specific *Burkholderia* strain combinations or relative abundances, dependent on the local community of natural enemies (38).

BURKHOLDERIA IN THE GUT MICROBIOTA OF HERBIVOROUS ANTS

In *Tetraponera binghami* ants, a *Burkholderia* strain is part of a bacterial consortium inhabiting a pouch-like organ at the junction between the midgut and the hindgut of the insect (164). The strain is closely related to members of the *Paraburkholderia* clade and resides extracellularly in this structure along with *Rhizobium*, *Methylobacterium*, and *Pseudomonas*, as well as a close relative of *Flavobacterium*. These strains are all related to bacteria involved in root-nodule formation in plants that are capable of nitrogen fixation. Additionally, the bacteria-containing sac is connected to the Malpighian tubules and the tracheae, indicating that they are exposed to nitrogen-rich metabolic waste as well as oxygen, and might therefore take part in nitrogen recycling. A later study on multiple *Tetraponera* species with and without the pouch-like organ showed that several nitrogen-fixing bacteria are predominant in the gut microbiota of these ants, although no *Burkholderia* strains were identified (152). In fact, nitrogen fixation by bacterial symbionts may have been important for adoption of an herbivorous lifestyle in numerous ant species (133). Thus, nitrogen-fixing *Burkholderia* strains that are common in the soil environment and rhizosphere could be suitable gut associates. However, further studies across different ant species are necessary to assess the occurrence and potential roles of *Burkholderia* as members of gut microbial communities in ants.

TRANSIENT BURKHOLDERIA IN INSECTS

There are several other reports on the presence of *Burkholderia* in the microbiota of insects or their nests. While most of these are presumably transient bacteria, their presence suggests exposure of insects to *Burkholderia* in the field. A strain with potent antifungal activity was isolated from 31 out of 57 samples of fungal garden material in *Atta sexdens rubropilosa* ant colonies, as well as from surrounding soil. However, it was not detected when placing leaf material from the nests, nor full ant bodies, directly on a microbial cultivation medium (139). Microbiota characterizations of two *Pseudomyrmex* species and *Formica exsecta* indicate the presence of *Burkholderia* in or on the bodies of the ants (29, 48, 63), yet the relative abundance and consistency across single individuals is unknown. Similarly, among other Hymenoptera, sequence-based microbial community analyses have detected the presence of *Burkholderia* strains in several bee and wasp families (104), and in the nest chambers of the red mason bee *Osmia bicornis* (69). There are additional reports from the guts of carabid (101) and cerambycid beetles (130, 174), as well as moth flies (35). However, the low infection frequencies across different individuals and/or unspecific localization are not indicative of consistent symbiotic associations. Instead, the widespread occurrence of *Burkholderia* in the soil and plants is likely to facilitate contact with the insects.

EVOLUTIONARY ECOLOGY OF BURKHOLDERIA-INSECT SYMBIOSES

Functional Benefits of *Burkholderia* for Insect Hosts

The evident ecological versatility of *Burkholderia* bacteria between and even within the newly defined genera translates into the potential for multiple relevant functional roles as host associates.

As a first example, complementing or optimizing nitrogen, amino acid, and vitamin metabolism with the aid of microorganisms can be particularly useful among herbivorous animals given the limited amounts of nitrogenous compounds in their diet. Insects feeding on wood, like termites (8), or even omnivores, like cockroaches (134) and carpenter ants (37), rely on gut- or bacteriome-associated symbionts to break down uric acid or urea and later integrate the products into amino-acid biosynthesis pathways. Nitrogen fixation might also be a valuable trait in symbionts of phytophagous insects, as suggested by studies on sap-feeding scale insects (19) and bark beetles (108), as well as termites (173). Although there is, to date, no direct evidence that any *Burkholderia* bacteria carry out these roles in an insect, studies on herbivorous ants mentioned above suggest a putative link between *Burkholderia* strains or related Burkholderiales with nitrogen metabolism (53, 133, 164). Given that diazotrophic *Burkholderia* have been found in different environments and hosts (32), and genes encoding for ureases have been identified in others (163), it is plausible that insect-associated *Burkholderia* participate in nitrogen metabolism, as has already been speculated for stinkbugs (73).

Burkholderia and other Burkholderiales are known for carrying genes associated with degradation of an array of aromatic compounds, including several pollutants of primary relevance in contaminated environments (16, 123, 146). Genomic analyses indicate the presence of multiple pathways associated with peripheral and ring-cleavage catabolic activity among these strains (123). For an insect host, one possible advantage of this bacterial ability is acquiring resistance to a specific pesticide, as demonstrated in *R. pedestris* and *C. saccharivorus* stinkbugs (59, 71, 159). Additionally, it might be advantageous for recycling plant-derived carbon of aromatic compounds resulting from the breakdown of lignin, or for detoxifying aromatic defense compounds that are abundant in plant exudates (123). Furthermore, plant-associated *Burkholderia* are known as producers of plant cell wall-degrading enzymes (167), which could confer the ability to access the plant cells' nutritious content and utilize the cell wall polysaccharides as carbon and energy sources to an insect host, as has been demonstrated for other symbiotic bacteria in insects (8, 135).

Last but not least, *Burkholderia* stand out as some of the most prolific producers of bioactive secondary metabolites among bacteria. Multiple strains have received considerable attention from natural product chemists, uncovering novel compounds of potential value for biotechnological or pharmaceutical applications (20, 100, 125, 167). This capacity might have also driven the evolution of symbiotic partnerships with a range of hosts, including fungi, plants, and insects. *B. rhizoxinica*, the intracellular symbiont of the fungus *R. microsporus*, produces the macrocyclic polyketide rhizoxin that causes disease symptoms in rice plants (120). Interestingly, the fungus is not capable of independent reproduction in the absence of the *B. rhizoxinica* symbiont (121), suggesting that this mutualistic strategy to infect a plant has led to a mutually dependent association between the fungus and the bacteria. A second endosymbiont of the *Rhizopus* fungus, *Burkholderia endofungorum*, is responsible for the production of the toxic cyclopeptide rhizonin, but the ecological role of this compound remains unknown (119). In plants, several *Burkholderia* have established symbioses with species within the families Rubiaceae and Primulaceae, in which the secondary metabolism of the bacteria likely plays an important role (126). The symbionts, which reside in leaf nodules and are vertically transmitted via the seeds, have partially eroded genomes yet conserve gene clusters that are thought to play a role in chemical protection of the host plant. Most symbionts of the Rubiaceae synthesize kirkamide, which is toxic to arthropods and thus likely confers an antiherbivore defense (127). *Candidatus Burkholderia crenata*, the symbiont of *Ardisia crenata* (Primulaceae), produces a cyclic depsipeptide that exhibits insecticidal activity and also carries a hypothetical polyketide synthase (PKS) gene cluster adding to its bioactive potential (12). Taken together, *Burkholderia* are candidates for defensive symbiosis given their ample repertoire of bioactive secondary metabolites, as observed in the *B. gladioli* symbiont strains from Lagriinae

beetles. However, it is noteworthy that the risk of toxic effects on the host from such bioactive molecules might hinder mutualistic partnerships. In the *Lagri* beetles, the chemical profile of the two described strains include compounds with antifungal and antibacterial activity that can protect the vulnerable egg stage in the soil (40, 41) yet might also have a negative impact on beetle health. There are demonstrated or assumed cytotoxic effects on animal cells for toxoflavin (97) and isothiocyanates (42), as well as etnangien (55) and bistramide A (131), which are structurally very similar to lagriene and lagriamide, respectively (40, 41). Such a scenario might represent a trade-off for the host and could help explain the presence or absence of multiple *Burkholderia* strains with different bioactivity. In addition, the localization of the bacteria in confined structures in the host might be crucial for the establishment of symbiosis with potentially noxious strains.

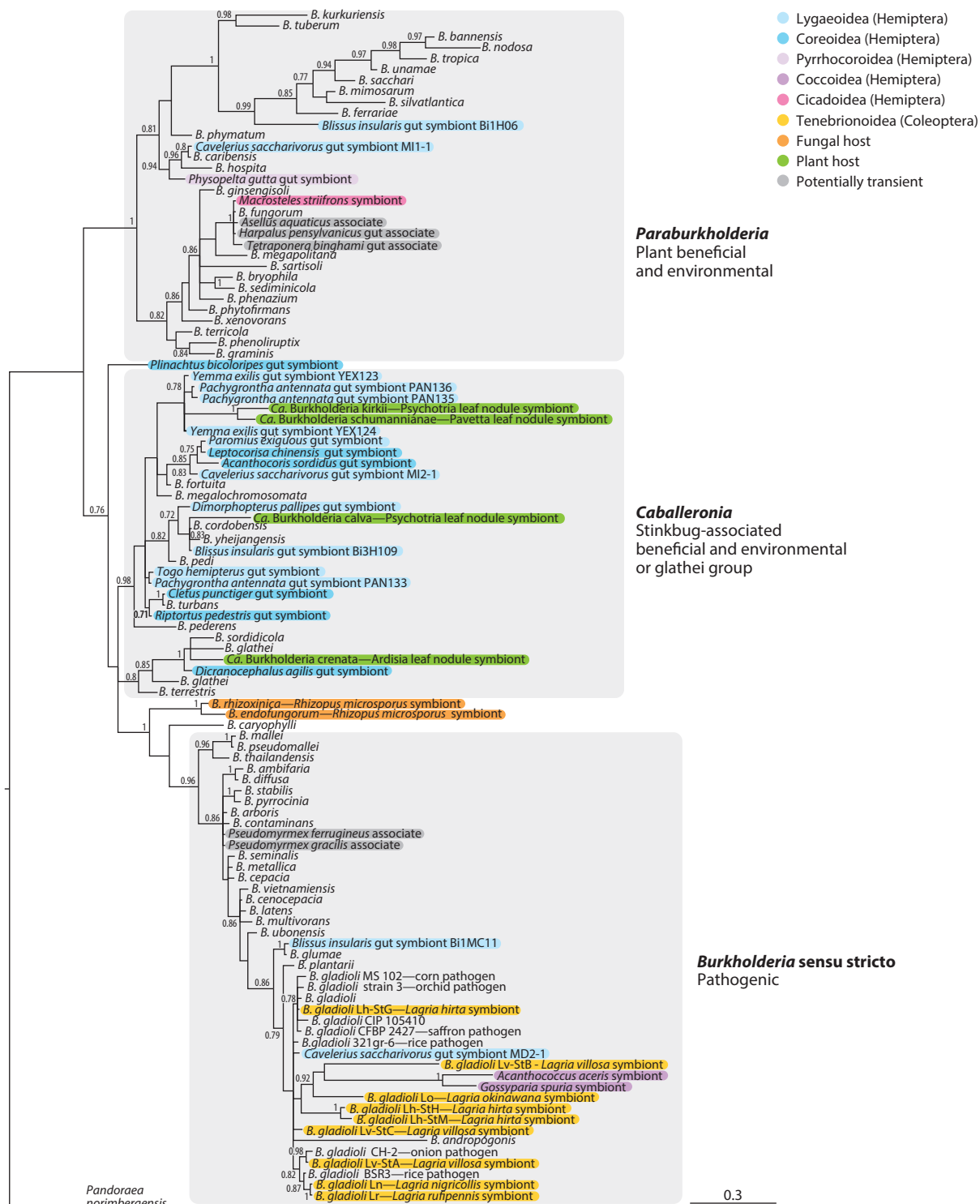
Localization in the Host and Transmission Routes

Most *Burkholderia* symbionts of insects are harbored extracellularly, as occurs in those symbiotically associated with plants. However, members of the group have the machinery to invade, persist in, and replicate in eukaryotic cells (117). While these mechanisms have been mainly investigated in the context of virulence (169), there is also evidence that *Burkholderia* can invade the germline in scale insects without detrimental effects (105) and can be intracellular as mutualists in *Rhizopus* fungi (107). The implications of host cell localization in symbiosis can be profound. An extracellular localization might be favorable to reduce the risk of virulence for the host and might be more suitable for specific symbiont contributions. The site of production and distribution of defensive compounds, for example, could be pivotal to block the entry of pathogens or the attack of predators. In addition, an extracellular localization is in line with more flexible associations, in which symbiont loss or environmental acquisition of different strains seems more plausible and might be of advantage under certain conditions (38, 137). However, ensuring vertical transmission via an intracellular localization in the germ line can be a game-changing event in the evolution of symbiosis. Comparing closely related *Burkholderia* with the potential for intra- and/or extracellular lifestyles in a symbiotic context might be useful for identifying the molecular and ecological factors influencing this transition.

The mechanisms for transmission of *Burkholderia* symbionts in insects are also multifaceted. Most stinkbugs associated with these bacteria appear to fully rely on environmental acquisition (73), while representatives of Blissidae bugs (57) and Lagriinae beetles (39, 40) exhibit a combination of vertical and horizontal transmission. Although conclusive evidence for consistent intraovarian vertical transmission is lacking, it is likely to occur at least occasionally in eriococcids (105) and leafhoppers (56). It is possible that the increased adaptability of *Burkholderia* to diverse environments has contributed to this diversity of transmission modes. The recurrent availability of strains in plants and soil could support the perpetuation of environmental acquisition as a reliable strategy, while the capacity to closely interact with eukaryotes on a molecular level (106, 107) permits more intimate associations involving vertical transmission. At the same time, these characteristics are consistent with the broad host range of *Burkholderia* across three major eukaryotic lineages, namely fungi, animals, and plants, and with the potential for shifts between these, as observed in the *Lagri*–*B. gladioli* symbiosis (40).

Transitions Along the Pathogen–Mutualist Continuum

The three major clades of *Burkholderia* s.l. have been broadly categorized as pathogenic (*Burkholderia* s.str.), plant beneficial and environmental (*Paraburkholderia*), and stinkbug-associated beneficial and environmental (*Caballeronia*), respectively (Figure 4) (3, 22, 34, 140). However, it is widely recognized that the fitness implications of a *Burkholderia* infection for the host can be varied



(Caption appears on following page)

Figure 4 (Figure appears on preceding page)

Phylogenetic reconstruction of selected *Burkholderia* s.l., including insect-associated strains, as well as described symbionts of plants and fungi (highlighted by colored boxes). Strains are highlighted according to host superfamily in the case of insect associates. Gray shadings indicate the recently described genera *Paraburkholderia* and *Caballeronia*, as well as *Burkholderia* s.str., which are well supported by previous phylogenetic analyses based on more extensive genomic data (3). Partial 16S rRNA gene sequences were aligned using the online SINA Aligner (129) and curated using Gblocks 0.91b in Phylogeny.fr (21), resulting in a 1,215 bp alignment. The phylogenetic tree was constructed using MrBayes 3.2 with a GTR substitution model. The analysis was run for 50,000,000 generations, sampling every 1,000 generations, and a burn-in of 5,000 was applied. Posterior probabilities above 70% are reported at the nodes.

and context-dependent within each of these clades, so it is generally difficult to categorize taxa into beneficial and harmful based on their taxonomic affiliation (16, 28). Insects associate with *Burkholderia* s.l. symbionts from all three major clades (**Figure 4**), indicating multiple and dynamic transitions to an insect-associated lifestyle. Interestingly, even though the different stinkbug taxa and *Lagria* beetles have repeatedly recruited novel *Burkholderia* strains from the environment, most host taxa appear to be specialized on one of the *Burkholderia* clades, with the coreoid and lygaeoid stinkbugs being generally associated with *Caballeronia* strains, the bordered plant bugs (Largidae) with *Paraburkholderia*, and the *Lagria* beetles with *Burkholderia* s.str., specifically *B. gladioli* (**Figure 4**). The only exceptions are the two Blissidae bug species *Blissus insularis* and *C. saccharivorus*, which engage in a symbiosis with strains across all three *Burkholderia* s.l. clades (6). Because the predominant lifestyle under natural conditions remains difficult to determine for most *Burkholderia* species, lifestyle transitions are generally challenging to disentangle. Thus, experimental and phylogenetic approaches only allow for cautious speculations. The stinkbug symbionts are acquired from environmental soil isolates in every generation (72, 159), but their closest relatives often include plant pathogenic and/or endophytic strains, suggesting that both soil bacteria and plant associates are sources of symbiosis in stinkbugs (**Figure 4**). By contrast, the *Lagria* beetle symbionts are phylogenetically restricted to the *B. gladioli* clade, which is generally reported to be plant pathogenic, suggesting a dynamic transition between plant pathogenicity and insect defensive mutualism (40). However, recent studies also detected *B. gladioli* strains living endophytically in maize plants without causing any obvious symptoms of disease (64) or even providing protection against plant pathogenic fungi (148). Interestingly, the intracellular symbionts of eriococcids (105) and a symbiont strain from *Cavelerius saccharivorus* (57) are phylogenetically closely related to the *Lagria* symbionts within the *B. gladioli* group, suggesting that plant-associated *Burkholderia* are a common source for the recruitment of insect symbionts.

Given the difficulties in predicting the predominant lifestyle of *Burkholderia* taxa based on their phylogenetic affiliations, a detailed understanding of the molecular mechanisms governing their interactions with plants and animals is required to decipher transitions between pathogenicity and mutualism. Due to their medical and agricultural relevance, a multitude of virulence factors of human and plant pathogenic *Burkholderia* has been elucidated, including secretion systems (type II, III, IV, and VI), quorum sensing machinery, surface oligosaccharides, motility factors, extracellular enzymes, and secondary metabolites (26, 45, 110). Although the genes underlying some of these virulence factors (e.g., the secretion systems, extracellular enzymes, and bioactive secondary metabolites) are enriched in the pathogenic *Burkholderia* s.str. clade (1, 28), most also occur in plant beneficial and/or environmental strains, suggesting that the mechanisms underlying mutualistic and pathogenic associations are closely related (7, 50). This may not be entirely surprising, as both pathogens and mutualists need to reach, enter, and colonize their host and overcome or evade its immune defenses (7). In this context, it is tempting to speculate that some virulence factors mediating colonization and establishment of a pathogenic infection in one particular host may not only preadapt the strain to colonize other host taxa as well, but even exert beneficial effects in a different host. Concordantly, the arsenal of bioactive secondary metabolites produced by *B. gladioli*

promotes plant virulence (62, 147) but also provides defense to insects (40, 41) and, possibly, plants (148) against detrimental fungi. Thus, although the ability to infect certain hosts is reflected in specific adaptations, these adaptations may expand rather than constrain host range, as well as the potential for both benign and detrimental infections. This versatility in engaging with a multitude of different hosts likely contributes to the ecological success of *Burkholderia* and their dynamic transitions along the pathogen-to-mutualist continuum on ecological and evolutionary timescales.

What Is Special—or Not—About *Burkholderia* as Symbionts?

The characteristics of *Burkholderia* symbioses with insects and other eukaryotes discussed above raise the question of whether *Burkholderia* is special among bacteria in its metabolic and ecological versatility, its transmission routes, and the types of interactions it engages in (mutualistic and pathogenic). This versatility is reminiscent of many Actinobacteria, in particular the genus *Streptomyces* (51), which is likewise abundant in terrestrial and aquatic environments and also interacts with plants and animals (145). Like the *Lagria*-associated *Burkholderia*, members of the genus *Streptomyces* confer protective benefits upon different insect hosts (13, 66, 145), including solitary beewolf wasps (30, 67, 93), leaf-cutter ants (141), and bark beetles (142). Concordantly, both *Burkholderia* (in particular the genus *Burkholderia* s.str.) and *Streptomyces* are of considerable pharmaceutical interest due to the production of a large number of bioactive secondary metabolites and extracellular enzymes (13, 167). Interestingly, the symbiotic associations between insects and *Streptomyces*, as well as the related genus *Pseudonocardia*, share additional characteristics with most *Burkholderia*-insect symbioses, in particular the extracellular localization and the potential for horizontal transmission on ecological or evolutionary timescales (11, 68, 92). Conceivably, the abundance of *Streptomyces*, *Pseudonocardia*, and *Burkholderia* in the soil facilitates environmental acquisition and horizontal exchange between host individuals and species. Additionally, the large genomes of all three genera and the capability to survive unfavorable environmental conditions by forming spores (51) or biofilms (81) and/or by producing storage polymers (89) likely enhance the chances for encountering novel hosts, providing the opportunity for dynamic associations with different host taxa (66).

The versatility of *Burkholderia* is also reflected in its pathogenic and mutualistic associations with host taxa from three different kingdoms: plants, animals (including vertebrates and invertebrates), and fungi (17, 166). Although other bacterial taxa, including *Rickettsia* (124), some *Enterobacteriaceae* (e.g., 52), and some Actinobacteria (145) show similarly broad host ranges, the occurrence of intra- and extracellular lifestyles, as well as vertical and horizontal transmission routes in both animals and plants, highlights *Burkholderia* as a particularly versatile group. This is especially true given that specific environmental symbiont acquisition routes are unusual in insects (137), while vertical transmission is generally thought to be rare in plants (17). As discussed above, these diverse associations entail the potential for a multitude of benefits to the respective host, including metabolic, digestive, detoxifying, and defensive functions, but can also be detrimental to host fitness in pathogenic interactions, with frequent and dynamic transitions along this continuum. Such phenotypic or genotypic transitions between mutualism and parasitism also occur in other animal (31, 113, 124, 144) and plant (50) associates and provide interesting glimpses into the often context-dependent outcomes of interactions between eukaryotes and bacteria.

CONCLUSIONS AND PERSPECTIVES

Symbiotic microbes fundamentally affect the physiology, ecology, and evolution of multicellular organisms, and insects present valuable model systems to understand the functions, ecological and evolutionary dynamics, and molecular interactions of symbiotic associations. In most cases,

however, particular host and symbiont taxa are confined in the ecological and evolutionary characteristics that they represent, limiting conclusions about general principles underlying the nature and dynamics of symbiotic alliances. Bacteria of the genus *Burkholderia* s.l. constitute an interesting group, as they engage in mutualistic and pathogenic associations with animals, plants, and fungi, and the associations with insects in particular cover a large fraction of the diversity of functions, localizations, and transmission routes represented in insect symbioses. As such, these interactions can help to assess the costs and benefits associated with vertical versus horizontal transmission, to understand the dynamics of host switching and lifestyle transitions between mutualism and pathogenicity, and to gain deeper insights into the early stages of genome erosion upon the evolution of a host-dependent lifestyle. In addition, several of the insect–*Burkholderia* symbioses are suitable model systems to decipher the molecular basis of host–symbiont interactions and partner choice, as the symbioses are dynamic in nature, can be experimentally manipulated, and are genetically tractable.

SUMMARY POINTS

1. Bacteria of the genus *Burkholderia* s.l. are characterized by large genomes, high metabolic versatility and ecological flexibility. They occur in aquatic and terrestrial environments, as well as in association with animals, plants, and fungi.
2. *Burkholderia* symbionts are widespread in stinkbugs, where they are predominantly acquired from the environment in every generation and can enhance the hosts' fitness by providing nutritional supplementation and/or detoxifying capabilities.
3. Due to its experimental and genetic tractability, the bean bug (*R. pedestris*)–*Burkholderia* symbiosis yields contributions that are valuable for understanding the molecular mechanisms that regulate establishment and maintenance of symbiosis.
4. In darkling beetles of the subfamily Lagriinae, multiple *B. gladioli* strains provide protection to the beetle eggs against pathogenic fungi by producing an arsenal of bioactive secondary metabolites.
5. The stinkbugs', Lagriinae's, and other insects' associations with *Burkholderia* span a considerable diversity of symbiont functions, localizations, and transmission routes, providing insights into the ecological and evolutionary implications of these symbiotic characteristics.
6. Different *Burkholderia* symbionts of insects are phylogenetically related to environmental, plant pathogenic, plant beneficial, and animal pathogenic bacteria, providing the opportunity to study lifestyle transitions in bacteria.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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