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The genus *Sodalis* as a resource for understanding the multifaceted evolution of bacterial symbiosis in insects

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Abstract
Insects can establish a variety of symbiotic associations with bacteria that can have a significant impact on their evolutionary ecology. Some bacterial lineages are particularly pervasive as symbiotic associates. This is the case of the *Sodalis* genus, whose members have established independent, maternally transmitted symbioses in diverse insect taxa. The first members of the genus were isolated and studied some thirty years ago in tsetse flies, where they evolved as heritable facultative symbionts. Since then, numerous symbiotic associations involving members of the genus have been documented, some of which have evolved into strictly host-dependent mutualistic associations. The genus also includes members circulating freely in the environment, which can be pathogenic, have extensive metabolic capabilities and constitute a potential reservoir of new insect symbionts. In this review, we cover more than thirty years of literature to highlight how the diversity of the *Sodalis* genus described so far embodies the different degrees of host dependence and anatomical integration that bacteria can experience over the course of their evolution with insects. We discuss the propensity of *Sodalis* bacteria to embrace an endosymbiotic lifestyle, how this feature can be used to understand the nascent stages of bacterial endosymbiosis, and how *Sodalis* bacteria can be used to address fundamental and applied research issues. Throughout the review, emphasis is placed on research gaps that need to be filled to better address these aspects. We also draw attention to previously overlook facets of the genus that deserve further investigation, such as the potential role of *Sodalis* bacteria in wood digestion in certain insects, or the nature of their interaction with plants.

Keywords Bacterial mutualism · Endosymbiosis · Facultative symbiont · Pathogen · Pectobacteriaceae · Trypanosome

1 Introduction

Insects are engaged in a wide range of interactions with symbiotic bacteria. These prokaryotic partners can play an important role in the evolutionary ecology of these invertebrates, as they can affect host phenotypes in a variety of ways (Zientz et al. 2004; Feldhaar 2011; Engel and Moran 2013; Sudakaran et al. 2017). The nature of these interactions can be diverse and evolve rapidly along the parasitism-mutualism continuum, particularly as a function of ecological context (Zytynska et al. 2021; Kaur et al. 2021; Drew et al. 2021). While some insect symbionts are transient partners, others have become host-dependent associates that pass faithfully from one host generation to the next by vertical transmission (Frago et al. 2020). Depending on the host insect's degree of dependence on heritable symbionts, these bacterial associates are generally classified as obligate or facultative. Obligate symbionts are essential for the survival and reproduction of their host, providing them with vital nutrients that are deficient in the host's diet (Zientz et al. 2004) or ensuring the feasibility of specific developmental mechanisms (Dedeine et al. 2001). Facultative symbionts, on the other hand, are not essential to the survival and reproduction of their host but can affect their adaptative phenotypes by producing beneficial or detrimental effects whose expression is conditioned by the ecological context, or by selfishly manipulating their reproduction (Oliver et al. 2010; Zytynska et al. 2021; Kaur et al. 2021). The

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diversity of these symbiotic associations is also reflected in the multiplicity of host tissues that symbiotic bacteria can colonize: while certain symbionts reside on the insect surface (ectosymbionts) (Bruner-Montero et al. 2021; Ganesan et al. 2023), others evolve within the host body (endosymbionts), sometimes living intracellularly in specialized host cells called bacteriocytes (Baumann 2005; Engel and Moran 2013). Moreover, other symbionts can colonize the host gut and other tissues (Salem et al. 2017; Pons et al. 2022a).

Over the past two decades, the sequencing of host and symbiont genomes from a myriad of symbiotic systems has revealed the extraordinary functional diversity of insect-bacterial interactions (Chellappan and Ranjith 2021). Studies of insect symbiont diversity have highlighted the propensity of some bacterial genera and species to embrace endosymbiosis, often members of the class Gammaproteobacteria and in particular Enterobacterales, an order that includes many pathogenic bacteria (Moran et al. 2008). This suggests that heritable symbionts derive from pathogenic bacteria whose virulence has faded during their evolution towards a host-dependent lifestyle. Some genera and species of symbionts are associated exclusively with specific insect groups. This is typically the case for many obligate nutritional symbionts such as *Buchnera aphidicola* (Gammaprotobacteria) found only in aphids, even though it is related to the Erwiniaceae family, which includes many plant pathogens (Nováková et al. 2013). These nutritional partners acquired tens of millions of years ago, have undergone significant divergence from their original progenitors and tend to form well-defined monophyletic clades. Conversely, some symbiont genera and species evolve with a wider range of host species, with which they have established a wide variety of associations along the parasitism-mutualism continuum. *Wolbachia pipientis* (Alphaproteobacteria) is arguably the most iconic species that illustrates the propensity of some symbionts to exploit a wide range of phylogenetically distant insect hosts. Indeed, *Wolbachia* is formally the only species in the genus, but is present in over 60% of insect species and is featured by a diversity of strains with distinct associated phenotypes, ranging from parasitism to mutualism (Weeks et al. 2007; Zug and Hammerstein 2015; Landmann 2019).

However, beyond *Wolbachia*, other bacterial genera and species have proved remarkably pervasive in insect populations (Jousselin et al. 2013; Tláskal et al. 2021; Pons et al. 2022b). This is the case of the *Sodalis* symbionts (family Pectobacteriaceae, order Enterobacterales, class Gammaproteobacteria), which were first isolated from tsetse flies more than thirty years ago (Welburn et al. 1987), and whose ubiquity and diversity in insects have been repeatedly demonstrated in recent years. For instance, the genus includes members that are free-living (i.e., live independently of the insect host), some of which may even be opportunistic pathogens for humans (Dale and Maudlin 1999; Clayton et al. 2012; Chari et al. 2015; Tláskal et al. 2021), but also members that have established symbiotic associations with insects, either facultative (Toh et al. 2006; Arp et al. 2014; Matsuura et al. 2014), or obligate (Heddi et al. 1999; Husnik and McCutcheon 2016; Santos-Garcia et al. 2017). This diversity reflects the different degrees of host dependence and anatomical integration that insect symbionts can experience over the course of their evolution.

The present article focuses on the diversity of the *Sodalis* genus. Covering more than thirty years of literature, it aims to provide an overview of the genus' tremendous diversity across the broad spectrum of interactions in which *Sodalis* bacteria can be involved, ranging from pathogenicity to mutualism and addressing the evolutionary transition from a free-living to a host-dependent lifestyle. This review focuses on what the multifaceted nature of *Sodalis* bacteria can tell us about the genesis of mutualistic symbiotic associations and their evolution, how symbiotic bacteria bypass host immunity to establish long-lasting relationships, and the role symbionts can play in the transmission of animal and human pathogens. We begin by briefly introducing the publicly available genomic resources for *Sodalis* members, which provides valuable information for exploring endosymbiosis evolution and will serve here as a roadmap for discussing the diversity of the genus and its evolution. We start with strains facultatively associated with insects, historically the first to have been discovered, and end with the free-living strains that have come to light more recently. Although *Sodalis* symbionts are pioneering models for the study of endosymbiosis in insects, there are still many gaps in knowledge regarding the exact nature of the associations these symbionts maintain with their insect hosts. We discuss these gaps and propose new avenues that leverage *Sodalis* members to grasp the many facets of bacterial symbiosis in insects.

2 Diversity and evolution of *Sodalis* bacteria: overview

The genus *Sodalis* has recently been classified in the family Pectobacteriaceae (Proteobacteria; Gammaproteobacteria, Enterobacterales), which consists mainly of plant pathogens, many members of which are characterized by their ability to hydrolyze pectin of the plants they infect (Adeolu et al. 2016; Motyka et al. 2017). *Sodalis* has repeatedly succeeded in entering new hosts to form maternally transmitted symbioses, and occurs in a wide variety of insect taxa, some of which are very distant from each other. Members of the genus were first described as symbiotic partners of tsetse flies (Diptera: Glossinidae) (Welburn et al. 1987), and

have subsequently been identified in various insect orders, involved in either facultative associations or obligate mutualistic associations (Nováková and Hypša 2007; Fukatsu et al. 2007; Toju et al. 2010; Grünwald et al. 2010; Kaiwa et al. 2010; Toju and Fukatsu 2011; Boyd et al. 2016; Santos-Garcia et al. 2017; Rubin et al. 2018). Phylogenetic analyses indicate that these infections are the result of independent events, most likely through horizontal insect-to-insect transfer and/or acquisition from free-living strains in the insect's immediate environment (Snyder et al. 2011; Clayton et al. 2012). In particular, the scenario of direct environmental acquisition is supported by the recent isolation of several free-living strains from non-insect material (Clayton et al. 2012; Tláskal et al. 2021). While certain *Sodalis*-allied insect symbionts are engaged in a long coevolution with their host, others have been acquired relatively recently, probably from free-living isolates, making the diversity of the genus a valuable resource for illuminating the different scenarios of bacterial symbiosis evolution in insects, in particular the transition from a free-living to a host-dependent lifestyle and from facultative to obligate symbiosis.

Sequencing and annotating the genome of bacterial symbionts is a pivotal step in deciphering the nature of the interactions in which they are involved and in sketching out the history they have with their host (Perreau and Moran 2022). To date, twenty-one genome assemblies referenced as belonging to the genus *Sodalis* are publicly available on the NCBI database. Table 1 documents the basic information of these assemblies, with the genomes ranked by size from largest to smallest. It should be noted, however, that the genus *Sodalis* may include other members whose genomes have been sequenced, but which have not been officially classified in the genus (Sloan and Moran 2012; Husnik and McCutcheon 2016; Santos-Garcia et al. 2017). This indicates that the taxonomy of the genus is not fully resolved. The diversity in genome size among *Sodalis* members reflects different degrees of genomic reduction, a typical process undergone by heritable symbionts, starting with a formerly free-living bacterium towards a host-dependent lifestyle involving a progressive genome remodeling (Fig. 1) (Moran and Bennett 2014). Free-living members are featured by a large genome (about 6 Mb) while ancient obligate symbionts have extremely small genomes, which can be less than 1 Mb. The genomes of facultative and recent obligate symbionts show an intermediate value.

The diversity of available *Sodalis* genomes provides a valuable data resource to examine the evolution of bacterial symbioses in insects (Santos-Garcia et al. 2017). We performed a maximum likelihood phylogenetic analysis (1000 bootstraps) with the 21 assembled genomes of *Sodalis* bacteria publicly available in NCBI (Fig. 2). We included in the analysis other bacterial species that are not officially part of the genus but are considered "*Sodalis*-allied symbionts" (Sloan and Moran 2012; Husnik and McCutcheon 2016; Santos-Garcia et al. 2017). Phylogenomic analysis shows no evidence of co-speciation events: *Sodalis* symbionts associated with taxonomically close insect lineages do not form distinct clades and are scattered throughout the tree, suggesting that symbioses with *Sodalis* bacteria are the result of multiple independent acquisitions in different insect lineages, as previously reported (Husnik and McCutcheon 2016; Santos-Garcia et al. 2017). This raises the question of how *Sodalis* bacteria are acquired, their ability to pass from one insect lineage to another and to undergo horizontal transfers. Members of the genus *Sodalis* are also remarkable for exhibiting highly variable degrees of anatomical integration with their host (Table 1): while some members live independently of an insect host, others are host-dependent. Free-living strains of *Sodalis* can be cultured, providing access to experimental approaches to examine the processes governing the establishment of new symbiotic associations and their functioning, but also to develop paratransgenic approaches for disease vector control (Maltz et al. 2012; Enomoto et al. 2017; Munoz et al. 2020; Elston et al. 2022).

3 *Sodalis* bacteria as facultative symbionts of insects

3.1 *Sodalis glossinidius*: a facultative associate of tsetse flies

3.1.1 An invasive heritable symbiont

The genus *Sodalis* was first established in the context of the study of the microbiota of tsetse flies (Welburn et al. 1987; Beard et al. 1993; Aksoy et al. 1997; Dale and Maudlin 1999). These insects are the vectors of trypanosomes, which cause Human African Trypanosomiasis (HAT, sleeping sickness) and Animal African Trypanosomiasis (AAT, Nagana). Tsetse flies (*Glossina* sp.) harbor an obligate nutritional symbiont, *Wigglesworthia glossinidia* (Gammaproteobacteria; Enterobacterales; Erwiniaceae), which supplements their host blood meal with folate (vitamin B_9) and other B vitamins that are deficient in vertebrate blood (Aksoy 1995; Akman et al. 2002; Michalkova et al. 2014; Snyder and Rio 2015). *W. glossinidia* has a highly eroded genome (0.72 Mb) containing only those genes strictly necessary for the functioning of the symbiotic association, and resides intracellularly in bacteriocytes, which form the bacteriome organ in the anterior midgut, although it can also reside extracellularly in the milk gland lumen (Rio et al. 2012; Balmand et al. 2013) (Fig. 3). *W. glossinidia* is universally present in tsetse flies and is essential for multiple aspects of their

Table 1 General features of *Sodalis* strains ranked by genome size (largest to smallest). **S. ligni* (strain 159R), *S. glossinidius* (strain M1) and *S. praecaptivus* (strain HS1) are publicly available in pure culture (https://www.dsmz.de/). At the bottom of the table, highlighted in blue, are bacterial species that do not formally belong to the genus *Sodalis*, but which are considered as "*Sodalis*-allied symbionts"

Host organisms	Intraspecific name	Genome size (Mb)	Life-style/ symbiotic status	Host	Symbiotic niche	Proven cultivability	GenBank assembly accession n°	Reference
Sodalis ligni	dw_23	6.44	Free-living bacterium	Deadwood habitat	Extracellular	Yes: in pure culture	GCA_016865525.2	Tláskal et al. (2021)
Sodalis ligni	159R	6.38	Free-living bacterium	Deadwood habitat	Extracellular	Yes: in pure culture*	GCA_004346745.1	Tláskal et al. (2021)
Sodalis ligni	dw_96	5.93	Free-living bacterium	Deadwood habitat	Extracellular	Yes: in pure culture	GCA_018449575.1	Tláskal et al. (2021)
Sodalis praecaptivus	HS1	5.16	Free-living bacterium	Human wound	Extracellular	Yes: in pure culture*	GCA_000517425.1	Oakeson et al. (2014)
Sodalis pierantonius	SOPE	4.51	Obligate symbiont	*Sitophilus oryzae* (weevil)	Intracellular (in bacteriocytes)	No	GCA_000517405.1	Oakeson et al. (2014)
Sodalis glossinidius	morsitans B4	4.31	Facultative symbiont	*Glossinidius morsitans* (tsetse fly)	Extracellular	Yes: in pure culture*	GCA_900004845.1	Goodhead et al. (2020)
Sodalis glossinidius	morsitans	4.29	Facultative symbiont	*Glossinidius morsitans* (tsetse fly)	Extracellular	Yes: in pure culture*	GCA_000010085.1	Toh et al. (2006)
Sodalis endolongispinus	SOD	3.73	Obligate symbiont	*Pseudococcus longispinus* (mealybug)	Intracellular (in *Tremblaya* cells, in bacteriocytes)	No	GCA_018777395.1	Garber et al. (2021)
Sodalis sp.	TME1	3.42	Presumed obligate symbiont	*Llaveia axin axin* (scale insect)	Unknown (presumably in bacteriocytes)	No	GCA_001879235.1	Rosas Pérez et al. (2017)
Sodalis sp.	SoCistrobi	3.07	Obligate symbiont	*Cinara strobi* (aphid)	Unknown (presumably in bacteriocytes)	No	GCA_900143145.1	Manzano-Marin et al. (2018)
Sodalis sp.	spu	2.24	Obligate symbiont	*Philaenus spumarius* (spittlebug)	Intracellular (in bacteriocytes)	No	GCA_002261105.1	Ankrah et al. (2018)
Sodalis sp.	SPI-1	2.19	Presumed obligate symbiont	*Proechinophthirus fluctus* (seal louse)	Intracellular (in bacteriocytes)	No	GCA_001602625.1	Boyd et al. (2016)
Sodalis baculum	HBA	1.62	Obligate symbiont	*Henestaris halophilus* (seed bug)	Intracellular (in bacteriocytes)	No	GCA_900161835.1	Santos-Garcia et al. (2017)
Sodalis sp.	IL	1.57	Obligate symbiont	*Bactericera trigonica* (psyllid)	Intracellular (in bacteriocytes and intranuclear)	No	GCA_003668825.1	Ghosh et al. (2020)
Sodalis sp.	PSPU	1.39	Obligate symbiont	*Philaenus spumarius* (spittlebug)	Intracellular (in bacteriocytes)	No	GCA_000647915.1	Koga et al. (2014)
Sodalis sp.	Fle	1.38	Obligate symbiont	*Formica lemani* (ant)	Intracellular (in bacteriocytes)	No	GCA_024748575.1	Jackson et al. (2022)
Sodalis sp.	Fse	1.37	Obligate symbiont	*Formica selysi* (ant)	Intracellular (in bacteriocytes)	No	GCA_024748555.1	Jackson et al. (2022)
Sodalis sp.	Ffu	1.37	Obligate symbiont	*Formica fusca* (ant)	Intracellular (in bacteriocytes)	No	GCA_024748595.1	Jackson et al. (2022)
Sodalis sp.	Ppy	1.15	Obligate symbiont	*Plagiolepis pygmaea* (ant)	Intracellular (in bacteriocytes)	No	GCA_024648725.1	Jackson et al. (2022)
Sodalis sp.	Psp	1.15	Obligate symbiont	*Plagiolepis sp.* (ant)	Intracellular (in bacteriocytes)	No	GCA_024648745.1	Jackson et al. (2022)

Table 1 (continued)

Host organisms	Intraspecific name	Genome size (Mb)	Life-style/ symbiotic status	Host	Symbiotic niche	Proven cultivability	GenBank assembly accession n°	Reference
Sodalis sp.	CWE	0.80	Presumed obligate symbiont	*Columbicola wolffhuegeli* (feather feeding louse)	Unknown (presumably in bacteriocytes)	No	GCA_019646055.1	Alickovic et al. (2021)
Secondary endosymbiont of *Ctenarytaina eucalypti*	Ceuc_S	1.4	Obligate symbiont	*Ctenarytaina eucalypti* (psyllid)	Unknown (presumably in bacteriocytes)	No	GCA_000287335.1	Sloan and Moran (2012)
Secondary endosymbiont of *Heteropsylla cubana*	Hcub_S	1.1	Obligate symbiont	*Heteropsylla cubana* (psyllid)	Unknown (presumably in bacteriocytes)	No	GCA_000287355.1	Sloan and Moran (2012)
Gullanella endobia	FVIR	0.94	Obligate symbiont	*Ferrisia virgata* (mealybug)	Unknown (presumably in *Tremblaya* cells, in bacteriocytes)	No	GCA_900048045.1	Husnik and McCutcheon (2016)
Doolittlea endobia	MHIR	0.85	Obligate symbiont	*Maconellicoccus hirsutus* (mealybug)	Intracellular (in *Tremblaya* cells, in bacteriocytes)	No	GCA_900039485.1	Husnik and McCutcheon (2016)
Hoaglandella endobia	TPER	0.64	Obligate symbiont	*Trionymus perrisii*	Unknown (presumably in *Tremblaya* cells, in bacteriocytes)	No	GCA_900044015.1	Husnik and McCutcheon (2016)
Moranella endobia	PCIT	0.54	Obligate symbiont	*Planococcus citri*	Unknown (presumably in *Tremblaya* cells, in bacteriocytes)	No	GCA_000219175.1	Husnik and McCutcheon (2016)
Mikella endobia	PMAR	0.35	Obligate symbiont	*Paracoccus marginatus*	Intracellular (in *Tremblaya* cells, in bacteriocytes)	No	GCA_900048045.1	Husnik and McCutcheon (2016)

biology, including digestion, reproduction, and immunity (Pais et al. 2008; Attardo et al. 2020). However, tsetse flies can harbor additional endosymbionts, including the reproductive manipulator *W. pipientis* that infects germ cells (Alam et al. 2011), and *Sodalis glossinidius*, a relatively recently facultative symbiont that can infect various host tissues (Dale and Maudlin 1999; Toh et al. 2006).

Historically, *S. glossinidius* was the first reported member of the genus and the first insect endosymbionts to be isolated and cultured, first in the C6/36 cell line of *Aedes albopictus* (Welburn et al. 1987), then in pure culture (Dale and Maudlin 1999). The *S. glossinidius* genome is 4.3 Mb in size (Toh et al. 2006; Goodhead et al. 2020), which is quite large for a heritable insect symbiont. However, it contains many pseudogenes and is featured by a reduced coding capacity of around 50%, with a substantial number of pseudogenes in pathways that are no longer of critical importance in the context of the association with tsetse flies (Belda et al. 2010). With moderate genomic reduction and the ability to survive outside the host, *S. glossinidius* symbionts are in an early/intermediate stage between a free-living and a host-dependent lifestyle (Toh et al. 2006; Goodhead et al. 2020).

S. glossinidius is also featured by an invasive phenotype: it can exhibit both an extracellular and intracellular lifestyle and can colonize a wide range of host tissues, including the midgut, fat body, milk gland, uterus, and oviduct (Fig. 1) (Attardo et al. 2008; Balmand et al. 2013). Like *W. glossinidia*, *S. glossinidius* is transmitted vertically through the milk secretions produced by the female in the uterus, which are rich in the nutrients required for larval development (Attardo et al. 2008; Balmand et al. 2013). However, De Vooght et al. (2015) found that *S. glossinidius* can also be sexually transmitted to females through the male's seminal secretions during mating in viviparous tsetse flies, and then passed on to the offspring (De Vooght et al. 2015). This paternal transmission pathway may partly explain the lack of phylogenetic congruence in tsetse-*Sodalis* associations and may drive genetic diversity in the symbiont via horizontal gene transfers (HGTs) between distinct strains co-infecting the same host that then spread within host populations

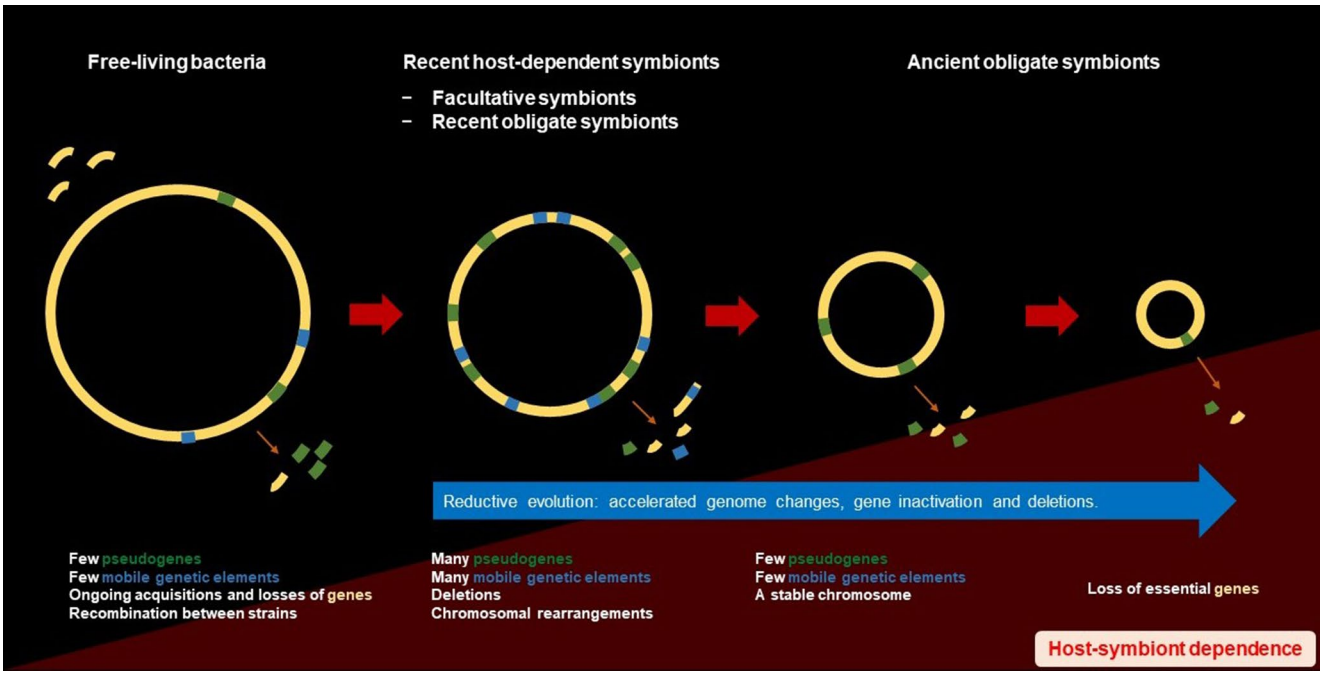
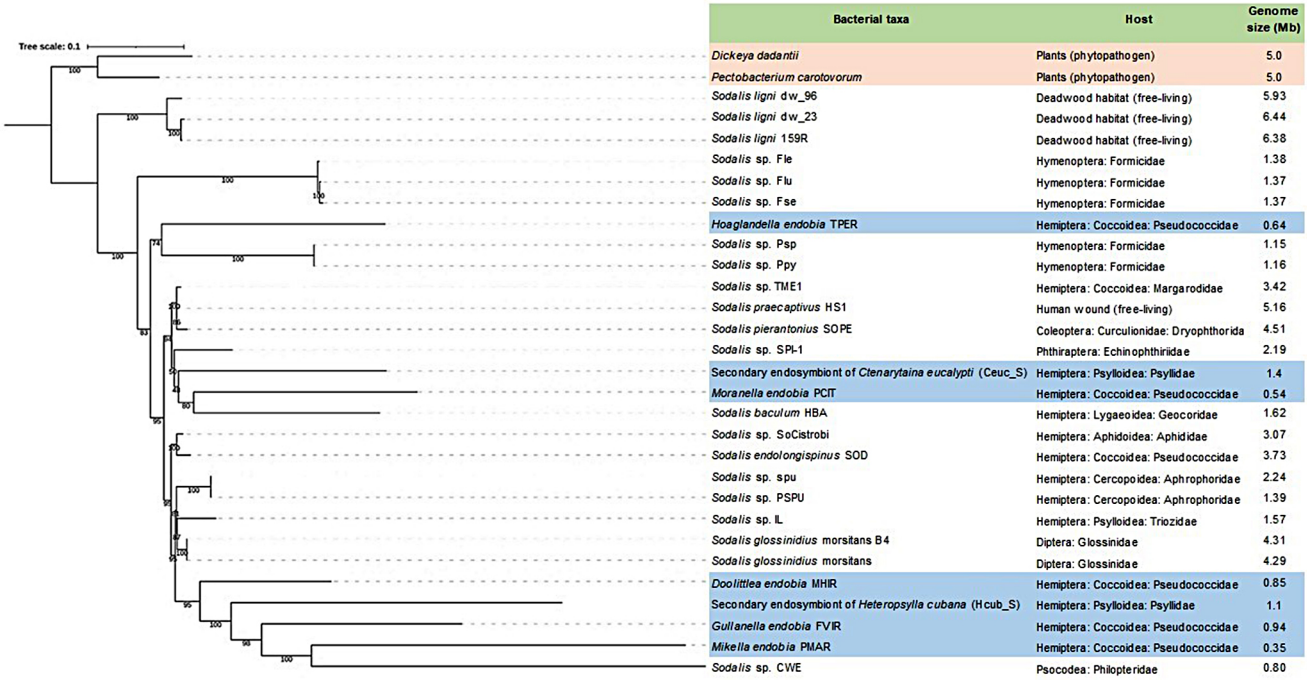


Fig. 1 Overview of the genome reduction process in endosymbionts. Free-living bacteria have few pseudogenes and mobile elements and regularly acquire new genetic material from the environment or from exchanges with other bacteria. Integration into the host of bacteria evolving as heritable symbionts leads to the accumulation of pseudogenes and mobile elements in their genome, which undergoes rearrangements. Over time, genes subject to relaxed selection are lost, while genes that play an essential role in the association are conserved. The result is an increase in host dependence for these bacteria. Gradually, the genomes become enriched in adenine and thymine (AT) nucleotides. Endosymbionts that evolve a high level of interdependence with the host end up with an extremely eroded genome, purged of mobile genetic elements and pseudogenes, retaining only the genetic information essential for symbiosis. As the level of interdependence between host and symbiont increases, so does the anatomical integration of the symbiont: it is condemned to an almost exclusively intracellular lifestyle in host-specific cells called bacteriocytes. By hindering genetic exchanges with other bacterial conspecifics, this intracellular lifestyle leads to asexuality, which also contributes to genomic degeneration

via mixed modes of transmission (De Vooght et al. 2015). These genetic exchanges may be facilitated by the extracellular lifestyle of *S. glossinidius*.

3.1.2 Associated effects that are still unclear

The facultative nature of *S. glossinidius* has been evidenced both by field and experimental studies. Being an obligate symbiont (such as *W. glossinidia*) implies being fixed in the host species, i.e. having a 100% prevalence in host populations. On the other hand, facultative symbionts are not fixed in host species and have fluctuating prevalence in insect populations, as is the case with *S. glossinidius*. The symbiont exhibits a heterogeneous prevalence in tsetse fly field populations, which also varies among tsetse fly species (Maudlin et al. 1990; Farikou et al. 2010; Lindh and Lehane 2011; Alam et al. 2012; Wamwiri et al. 2013; Aksoy et al. 2014; Dennis et al. 2014; Tsagmo Ngoune et al. 2019; Mfopit et al. 2023; Malulu et al. 2023; El Yamlahi et al. 2023). While the effects associated with facultative symbionts have been clarified in some insects, for example aphids (Oliver et al. 2010; Zytynska et al. 2021), the exact nature of the relationship between *S. glossinidius* and tsetse flies remains unclear. Dale and Welburn (2001) reported that the selective elimination of the symbiont by antibiotics has a negative impact on the host lifespan (Dale and Welburn 2001). However, Weiss et al. (2006) found that *S. glossinidius* has neither negative nor positive effects on host fitness (Weiss et al. 2006). Studies about the impact of *S. glossinidius* on host fitness are quite limited, perhaps because antibiotic treatment of fertile flies often results in the elimination of the obligate symbiont *W. glossinidia*, leading to host sterility and making it challenging to generate fly lines lacking a specific symbiont. Furthermore, the nature of the relationship between *S. glossinidius* and tsetse fly species likely depends on a variety of factors, including strain-specific genotype, host species, coevolutionary processes that shaped the interaction, and environmental conditions, as has been shown in other well-studied model insects (Oliver et al. 2010; Zytynska et al. 2021). These aspects have been little studied in tsetse flies, although some *S. glossinidius* genomes are now available and some strains have been isolated and cultured, making it possible to experimentally test the symbiotic status of the facultative symbiont. This lack of knowledge is further



Bacterial taxa	Host	Genome size (Mb)
Dickeya dadantii	Plants (phytopathogen)	5.0
Pectobacterium carotovorum	Plants (phytopathogen)	5.0
Sodalis ligni dw_96	Deadwood habitat (free-living)	5.93
Sodalis ligni dw_23	Deadwood habitat (free-living)	6.44
Sodalis ligni 159R	Deadwood habitat (free-living)	6.38
Sodalis sp. Fle	Hymenoptera: Formicidae	1.38
Sodalis sp. Flu	Hymenoptera: Formicidae	1.37
Sodalis sp. Fse	Hymenoptera: Formicidae	1.37
Hoaglandella endobia TPER	Hemiptera: Coccoidea: Pseudococcidae	0.64
Sodalis sp. Psp	Hymenoptera: Formicidae	1.15
Sodalis sp. Ppy	Hymenoptera: Formicidae	1.16
Sodalis sp. TME1	Hemiptera: Coccoidea: Margarodidae	3.42
Sodalis praecaptivus HS1	Human wound (free-living)	5.16
Sodalis pierantonius SOPE	Coleoptera: Curculionidae: Dryophthorida	4.51
Sodalis sp. SPI-1	Phthiraptera: Echinophthiriidae	2.19
Secondary endosymbiont of *Ctenarytaina eucalypti* (Ceuc_S)	Hemiptera: Psylloidea: Psyllidae	1.4
Moranella endobia PCIT	Hemiptera: Coccoidea: Pseudococcidae	0.54
Sodalis baculum HBA	Hemiptera: Lygaeoidea: Geocoridae	1.62
Sodalis sp. SoCistrobi	Hemiptera: Aphidoidea: Aphididae	3.07
Sodalis endolongispinus SOD	Hemiptera: Coccoidea: Pseudococcidae	3.73
Sodalis sp. spu	Hemiptera: Cercopoidea: Aphrophoridae	2.24
Sodalis sp. PSPU	Hemiptera: Cercopoidea: Aphrophoridae	1.39
Sodalis sp. IL	Hemiptera: Psylloidea: Triozidae	1.57
Sodalis glossinidius morsitans B4	Diptera: Glossinidae	4.31
Sodalis glossinidius morsitans	Diptera: Glossinidae	4.29
Doolittlea endobia MHIR	Hemiptera: Coccoidea: Pseudococcidae	0.85
Secondary endosymbiont of *Heteropsylla cubana* (Hcub_S)	Hemiptera: Psylloidea: Psyllidae	1.1
Gullanella endobia FVIR	Hemiptera: Coccoidea: Pseudococcidae	0.94
Mikella endobia PMAR	Hemiptera: Coccoidea: Pseudococcidae	0.35
Sodalis sp. CWE	Psocodea: Philopteridae	0.80

Fig. 2 Rooted maximum likelihood phylogeny of the 21 *Sodalis* strains whose assembled genomes are publicly available in NCBI. Single-copy genes conserved between these *Sodalis* bacteria, bacteria considered as "*Sodalis*-allied symbionts" and the outgroups *Dickeya dadanti* and *Pectobacterium carotovorum* were obtained using OrthoFinder (Emms and Kelly 2019), and the concatenated sequences were aligned using MAFFT (Katoh and Standley 2013). The phylogenetic tree was constructed from the 50 conserved single-copy genes using maximum likelihood with 1,000 bootstrap replicates via IQ-Tree (http://iqtree.cibiv.univie.ac.at/). Bacterial species highlighted in blue do not formally belong to the *Sodalis* genus but are considered as "*Sodalis*-allied symbionts". Bacteria used for the outgroup are highlighted in orange

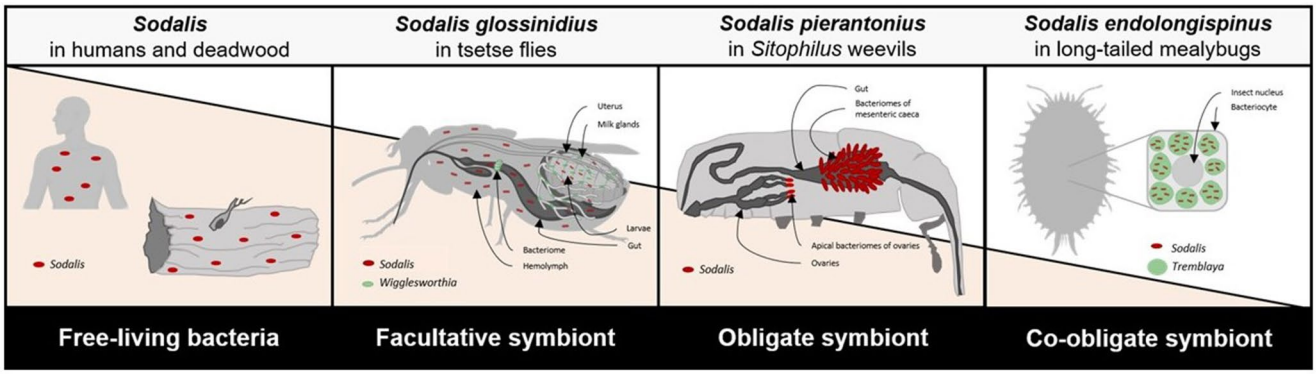


Fig. 3 Schematics showing *Sodalis* bacteria's localization and the diversity of its lifestyle. Free-living strains of the *Sodalis* bacterium have been found in humans and decomposing deadwood. In tsetse flies, *S. glossinidius* is a facultative symbiont localized intra- and extracellularly throughout the whole fly (red). *W. glossinidia* is the obligate symbiont found intracellularly in a bacteriome around the midgut, as well as extracellularly in the lumen of the milk glands (green). Both symbionts are maternally transmitted through milk feeding. In *Sitophilus* weevils, *Sodalis pierantonius* is an obligate symbiont localized in bacteriomes found at the apex of midgut mesenteric caeca, as well as at the apex of female ovaries, from which maternal transmission occurs (red) (inspired by (Zaidman-Rémy et al. 2018). In long-tailed mealybugs, *Sodalis endolongispinus* is a co-obligate symbiont (red) localized within the cytoplasm of the ancestral symbiont *Tremblaya princeps* (green). The nested endosymbiotic system is contained in bacteriocytes, and the *Sodalis* genome complements those of *Tremblaya* and its host for amino acid and vitamin biosynthesis pathways

compounded by the fact that most studies have focused on *Glossina morsitans*, whereas it is now clear that the nature of the relationship between a symbiont species and a host species can differ radically depending on the host-symbiont combination (Niepoth et al. 2018; Stoy et al. 2020). The *Glossina* genus is diverse (Moloo 1993; Dyer et al. 2008; Shaida et al. 2018) and it is likely that *Sodalis* symbionts hosted by tsetse flies have evolved differently according to host species and exhibit distinct associated phenotypic effects (Geiger et al. 2005).

3.1.3 A rich array of virulence factors

S. glossinidius was the first member of the genus to be documented (Dale and Maudlin 1999). Since it can be isolated and cultured, it is possible to used genetic engineering approaches to decipher the mechanisms used by symbiotic bacteria to infect their host and overcome immune barriers (Dale et al. 2002, 2005). Indeed, despite ongoing genome streamlining, the *S. glossinidius* genome retains genes encoding a wide range of virulence factors, which may explain the propensity of the symbiont to invade a wide range of host tissues (Akman et al. 2002; Toh et al. 2006). These virulence factors include several type III secretion systems similar to those found in free-living and parasitic bacterial species, but which have been altered during degenerative genome evolution (Dale et al. 2005; Toh et al. 2006). These modifications are assumed to be adaptive for a stabilized symbiotic relationship (Dale et al. 2002, 2005). Iron is an essential nutrient for bacterial survival and proliferation (Ratledge and Dover 2000). Genome analysis of *S. glossinidius* has also highlighted the role of iron chelation systems in host colonization by symbiotic bacteria. The *S. glossinidius* genome retains a series of genes involved in the biosynthesis of these systems, including siderophores, TonB-dependent transporters (TBDTs) and the hemin receptor HemR (Akman et al. 2002; Toh et al. 2006; Runyen-Janecky et al. 2010; Smith et al. 2013a; Hrusa et al. 2015). Using an RNA-seq approach complemented by a mutagenesis approach, Runyen-Janecky et al. (2022) recently demonstrated that *S. glossinidius* uses iron-uptake systems to colonize the host digestive tract, highlighting, in particular, the role of a phosphotransferase sugar (PTS) system component, a fucose transporter and bacterioferritin in the symbiont's ability to reside in the heme-rich environment of the tsetse host's midgut (Runyen-Janecky et al. 2022). Analysis of the *S. glossinidius* genome also suggests the importance of motility during host colonization. However, although the symbiont is virtually capable of biosynthesizing a complete flagellum structure, no apparent swimming motility has been observed (Akman et al. 2002; Toh et al. 2006; Goodhead et al. 2020). *S. glossinidius* is also endowed with an acylated homoserine lactone (AHL)-based quorum sensing system to modulate gene expression in a density-dependent manner and ensure coordination between symbiotic bacteria during host tissue invasion (Pontes et al. 2008). The key genes regulated by the quorum sensing system, although truncated, appear to have acquired a functionality that also reflects the adaptation of *S. glossinidius* to a stabilized endosymbiotic lifestyle. In addition, the lipopolysaccharide (LPS) structure of *S. glossinidius* is truncated and lacks the O-antigen, a major effector protein in Gram-negative bacteria recognized by the insect immune system (Toh et al. 2006). This may partly explain why, despite residing in the hemolymph and digestive tract, *S. glossinidius* elicits no apparent systemic or epithelial immune response (Hao et al. 2001; Trappeniers et al. 2019). Finally, the symbiont harbor a modified outer membrane protein (OmpA) that allows it not to be sensed as a foe by the host and is essential for the establishment of gut infections in tsetse flies through biofilm formation (Weiss et al. 2008; Maltz et al. 2012). In conclusion, examination of the role of *S. glossinidius* virulence factors in host colonization revealed that the bacterium's propensity to invade a wide range of host tissues is explained in part by the conservation of genes encoding a wide range of colonization factors, but which appear to have been shaped for moderate virulence during genome streamlining to enable the bacterium to enter a long-lasting symbiotic lifestyle.

While *S. glossinidius* has been used as a model for understanding the arsenal of virulence factors that a facultative symbiont can use for host colonization, less attention has been paid to how the host regulates populations of this symbiont and how bacterial population dynamics evolve over the host's life cycle (at least compared to other model insects such as aphids, stink bugs and weevils). Populations of *W. glossinidia* and *S. glossinidius* symbionts appear to fluctuate dramatically during *Glossina morsitans morsitans* development, with a rapid increase in the adult just after pupal hatching over a two-week period, before gradually declining (Rio et al. 2006). This dynamic is quite similar to that observed in other model insects (Vigneron et al. 2014; Simonet et al. 2018; Maire et al. 2020). However, the tissue distribution of *S. glossinidius* symbionts during tsetse fly development remains unknown and there is little data concerning factors that may influence symbiont population dynamics such as, for example, host genotype, host sex and environmental conditions (Rio et al. 2006; Hamidou Soumana et al. 2013). Rio et al. (2006) reported that fluctuations in relative humidity can have dramatic effects on the transmission and maintenance of tsetse-associated symbionts in subsequent generations (Rio et al. 2006). Temperature can play a major role in the dynamics of symbionts and symbiotic host organ development, and some facultative symbionts have protective effects for their host against heat stress (Burke et al. 2010; Kikuchi et al. 2016). However, nothing is known about the influence this environmental factor may have on *S. glossinidius*. Roma et al. (2019) examined the thermal stress responses of *S. glossinidius*, but only under in vitro conditions (Roma et al. 2019). Yet examining the impact of temperature on bacterial symbioses in tsetse flies is particularly crucial for understanding how climate change may affect the health of these insects, and for determining whether *S. glossinidius* may be associated with protective effects against heat stress, as is the case for some facultative symbionts in other insect species (Burke et al. 2010;

Heyworth et al. 2020). Finally, as *S. glossinidius* may depend on *W. glossinidia* for access to certain nutrients such as thiamine (vitamin B_1), the dynamics of the facultative symbiont during host development are assumed to mirror those of the obligate symbiont (Belda et al. 2010; Snyder et al. 2010; Wang et al. 2013; Hall et al. 2019). However, the study of the interaction between *W. glossinidia* and *S. glossinidius* during host development remains limited, and it is unclear how the two symbionts influence each other and are regulated. These are key aspects that future work should address, in particular to optimize *S. glossinidius*-based paratransgenic strategies for controlling trypanosome infection in tsetse flies.

3.1.4 *S. glossinidius* and trypanosome infections

S. glossinidius is a remarkable model for addressing fundamental questions concerning bacterial symbiosis, but also for applied purposes, as there appears to be a correlation between the presence of the symbiont and trypanosome infection in tsetse flies. Indeed, *S. glossinidius* influences the ability of tsetse flies to carry and transmit pathogenic trypanosomes (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*): (1) the facultative symbiont increases tsetse fly susceptibility to trypanosomes (Welburn et al. 1993; Welburn and Maudlin 1999), (2) its selective elimination by antibiotics makes tsetse flies more resistant to trypanosome infection (Dale and Welburn 2001) and (3) the prevalence of parasitic infection is positively correlated with the presence of *S. glossinidius* in tsetse populations (Farikou et al. 2010; Hamidou Soumana et al. 2013; Kallu et al. 2023). However, the mechanisms behind this possible correlation remain uncertain, and the actual influence of *S. glossinidus* in the ability of tsetse flies to acquire and transmit the parasite is questioned by some studies (Geiger et al. 2005; Kanté Tagueu et al. 2018; Channumsin et al. 2018).

Despite these controversies, paratrangenic approaches (i.e. the genetic transformation of an organism's symbionts to confer one or more specific functions that reduce the vector's competence against pathogens) using *S. glossinidus* to control trypanosome transmission by tsetse flies have been set up (Aksoy et al. 2008; De Vooght et al. 2012; Vooght et al. 2014; Medlock et al. 2013; Ratcliffe et al. 2022). As the symbiont can be cultivated, genetic engineering manipulations are easily achievable. Paratransgenesis consists in genetically transforming cultivated *S. glossinidius* with genes encoding antitrypanosomal factors and reintroducing the modified symbiont into tsetse flies. The presence of *S. glossinidius* in host tissues is expected to establish an unsuitable environment for trypanosome multiplication, thus reducing their transmission capacity. Genetically modified *S. glossinidius* symbionts can deliver functional anti-trypanosome nanobodies (Nbs) in *Glossina morsitans morsitans* (De Vooght et al. 2012; Vooght et al. 2022). Their presence in the tsetse gut, in immediate proximity to pathogenic trypanosomes, promotes parasite exposure to anti-trypanosomal factors (Geiger et al. 2015). However, this manipulation remains a proof of concept, not least because of the challenge of generating genetically modified strains that transmit vertically in a stable manner (De Vooght et al. 2012; Vooght et al. 2014, 2018). Bacteria expressing transgenes often suffer a fitness cost limiting the establishment of a stable relationship between the host insect and the engineered symbiont. This is an obstacle to be overcome for implementing effective biological control based on paratransgenesis (Elston et al. 2022). In this context, it is therefore crucial to clearly establish the nature of the interaction between *S. glossinidius* and tsetse flies because the impact of the symbiont on host fitness could influence the effectiveness of this type of approach.

3.2 Other cases of facultative *Sodalis* symbionts

S. glossinidius is undoubtedly the best-studied facultative symbiont of the genus and it is the only facultative symbiont of the genus whose genome has been sequenced and annotated. However, several studies report members of the genus as putative facultative partners, sometimes resembling *S. glossinidius* in terms of tissue tropism and cultivability. This is the case of *Sodalis melophagi*, a presumable facultative symbiont of the sheep ked *Melophagus ovinus* (Diptera: Hippoboscidae), a blood-feeding parasite of sheep related to tsetse flies (Chrudimský et al. 2012; Nováková et al. 2015). *S. melophagi* resembles *S. glossinidius* in many ways: (1) it cohabits with a bacteriocyte-associated endosymbiont of the genus *Arsenophonus* (*A. melophagi*), (2) it infects various host tissues, including the milk gland, the gut and the contiguous bacteriome and (3) it can live in insect cell culture as well as in cell-free medium. Unfortunately, there is a lack of experimental data to clarify the nature of the interaction between *S. melophagi* and *M. ovinus* and no complete annotated genome is available. Interestingly, given that *S. melophagi* encodes the same complete set of vitamin B pathways as the obligate endosymbiont *A. melophagi*, it has been suggested that it might be able to switch from a facultative to an obligate lifestyle and replace *A. melophagi* (Šochová et al. 2017). In the bloodsucking fly *Craterina melbae* (Diptera, Hippoboscoidea), the *Sodalis* symbiont is also a putative facultative associate (Nováková and Hypša 2007), but unfortunately no genomic data are yet publicly available. Finally, the 30% prevalence of *Sodalis* in *Guimaraesiella* lice feeding on songbirds also suggests a facultative relationship with these insects (Grossi et al. 2024).

Sodalis symbionts have been detected in a whole range of insects, sometimes belonging to distant taxa, and in which the biological significance of the symbiont's presence is unclear. This is the case in stink bugs (Hemiptera), where members of *Sodalis* have been detected in different families (Acanthosomatidae, Pentatomidae, Scutelleridae, Urostylididae, Rhopalidae and Pyrrhocoridae), but sporadically, indicating that the bacteria are not fixed in these insect hosts and are facultative or even commensal partners (Kaiwa et al. 2010, 2011, 2014; Matsuura et al. 2014; Hosokawa et al. 2015; Fourie et al. 2023). Similarly, *Sodalis* bacteria have been sporadically detected in the potato psyllid *Bactericera cockerelli* Sulc (Hemiptera: Psylloidea: Triozidae), suggesting the facultative nature of the symbiont in this insect species (Arp et al. 2014; Cooper et al. 2022). Grünwald et al. (2010) investigated the gut flora of several species of bark- and wood-inhabiting cerambycid beetle larvae (Coleoptera: Chrysomeloidea: Cerambycidae) (Grünwald et al. 2010). Interestingly, they discovered that *Sodalis* massively colonizes the gut of the longicorn beetle *Tetropium castaneum*, a wood-boring insect that burrows into conifers. Although the authors describe the endosymbiont as a facultative partner, its associated effects are unknown. However, the massive presence of the symbiont in the insect's digestive tract raises several questions. Is it a potentially pathogenic invasive partner, or, alternatively, does it fulfill a specific function in the digestive tract (e.g. a contribution to wood digestion)? The microbiota of wood-destroying beetles remains little studied, even though they could be teeming with bacteria associated with wood-decomposing properties. *Sodalis* bacteria have also been sporadically reported in archaeococcoid scale insects (Hemiptera: Coccoidea) (Dhami et al. 2013) and cicadas (Hemiptera: Cicadoidea: Cicadidae) (Matsuura et al. 2018; Zhang et al. 2023), again without the biological meaning of the symbiont's presence in these insects being established. Interestingly, *Sodalis* bacteria have been detected in bacteriomes, fat bodies and spermatic tissue of the cicada *Angamiana fuscula*, but not in oocytes and sperms, suggesting that these are opportunistic strains acquired through horizontal transmission (Zhang et al. 2023). *Sodalis* bacteria are often found associated with social bees (Hymenoptera), but the nature of these interactions also remains unknown (Rubin et al. 2018). Finally, *Sodalis* symbionts have been frequently found associated with acorn weevils (Coleoptera: Curculionidae) (Toju et al. 2010, 2013; Toju and Fukatsu 2011). However, unlike *Sodalis* symbionts obligatorily associated with cereal weevils of the genus *Sitophilus*, in this case, they appear to be facultative associates.

4 *Sodalis* symbionts as obligate partners of insects

Most of the *Sodalis* symbionts whose genomes have been sequenced to date have been identified as obligate nutritional associates (Table 1). This means that they are permanent residents necessary for the survival and reproduction of the host insect, as they provide essential nutrients that the insect cannot find in sufficient quantities in its diet. These obligate *Sodalis* symbionts typically reside in bacteriocytes (Nováková and Hypša 2007; Fukatsu et al. 2007; Gruwell et al. 2010; Chrudimský et al. 2012; Koga et al. 2013; Smith et al. 2013b; Koga and Moran 2014). These symbiotic host cells, which often assemble to form an organ called a bacteriome, have specifically evolved to house nutritional symbionts and mediate metabolic exchanges between them and the insect host (Douglas 2014). This is achieved by a fine-tuning of the symbiont populations in these eukaryotic cells according to the insect's nutritional needs (Login et al. 2011; Simonet et al. 2016; Whittle et al. 2021).

4.1 *Sodalis pierantonius*: the nutritional obligate symbiont of *Sitophilus* weevils

4.1.1 A recent obligate symbiont

The first cases of obligate symbioses involving associates of the genus *Sodalis* were identified in cereal weevils of the genus *Sitophilus* (Coleoptera: Curculionidae: Dryophthoridae) (Heddi et al. 1999; Plarre 2013). These cosmopolitan insects harbor an obligate nutritional endosymbiont, *Sodalis pierantonius* (formerly SOPE for *Sitophilus oryzae* primary endosymbiont), which is mainly studied in the context of its association with the rice weevil *S. oryzae*. *S. pierantonius* provides its host with vitamins (especially B vitamins) and amino acids (especially tyrosine and phenylalanine) that are lacking in cereals (Wicker and Nardon 1982; Wicker 1983; Oakeson et al. 2014; Vigneron et al. 2014). *S. pierantonius* boosts cuticle development, increasing its thickness and melanization. This cuticle-enhancing symbiont has been shown to provide desiccation resistance to its host (Kanyile et al. 2023). *S. pierantonius* is hosted in bacteriocytes that form a bacteriome surrounding the midgut of the insect (Fig. 3) (Zaidman-Rémy et al. 2018; Maire et al. 2020). In females, the symbiont also infects the apex of female ovaries, from which maternal transmission takes place.

The *S. pierantonius* genome has not undergone a drastic reduction in size: at 4.51 Mb (Table 1) it remains quite large compared to the genomes of most ancient obligate symbionts, which are typically between 2.0 and 0.4 Mb in size (Oakeson et al. 2014). Ancient obligate symbionts share specific genomic features, including small genomes,

extreme biases in nucleotide composition, high coding density, and scarcity of mobile DNA (McCutcheon and Moran 2012) (Fig. 1). In *S. pierantonius*, these genomic features are less extreme. Almost half of the protein-coding genes of its genome are pseudogenes, indicating relaxed selection on many genes and ongoing genomic erosion (Oakeson et al. 2014). In addition, the symbiont has lost the ability to synthesize several essential amino acids and vitamins. However, similarly to *S. glossinidius*, retains many genes encoding virulence factors that have been tuned to suit the establishment of a mutualistic relationship. Moderate genome erosion, continuous gene inactivation with accumulation of pseudogenes, and strong modulation of genome dynamics with chromosomal rearrangements and proliferation of mobile genetic elements are genomic traits typically associated with facultative symbionts (Gil et al. 2008; Oliver et al. 2010; Lo et al. 2016). Despite differences in metabolic capabilities, *S. pierantonius* and *S. glossinidius* are very similar in terms of genomic evolution (Rio et al. 2003; Oakeson et al. 2014), and although obligate, *S. pierantonius* resembles a facultative symbiont. However, unlike the facultative symbiont of tsetse flies (and other facultative insect symbionts), *S. pierantonius* is compartmentalized into bacteriocytes, an anatomical integration that promotes metabolic exchange between the symbiont and the host and allows the insect to fine-tune symbiont populations (Masson et al. 2016). It is estimated that the free-living ancestor of *S. pierantonius* was acquired by cereal weevils less than 1 million years ago (Clayton et al. 2012), likely as a result of the replacement of Candidatus *Nardonella*, the ancestor endosymbiont of the Dryophthoridae family (Lefèvre et al. 2004; Conord et al. 2008). The weevil-*S. pierantonius* association is therefore a fairly recent symbiosis involving a bacteriocyte-associated nutritional mutualist. By comparison, the symbiosis between aphids and *Buchnera* was established over 100 million years ago (Chong et al. 2019). This makes *S. pierantonius* a valuable model for examining the early stages of obligate nutritional mutualism (Heddi et al. 1998, 1999; Gil et al. 2008).

4.1.2 A model for understanding the dialogue host-symbiont

The weevil-*S. pierantonius* system has enabled major advances to be made in elucidating the key mechanisms by which the insect controls symbionts, notably via its bacteriocytes. In association with *S. oryzae*, *S. pierantonius* produces amino acids that the insect needs to manufacture its cuticle, and which it cannot find in sufficient quantities in the grain (Vigneron et al. 2014). Interestingly, although the symbiont is an obligate partner, it has only a temporary nutritional function, multiplying primarily in young adults whose protective exoskeleton is developing, before being rapidly eliminated within a few days. The multiplication of *S. pierantonius* corresponds to the host's physiological need for the amino acids tyrosine (Tyr) and phenylalanine (Phe). Indeed, these amino acids are precursors of the dihydroxyphenylalanine (DOPA) molecule, an essential component in cuticle synthesis. Once the cuticle is formed, DOPA reaches high levels in insects, triggering the elimination of the endosymbiont through apoptosis and the activation of autophagy (Vigneron et al. 2014; Ferrarini et al. 2023).

The *S. pierantonius* symbionts are contained in large bacteriocytes which, at the larval stage, form a specialized organ (the bacteriome) at the junction of the foregut and the midgut. The molecular dialogue between *S. oryzae* and *S. pierantonius* has been extensively studied (Galambos et al. 2023). Maire et al. (2020) found that the symbiont uses its virulence factors, in particular a type III secretion system (T3SS), during metamorphosis: the stem cells are infected by the symbiont before bacteriocytes develop and assemble into a bacteriome (Maire et al. 2020). The *S. pierantonius* genome also encodes genes required for the synthesis of microbe-associated molecular patterns (MAMPs), including peptidoglycans (PGs), which can activate insect immune responses through their interaction with host pattern recognition receptors (Zaidman-Rémy et al. 2018). Injection of *S. pierantonius* into insect hemolymph triggers a plethora of antimicrobial peptides (AMPs), suggesting that the nutritional symbiont is perceived as a foe in the host body (Anselme et al. 2008). However, several studies indicate that the immune response within bacteriocytes differs markedly from that expressed outside these cells and is conducive to the multiplication of *S. pierantonius* (Anselme et al. 2008; Login et al. 2011; Maire et al. 2019). For instance, the antimicrobial peptide coleoptericin A (ColA) keeps *S. pierantonius* within the bacteriocytes by regulating their multiplication through the inhibition of cell division (Login et al. 2011). The expression of *colA* is dependent on *relish* and *imd*, two genes belonging to the immune deficiency (IMD) pathway. Maire et al. (2019) also found that the weevil peptidoglycan recognition protein LB (PGRP-LB) plays a key role in this homeostasis by cleaving tracheal cytotoxin (TCT), a monomeric form of DAP-type peptidoglycan constantly produced in the bacteriome (Maire et al. 2019). This cleavage prevents the outflow of TCT into the hemolymph and thus the activation of a chronic immune response at this level. Other work has shown that bacteriocytes play a central role in the insect's general immune response, while providing a safe haven for the internalized symbionts during the period in which they are required for insect development, i.e. during the development of its cuticle (Ferrarini et al. 2022; Galambos et al. 2023). As *S. pierantonius* is a "young" obligate symbiont that still exhibits a wide range

of virulence factors and may be perceived as a pathogen, its interaction with *Sitophilus* weevils gives the opportunity to decipher mechanisms by which an antagonistic interaction between a eukaryote and a prokaryote can be circumvented during the evolution of bacterial mutualism.

4.2 Other cases of obligate *Sodalis* symbionts

4.2.1 *Sodalis* symbionts in Hemiptera

Sodalis bacteria have been identified as obligate partners in many other insect species, particularly hemipterans, although these cases have not yet been studied as thoroughly as in *Sitophilus* weevils. An example is the long-tailed mealybug *Pseudococcus longispinus* (Hemiptera: Coccoidea: Pseudococcidae) where *Sodalis endolongispinus* is a recent obligate partner involved in an interdependent system based on four partners: the insect and its ancestral obligate symbiont *Tremblaya princeps* and two additional gammaproteobacterial (*Symbiopectobacterium endolongispinus* and *S. endolongispinus*). These two additional partners acquired more recently have rather large genomes (3.73 Mb) (Table 1), and exhibit an atypical localization as they live within the cytoplasm of the ancestral symbiont *T. princeps* (Gatehouse et al. 2012; Garber et al. 2021). The two gammaproteobacterial genomes complement those of *T. princeps* and the host for biosynthetic pathways of amino acids and vitamins (Garber et al. 2021). Similarly, the mealybug species *Maconellicoccus hirsutus*, *Planococcus citri*, *Trionymus perrisii*, *Paracoccus marginatus* and *Ferrisia virgata* (Hemiptera: Coccoidea: Pseudococcidae) are associated with obligate symbionts with highly reduced genomes and considered *Sodalis*-allied insect endosymbionts, but which are not officially part of the genus, although they form a unique clade with members of the genus *Sodalis* (Husnik and McCutcheon 2016).

Sodalis symbionts have also evolved with certain aphid species. Virtually all aphid species (Hemiptera: Aphidoidea: Aphididae) host the ancestral obligate symbiont *Buchnera aphidicola*. But some species host an additional, more recently acquired, co-obligate symbiont that *complements B. aphidicola* in the biosynthesis of certain essential amino acids and vitamins (Monnin et al. 2020; Renoz et al. 2022; Manzano-Marín et al. 2023). These nutritional di-symbiotic systems have notably evolved in aphids of the subfamilies Lachninae and Chaitophorinae. In the white pine aphid *Cinara strobi*, a recently acquired *Sodalis* symbiont having a genome size of 3.07 Mb (Table 1) metabolically complements *B. aphidicola* for vitamin biosynthesis (biotin and riboflavin), a function previously fulfilled by a co-obligate but now obsolete *Serratia symbiotica* symbiont (Manzano-Marín et al. 2018). Interestingly, Manzano-Marín et al. (2020) have shown that genes of *Sodalis*-related bacteria providing the basis for the synthesis of certain amino acids and vitamins are found in other co-obligate symbiont species as a result of horizontal transfers as is the case in *Erwinia* symbionts associated with *Cinara* aphids (Manzano-Marín et al. 2020). This suggests that circulating *Sodalis* strains can be integrated as nutritional partners in aphids and exchange genetic material with other bacteria, thus acting as a source of metabolic innovations.

In sap-feeding insects of hemipteran suborder Auchenorrhyncha, which feed exclusively on xylem sap, the production of essential nutrients is ensured by a consortium of obligate symbionts, originally *Sulcia muelleri* (Bacteroidetes) and *Zinderia insecticola* (Betaproteobacteria), each confined to distinct bacteriocytes (Koga et al. 2013). However, in the meadow spittlebug *Philaenus spumarius* (Hemiptera: Cercopoidea: Aphrophoridae), a third symbiont, more recently acquired and belonging to the genus *Sodalis*, has replaced the *Zinderia* symbiont (Koga and Moran 2014; Ankrah et al. 2018). This co-obligate *Sodalis* symbiont with a genome size of 2.24 Mb (Table 1) is located in syncytial bacteriocytes near the bacteriocytes specifically hosting *Sulcia*. Genes replacing its predecessor's functions for amino acid biosynthesis have been selectively maintained in *Sodalis* genome. For instance, the genome retains genes supporting efficient energy production pathways, including a complete tricarboxylic acid (TCA) cycle, which could alleviate severe energy limitations in the host due to its xylem-based diet (Koga and Moran 2014).

A *Sodalis* bacterium is also an obligate partner of the seed bug species *Henestaris halophilus* (Hemiptera: Lygaeoidea: Geocoridae), where it is found in bacteriocytes forming tubular-shaped bacteriomes on the abdominal flanks (Santos-Garcia et al. 2017). In this insect, *Sodalis baculum*, is a mutualistic partner capable of providing the amino acids tyrosine, lysine, and some cofactors. The size of its genome (1.62 Mb) is small compared with other *Sodalis* genomes (Table 1). Interestingly, it still includes many pseudogenes but few mobile elements, indicating an intermediate stage in reductive evolution.

Other *Sodalis* symbionts are potential obligate insect partners, although they have been less studied to date. For this reason, they are referred to as putative obligate symbionts (Table 1). Among them the *Sodalis* symbiont strain TME1 (genome size: 3.42 Mb) associated with the wax cochineal *Llaveia axin axin* (Hemiptera: Coccoidea: Margarodidae), probably a co-obligate symbiont that metabolically complements the more ancient symbiont *Walczuchella monophlebidarum* endowed with a 0.3 Mb genome (Rosas-Pérez et al. 2017). However, this metabolic complementation has yet to be formally demonstrated. Another case is the *Sodalis* SPI-1 strain associated with the sucking lice *Proechinophthirus*

fluctus (Phthiraptera: Echinophthiriidae) and featured by a genome of 2.19 Mb (Table 1). The role of this *Sodalis*-allied symbiont has yet to be elucidated. It appears to be a recently acquired obligate symbiont, capable of infecting a wide range of tissues, exhibiting both an intracellular and extracellular lifestyle. Indeed, it infects bacteriocytes, but also other tissue types such as fat bodies, oviduct and oocytes, a rather singular tissue tropism for an obligate symbiont. Another putative obligate *Sodalis* symbiont is the strain IL reported in the carrot psyllid *Bactericera trigonica* (Hemiptera: Psylloidea: Triozidae) (Ghosh et al. 2020). Despite the availability of its genome sequence (1.57 Mb) (Table 1), the function associated with this *Sodalis* symbiont has yet to be established. However, this strain offers a rare case of intranuclear symbiont since the *Sodalis* bacteria in this psyllid species are localized inside the nuclei of midgut cells, in addition to being internalized in bacteriocytes. This atypical localization raises the question of the influence that this *Sodalis* symbiont could have on biological processes taking place in the nuclei of the midgut cells, such as gene regulation and cell division. In the psyllid species *Ctenarytaina eucalypti* and *Heteropsylla cubana* (Hemiptera: Psylloidea: Psyllidae), a *Sodalis*-allied symbiont metabolically complements the ancient obligate symbiont *Carsonella ruddii* for the biosynthesis of essential amino acids such as tryptophan and arginine (Sloan and Moran 2012).

4.2.2 *Sodalis* symbionts in blood-feeding insects

The award for the smallest genome for a symbiont officially belonging to the genus *Sodalis* goes to the symbiont associated with the slender pigeon louse *Columbicola wolffhuegeli* (Psocodea: Philopteridae) that parasitizes the pied imperial pigeon (*Ducula bicolor*). Genome analysis reveals features similar to other obligate symbionts in insects, namely a sharp reduction in size and a GC content shift (0.8 Mb, 31.4% of GC) (Table 1), but provides no clear evidence of its function in the host (Alickovic et al. 2021).

4.2.3 *Sodalis* symbionts in ants

Until recently, the biological significance of *Sodalis* bacteria in ants was unknown. However, Jackson et al. (2022) recently discovered that *Sodalis* symbionts have evolved obligate symbioses with ant species of the genera *Formica* and *Plagiolepis* (Hymenoptera: Formicidae) (Jackson et al. 2022). All these *Sodalis*-allied insect symbionts exhibit genomes between 1.0 and 1.5 Mb (Table 1) and are obligate associates involved in the biosynthesis of tyrosine, an amino acid involved in cuticle synthesis. They are found in the bacteriocytes surrounding the midgut of adult queens, but also infect eggs and ovaries. Interestingly, the authors found that the prevalence of *Sodalis* is not 100% in different species of these ant genera. The retention of *Sodalis* symbionts in queens and workers of some species, but not in others, suggests that species differ in their dependence on symbiont-derived nutrients, or that *Sodalis* has lost the ability to produce nutrients in certain host lineages (Jackson et al. 2022). The authors hypothesize that these differences are linked to the feeding ecology of the species examined. Ant species with a predominantly plant-based diet would be more likely to have evolved a dependence on nutritional symbionts than species with an omnivorous diet.

5 The free-living *Sodalis* bacteria

5.1 *Sodalis praecaptivus*: a human pathogen

The genus *Sodalis* also include free-living members. *Sodalis praecaptivus*, originally known as the HS strain, was isolated from a human hand wound caused by impalement on a crabapple branch (Clayton et al. 2012; Chari et al. 2015). It is assumed, however, that the tree was the original source of the *S. praecaptivus* infection (Fig. 3) (Clayton et al. 2012). The bacterium is a prototroph capable of growing in minimal media at 37 °C, whose genome has been sequenced and annotated (Clayton et al. 2012; Chari et al. 2015). Comparative genomic analyses revealed that the free-living *S. praecaptivus* strain has a large and less altered genome (5.16 Mb) than *S. glossinidius* (4.3 Mb), the facultative symbiont of tsetse flies, and *S. pierantonius* (4.5 Mb), the obligate symbiont of cereal weevils *Sitophilus* sp. (Table 1) (Clayton et al. 2012; Oakeson et al. 2014). The free-living strain has preserved many genes inactivated and lost in *Sodalis*-allied insect symbionts due to rapid genome degeneration, consistent with the hypothesis that *S. praecaptivus* evolves under strong stabilizing selection in a free-living lifestyle (Clayton et al. 2012; Chari et al. 2015). For instance, compared with *S. glossinidius*, *S. praecaptivus* has a more extensive metabolic capability and can utilize carbon sources contained in plants and animals (Clayton et al. 2012). In contrast to the free-living strain genome, those of *Sodalis*-allied insect symbionts has undergone evolutionary adaptations during the transition to their symbiotic lifestyle: pseudogenes thus account for a significant fraction of their total genomic coding capacity, the result of relaxed selection on non-essential loci (Clayton et al. 2012; Chari et al. 2015). Importantly, comparative genomic and phylogenetic analyses have shown that *S. praecaptivus* and *Sodalis*-allied insect symbionts are closely related (Clayton et al. 2012; Oakeson et al. 2014), suggesting that *Sodalis* symbionts could evolve independently and repeatedly from *S. praecaptivus*-like environmental bacteria. This makes *S.*

praecaptivus an ideal model for examining the early stages of bacterial mutualism in insects.

5.2 *Sodalis* members as ubiquitous environmental bacteria

The existence of free-living *S. praecaptivus* suggests a diverse spectrum of living strategies among *Sodalis* members that extend beyond associations with insects. Recently, Tláskal et al. (2021) described two free-living isolates from the *Sodalis* genus, *Sodalis ligni* sp. nov. strain dw23 and *Sodalis* sp. strain dw96, which inhabit decomposing deadwood and possess genome characteristics consistent with a non-symbiotic lifestyle (Tláskal et al. 2021). They can grow on laboratory media and have a larger genome relative to other *Sodalis* strains (6.44 and 5.93 Mb respectively, Table 1). These strains also have a higher number of longer genes and a lower coding density. A pangenome analysis revealed genomic differences between the various *Sodalis* genomes, showing that deadwood-associated strains encode genes for the active decomposition of biopolymers of plant and fungal origin and can use more diverse carbon sources than the symbiotic strains associated with insects (Tláskal et al. 2021). Accessory genes specific to deadwood-associated strains account for a significant portion of the total identified genes and are involved, for example, in amino acid and carbohydrate metabolism (Tláskal et al. 2021). It has also been suggested that deadwood-associated strains encode multiple nifHDK operons expressing nitrogenase, a key enzyme for metal cofactor-dependent nitrogen fixation (Tláskal et al. 2021). Their genetic potential for nitrogen fixation makes them one of the few members of the nitrogen-fixing Enterobacterales described so far.

Another novel free-living *S. ligni* strain, called *Sodalis ligni* strain 159R, has been isolated from an anaerobic enrichment culture of temperate forest soils (Tláskal et al. 2021; Chaput et al. 2022). Whole-genome sequencing revealed a genome size of 6.38 Mb, which appears similar in genome size to *S. ligni* strain dw23 (Table 1) (Tláskal et al. 2021). This strain is closely related phylogenetically to the deadwood-associated *Sodalis* strains (Chaput et al. 2022). The authors suggest that *S. ligni* strain 159R has a genetic potential for anaerobic lignin degradation and aromatic catabolism (Chaput et al. 2022). This strain has a potentially applied dimension, as microorganisms with the ability to depolymerize and catabolize lignin are relevant candidates for lignocellulosic biofuel applications. Removing lignin from lignocellulosic materials is a generally unsustainable and expensive process, but using microorganisms and their enzymes to break down lignin could be a more environmentally-friendly approach (Chaput et al. 2022). However, the process has its limitations, not least the need for constant aeration and mixing. Anaerobic bacteria could therefore be a substitute for lignin depolymerization and conversion into valuable by-products.

5.3 A potential reservoir for new symbiotic associations

The presence of *Sodalis* in phylogenetically distant insect taxa and the isolation of free-living strains from non-insect material suggest that associations involving the bacterial genus arose during multiple independent infectious events, such as through horizontal transfers or direct acquisition of free-living bacteria from the environment (Clayton et al. 2012; Tláskal et al. 2021). The close relationship between the free-living *S. praecaptivus* bacterium and *Sodalis*-allied insect symbionts suggests that this free-living bacterium may be a close relative and putative environmental progenitor of *Sodalis*-allied insect symbionts (Clayton et al. 2012; Tláskal et al. 2021) (Fig. 2). All this information constitutes a rich data set from which to examine the origin of the insect-*Sodalis* symbiosis.

Over the past decade, studies have examined how *Sodalis*-allied insect symbionts have evolved in their transition to symbiosis, using the free-living *S. praecaptivus* bacterium as a model. One study computationally examined the pathway by which the *S. glossinidius* symbiont may have taken in its transition to symbiosis with tsetse flies (Hall et al. 2020). The authors used the free-living *S. praecaptivus* and metabolic modeling in combination with a multi-objective evolutionary algorithm to investigate the evolutionary trajectories of the bacterial symbiont after being internalized (Hall et al. 2020). They found that the order in which key genes are lost influence the evolved populations. Although *S. praecaptivus* has a free-living lifestyle, it can colonize insects known to be associated with *Sodalis*-allied insect symbionts by suppressing its virulence through quorum sensing (QS) (Enomoto et al. 2017; Munoz et al. 2020). QS can play a pivotal role in the regulation of symbiotic relationships, including the coordination of group behavior, as demonstrated by the symbiont *Vibrio fischeri*, which uses this mechanism to regulate bioluminescence in the light organ of its squid host, *Euprymna scolopes* (Visick et al. 2000). QS can also control the production of extracellular factors, such as antibiotic production in the plant-associated pathogen *Erwinia carotovorum* (McGowan et al. 1995). Enomoto et al. (2017) identified three genic components of a QS system within the *S. praecaptivus* genome: a luxI-like synthase homolog (*ypeI*), which produces N-(3-oxohexanoyl) homoserine lactone, and two luxR-like response regulators. They created mutant strains of *S. praecaptivus* lacking these genetic mechanisms and showed that the bacterium then expresses an extremely virulent ("killing") phenotype in the

cereal weevil *Sitophilus zeamais* (Enomoto et al. 2017). The authors thus established the importance of QS in promoting the symbiotic domestication process by repressing virulence factors after infection. Attenuation of virulence by QS occurs when the bacterial population reaches a certain density (Enomoto et al. 2017). Munoz et al. (2020) performed similar experiments with tsetse flies and showed that QS virulence suppression also facilitates the establishment of *S. praecaptivus* infection in tsetse flies (Munoz et al. 2020). In addition, *S. praecaptivus* can infect the reproductive tissues of these insects, enabling vertical transmission within a single host generation (Munoz et al. 2020). The modulation of virulence by QS could be a mechanism enabling *Sodalis* bacteria to establish themselves in insect hosts with attenuated virulence and could explain their propensity to establish symbiotic associations with a wide range of insect hosts. These results therefore suggest that QS modulation may represent a crucial evolutionary step in the early stage of symbiosis between insects and bacteria.

In continuation of these studies, researchers have set up a protocol for microinjecting *S. praecaptivus* into grain weevil eggs to study its transmission and maintenance through host generation (Su et al. 2022). They showed that the free-living strain can colonize prototypical somatic and germinal bacteriomes after microinjection, leading to its maternal transmission over several generations. The finding that *S. praecaptivus* can be found in weevil bacteriocytes along with the native symbiont *S. pierantonius* and can be co-transmitted to offspring (albeit at a low rate) offers the possibility of exploring the evolution of the interaction between an established symbiont and a new bacterial associate (Su et al. 2022). Indeed, studies have suggested that *S. pierantonius* has recently replaced *Nardonella*, the ancestral endosymbiont of the Dryophthoridae family to which cereal weevils belong (Charles et al. 2001; Lefèvre et al. 2004; Conord et al. 2008). The ancestor of *S. pierantonius* could be functionally close to *S. praecaptivus*, given their phylogenetic proximity. The replacement of *S. pierantonius* by *S. praecaptivus* could mimic the way in which *Nardonella* was replaced by *S. pierantonius* (Vigneron and Kaltenpoth 2022).

The close relationship of the free-living *S. praecaptivus* bacterium with *Sodalis*-allied insect symbionts, as well as its ability to colonize and associate with insect hosts, raise questions about its principal habitat and ecological functions (Clayton et al. 2012; Enomoto et al. 2017; Munoz et al. 2020). Based on virulence factors in its genome and its metabolic capability to use animal and plant hosts, *S. praecaptivus* could be an opportunistic plant or animal pathogen that could develop associations with insects to achieve vector-based transmission (Su et al. 2022). That is consistent with the fact that *S. praecaptivus* uses quorum sensing to reduce its virulence towards insects and, in general, natural selection favors bacteria that minimize the fitness costs associated with their transmission by vectors.

Unlike the *S. praecaptivus* strain, free-living strains associated with deadwood and temperate forest soils do not belong to the same phylogenetic clade as *Sodalis*-allied insect symbionts. However, a study identified a *Sodalis* bacterium massively infecting the epithelial cells of the larva's proximal midgut of the longicorn beetle *T. castaneum* (Grünwald et al. 2010). No genomic or phylogenetic analysis has been carried out, making it impossible to position this *Sodalis* member phylogenetically and to study its biological role. It is possible that the ancestor of this bacterium came from the wood on which the beetle's lava feeds, and resembles the strains living in dead wood (Tláskal et al. 2021; Chaput et al. 2022). It would be interesting to test this hypothesis to confirm that *Sodalis*-allied insect symbionts originate from different sources and multiple, independent infectious events.

Overall, the data provide evidence that the *Sodalis* genus is predisposed to evolve associations with insects due to its ability to maintain minor infections, colonize many different insect tissues, and undergo vertical transmission in diverse insect hosts (Clayton et al. 2012; Tláskal et al. 2021). They also corroborate that mutualism can be initiated in insects by the rapid acquisition of environmental bacteria, echoing a recent study showing the quick establishment of a novel symbiosis in the stinkbug *Plautia stali* from an *Escherichia coli* strain (Koga et al. 2022). This study demonstrated that a non-symbiotic bacterial strain can be converted into a mutualistic insect associate via a single mutation that disrupts the carbon catabolite repression global transcriptional regulator system.

6 Concluding remarks and future directions

The *Sodalis* genus is highly diverse and undeniably a powerful tool for studying the many facets of bacterial symbiosis in eukaryotes, as its members embody the different degrees of host dependence and anatomical integration that bacteria can experience during their evolution with insects. Members of the *Sodalis* genus have repeatedly succeeded in entering new hosts and forming heritable symbioses in a variety of insects. This is reflected in the incongruence between *Sodalis* phylogeny and insect host taxonomy. Associations between insects and *Sodalis* symbionts have been studied mainly in tsetse flies and weevils, but their study has been extended to a range of other insect taxa (e.g. aphids, spittlebugs, mealybugs). This repeated success for an endosymbiotic lifestyle reflects a pre-adaptation of *Sodalis* bacteria to associate with insects, as demonstrated by the

use of free-living members in the context of groundbreaking experiments aimed at understanding the initial stages of bacterial endosymbiosis in insects (Munoz et al. 2020; Su et al. 2022). In particular, the *Sodalis* genus offers an exciting playground for understanding how genes encoding virulence factors are shaped to foster the entry of a pathogenic bacterium into a stabilized symbiotic lifestyle. However, many questions remain unanswered about the interactions that the *Sodalis* members may have with insects (and beyond). Here, we develop a few of them by way of perspective. One key issue is the exact nature of the relationship between *S. glossinidius* and tsetse flies. Although this is undoubtedly the most extensively studied *Sodalis* species, it remains unclear whether the symbiont maintains a mutualistic relationship with its host, or whether it is an opportunistic partner. Nor is it known whether environmental conditions can influence the nature of the interaction between the two partners on the parasitism-mutualism continuum, as has been demonstrated in aphids (Oliver et al. 2010). These are important issues because *S. glossinidius* could influence the transmission of pathogenic trypanosomes and is used in emerging paratransgenic approaches to counter their transmission. Further experimental efforts are therefore essential to clarify the symbiotic nature of *S. glossinidius*. Omics approaches could, for example, be used to understand how *S. glossinidius* populations are regulated in the various infected host tissues, and to determine the impact of the symbiont on host physiology. Furthermore, the diversity of *S. glossinidius* has been little studied in the different species of tsetse fly that host the symbiont. One hypothesis is that the strains associated with these insects are the result of independent acquisitions and are associated with different phenotypic effects. Genome sequencing and analysis of *S. glossinidius* strains associated with different tsetse species should be undertaken in the future to address these aspects.

The puzzling tissue localization of *Sodalis* associates in certain insects also raises questions about their associated phenotypic effects. This is the case of *Sodalis* bacteria that massively infect the digestive tract of certain Cerambycid beetles (Grünwald et al. 2010). Are these *Sodalis* bacteria opportunistic associates or do they play a potential role in wood digestion in host insects? Are these *Sodalis* bacteria systematically associated with these insects? The existence of free-living strains capable of degrading lignin raises the question of the ability of certain wood-eating insects to associate with strains of this type to benefit from their remarkable metabolic properties. Future work will need to examine the associations between wood-eating insects and *Sodalis* bacteria. Once again, sequencing the genome of these strains is an essential step towards understanding their metabolic capacities and the nature of their relationship with their host. In a similar vein, the atypical intranuclear localization of *Sodalis* symbionts in the cell nuclei of the midgut of the carrot psyllid *B. trigonica* also raises the question of the influence the symbiont may have on the molecular and cellular processes taking place in this organelle (Ghosh et al. 2020).

Interactions between *Sodalis* bacteria and plants are another aspect worth investigating. Indeed, the *Sodalis* genus is part of the Pectobacteriaceae family, which largely consists of plant pathogens (Adeolu et al. 2016; Motyka et al. 2017). Symbioses with *Sodalis* bacteria in different insect lineages are the result of multiple independent infections, and it is likely that *Sodalis* bacteria circulate in plants that likely mediate horizontal transfers to insects. This raises the question of the nature of interactions between *Sodalis* bacteria and plants. Do *Sodalis* bacteria have phytopathogenic properties? Are they beneficial endophytes or commensal associates? Can they circulate in plant sap like other insect symbionts (Caspi-Fluger et al. 2011; Pons et al. 2019)? Does interaction with the plant pre-adapt *Sodalis* bacteria to becoming insect symbionts? These questions need to be addressed, as they will provide a better understanding of the origin of bacterial symbioses in insects and, in the case of *Sodalis*, how independently established symbioses arose. The possibility of culturing certain *Sodalis* strains (especially the free-living members *S. praecaptivus* and *S. ligni* strains dw23, dw96, and 159R) is conducive to infection experiments on plants to examine these questions. At the same time, it is also essential to decipher in depth the metabolic capabilities of the free-living strains to determine whether they can be used for industrial and public health purposes.

An important technical point to consider is that several *Sodalis*-allied insect symbionts (e.g. *S. glossinidius* and *S. praecaptivus*) have been isolated in pure culture (Dale and Maudlin 1999; Matthew et al. 2005; Chari et al. 2015), opening the door to genetic engineering approaches applied to insect symbionts for fundamental and applied purposes. Indeed, most heritable insect symbionts are recalcitrant to culture approaches, given the dependence they have evolved towards their insect host. Despite these advantages, cultivable *Sodalis* bacteria, especially the facultative symbiont *S. glossinidius*, are reluctant to DNA transformation by the standard techniques (heat shock and electroporation) usually used on *E. coli* and other model bacteria (Pontes and Dale 2006, 2011; Kendra et al. 2020). However, it has recently been discovered that phage-mediated DNA transduction can be used to deliver exogenous DNA to *S. glossinidius* and *S. praecaptivus* (Keller et al. 2021). This is an important technical step, as it bypasses the culture requirement for genetically manipulating insect endosymbionts. In future research, this approach could greatly facilitate analyses aimed at identifying the genetic component and pathways governing interactions between bacteria and their

eukaryotic hosts (Elston et al. 2022). This approach could also be used for paratransgenesis to control insect-borne microbes that pose economic and health concerns (Elston et al. 2022; Ratcliffe et al. 2022).

Finally, like Santos-Garcia et al. (2017), we recommend a taxonomic re-evaluation of the genus *Sodalis* (Santos-Garcia et al. 2017). Indeed, phylogenomic analyses suggest that insect symbionts affiliated with other genera actually form a single clade with members of the genus *Sodalis* (Sloan and Moran 2012; Husnik and McCutcheon 2016; Santos-Garcia et al. 2017). The unification of *Sodalis*-allied species into the same genus would undoubtedly facilitate further work on the genus and shed light on its tremendous diversity.

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Declarations

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