Lyme Borreliosis

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Editors

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ISBN 978-3-030-93679-2 ISBN 978-3-030-93680-8 (eBook)
https://doi.org/10.1007/978-3-030-93680-8

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The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Introduction

26.03.2022

Historically, tick-associated pathogens can be tracked back to the book of Exodus in the Hebrew Bible. The plague ("murrain") visited upon the cattle of Pharaoh Ramses II is probably the first historical reference to a disease transmitted by ticks [1]. It took until the late eighteenth century, however, to gather scientific evidence for the existence of tick-borne microorganisms and to attain a better understanding of the circumstances of transmission and the life cycles of such pathogens. In 1893, Smith and Kilbourne discovered that the causative agent of Texas cattle fever, now known as the protozoan *Babesia bigemina*, is transmitted by ticks and were the first to determine these arthropods as important vectors of pathogens [2]. McCalla and Brereton further substantiated the importance of ticks in the transmission of disease in 1908 in the USA. A tick from a patient with Rocky Mountain spotted fever was at that time used to transmit the infection to two healthy volunteers [3]. In 1909, Ricketts discovered the eponymous genus of bacteria responsible for Rocky Mountain spotted fever—*Rickettsia* [4]. Up to now, more than 50 tick-borne pathogens—parasites, bacteria and viruses—have been found to be of considerable concern to humans exposed to tick bites in Europe [5].

Today, Lyme borreliosis (LB)—an infectious disease caused by tick-borne spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex—is the most commonly reported vector-borne infection in the northern hemisphere [6, 7]. The geographical presence of the disease thereby follows a belt-like distribution and mirrors the distribution of ixodid ticks in this part of the world [6]. In central Europe, *Ixodes ricinus* is primarily known as the main vector of *B. burgdorferi* s.l. and tick-borne encephalitis (TBE) virus, which taken together are estimated to infect some 100,000 individuals per year. Moreover, studies based on polymerase chain reaction (PCR) and DNA sequence analyses have shown that tick-borne pathogens other than *B. burgdorferi* s.l., such as *Borrelia miyamotoi, Anaplasma phagocytophilum, Neoehrlichia mikurensis*, *Rickettsia* spp. and *Babesia* spp., are also widely prevalent in the three-host tick *I. ricinus*, whose larvae, nymphs, and adults feed on different hosts, including virtually any warm-blooded animal and humans [5, 8].

According to the Centers for Disease Control and Prevention (CDC), the incidence in the USA was 7.9/100,000 in 2014, with the majority of cases reported in the Northeastern and upper Midwestern States [9]. In the USA, 30,000–40,000 cases are reported annually through surveillance each year with an estimated

476,000 patients treated during 2010–2018 [7]. In Europe, incidence ranges of 0.001/100,000 in Italy (2001–2005) up to 188.7/100,000 in Slovenia (2014) have been published [8]. "Lyme disease", as it was called, emerged when Steere et al. [10] investigated an arthritis epidemic among young children in the community of Old Lyme, Connecticut, USA, in the late 1970s [10], but the infection was known to medicine in Europe much earlier.

Typical cutaneous manifestations are the most frequent signs of the disease and was described at the end of the nineteenth century and the beginning of the twentieth century by physicians such as Buchwald, Pick, Herxheimer, Hartman, Afzelius and Lipschütz [11]. Additionally, two French physicians, Garin and Bujadoux, in a landmark paper published in 1922, reported a patient who developed erythema chronicum migrans followed by painful meningoradiculitis [12]. This patient was reportedly bitten by a tick and had a positive Bordet-Wasserman test, which was used at that time to diagnose syphilis. Although the test was positive, the patient obviously did not have syphilis, and the authors concluded he had a tick-borne disease caused by a spirochete that induced cutaneous and neurological manifestations and was different from the causative agent of syphilis, *Treponema pallidum* [12]. The causative agent of LB, however, remained a mystery until the discovery of spirochetal bacteria in the midgut of ticks collected from Long Island, New York, in 1982 by the Swiss-borne entomologist Willy Burgdorfer [13]. The subsequent epidemiological and laboratory establishment of LB as a new multi-system infectious disease entity is one of the most important biomedical discoveries of the twentieth century [14]. In the years after the isolation of the causative bacterium, it was quickly shown that there were significant differences in disease expression between North America and Europe. Furthermore, it was established that in North America there was just one predominant pathogenic species of borrelia (*B. burgdorferi* sensu stricto) and one recently discovered minor one (*B. mayonii*), while there were at least four different pathogenic species in Europe [6]. The infection may occur without signs and symptoms, but in clinically apparent cases, typical symptoms associated with infection include erythema migrans (EM), neurological manifestations (e.g. polymeningoradiculoneuritis, also known as Bannwarth's syndrome), Lyme arthritis (LA) and acrodermatitis chronica atrophicans (ACA). Such manifestations, together with some other rare ones, had been well recognized in Europe years before the final discovery of the causative pathogen *B. burgdorferi* s.l. [11]. Over the last few decades, tremendous progress has been achieved in well-recognized treatment options [6]. Nevertheless, LB, like syphilis, can behave as a chameleon of clinical medicine for inexperienced clinicians, resulting in a cornucopia of problems, especially when it comes to direct and indirect laboratory diagnosis of the pathogen and consideration of the many potential differential diagnoses [15].

This is why we see an urgent need for a practical medical textbook for doctors and students devoted to all the different facets of the diagnosis and clinical management of Lyme borreliosis. In addition, special chapters cover differences in disease manifestations between Europe and North America, the pathogenicity of the pathogens, the life cycle and biology of the vectors, and also important tick-borne pathogens other than *B. burgdorferi* s.l. that are important for the differential diagnosis of

tick-borne diseases in Europe. The contributors to this book are all internationally well-known specialists in the field of infectious diseases and tick-borne pathogens. We aim to provide a well-structured and practice-oriented presentation of clinical management, and laboratory diagnosis of LB, and other important tick-borne diseases in Europe. We also discuss the current pitfalls and limitations, as well as future prospects in this challenging and rapidly moving area of medicine.

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Characteristics of *Borrelia burgdorferi* sensu lato

1

Gabriele Margos, Sabrina Hepner, and Volker Fingerle

1.1 Introduction

The microorganisms that can cause Lyme borreliosis in humans are spirochetal bacteria (Fig. 1.1) that comprise the *Borrelia burgdorferi* sensu lato (s.l.; Latin: in the broad sense) species complex. The bacteria live a parasitic lifestyle and are maintained in natural transmission cycles between tick vectors of the *Ixodes ricinus–persulcatus* species complex and small- to medium-sized vertebrate reservoir hosts [1–3].

It had been suspected since the beginning of the last century that tick-borne pathogens may cause symptoms that are now known as Lyme borreliosis (reviewed by [4]). However, it was not until the early 1980s that the causative agent was shown to be a spirochetal bacterium that utilizes ticks as vectors [5]. The bacterium was named *Borrelia burgdorferi* Johnson et al. 1984 [6]. Subsequent studies unraveled the genetic and ecological heterogeneity of borreliae in Europe, Asia, and North America and several new genospecies were named, e.g., *Borrelia garinii* Baranton et al. 1992 and *Borrelia afzelii* Baranton et al. 1992; (Table 1.1) [7–25]. Since then, the name *B. burgdorferi* s.l. has been used to refer to the species complex, while *B. burgdorferi* sensu stricto (s.s.; Latin: in the strict sense) refers to the species first discovered by W. Burgdorfer and colleagues [5, 6]. Today the species complex contains 23 named and proposed genospecies (Table 1.1). The species are non-uniformly distributed mainly between the northern 40° and 60° latitude (Fig. 1.3). This distribution reflects the presence of competent tick vector and reservoir host species [26].

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K.-P. Hunfeld, J. Gray (eds.), *Lyme Borreliosis*,
https://doi.org/10.1007/978-3-030-93680-8_1

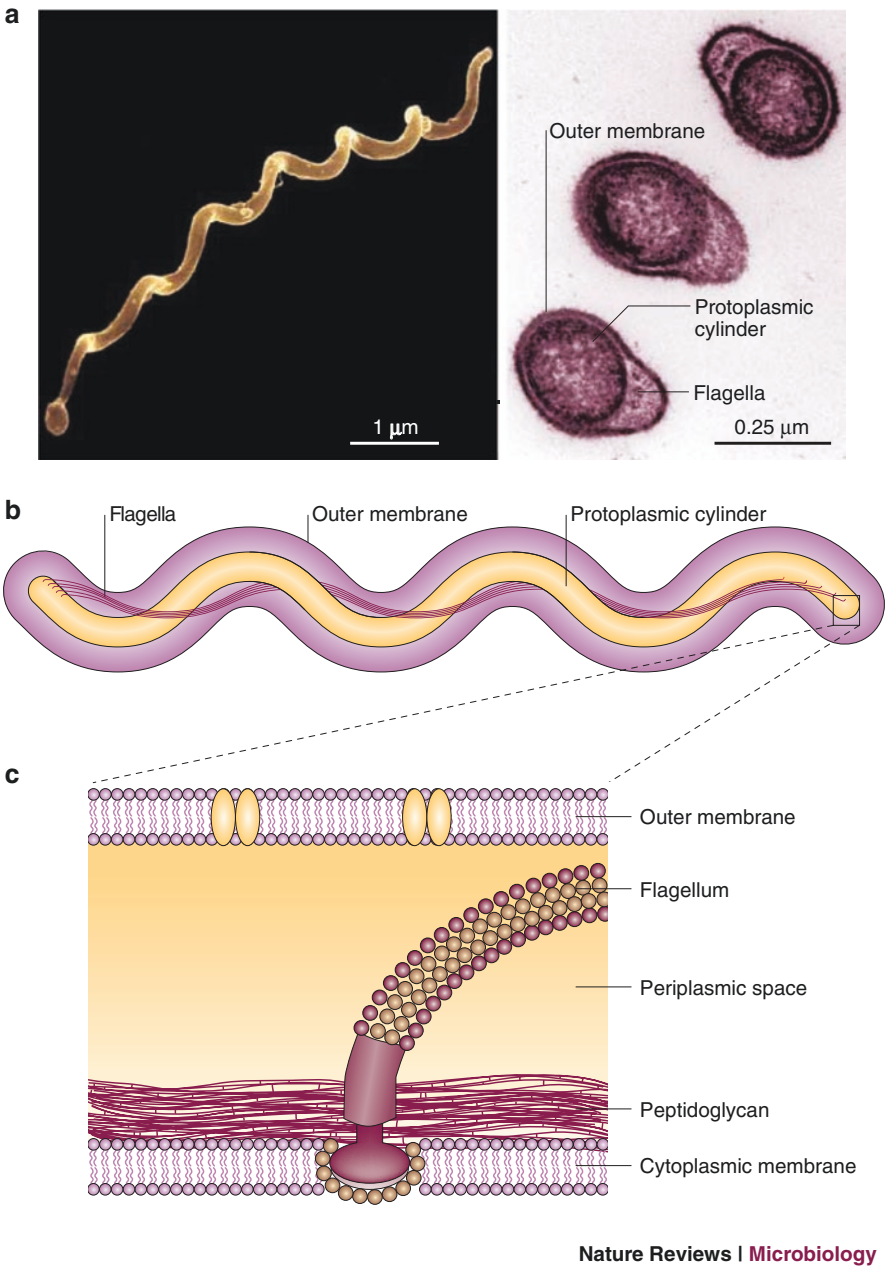


Fig. 1.1 Morphology of *Borrelia* (adapted from [54] with permission from Nature Reviews Microbiology). (**a**) light microscopy of *Borrelia* and schematic drawing of transection of a spirochete; (**b**) schematic representation of a spirochete showing the protoplasmic space with inserted flagella; (**c**) magnification from (**b**) of the insertion site of a flagellum into the cytoplasmic membrane

Table 1.1 The *Borrelia burgdorferi* sensu lato species complex

Borrelia species	Type strain	Reservoir hosts	Vector species	Distribution	Human pathogenicity
B. afzelii	VS461	Rodents, insectivores	*Ixodes ricinus, Ixodes persulcatus, Ixodes hexagonus*	Asia, Europe	Yes
B. americana	SCW-41	Birds, rodents	*Ixodes minor, Ixodes pacificus*	North America	Unknown
B. andersonii (p)	21,038	Birds, rabbits	*Ixodes dentatus*	North America	Unknown
B. bavariensis	PBi	Rodents	*Ixodes ricinus, Ixodes persulcatus*	Asia, Europe	Yes
B. bissettiae [a]	DN-127	Rodents	*Ixodes spinipalpis, Ixodes pacificus, Ixodes ricinus*	Europe, North America	Potentially
B. burgdorferi sensu stricto	B31	Birds, rodents, insectivores, carnivores	*Ixodes ricinus, Ixodes scapularis, Ixodes affinis, Ixodes pacificus, Ixodes minor, Ixodes hexagonus*	Europe, North America	Yes
B. californiensis	CA446	Rodents	*Ixodes pacificus, Ixodes spinipalpis, Ixodes jellisoni*	North America	Unknown
B. carolinensis	SCW-22	Rodents	*Ixodes minor*	North America	Unknown
B. chilensis (p)	VA1 (p)	Rodents	*Ixodes stilesi*	South America	Unknown
B. garinii	20047	Birds	*Ixodes ricinus, Ixodes persulcatus, Ixodes uriae* [b]	Asia, Europe	Yes
B. japonica	HO14	Rodents	*Ixodes ovatus*	Asia	Unknown
B. kurtenbachii	25015	Rodents	*?*	North America	Potentially
B. lanei	CA28–91	Lagomorphs?	*Ixodes spinipalpis, Ixodes pacificus*	North America	Unknown
B. lusitaniae	PoTiB2	Lizards	*Ixodes ricinus*	Europe	Potentially
B. maritima	CA690	?	*?*	North America	Unknown
B. mayonii	M14–1420	Rodents?	*Ixodes scapularis*	North America	Yes

(continued)

Table 1.1 (continued)

Borrelia species	Type strain	Reservoir hosts	Vector species	Distribution	Human pathogenicity
B. sinica	CMN3	Rodents	*Ixodes ovatus*	Asia	Unknown
B. spielmanii	PC-Eq17	Rodents	*Ixodes ricinus, Ixodes hexagonus*	Europe	Yes
B. tanukii	Hk501	Rodents	*Ixodes tanuki*	Asia	Unknown
B. turdi	Ya501	Birds	*Ixodes turdus, Ixodes frontalis, Ixodes ricinus*	Asia, Europe	Unknown
B. valaisiana	VS116	Birds	*Ixodes ricinus*	Europe	No
B. yangtzensis	Okinawa CW62	Rodents	*Ixodes granulatus*	Asia	Potentially
Candidatus B. aligera	NA	Birds?	*?*	Europe	Unknown

(p) proposed, *a* formerly *B. bissettii, b* also in sea bird colonies in Canada, *NA* not applicable

1.2 *Borrelia* Genomics and Cell Biology

Genomics. The first genome of Lyme borreliosis group spirochete to be completely sequenced was that of *B. burgdorferi* s.s. isolate B31 [27]. The genome turned out to be unusual for bacteria: it consisted of a large linear chromosome of about 910 kbp and of 12 linear and 9 circular plasmids which make up another 600 kbp of DNA sequence, a substantial contribution to the total genome of *B. burgdorferi* s.s. [27–29]. The genomic structure, i.e., consisting of a linear chromosome and circular as well as linear plasmids, was found to be maintained in all species investigated so far [19, 24, 30–33]. In B31, the main chromosome contains 820 open reading frames (803 protein-coding sequences, 17 pseudogenes; 5 rRNA, 32 tRNA, 3 ncRNA), 10% of which match hypothetical proteins and 29% have no match in a database. The G + C content of the chromosome is around 28% [27, 34]. The plasmids in B31 range in size from 5 to 60 kbp, contain additional 700 coding sequences of which >90% have no convincing database match outside the genus *Borrelia* [27, 28]. Main chromosome and linear plasmids are terminated by covalently closed hairpin structures [35–37] which are created involving a telomere resolvase, ResT, an enzyme encoded on plasmid cp26 [38, 39]. Plasmids may be lost under *in vitro* culture conditions [40–43], but they are essential for completion of the complex *B. burgdorferi* s.l. life cycle in nature [44, 45].

Initially, plasmids have been named according to whether they are linear or circular and according to size, e.g., lp54 for a 54 kbp linear plasmids, cp26 for a 26 kbp circular plasmid [28]. However, since several plasmids of similar size have been found in a single isolate, and size differences of the same plasmid have been noticed in different isolates, recently plasmids are typed according to their PFam32 locus, which supposedly is homologous to plasmid partitioning protein (ParA) encoding

sequences in other bacteria [29]. Apart from PFam32, related loci (PFam49, PFam52, PFam57/60) may be involved in autonomous plasmid replication and maintenance but their function is yet to be confirmed [39, 46].

Perhaps as a result of the parasitic lifestyle, *B. burgdorferi* s.l. has very few genes for biosynthesis of cell constituents [27]. The majority of chromosomal genes encode proteins for housekeeping and metabolic functions, while many of the genes encoding outer surface proteins required for interaction with host or vector are located on plasmids. Analyses of plasmid sequences showed that there have been extensive rearrangements, and plasmid numbers and structures vary not only between genospecies but also between strains of a single species [29, 30, 46, 47]. Plasmids of the cp32 family have been shown to contain prophages, perhaps facilitating rearrangements and/or exchange of genetic material [46, 48, 49]. Information on *B. burgdorferi* s.l. genome content and structure has been largely gained from strains of the genospecies *B. burgdorferi* s.s. [29, 47, 50]. Although for other *Borrelia* genospecies genomes have been sequenced, the whole complement of plasmids has not been completed for all of them [30, 32, 33, 46, 51], (http://BorreliaBase.org).

Cell biology. Borreliae are helical bacteria. Their size is 0.2–0.3 μm wide and 10–30 μm long. *Borrelia* are not gram-negative, they lack the lipopolysaccharide (LPS) and the protein richness that are typical for the cell surface membrane of gram-negative bacteria [52, 53]. Instead, they have a diderm cell envelope consisting of an outer surface membrane separated by a periplasmic space from the cytoplasmic membrane, which is covered by a peptidoglycan layer. Usually 7–11 flagella are inserted near the end of the protoplasmic cylinder of the cell extending into the periplasmic space (Fig. 1.1) [54]. These endoflagella give the bacteria a unique form of motility permitting them to move in viscous media. They can flex and bend, propel themselves forwards and backwards and rotate (non-translational mode of motility) [55, 56] and this motility is crucial for host/vector infection [57].

Inserted in the outer surface membrane via lipid moieties are outer surface membrane proteins (Osps); >150 potential Osps have been identified [27]. They have been named alphabetically in order of their identification, e.g., OspA, OspB, OspC, etc. Many of these proteins have functions in the interaction of the bacteria with their environment (host or vector). Table 1.2 provides a non-exhaustive list.

Apart from these Osps, there are outer membrane proteins (OMPs) that are integral membrane proteins and may serve as transporters for nutrients or other essential molecules that borreliae take up from the host environment. Freeze fracture electron microscopy has shown that the outer membrane contains relatively few transmembrane proteins [53]. These studies also provided evidence that blebs, surrounded by a membrane(s) resembling the outer membrane and/or the cytoplasmic membrane, are shed from *Borrelia* cells suggesting that blebs are pinched off sections of the cells.

Many other outer membrane and internal proteins are important for the life cycle of *B. burgdorferi* s.l. and intensive research efforts are being made to understand their function and role in the life cycle of these bacteria (e.g., [44, 58–60]).

Table 1.2 A non-exhaustive list of outer surface proteins and transmembrane proteins of *B. burgdorferi* s.l.

Protein name		Gene designation B31	Proposed biological role	Size
Outer surface proteins (Osp)				
OspA/ OspB	Outer surface protein A/B	BB_A15/ BB_A16	Interaction with tick receptor TROSPA	31 kDa/34 kDa
OspC	Outer surface protein C	BB_B19	Early infection of vertebrate host	22 kDa
OspD	Outer surface protein D		Unknown, potentially adherence to the tick midgut	28 kDa
BptA	Borrelial persistence in ticks A	BBE16		
P35		BBA64	Unknown, tick-to-host transmission or vertebrate infection	35 kDa
DbpA/ DbpB	Decorin-binding protein A/B	BBA24/BBA25	Interaction with collagen fibers; decorin binding	18 kDa/17 kDa
BBK32		BBK32	Binding to fibronectin	47 kDa
OspF	Outer surface protein F protein family	BBM38/ BBO39/ BBR42	Unknown, potential adhesin	29 kDa/26 kDa /25 kDa
VLsE	Variable major protein-like sequence expressed	BB_F0041	Immune evasion	35 kDa
OspE	Outer surface protein E protein family	BBL39/ BBN38, (BBP38 identical to BBL39)	Evasion of complement lysis (CRASP)	
ErpG, ErpL, ErpX, ErpY	OspE-related proteins		Complement evasion?	
CspA	CRASP-1	BBA68	Evasion of complement lysis	27 kDa
CspZ	CRASP-2	BBH06	Evasion of complement lysis	27 kDa
BBA36		BBA36	Unknown	
BBA65		BBA65	Unknown	
BBA66		BBA66	Unknown	
BBA69		BBA69	Unknown	
BBA71		BBA71	Unknown	
BBA73		BBA73	Unknown	

Table 1.2 (continued)

Protein name		Gene designation B31	Proposed biological role	Size
BBI42		BBI42	Unknown	
Integral outer membrane proteins (OMP)				
P66		BB0603	Putative porin	66 kDa
P13		BB0034	Putative porin	13 kDa
Lmp1	Surface-located membrane protein 1	BB0210	Protection from host adaptive immunity	128 kDa
BesA/ BesB/ BesC	*Borrelia* efflux system proteins A, B, C	Bb0141/ Bb0140/ Bb0142	Putative bacterial resistance-nodulation-division (RND)-type multidrug-efflux system	
BamA	β-Barrel assembly machine protein	bb0795	β-Barrel assembly machine	94 kDa
BB0405		BB0405	Unknown	22 kDa
Bgp	*Borrelia* glycosaminoglycan-binding protein	bb0588	Glycosaminoglycan (GAG)-binding protein; cell adhesion	

1.3 The *Borrelia burgdorferi* Sensu Lato Species Complex

The phylum Spirochaetes Cavalier-Smith 2002 comprises a group of helically shaped bacteria, several of which cause human diseases such as *Leptospira*, *Treponema*, *Brachyspira,* and *Borrelia*. The genus *Borrelia* contains the relapsing fever group of spirochetes (e.g., *Borrelia recurrentis* causing louse-borne human relapsing fever and several species causing tick-borne relapsing fever), the Lyme borreliosis group of spirochetes (*B. burgdorferi* s.l. complex), and a group of reptile- and echidna-associated spirochetes [61–64]. In 2014, based on investigations on conserved signature proteins (CSP), conserved signature insertions/deletions (indels) (CSI), and average nucleotide identity (ANI), the genus was divided into two genera: *Borrelia* containing the relapsing fever species and *Borreliella* for the Lyme borreliosis species [65]. The third clade, reptile- and echidna-associated species were not considered. Using different methodology of genus delimitation, namely the percentage of conserved proteins (PCOP) [66], recently all groups were reunited in the genus *Borrelia* [62]. This work also showed that reptile- and echidna-associated species do not genetically resemble relapsing fever species but take a somewhat intermediate position between relapsing fever and Lyme borreliosis spirochetes [64].

The *B. burgdorferi* s.l. species complex currently consists of 23 named species (Table 1.1), six of which are assured human pathogens. Five of the species

pathogenic to humans occur in Europe including *B. afzelii*, *Borrelia bavariensis* Margos et al. 2013, *B. burgdorferi* s.s., *B. garinii*, and *Borrelia spielmanii* Richter et al. 2006 [67, 68]. *Borrelia afzelii*, *B. bavariensis,* and *B. garinii* also occur in Eastern Europe and Asia [69–71].

In North America, two species are the cause of human Lyme disease, these are *B. burgdorferi* s.s. and *Borrelia mayonii* Pritt et al. 2016 [72–75]. The latter species was only discovered in 2016 in patients visiting the Mayo Clinic in Wisconsin [73]. Since then more symptomatic patients have been found to be infected with *B. mayonii* [72].

Two additional species have been discussed as putative human pathogens; these are *Borrelia lusitaniae* Le Fleche et al. 1997 and *Borrelia bissettiae* Margos et al. 2016. *Borrelia lusitaniae* can be commonly found in questing ticks in countries neighboring the Mediterranean Sea [76–81], and so far two cases have been described in the literature incriminating *B. lusitaniae* as a suspected human pathogen [82, 83]. On the other hand, *B. bissettiae* has rarely been found in questing ticks in Europe [84–86]. So far one human case (where an isolate was obtained) of *B. bissettiae* causing symptoms resembling mild neuroborreliosis has been described [11, 67]. In North America where *B. bissettiae* can be commonly found at a regional scale and in certain habitat types [87–91], no patient isolates have been obtained from humans although *B. bissettiae* DNA was recovered from serum [92]. *Borrelia valaisiana* Wang et al. 1997, has been asserted to be nonpathogenic for humans [93]. This *Borrelia* species is transmitted by *Ixodes ricinus* Linnaeus 1758, the main vector of human pathogenic *Borrelia* species in Europe (reviewed by [94, 95], see chapter "Pathogenesis and Immune Defense"), it utilizes avian reservoir hosts and is being found as frequently as *B. garinii* in certain regions [96]. Although it is found commonly in ticks, to date not a single human isolate of *B. valaisiana* has been acquired [93]. For the remaining species shown in Table 1.1, the human pathogenic potential is unknown. Many of these species are transmitted by ticks that do not bite humans, which may explain why these spirochetes have not emerged as pathogens, although their lack of human pathogenicity may be because of their genetic makeup.

1.4 Ecology and Transmission Cycles

As the geographical distribution of the different *Borrelia* species depends on vector and host associations (putatively also their pathogenic potential), it may be worth to briefly consider the biology of ticks and hosts, both of which will be discussed in more detail in chapter "Tick ecology and the eco-epidemiology of *Borrelia burgdorferi* sensu lato" in this book.

Only hard ticks of the genus *Ixodes* serve as vectors for *B. burgdorferi* s.l. (reviewed by [71, 94, 95, 97, 98]. *Ixodes* ticks have three life stages that require a blood meal from a host: larvae, nymphs, and adult females. In between blood meals, the ticks drop off the host, digest the blood meal, and molt into the next developmental stage in the undergrowth or leaf litter of their habitats. Ticks with a generalist

feeding behavior serve as bridge vectors for agents of human Lyme borreliosis. The most important vectors for *B. burgdorferi* s.l. include *I. pacificus* (west of the Rocky Mountains) and *I. scapularis* (east of Rocky Mountains, Northeast, Midwest and Southeast USA, and Canada) in North America, *I. ricinus* in Europe, and *I. persulcatus* in Eastern Europe and Asia [99]. Host-specific or nidicolous ticks such as *I. uriae* [100], *I. hexagonus* [101], *I. frontalis* [102, 103], or *I. spinipalpis* [104], have more or less strong host preferences and are thus less prone to bite (and therefore only rarely transmit *Borrelia* to) humans. However, these specialist ticks in many cases use identical hosts to more generalist vectors (such as *I. ricinus*, *I. scapularis*, *I. pacificus*, and *I. persulcatus*); in this way, a potential connection arises between *Borrelia* transmission cycles of nonhuman-biting and human-biting ticks [105].

Ticks are armed with a cocktail of components that deflect adverse reactions by the host to the attached tick [106–110]. Microorganisms that utilize ticks as vectors can use tick salivary molecules to their own advantage during transmission, e.g., not being recognized by the host's immune system (reviewed by [110–113]). This phenomenon has been termed saliva-assisted transmission or SAT [114]. Nevertheless, some natural hosts are able to develop immune responses toward ticks leading to premature detachment of the feeding tick [115] and that can have an effect on pathogen transmission (see section *Reservoir hosts*).

Tick immunity to pathogens. In recent years, progress has been made in recognizing the complexity of the tick's immune system (reviewed in [113, 116–118]). *Ixodes* possess a number of immune effectors and modulators such as recognition molecules that serve as lectins labeling foreign cells for immune attack, phagocytotic hemocytes, antimicrobial peptides, lysozymes, defensins, and a dityrosine network (DTN) [119]. Signaling pathways such as Toll, an atypical IMD (Immunodeficiency), and JAK-STAT (Janus Kinase/Signal Transducers and Activators of Transcription) regulate the immune system and, interestingly, ticks also possess an indirect, cross-species signaling pathway that recognizes the cytokine interferon gamma in the blood of the host [113, 116, 120–122]. The tick's immune system may even be exploited by *Borrelia* as RNA interference studies of genes involved in the tick's immune response have shown that depletion of expression may lead to suppression of *Borrelia* colonization in ticks [123]. Furthermore, induction of a protein of *I. scapularis* with a Reeler domain (PIXR) by *Borrelia* limits bacterial biofilm formation in the tick's gut, thereby preventing alterations in the microbiome and promoting colonization by *Borrelia* [123]. Thus, it is likely that immune effectors play an important role in determining the competence of *Ixodes* species for *Borrelia* species and/or *vice versa*.

The microbiome of ticks. In the past decade, efforts have been devoted to study the tick's microbiome in detail. Using high-throughput sequencing methods, initial studies on different *Ixodes* species (e.g., *I. scapularis, I. ricinus, I. pacificus,* and *I. persulcatus*) discovered a whole range of bacterial taxa associated with ticks. It showed that the microbiome of ticks consists of microorganisms associated with the outer surface of ticks, the gut, and endosymbiotic bacteria (reviewed by [124]). Bacterial genera that were found constituted known tick symbionts like *Arsenophonus*, *Cardinium*, *Coxiella*, *Francisella*, *Lariskella, Midichloria*,

Rickettsia, *Rickettsiella*, *Spiroplasma*, and *Wolbachia* [125–131]. A more recent study used dissected tick tissues of questing *I. scapularis* to determine the "internal" microbiome and the "surface" microbiome. The authors found that in the majority of adults the gut microbiome of *I. scapularis* was limited in diversity [132]. The dominating bacteria were *Rickettsia* and *B. burgdorferi*. Only a minority of samples showed a high microbiome diversity with bacteria of the genera *Bacillus* and *Pseudomonas*, and the family Enterobacteriaceae in their midguts [132]. It remains to be investigated what impact the different "layers" of the microbiome have on the tick itself and the microorganisms it transmits.

Reservoir hosts (see also chapter "Pathogenesis and Immune Defense"). More than 100 vertebrate species can serve as host for generalist *Ixodes* ticks such as *I. ricinus*. Most of these species belong to the orders Rodentia, Eulipotyphla (formerly part of the Insectivores), Carnivores, Lagomorphs, as well as the classes Aves (here mostly Passeriformes and sea birds) and Reptiles. A fraction of these tick hosts can serve as hosts for *Borrelia*, among them various species of mice (genera *Apodemus*, *Peromyscus*, *Neotoma*), voles (genus *Myodes*, *Microtus*), shrews (genera *Sorex*, *Blarina*), squirrels (*Tamias, Sciurus*), lizards, and ground-feeding passerine birds (genera *Turdus*, *Parus*) (e.g., [25, 71, 78, 88, 133–147]).

However, experimental studies have shown that not all hosts that become infected with *Borrelia* species also serve as reservoirs (e.g., [147–149]. Complement sensitivity or resistance matches the reservoir host association of *Borrelia* species well, with *B. garinii* surviving bird complement but lysed by rodent complement, while rodent-associated species such as *B. afzelii* survive rodent complement but are lysed by bird complement. Complement-active deer serum lysed all tested *Borrelia* species suggesting that deer are nonpermissive as hosts for *Borrelia* [150–152]. The expression "host association" has been used to refer to "true" reservoir hosts of *Borrelia* as defined by Kahl and co-authors and Martin and co-authors [153, 154], i.e., only those hosts are considered reservoir competent that are able to acquire the bacteria from a competent vector tick and (critically) also to transmit it back to new vector ticks [1, 155]. The term "host association" was used instead of "host specialization" because *Borrelia* spirochetes are not "specialized" to infect *only* their reservoir hosts, as may be the case for other directly transmitted or vector-borne infectious agents, e.g., [148].

The development of resistance to tick bites by a host may reduce the ability to transmit tick-borne pathogens to vector ticks [109, 156, 157]. One such example is the bank vole, *Myodes glareolus*. In comparison to the wood mouse, *Apodemus sylvaticus*, repeated exposure of *M. glareolus* to tick bites reduced the engorgement time and weight of ticks making them drop-off the host prematurely (i.e., before complete engorgement) [115]. Reduction of engorgement time limits the transmission of tick-borne pathogens [158–161].

Some studies have suggested that hosts, once infected with *Borrelia*, carry the infection lifelong [162]. However, experimental transmission studies using different isolates of *B. burgdorferi* s.s. have shown that the duration of infection may differ between strains of *Borrelia* [163, 164].

1.4.1 Infection of Ticks by *Borrelia burgdorferi* s.l.

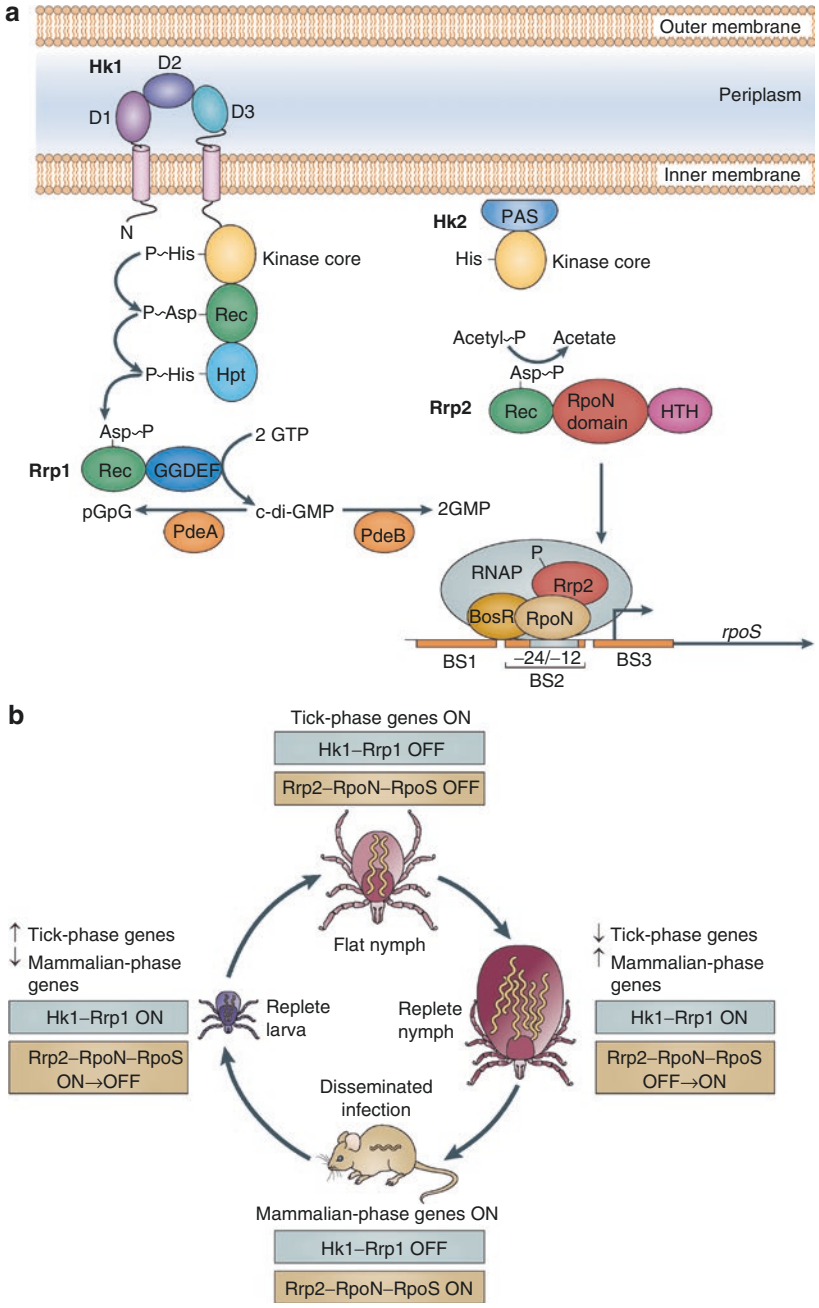
Infection of ticks by Borrelia burgdorferi s.l. Borreliae are taken up by the tick during the blood meal although the transmission efficiency may be variable depending on tick species, *Borrelia* species, or concomitant infections [161, 165–172]. The tick may feed for 16–48 h before the bacterium enters the tick gut [160, 173]. In the tick gut, the bacteria adhere to midgut cells via outer surface proteins. It has been suggested that OspA interacts with a tick midgut protein that was named tick receptor for OspA (TROSPA) [118, 174]. Upon entering the tick midgut, during blood meal digestion, molting, and questing periods, the bacteria remain adhered to the midgut. When the tick takes the next blood meal, changes in environmental conditions and the provided nutrients prompt the bacteria to divide and migrate through the midgut into the hemocoel and the salivary glands [175]. This is accompanied by changes in patterns of protein expression [45] due to regulatory factors responding to environmental cues, e.g., temperature and other physiological changes (reviewed by [3, 176]) (Fig. 1.2).

Although some studies have suggested that *B. burgdorferi* s.l. may create a biofilm in vitro and in vivo [189, 190], biofilm production seems not to be required in the ticks' midgut for spirochete colonization [123]. The spirochetes induce the expression of a tick protein of *I. scapularis* with a Reeler domain (PIXR), which prevents biofilm formation and appears to inhibit changes in the gut microbiome, supposedly giving *Borrelia* an advantage during the tick phase of their development [123].

When characterization of the first genome of *Borrelia* isolate B31 was completed, it was quite astonishing to find that many of the genes encoded hypothetical proteins with unknown functions and no match in databases [27, 28]. In spite of intensive research efforts, the genetic basis for the host- or vector association is still not clear [3, 26, 98, 130, 187]. In contrast to other human pathogenic bacteria, *B. burgdorferi* s.l. lack pathogenicity islands or virulence factors and although several proteins have been identified as virulence determinants, which factor exactly trigger human pathogenicity is currently still unknown (reviewed by [191, 192]).

1.5 Geographic Ranges of the Lyme Borreliosis Spirochetes

The interplay between competent vector ticks and reservoir hosts, their ecology, and migration pattern determines the geographic distribution of LB species (Fig. 1.3). The geographic ranges of the various *B. burgdorferi* s. l. species [193] are in each case limited to those locations in which both reservoir hosts and vector ticks are able to maintain natural transmission cycles [1, 2, 155, 194] (Fig. 1.3). Thus, one should be able to define the fundamental niche of each *Borrelia* species simply by taking account of where its vectors and hosts occur. However, many *B. burgdorferi* s.l. species can utilize multiple vertebrate host species and a number can utilize more than one vector. In addition, ecological associations between borreliae, ticks, and


a
Outer membrane
Periplasm
Inner membrane
Hk1
D2
D1
D3
N
P~His
Kinase core
P~Asp
Rec
P~His
Hpt
Asp~P
Rrp1
Rec
GGDEF
2 GTP
pGpG
PdeA
c-di-GMP
PdeB
2GMP
Hk2
PAS
His
Kinase core
Acetyl~P
Acetate
Asp~P
Rrp2
Rec
RpoN domain
HTH
P
RNAP
Rrp2
BosR
RpoN
rpoS
BS1
–24/–12
BS2
BS3
b
Tick-phase genes ON
Hk1–Rrp1 OFF
Rrp2–RpoN–RpoS OFF
Flat nymph
↑ Tick-phase genes
↓ Mammalian-phase genes
Hk1–Rrp1 ON
Rrp2–RpoN–RpoS ON→OFF
Replete larva
Replete nymph
↓ Tick-phase genes
↑ Mammalian-phase genes
Hk1–Rrp1 ON
Rrp2–RpoN–RpoS OFF→ON
Disseminated infection
Mammalian-phase genes ON
Hk1–Rrp1 OFF
Rrp2–RpoN–RpoS ON

reservoir hosts are not all equivalent in strength, thus, the realized niche actually occupied by each *B. burgdorferi* s.l. species is likely to be less than its fundamental niche [26, 155]. The actual spatial limitation for each spirochete species (i.e., its realized niche) will be roughly equivalent to the sum of all those areas in which both at least one vector species and one host species occur at sufficiently high density to maintain its transmission cycle. The basic reproduction number R_0 presents a quantification of the biological framework and efficiency of the transmission cycle and its value can serve as a measure for population fitness [195]. For every local population of the bacterium, the value of R_0, summed over all its hosts and vectors, must be >1 for transmission cycles to be sustained [155, 196, 197]. As the presence of less efficient vectors and hosts will impact negatively on the value of R_0 achieved by the "best" vectors and hosts, one cannot simply add up values of R_0 that have been determined for each vector and each host under laboratory conditions [195, 198]. The effects caused by nonpermissive vectors and/or hosts are very important to consider as they can influence the success of the bacterium in entirely opposite ways

Fig. 1.2 Regulation of gene expression in *Borrelia burgdorferi* sensu lato (modified from [3] with permission from Nature Reviews Microbiology, and with special thanks to Melissa Caimano). (**a**) The histidine kinase 1 (Hk1)–response regulatory protein 1 (Rrp1) and alternative RNA polymerase σ-factor RpoS global regulatory systems. Binding of ligands to the periplasmic sensor domains (D1, D2, and D3) of the hybrid histidine kinase Hk1 initiates the activation of the diguanylyl cyclase activity of Rrp1, resulting in the production of cyclic di-GMP (c-di-GMP) [177–179]. Phosphodiesterase A (PdeA) and PdeB degrade c-di-GMP to 5′-phosphoguanylyl-(3′–5′)-guanosine (pGpG) and GMP, respectively [180, 181]. Activation of Rrp2 *in vitro* and *in vivo* occurs via the high-energy phosphoryl donor acetyl-phosphate rather than by its presumptive cognate histidine kinase, Hk2 [182]. The function of Hk2 is currently unknown. Phosphorylated Rrp2, *Borrelia* oxidative stress regulator (BosR), and RpoN initiate transcription of *rpoS* ([183, 184] and references therein). This is depicted as a trimeric complex, but the precise interactions between these proteins have yet to be determined. Putative BosR-binding sites (BSs) containing the direct repeat sequence TAAATTAAAT are shown; −24/−12 is the RpoN-binding site in the *rpoS* promoter [185]. RpoS in turn induces the expression of genes that are required during the mammalian-host phase of the spirochaete life cycle and represses the expression of tick-phase genes. (**b**) Expression of the Hk1–Rrp1 and RpoS global regulatory systems during the *B. burgdorferi* life cycle [177–179, 183, 184, 186]. In the flat nymph, both the Hk1–Rrp1 and the Rrp2–RpoN–RpoS systems are inactive and only tick-phase genes are expressed. The nymphal blood meal activates both the Hk1-Rrp1 and Rrp2–RpoN–RpoS pathways. Expression of mammalian phase genes begins in concert with downregulation of tick-phase genes. Following inoculation into a mammalian host, the spirochaetes complete the process of adaptation; the Hk1–Rrp1 pathway is inactive, the Rrp2–RpoN–RpoS pathway is active, mammalian phase genes are expressed, and tick-phase genes are repressed. During larval acquisition of spirochaetes, Hk1–Rrp1 is activated, probably at the feeding site, whereas the Rrp2–RpoN–RpoS system is inactivated. Mammalian-phase genes are repressed, expression of tick-phase genes begins, and ingested spirochaetes bind to the larval midgut epithelium via OspA and possibly other receptors [186–188]. GGDEF, a conserved motif present in diguanylyl cyclases; Hpt, histidine-containing phosphotransfer domain; HTH, helix–turn–helix domain; N, amino; PAS, putative sensor domain for Hk2; Rec, receiver domain

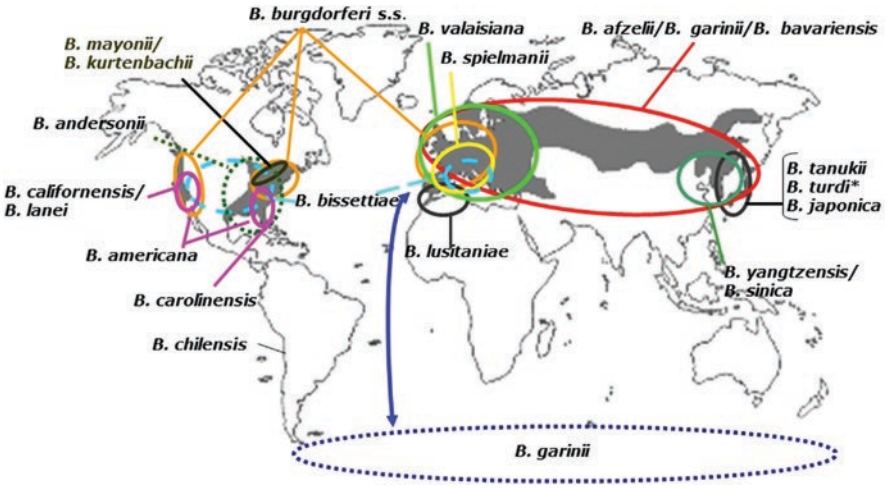


Fig. 1.3 Global distribution of *B. burgdorferi* sensu lato (from [26]). *In recent years, *B. turdi* has also been found in Europe in enzootic cycles driven by *I. frontalis* and passerine bird species [145]. For sake of clarity this is not indicated in the figure

[194]. For example, some potential mammalian hosts (e.g., large animals such as deer) may be colonized by *B. burgdorferi* s.l. spirochetes when bitten by an infected tick vector. They are, however, nonpermissive when it comes to transmission of the bacteria to a new tick and feeding on a deer may actually clear a *B. burgdorferi* s.l. infection in a tick [150, 199]. Following this, the presence of large numbers of deer may actually suppress the spirochete infection rate of true reservoir hosts in that location because ticks are more likely to feed on deer than on small mammals. On the other hand, the presence of deer in a particular geographic region may permit the population density of vector ticks to rise, which would increase the likelihood of successful transmission of spirochetes from infected reservoir hosts to ticks and thus increase R_0 [200–203].

The nonuniform distribution pattern of *Borrelia* genospecies observed in field studies suggests that apart from host associations, vector associations do indeed play an important role in limiting their geographic distribution ranges [193]. Some *Borrelia* species are able to utilize a wide range of vectors [71, 204], for example, *B. burgdorferi* s.s. are able to utilize *I. scapularis*, *I. pacificus*, *I. spinipalpis,* and *I. affinis* as vector in North America, as well as *I. ricinus* in Europe but they have not been found in *I. persulcatus* [69, 138]. *Borrelia garinii* can be vectored by *I. persulcatus, I. pavlovskyi, I. ricinus*, and *I. uriae*. Consequently, *B. garinii*'s geographic distribution ranges from France to Japan and it can be found in sea bird colonies in the Northern and Southern Hemisphere. *Borrelia garinii* has been found in sea bird colonies in Newfoundland [205] but it has not been discovered in North America in *I. scapularis* dominated regions or in *I. pacificus* [90, 206–209]. *Borrelia valaisiana*, also a bird-adapted *Borrelia* species, is frequently found in Europe associated with *I. ricinus* but only a single occurrence in Russia has been recorded [210]

suggesting that *I. persulcatus* is not a competent vector. Accordingly, in the overlapping zone of *I. ricinus* and *I. persulcatus* in Eastern Europe, the prevalence of *B. valaisiana* is higher in *I. ricinus* than in *I. persulcatus* [211].

A particular interesting case showing that differential vector adaptation plays an essential role in the geographic distribution of *Borrelia* species is that of *B. bavariensis* [13]. The *B. bavariensis* population in Western Europe differs genetically from that in Eastern Europe and Asia and they form sister clades in phylogenies not only based on MLST housekeeping genes but also based on >100 single-copy genes [212]. In addition, the Eastern population of *B. bavariensis* appears to be present only in regions where *I. persulcatus* serves as vector and it shows much higher genetic diversity than the populations in Western Europe. The population that is adapted to *I. ricinus* (Western Europe) shows very little genetic heterogeneity and appears almost clonal suggesting that this population arose recently via a vector switch [13, 26, 32].

1.6 Molecular Typing of *B. burgdorferi* s.l.

Because species of the genus *Borrelia* are difficult to distinguish by morphological criteria, approaches that can accurately identify species and strains within species are critical for epidemiological, clinical, and evolutionary studies. Early tools to discriminate between different *Borrelia* species included DNA-DNA hybridization, ribotyping, DNA sequencing of 16S rRNA or other conserved genes, PCR-based restriction fragment length polymorphism (RFLP) analysis, random amplified polymorphic DNA (RAPD) fingerprinting, or pulsed-field gel electrophoresis (RFLP) [213]. Single loci such as the outer surface proteins A (OspA), outer surface protein C (OspC), the intergenic spacer (IGS) region between the duplicated 5S and 23S rRNA [214], the 23S rRNA locus or flagellin (*flaB*) have been used for species and strain discrimination and are still popular targets for diagnostic purposes, e.g., [7, 23, 89, 215–223]. These targets have been used either individually or in combination for molecular characterization of *B. burgdorferi* s.l. from cultured isolates or directly on clinical samples, samples from mammalian hosts or ticks.

Since 2006/2007 multilocus sequence analysis (MLSA) has replaced DNA–DNA hybridization for species delimitation, epidemiological studies, or strain identification in *B. burgdorferi* s.l. and various multilocus sequence typing (MLST) schemes have been proposed (e.g., [14, 16, 224–227]). Not all of them use exclusively housekeeping genes as originally proposed for bacterial epidemiology and population-level studies [228, 229]. The system currently maintained at the Pubmlst database (http://pubmlst.org/borrelia/) at the University of Oxford [230] uses eight housekeeping loci that are encoded on the main chromosome; these are *clpA, clpX, nifS, pepX, pyrG, recG, rplB,* and *uvrA* [224, 225]. This MLST scheme has been shown to have great potential not only for *Borrelia* species discrimination [10–13, 15, 19, 24, 73, 90] but also for dissecting relationships of bacterial populations [25, 69, 70, 81, 205, 208, 209, 231–237].