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Genus

Spirochaetes/Spirochaetes/Spirochaetales/Borreliaceae/

Borreliella

Adeolu and Gupta 2014, VL163

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Bor.rel.i.el'la. N.L. fem. dim. n. *Borreliella*, named after Amédée Borrel (1867-1936).

Cells are helical, 0.2–0.3 μm in diameter, 15–30 μm in length, and do not have hooked ends. Coils are regular or irregular in spacing and amplitude. Motile. Inner and outer membrane with overlapping periplasmic flagella; 7 to 11 subterminal insertion points in most species. Aniline-stain-positive. Microaerophilic. Ferments glucose. Most species are cultivable in complex, serum-containing media that includes N-acetylglucosamine. Optimum growth is between 33 and 38° C. Cells are polyploid, with each genome comprising a linear chromosome and one or more linear and circular plasmids. All known species are host-associated organisms that are transmitted between mammalian, avian, and reptile reservoirs by a tick of the prostriate genus *Ixodes*. In unfed ticks the organisms are located in the midgut and only travel to the salivary glands once the next blood meal commences. Transovarial transmission in tick vector does not occur. Members of this genus include all known agents of Lyme disease (Lyme borreliosis) as well as several species not associated with human disease.

DNA G + C content (mol %): 28-29

Type Species: **Borreliella burgdorferi** (Adeolu and Gupta 2014, VL163) (basonym: *Borrelia burgdorferi* Johnson et al. 1984b[VP])

Cells are helical, 0.2–0.3 μm in diameter, and 15-30 μm in length. Lengths greater than 40 μm represent two daughter cells temporarily sharing a membranous connection at their ends. The coils, which usually are observed as flat waves under the microscope, vary in amplitude according to conditions and growth and may be regular or irregular in spacing (Figure 1). In most species, 7-11 unsheathed flagella are inserted sub-terminally in the protoplasmic cylinder and overlap centrally as a periplasmic bundle. The cells are actively motile in a liquid media, but the movements generally are slower and more languid than those of *Borrelia* species under the same conditions. *Borreliella* spp. cells in culture seldom manifest the rapid tight contraction and reversal movements characteristic of *Borrelia* spp. The spirochetes in culture, in particular low-passage

isolates, form large aggregates and mats visible to the eye (Figure 1) (Barbour, 1984). When *Borreliella* spirochetes are disseminating in the blood, most species are at densities too low to be visible in a blood smear by microscopy. *Borreliella* spirochetes are identified in skin and other tissues with silver stains, such as Warthin-Starry, but this reaction is not specific for this genus.

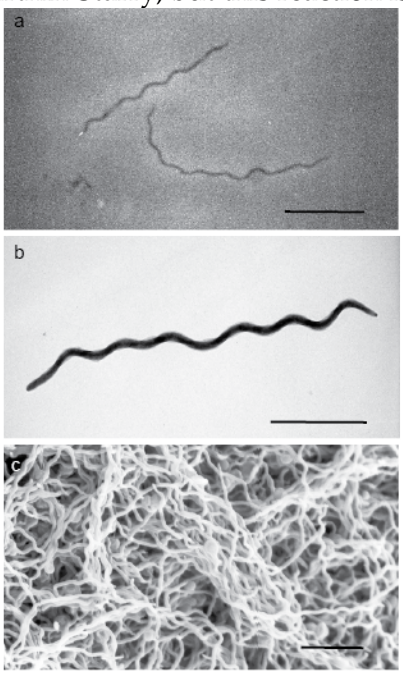

Figure 1. Morphology of *Borreliella burgdorferi* in broth medium. (a) Phase contrast photomicrograph of a wet mount of log-phase spirochetes in BSK II broth medium. Bar, 10 µm. (b) Electron photomicrograph of a spirochete negatively-stained with osmium tetroxide. Bar, 5 µm. (c) Scanning electron photomicrograph of a large aggregate of the spirochetes growing in broth medium and then fixed with glutaraldehyde,

Borreliella organisms are chemoorganotrophic. Ferment the monosaccharides glucose and mannose and utilize glycerol and the disaccharides maltose and chitobiose (von Lackum and Stevenson, 2005). They grow under microaerophilic conditions and elevated levels of CO_2 in a complex broth medium, which includes N-acetylglucosamine, long-chain fatty acids, short peptides and amino acids, nucleosides, albumin, and a serum source or substitute (Barbour, 1984, 1988). The diamino acid of the peptidoglycan is L-ornithine. Optimum growth between 33 and 38° C. Growth ceases above 42° C. *B. burgdorferi* remains viable in broth medium cultures stored at 4° C for a month or more. *B. burgdorferi* is cultivable as discrete single colonies within a soft agar overlay on a firmer layer (Kurtti et al., 1987).

Antibiotics that are routinely used in media to select from clinical or environmental sources *Borreliella* species from among other bacteria include the combination of rifampicin and phosphomycin. Under growth-favoring conditions, the spirochetes are also insusceptible to sulfamethoxazole, metronidazole (Caol et al., 2017), and 5-fluorouracil (Johnson et al., 1984a, b). The spirochetes are relatively resistant to inhibitors of DNA gyrase A, like nalidixic acid and fluoroquinolones, but susceptible to coumermycins, inhibitors of DNA gyrase B (Samuels and Garon, 1993). Minimal inhibitory concentrations of aminoglycosides are generally higher than considered safe for human use, but low enough to employ aminoglycosides for selection of transgenic organisms bearing the resistance marker (Stevenson et al., 1998). Unlike most other types of bacteria with two cell membranes, *Borreliella* species are susceptible to vancomycin (Dever et al., 1993).

The genome comprises a single linear chromosome of 900-1000 kb, several linear plasmids, usually in the range of 15-60 kb, and one or more circular plasmids of between 6 and 32 kb, each in approximate equal copy numbers to the chromosome (Hinnebusch and Barbour, 1992). The chromosome and linear plasmids have hairpin telomeres (Barbour and Garon, 1987). There is one 23S (*rrl*) gene in the chromosome, but in contrast to *Borrelia* species there are two 16S rRNA (*rrs*) genes (Schwartz et al., 1992). The multiple genomes of these polyploid cells are linearly-arrayed as nucleoids over the cell's length. Some circular plasmids are either lysogenic prophages that can be induced to form complete virions or are derived from bacteriophage that are now inactive (Eggers et al., 2000). Like other members of the family *Borreliaceae*, *Borreliella* species produce many types of bacterial lipoproteins (Bergström and Zückert, 2010; Dowdell et al., 2017), but not any lipopolysaccharides of the endotoxin type (Takayama et al., 1987).

DNA G + C content (mol %): 28-29

Type Species: **Borreliella burgdorferi** (Adeolu and Gupta 2014, VL163) (*Borrelia burgdorferi* Johnson et al., 1984b^{VP})

Number of species with validated names: 19

Number of other species: 4

Further descriptive information

The documented arthropod vectors of *Borreliella* species are hard ticks of the family Ixodidae, subfamily Ixodinae, and genus of *Ixodes*. Claims of carriage of *Borreliella burgdorferi* by hard ticks of other subfamilies of Ixodidae were either based on mistaken identification of a *Borrelia* species for *B. burgdorferi*, for example, *Borrelia lonestari* in *Amblyomma americanum* ticks (Barbour et al., 1996), or unconfirmed by other investigators (Stromdahl et al., 2018). Some members of the genus *Borreliella* are transmitted by two or three species of *Ixodes* ticks, as in the examples of *B. burgdorferi* by *I. scapularis*, *I. pacificus*, or *I. ricinus* ticks, or *B. afzelii* by *I. ricinus* or *I. persulcatus* ticks. Other species, such as *B. turdi* by *I. turdus* ticks, have more restricted ranges among vectors. With the exception of one species, *B. chilensis*, the genus members have been documented to date only in the temperate latitudes of the northern hemisphere.

The usual vertebrate reservoirs for member species are rodents, but other types of vertebrates may serve as competent reservoirs as well. Shrews are reservoirs for some species (Brisson and Dykhuizen, 2004), and other species are adapted to birds (Kurtenbach et al., 2002; Munro et al., 2017) or to lizards (Majlathova et al., 2006). Large ungulates, like deer, sheep, and cows, are important hosts for the adult stage of the tick vectors, but these animals generally are not effective as reservoirs of *Borreliella* spp.

The life cycles of the spirochetes and infections of hosts are best characterized for the three most frequent causes of Lyme disease: *B. burgdorferi*, *B. afzelii*, and *B. garinii* (Tsao, 2009; Radolf et al., 2012). *Borreliella* spirochetes are characteristically absent from eggs of ticks and from larvae until they feed (Rollend et al. 2013). After ticks as larvae or nymphs acquire spirochetes from an infected mammalian, avian, or reptilian reservoir, the bacteria remain in the tick's intestine after feeding and then through the molt to the next stage: nymph from larva, and adult from nymph (Piesman and Schwan, 2010). When an infected nymph embeds in the skin of vertebrate and begins its meal of blood and tissue fluid at the bite site, the spirochetes over the next 1-2 days translocate from the intestinal lumen into the hemolymph space and then travel to the salivary glands, from which enter into the host in the saliva. There is no transmission through regurgitation of the spirochetes in intestinal contents.

In the embedded tick, as the spirochetes make their way to the salivary glands and then into the skin, they decrease expression of the tick-associated, outer membrane lipoproteins OspA and OspB and increase expression of another outer membrane lipoprotein, OspC, and a suite of other proteins (Schwan and Piesman, 2000). Vertebrate hosts are seldom are exposed to cells expressing OspA or OspB, and consequently in the absence of selection by an adaptive immunity against these proteins OspA and OspB differ little in sequence between strains (Wilske et al., 1993). In contrast, OspC is abundantly expressed in the vertebrate host. As an immunodominant antigen of the invading spirochetes, OspC elicits an antibody response. Adaptive immunity and possibly host-specific innate immunity provides positive selection on OspC, and this probably accounts for the considerable variation in OspC sequences in sympatric populations of these organisms (Barbour and Travinsky, 2010). The polymorphisms are sufficiently specific for different strains that *ospC* sequences can be used for genotyping (Barbour and Cook, 2018).

After the spirochetes are cleared from the blood by antibodies to OspC and perhaps other strain-specific antigens, they can persist in the skin, as well as other tissues, of the reservoir host animal for the rest of its life. In this later, persistent phase of the infection a dominant surface antigen is the VlsE protein, which is homologous to the Vlp proteins of *Borrelia* species (Zhang et al., 1997a, b). A feeding tick acquires spirochetes from the blood directly during the bacteremic phase or from tissue fluid in the skin in the post-bacteremic phase.

Taxonomic comments

After the discovery of *B. burgdorferi* in the United States in 1981 and its first published description in 1982 (Burgdorfer et al., 1982), the occurrences in patients, ticks, and other vertebrates of *B. burgdorferi*-like organisms in Europe and subsequently in Asia were reported. These isolates were originally considered different strains of "*Borrelia burgdorferi*" in the formal description (Johnson et al., 1984a, b). While strains similar to the *B. burgdorferi* strains of North America were identified in Europe (Barbour et al., 1983; Postic et al., 1999), other strains from Europe differed from their North American counterparts in their electrophoretic protein patterns, plasmid profiles, and antibody reactivities (Barbour et al., 1985; Wilske et al., 1992). Two clusters of such isolates were eventually recognized as sufficiently distinct from *B. burgdorferi* to merit their own species designations: *B. afzelii* and *B. garinii* (Baranton et al., 1992). After this reclassification, the species *B. burgdorferi* kept its special status by an appended "sensu stricto", thereby distinguishing it both from other named agents of Lyme disease and yet-to-be classified spirochetes (Baranton et al., 1992). But all were grouped in the larger species complex under the term "*Borrelia burgdorferi* sensu lato", a *de facto* designation that served to distinguish this monophyletic group from all other *Borrelia* species, a set that included the known agents of relapsing fever (*see Borrelia*). Over time several other species, most of which were not associated with Lyme disease or any other human disease, were identified in Eurasia, North America and, eventually, South America (Ivanova et al., 2014). These were also informally included in the "*Borrelia burgdorferi* sensu lato" group (Stanek and Reiter, 2011).

In 2014 the species constituting the clade "*Borrelia burgdorferi* sensu lato" were assigned to a new genus, *Borreliella* (Adeolu and Gupta, 2014). In addition to the genome-wide analyses of coding sequences supporting the classification, Adeolu and Gupta (2014) distinguished *Borreliella* species from *Borrelia* species on the basis several conserved signature proteins (CSPs) and several conserved signature indels (CSIs), which are exclusively present in different species from these two groups of spirochetes (Gupta, 1998). A subsequent paper updated the list of distinguishing CSPs and CSIs and also reviewed the morphologic, ecologic, and epidemiologic features that characterize each genus (Barbour et al., 2017). These phylogenetic and phenotypic characteristics were then summarized in the description of the family *Borreliaceae*.

The split into two genera was challenged by Margos et al. (2018), whose claim largely relied on a metric called "percentage of conserved proteins" (POCP) (Qin et al. 2014). Qin et al. (2014) proposed a POCP value of 50% as a genus boundary for prokaryotes, but in the original article there were several instances of inter-generic pairs of species with POCP values that exceeded 50%. Gupta (2019) in a rebuttal to the Margos et al. (2018) paper provided a broad analysis of a variety of bacteria and further called in question the informativeness or utility of the 50% value as a genus boundary. Gupta (2019) also reaffirmed the phylogenetic informativeness of the majority of original CSPs and CSIs as the set of available Borreliaceae genomes further expanded to include more diverse members of the family, including *Borrelia turcica* and *Candidatus* Borrelia tachyglossi (Gofton et al., 2018).

The species excluded from the "*Borrelia burgdorferi* sensu lato" group retained the emended genus name *Borrelia* on the basis of priority of its type species, *Borrelia anserina* (*see Borrelia*). The genus name *Borreliella*, which means "Borrelia-like" in this context, indicated the relatedness of these organisms to *Borrelia* species and allowed retention of the abbreviation "*B. burgdorferi*" (or "*B. afzelii*", etc) in the scientific and medical literature as well as the popular press and other news media. The family name *Borreliaceae* permits continued usage of "borreliosis", as in "Lyme borreliosis", "neuroborreliosis", and "bovine borreliosis", for diseases caused by members of the family.

Phylogeny

The demarcation of the various *Borreliella* species described below and listed in Table 1 was primarily based on molecular sequences, initially individual genes, such as 16S rDNA (Figure 2) or flagellin; then combinations of genes, such as those used for multi-locus sequence typing (MLST); and more recently whole genome sequences (Figure 3). Some formal descriptions of *Borreliella* species also include tick vector, reservoir host associations, geographic distribution, and whether or not human infections have been documented. Most *Borreliella* or "*Borrelia burgdorferi* sensu lato" species have not generally been distinguished from other species in the genus on the basis of differences in morphology or physiology.

As described earlier, there are several CSPs and CSIs that distinguish *Borreliella* from *Borrelia* species (Adeolu and Gupta 2014; Barbour et al., 2017; Gupta, 2019). Table 2 lists protein-encoding genes that that are present in one genus but not the other. Table 3 list CSIs that distinguish between the two groups (Gupta, 2019). One gene absent from the genus *Borreliella* but present in all species of the genus *Borrelia* characterized to date, including the reduced genome species *Borrelia anserina* (Elbir et al., 2017) (Figure 3), is *glpQ*, whose protein product is glycerophosphodiester phosphodiesterase or GlpQ (Schwan et al., 2003). GlpQ commonly elicits an antibody response during the course of relapsing fever and has been used as the antigen of a serological assay that can used to discriminate relapsing fever from Lyme disease (Schwan et al., 1996).

With the exception of "*B. chilensis*", which was discovered in South America, all known *Borreliella* species occur in temperate regions of the Northern Hemisphere. The basal position of *B. chilensis* in Figure 3 and the distance of its chromosome sequence from other species (Table 4) are consistent with a biogeographic history of a refugia population directly descended from an ancestral *Borreliella* population that existed before further diversification.

While there is a general trend for the phylogeny of a species to correspond to the continent on which they are most abundant, there are exceptions (Table 1). The finding of *B. garinii* and *B. bavariensis* in *I. uriae* ticks of seabird colonies on the Atlantic coast of North America is consistent with the migration patterns of these birds and the frequent interactions between seabirds in the Arctic and Antarctic regions (Olsen et al., 1995). Another exception is "*Borreliella finlandensis*", which was identified in Finland, but its chromosome sequence is in a clade mainly comprising North American species (Casjens et al., 2011) (Figure 3).

The presence of some strains of the North America-associated species *B. burgdorferi* in Europe is also enigmatic (Postic et al., 1999) (Table 1). Initial phylogenetic studies using MLST with partial sequences of 8 chromosomal genes concluded that *B. burgdorferi* has an European origin, primarily based on the geographic range of its closest outgroup *B. finlandensis* (Margos et al., 2008; Qiu et al., 2008). But more recent genome-based phylogenies revealed much expanded lineages, one of which mainly comprised *B. burgdorferi* isolates but also included "*B. finlandensis*" (Qiu and Martin, 2014; Becker et al., 2016). By parsimony reasoning, it is plausible that European *B. burgdorferi* lineages and "*B. finlandensis*" were founded by trans-Atlantic dispersal of what originated in North America, perhaps by migratory birds breeding in high latitudes.

Methods for genotyping strains within a species have mainly been implemented for *B. burgdorferi, B. afzelii*, and *B. garinii*. Strains originally were defined by whole lysate protein profiles in electrophoretic gels, antibody reactivities, and restriction fragment length polymorphisms of chromosomes (Barbour and Cook, 2018). Genotyping for strain identification is now sequence-based, most commonly of the following loci, alone or in combination (Barbour and Cook, 2018): the *ospC* gene of the circular plasmid that carries the essential telomerase gene *resT* , the 16S-23S intergenic spacer (Bunikis et al., 2004), or MLST of 8 housekeeping genes on the chromosome (Margos et al., 2008). Increasingly, whole chromosome sequences are being used for genotyping strains.

Table 1. List of species

***Borreliella* species**	**Geographic distribution**	**Primary *Ixodes* tick vectors**	**Primary vertebrate reservoirs**	**Documented human disease**	**Major phylogenetic clade**	**Genome sequence (2018)**
B. afzelii	Europe, Asia	*I. ricinus, I. persulcatus*	Rodents	Lyme disease (Lyme borreliosis)	Eurasia	Yes
B. americana	Eastern and western United States (U.S.)	*I. minor, I. pacificus, I. spinipalpis*	Unknown	None	North America	No
"B. andersonii"	Eastern and central U.S.	*I. dentatus*	Rabbits, birds	None	North America	No
B. bavariensis	Europe, Asia, seabird colonies of Atlantic Canada	*I. ricinus, I. persulcatus, I. uriae*	Rodents, seabirds	Lyme disease	Eurasia	Yes
B. bissettiae	Western U.S.	*I. pacificus, I. spinipalpis*	Wood rats, black rats	None	North America	Yes
B. burgdorferi	Eastern, central, and western U.S., Canada, Europe	*I. ricinus, I. pacificus, I. scapularis*	White-footed mouse, other rodents, shrews, birds	Lyme disease	North America	Yes
B. californiensis	Far-western U.S.	*I. jellisoni, I. pacificus*	Kangaroo rats	None	North America	No
B. carolinensis	Southeastern U.S.	*I. minor*	Rodents	None	North America	No
"B. chilensis"	Chile	*I. stilesi*	Rodents	None	South America	Yes
"B. finlandensis"	Europe	*I. ricinus*	Unknown	None	North America	Yes
B. garinii	Europe, Asia, seabird colonies of Atlantic Canada and Maine (U.S.)	*I. ricinus, I. persulcatus, I. uriae*	Birds	Lyme disease	Eurasia	Yes
B. japonica	Japan	*I. ovatus*	Rodents, shrews	None	Eurasia	Yes
B. kurtenbachii	Eastern, central, and western U.S.	Unknown	Rodents	None	North America	No
B. lanei	California (U.S.)	*I. pacificus, I. spinipalpis*	Rabbits	None	North America	Yes
B. lusitaniae	Southern Europe, North Africa	*I. ricinus*	Lizards	One case of skin infection	Eurasia	No
B. mayonii	North-central U.S.	*I. scapularis*	Rodents	Lyme disease	North America	Yes
B. sinica	China	*I. minor*	Rodents	None	Eurasia	No
B. spielmanii	Europe	*I. ricinus*	Dormouse species	Lyme disease	Eurasia	Yes
B. tanukii	Japan	*I. tanuki*	Rodents	None	Eurasia	No
B. turdi	Japan, Taiwan	*I. turdus*	Birds	None	Eurasia	No
B. valaisiana	Europe, Asia	*I. columnae, I. granulatus, I. nipponensis, I.ricinus*	Rodents	None	Eurasia	Yes
B. yangtzensis	China, Japan	*I. minor*	Rodents, shrews	Possibly Lyme disease	Eurasia	No
Genomospecies CA690	California (U.S.)	*I. spinipalpis*	Unknown	None	North America	No

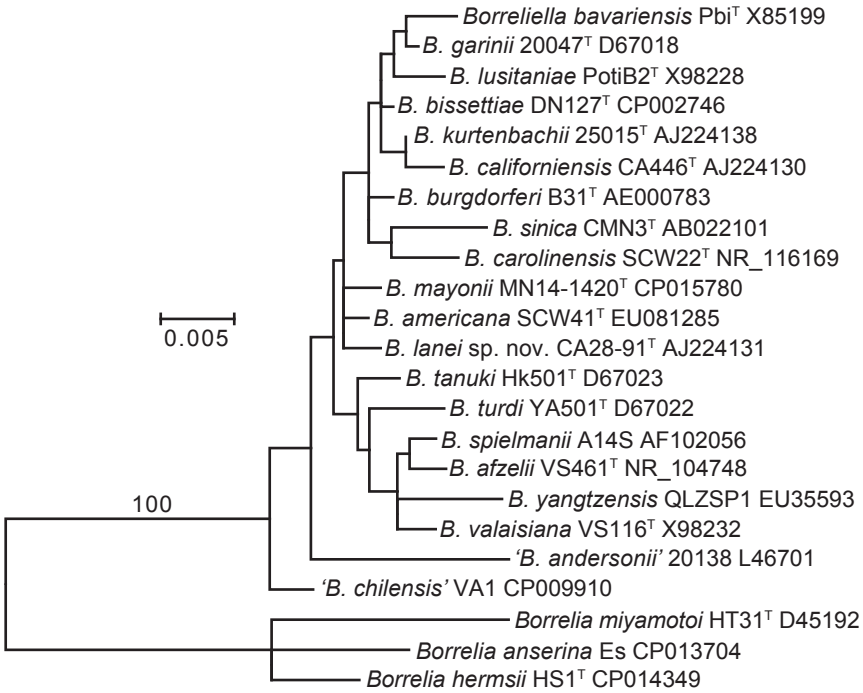

Figure 2. Neighbor-joining (NJ) distance tree based on nearly full-length 16S rRNA gene sequences showing the monophyletic cluster formed by members of the genus *Borreliella*, with selected members of the genus *Borrelia* as an outgroup, within the family *Borreliaceae*. The MUSCLE alignment included the 16S rDNA sequence of *Escherichia coli* K-12 substrain MG1655 (U00096; positions 4166659–4168200). The tree was based on ungapped nucleotide sequences between positions 56 and 1352 according to the *E. coli* sequence; for tree construction, the *E. coli* sequence was excluded. The numbers at the node represents bootstrap values by the distance protocol with the observed-differences criterion and by maximum likelihood (ML) under the General Time Reversible model as implemented with SeaView v. 4 (Gouy et al. 2010). GenBank/EMBL/DDBJ accession numbers follow the strain name of individually identified species or species candidates of Borreliaceae. The type strains are indicated by superscript "T." The bar indicates distance.

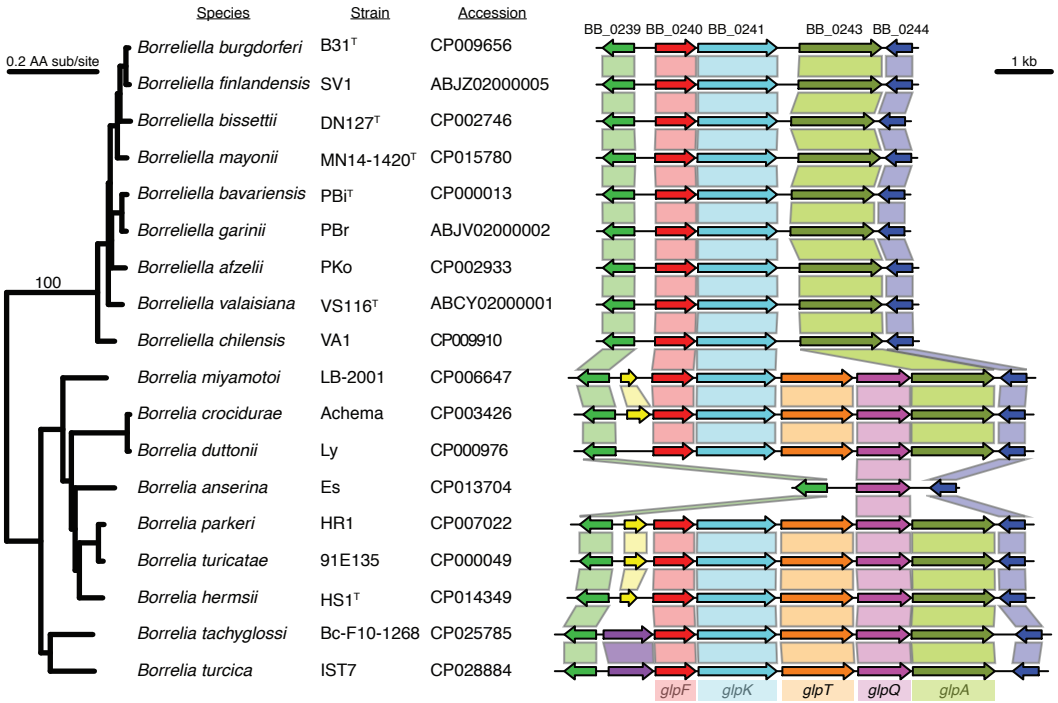

Figure 3. Chromosome phylogeny and a fixed chromosomal difference between *Borreliella* and *Borrelia*. On the left is a genome phylogram that is based on a concatenated protein-sequence alignment from 650 orthologous loci in 18 representative species of *Borreliella* and *Borrelia* (http//:borreliabase.org) (Di et al. 2014). Type (T) and other strain designations and corresponding chromosome sequence accession numbers are shown. Sequences were aligned with MUSCLE (Edgar 2004) and concatenated by BIOALN (Hernandez et al. 2018). The tree topology is inferred using FastTree (Price et al. 2010) and rooted at mid-point using BIOTREE (Hernandez et al. 2018). All branches are supported by bootstrap values of ≥ 90%. On the right is a schematic physical map of the glp region of the chrosomes of the species. The *glpT* gene (orange), encoding glycerol-3-phosphate transporter, is absent from all *Borreliella* species but present in all *Borrelia* species but *B. anserina*, and the *glpQ* gene (magenta), encoding glycerophosphodiester phosphodiesterase, are absent in *Borreliella* species but present in all *Borrelia* species, including *B. anserina*. Genes *glpF* (red), *glpK* (turquoise), and *glpA* (olive green) genes are present in all *Borreliaceae* but not *B. anserina*, which has a reduced genome (Elbir et al. 2017). In space between the BB_0239 and *glpF* loci are predicted coding sequences (yellow or purple) that are present in one or another of the *Borrelia* species but not the *Borreliella* species. Other fixed chromosomal differences are listed in Table 2.

Table 2. Fixed chromosomal ORF differences[a]

Locus	Predicted gene product
Genes present in *Borreliella* but not in *Borrelia*[b]	
BB_0364	Methylglyoxal synthase
BB_0576	Hypothetical protein (DUF1761; pfam08570)
Genes present in *Borrelia* but not in *Borreliella*[c]	
BH0123A	Septum formation protein Maf
BH0241A and B	Glycerol-3-phosphate transporter (GlpT; absent in *B.anserina*) and glycerophosphodiester phosphodiesterase (GlpQ) (Figure 3)
BH0253A	DNA repair protein RecO
BH0309A	DNA repair protein RecN
BH0398A	Hypothetical protein
BH0413A	Trigger Factor, a chaperone for nascent peptide
BH0421A, B and C	Uracil phosphoribosyltransferase, adenylosuccinate synthase, and argininosuccinate lyase
BH0438A	DNA replication/repair protein RecF
BH0445A	Hsp20/IbpA family protein (pfam00011)
BH0536A	Hypothetical protein
BH0541A	Hypothetical protein
BH0570A	Succinyl-diaminopimelate desuccinylase
BH0699A and B	Hypothetical protein and flagellar biosynthetic protein FlhB
BH0733A	Heme biosynthesis-associated TPR protein
BH0790A	RIP metalloprotease RseP/Periplasmic serine peptidase DegS

[a] Fixed genomic differences in absence and presence of chromosomal genes were obtained using the synteny view feature of BorreliaBase (Di et al. 2014)

[b] Locus names based on *Borreliella burgdorferi* B31 genome (accession number AE000783)

[c] Locus names based on *Borrelia hermsii* DAH genome (accession number CP000048)

Table 3. Conserved signature indels distinguishing *Borreliella* from *Borrelia* species [a]

Protein Name	*Borreliella burgdorferi* B31 locus	*Borrelia hermsii* DAH locus	Amino acid (aa) indel	Indel position *B. burgdorferi* [b]
Recombinase A	BB_0131	BH0131	1 aa insertion	250
Ferrous iron transporter A	BB_0347	BH0347	1 aa deletion	109
Chemotaxis protein CheY	BB_0415	BH0415	1 aa deletion	213
DNA polymerase III subunit beta	BB_0438	BH0438	1 aa deletion	114
Trigger factor Tig1	BB_0610	BH0610	2 aa insertion	129-130
Glucose-6-phosphate isomerase	BB_0730	BH0730	1 aa insertion	110
Translation factor Sua5	BB_0734	BH_0734	2 aa insertion	168-169

[a] Adeolu and Gupta, 2014; Gupta, 2019

[b] Indel in *B. burgdorferi* strain B31: at listed position for insertion and right after listed position for deletion

List of species of the genus *Borreliella*

Borreliella afzelii

Adeolu and Gupta 2014, VL182. (basonym: *Borrelia afzelii* Canica et al. 1993, VL48)

……...

af.ze'li.i. N.L. masc. gen. n. *afzelii* of Afzelius. The species was named in honor of Arvid Afzelius (1857-1923), who at a medical conference in Stockholm in 1909 presented a case of erythema migrans and attributed this skin rash to a bite of an *Ixodes* tick.

Another skin manifestation of Lyme disease, acrodermatitis achronica atrophicans (ACA), is more commonly associated with *B. afzelii* than either *B. garinii* or *B. burgdorferi*. The vectors of *B. afzelii* are *Ixodes ricinus* (the sheep tick) in Europe and *I. persulcatus* (the taiga tick) east of the Ural mountains in Russia and in Asia. *B. afzelii* is less likely than *B. garinii* to be isolated from the cerebrospinal fluid in areas where both species are endemic.

Primary natural reservoirs are rodents (Hanincova et al., 2003). The distinction between *B. afzelii* and *B. garinii* (see entry below) in their host ranges among vertebrates has been attributed to differential susceptibilities to serum complement and affinities for immune regulators, like Factor H, of various vertebrate species (Kurtenbach et al., 2002).

This clade was originally called "group VS461" (Baranton et al., 1992), and VS461, which was isolated from an *I. ricinus* tick in Switzerland, is the type strain. But the *de facto* representative strain of the species in many studies has been PKo, which was isolated from a skin biopsy of an ACA skin lesion in Germany (Preac-Mursic et al., 1986). *B. afzelii* is infectious for laboratory mice (Barthold et al., 2010). There are many isolates from ticks, mammals, and specimens from patients with Lyme disease. Several genome sequences are publicly available. For three strains (accession number for chromosome) there are sequences for complete chromosomes and 9 or more plasmids: PKo (CP00395 and CP002933), K78 (CP009058), and ACA-1 (CP018262)

DNA G + C content (mol %): 28.0 (median of 11 genome sequences).

Type strain: strain VS461, ATCC 51567, CIP 103469, DSM 10508.

Sequence accession no. (16S rRNA gene): U78151 (strain VS461) and CP000395 (strain PKo)

Borreliella americana

Adeolu and Gupta 2014, VL182. (basonym: *Borrelia americana* Rudenko et al., 2009 VL135)

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a.mer.i.ca'na. N.L. fem. adj. *americana* American, referring to the United States of America, where the organism was first isolated.

The taxon was first identified as "*Borrelia* genomospecies 1" as a category of organisms, including strain CA-29-1, that were identified in ticks in California but distinguishable from known species at the time (Schwan et al., 1993; Postic et al., 2007). Associated with *Ixodes minor* ticks and birds in the eastern United States (Rudenko et al., 2009) and with *I. pacificus* and *I. spinipalpis* ticks in California (Fedorova et al., 2014). No documented cases of human infection. Little information about vertebrate reservoirs. Infectivity and pathogenicity for laboratory mice or other experimental models have not been reported. Similar organisms by partial 16S rRNA gene sequences have been identified in *Ixodes* spp. ticks in South America, but these have not been well-characterized (Barbieri et al., 2013).

DNA G + C content (mol %): not known

Type strain: SCW-41, ATCC BAA-1877, DSM 22541.

Sequence accession no. (16S rRNA gene): EU081285 (SCW-41).

Borreliella bavariensis
Adeolu and Gupta 2014, VL163 (basonym: Margos et al. 2013[VP])

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ba.va.ri.en'sis. N.L. fem. adj. *bavariensis* of or belonging to Bavaria, where the type strain was first isolated.

The type strain PBi, a human isolate from cerebrospinal fluid (Preac-Mursic et al., 1986), was originally classified as *B. garinii* and eventually in the sub-category "OspA type 4" or "serotype 4" of that species (Wilske et al., 1993). Type 4 strains of *B. garinii* were noted to be comparatively common among isolates from cases of Lyme disease with central nervous system involvement (Marconi et al., 1999). Type 4 isolates, unlike other types of *B. garinii*, were resistant to complement of rodent species, and rodents, mainly *Apodemus* spp., are their main reservoir in Europe (Huegli et al., 2002). These phenotypic differences along with some sequence differences from other *B. garinii* strains led to the reclassification of type 4 isolates as *B. bavariensis* (Margos et al., 2013), but on the basis of its chromosome sequence, it might still be considered a strain of *B. garinii* (Figure 3).

Strain PBi was representative of *B. bavariensis* isolates from *I. ricinus* ticks of Europe. A second cluster of strains, typified by strain NT29, of this species was isolated from *I. persulcatus* ticks of Russia and Asia (Gatzmann et al., 2015). This second clade, called "NT29-like" originally to distinguish it from "OspA type 4", is genetically more diverse. Another reference strain in the second clade is BgVir, which was isolated from a *I. persulcatus* of Russia and was previously categorized as *B. garinii* (Brenner et al., 2012).

B. bavariensis was also identified in *Ixodes uriae* ticks of seabird colonies of Newfoundland and Labrador, Canada, in association with *B. garinii* in the same colonies (Munro et al., 2017). These *I. uriae*-associated strains cluster with the Asian clade rather than the European cluster of species. Some organisms identified as "*B. garinii*" in *I. uriae* and seabirds in the Northeast Atlantic likely represent previously unrecognized examples of *B. bavariensis*.

While *B. bavariensis* is generally known for its etiologic role in erythema migrans and neuroborreliosis, it was also identified in the synovial fluid of child with arthritis in Austria (Markowicz et al., 2015).

Genome sequence is available for type strain PBi (CP000013-CP000015 for chromosome and cp26 and lp54 plasmids) and for strain BgVir of the NT29-like cluster (CP003151 for chromosome and CP003201-CP003202 for cp26 and lp54 plasmids).

DNA G + C content (mol %): 28.1 (chromosome and two plasmids of strain PBi).

Type strain: PBi, DSM 23469, BAA-2496

Sequence accession no. (16S rRNA gene): NR_074854 (strain PBi).

Borreliella bissettiae
Gupta 2019 (basonym: *Borrelia bissettiae* Margos et al. 2016[VP]; corrig "*Borrelia bissettii*" (Postic et al., 1998)

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bis.set'ti.ae N.L. gen. n. *bissettiae*, of Bissett. Named in honor of Marjorie Bissett, who with her co-worker Warren Hill, first isolated and described this organism from a *I. pacificus* tick collected in Del Norte County, California (Bissett and Hill, 1987).

Originally called "*Borrelia* group DN127". In California associated with *Neotoma* spp., like the dusky-footed wood rat (*Neotoma fuscipes*), and less commonly the black or "roof" rat *Rattus rattus* (Fedorova et al., 2014). The vectors are *I. pacificus* and *I. spinipalpis* in California. Also found in *I. spinipalpis* in Colorado

(Maupin et al., 1994; Burkot et al., 2000). *I. spinipalpis* ticks in their areas of distribution rarely bite humans. There are no documented cases of Lyme disease or other human infection with this species in North America and only one reported case in Europe (Margos et al., 2016). Organism is infectious for laboratory mice but with lower spirochete burdens and less joint pathology than is observed with *B. burgdorferi* in experimental infections (Leydet and Liang, 2015). Some strains from the eastern and central United States previously called "*B. bissettii*" were reclassified as *B. kurtenbachii* (Margos et al. 2014).

The genome sequence of the type strain is available (CP002746-CP002762 for chromosome and 17 plasmids).

DNA G + C content (mol %): 28.4

Type strain: DN127, DSM 17990, CIP 109136.

Sequence accession no. (16S rRNA): KT709291 (strain DN127).

Borreliella burgdorferi

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia burgdorferi* Johnson et al. 1984b[VP])

burg.dor'fe.ri. N.L. masc. gen. n. *burgdorferi* of Burgdorfer. Named in honor of Wilhelm (Willy) Burgdorfer (1925-2014) for his discovery of the organism in *Ixodes* ticks in North America and Europe.

The source of type strain B31 was a set of *Ixodes scapularis* (formerly *I. dammini*) ticks collected by Jorge Benach on Shelter Island, New York and sent to Dr. Burgdorfer of the Rocky Mountain Laboratories of the National Institutes of Health in 1981 for a study of rickettsiae. Dr. Burgdorfer serendipitously observed spirochetes in the midguts of some of the ticks and recognized their significance as a possible etiology of Lyme disease (Burgdorfer et al., 1982). Pooled dissected midguts were provided to one of us (A.G.B.), who isolated and propagated the organism, provisionally named the "*Ixodes dammini* spirochete", in pure culture (Barbour 1984).

Within two years, similar organisms were isolated from a variety of sources, including blood, skin, and cerebrospinal fluid from patients with Lyme disease, from the white-footed deermouse (*Peromyscus leucopus*) and other small mammals, and from *Ixodes* ticks collected from other locations in the United States. Although initially described as "treponeme-like" (Burgdorfer et al., 1982), these spirochetes were subsequently recognized as being more like the *Borrelia* species that cause relapsing fever than either treponemes or leptospires. On the basis of characteristics shared with relapsing fever agents and limited DNA-DNA hybridization studies, the original isolate was assigned to the genus *Borrelia* (Johnson et al., 1984a, b; Schmid et al., 1984).

The tick vectors are *I. scapularis* in the northeastern, mid-Atlantic, and north-central region United States and adjoining areas of Canada, *I. pacificus* in far-western North America, and *I. ricinus* in western and northern Europe. *B. burgdorferi* has not been documented in *I. persulcatus* or other *Ixodes* species ticks of Russia or Asia. (There is a report of "*Borrelia burgdorferi*" isolated from metastriate *Dermacentor* and *Hyalomma* ticks in China (Ni et al., 2014), but this is an unlikely tick association for *B. burgdorferi*, and identity of the organisms was unverified.)

The main reservoirs in North America and Europe are rodents, such as the white-footed deermouse, in North America and *Apodemus* spp. (e.g. the wood mouse *A. sylvaticus*) and the bank vole *Myodes glareolus* in Europe. But the range of competent hosts also includes chipmunks, voles, shrews, and birds, especially ground-foraging or ground-nesting songbirds . Medium-sized mammals such as racoons, opossums, and skunks may become infected, but are not important as reservoirs. In northern California tree squirrels, like the fox squirrel *Sciurus niger* and the western gray squirrel *S. griseus*, are important reservoirs (Eisen et al.,

2009). Deer are the principal hosts for the adult stages of the vector ticks but are insufficiently competent as reservoirs to maintain the pathogen in an environment.

Strains of *B. burgdorferi*, defined by different genotyping schemes (Barbour and Cook 2018), vary in their capacity to cause disseminated infections and inflammation in humans and laboratory mice (Hanincová et al., 2008; Wormser et al., 2008; Strle et al., 2011). *B. burgdorferi* strains generally are susceptible to lizard sera, which have a lytic effect (Lane and Quistad, 1998). This limits the prevalence of infection of *B. burgdorferi* in ticks in California and the southeastern United States, where lizards are a common host for *I. pacificus* and *I. scapularis*, respectively.

While *B. burgdorferi*, like *B. afzelii* and *B. garinii*, causes erythema migrans, this species is notable for its causal role in a proliferative oligoarthritis of large joints, which fully manifests weeks to months after an initial infection (Steere and Glickstein, 2004). *B. burgdorferi* elicits a more inflammatory profile of responses in in vitro assays than either *B. afzelii* or *B. garinii* (Strle et al., 2009).

The strain B31 was the first Lyme disease agent to have its genome (the chromosome and all plasmids) sequenced (Fraser et al., 1997; Casjens et al., 2000). There are also complete or draft genome sequences from several isolates representing the three geographic regions (northeastern, north-central, and far-western) the species is found in North America, as well as strains unique to Europe.

DNA G + C content (mol %): 28.6.

Type strain: B31, ATCC 35210, CIP 102532, DSM 4680.

Sequence accession no. (16S rRNA gene): M59293 (strain B31).

Borreliella californiensis
Gupta 2019 (basonym: *Borrelia californiensis* Margos et al. 2016[VP])

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cal.i.for.ni.en'sis. N.L. fem. adj. *californiensis*, belonging to California, the United States state of origin of the first isolate.

The source of the type strain was a California kangaroo rat (*Dipodomys californicus*) (Postic et al., 1998). Subsequently all other isolates from a vertebrate species have been from *D. californicus* (Margos et al., 2016). Isolates were from *Ixodes jellisoni* and *I. pacificus* ticks removed from kangaroo rats (Fedorova et al., 2014). All isolates to date are limited to northern California. Organism is cultivable, but there is no description of the morphology. Infectivity and virulence for laboratory mice are not reported. No documented human infections.

DNA G + C content (mol %): not known

Type strain: CA446, DSM 17989, ATCC BAA-2689.

Sequence accession no. (16S rRNA): KT709517 (strain CA446).

Borreliella carolinensis
Adeolu and Gupta 2014, VL163 (basonym: Borrelia carolinensis (Rudenko et al. 2011[VP])

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ca.ro.li.nen'sis. N.L. fem. adj. *carolinensis*, of or belonging to Carolina, referring to South Carolina, the United States state where the organism was first isolated.

Associated with the tick *Ixodes minor* and a rodent cycle in the southeastern United States. *I. minor* rarely if ever bites humans. Similar organisms by partial DNA sequences were reported in a subspecies of the California vole (*Microtus californicus*) that is parasitized by *I. minor*-like ticks in the Mojave Desert of California

(Foley et al., 2014). Organism was cultivated, but there is no description of the morphology. Infectivity and virulence for laboratory mice are not known. No documented human infections.

DNA G + C content (mol %): not known

Type strain: SCW-22, ATCC BAA-1773, DSM 22119.

Sequence accession no. (16S rRNA): EU085407 (strain SCW-22).

Borreliella garinii

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia garinii* Baranton et al. 1992[VP])

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ga.ri'ni.i. N.L. masc. gen. n. *garinii* of Garin.

Named in honor of Charles Garin (- 1971), a French physician, who with Bujadoux reported in 1922 a case of erythema migrans that was associated with radiculopathy and meningitis (Garin and Bujadoux, 1922). First distinguished from *B. burgdorferi* as "Borrelia genomic group 20047" on the basis of whole cell protein profiles and antibody typing of the OspA protein (Wilske et al., 1993). One of the OspA-based serotypes, type 4, has since been split off as *B. bavariensis* (see entry above). There are multiple isolates, primarily from *Ixodes* ticks, human specimens, and less commonly birds in Europe, Asia, and sub-polar regions. The species is more diverse than other known species of the genus (Jacquot et al., 2014). The important tick vectors for transmission to humans are *I. ricinus* in Europe and *I. persulcatus* in Russia east of the Ural mountains and in Asia. In seabird colonies on maritime islands and sub-polar coastal regions, the vector is *I. uriae*, which rarely encounters humans (Olsen et al., 1995). Birds, especially ground-foraging and ground-nesting varieties, are the primary reservoirs (Comstedt et al., 2006). The species has also been recorded in North America, but this has been restricted to *I. uriae* and seabird colonies of Maine and Atlantic Canada (Smith et al., 2006; Munro et al., 2017).

B. garinii is more commonly associated with neuroborreliosis and the symptoms and signs of radiculopathy and meningitis than either *B. afzelii* or *B. burgdorferi* (Strle et al. 2006). Although "*B. garinii*" was said to be infectious for laboratory mice (Barthold et al., 2010), documented instances of experimental infection were often with a OspA type 4 strain (Hovius et al., 2007; Krupka et al., 2009), which was reclassified as *B. bavariensis* and, unlike *B. garinii*, known to be resistant to rodent complement. In a study of infectivity for mice of different strains of "B. garinii", the Pbi strain (now the *B. bavariensis* type strain) produced an infection, but the PBr strain, which remains *B. garinii*, did not (Pachner et al. 2004). *B. garinii* infections of mice may be achieved, but only with large inocula (Yrjanainen et al., 2006) (A.G.B., unpublished findings). Under these conditions rodent complement may select for complement-resistant mutants from complement-susceptible wild-type population. The comparative resistance of *Mus musculus* to experimental infection with *B. garinii* is an important phenotype that serves to distinguish this species from *B. burgdorferi*, *B. afzelii*, and *B. bavariensis*.

Genome sequence accession is available for the type strain (CP018744-CP018755 for complete chromosome, 5 complete plasmids, and 6 plasmid contigs). There are also draft or complete genome sequences available for strains PBr (human cerebrospinal fluid, Germany), Fa04 (puffin blood, Faroe Islands), and 935T (*Ixodes persulcatus* tick from South Korea).

DNA G + C content (mol %): 28.2%

Type strain: 20047, ATCC 51383, DSM 10534, CIP 103362.

Sequence accession no. (16S rRNA): D67018 (strain 20047).

Borreliella japonica

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia japonica* Kawabata et al. 1993, VL50)

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ja.po'ni.ca. N.L. fem. adj. *japonica* pertaining to Japan.

In Hokkaido, Japan where *B. garinii*, *B. afzelii*, and *B. japonica* co-exist, *B. japonica* was only identified in *I. ovatus* ticks, and *B. garinii* and *B. afzelii* were only identified in *I. persulcatus*, the predominant *Ixodes* species in the study areas (Murase et al., 2013). *I. ovatus* is distributed across Japan (Fournier et al., 2002). *B. japonica* has been isolated from rodents and shrews (Nakao et al., 1994; Yano et al., 1997). *B. japonica* infects laboratory mice but under the same conditions is comparatively less infectious than *B. afzelii* (Ishii et al., 1995) and less pathogenic than *B. burgdorferi* (Kaneda et al., 1998). There is serological evidence of *B. japonica* infection in one case of lymphadenitis after a tick bite in Japan, but no isolation from or direct detection in humans (Masuzawa et al., 1996). The species has not been documented outside of Japan (Masuzawa, 2004).

There is a draft genome sequence available for the type strain (FMTE00000000).

DNA G + C content (mol %): 27.9%.

Type strain: strain HO14, ATCC 51557, JCM 8951.

Sequence accession no. (16S rRNA): L40597 (strain HO14).

Borreliella kurtenbachii

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia kurtenbachii* Margos et al. 2014[VP])

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kur.ten.bach'i.i. N.L. gen. n. *kurtenbachii*, of Kurtenbach.

Named in honor and the memory of Klaus Kurtenbach (1959-2009) for his contributions to the ecology of Lyme disease. The type strain 25015 was originally isolated from an engorged *I. scapularis* larva feeding on *P. leucopus* in New York state in 1987 (Anderson et al., 1990). It was infectious for mice but less pathogenic than *B. burgdorferi* under the same conditions. It was recognized as phenotypically distinct from *B. burgdorferi* in other respects, including expression of an antigenically-divergent OspA protein sequence (Fikrig et al., 1992). But it has otherwise rarely been noted in *I. scapularis* ticks in North America. Similar organisms were later identified in California, and on the basis of limited sequence data, strain 25015 was included in "*Borrelia bissettii*" sp. nov. (Postic et al. 1998). Other isolates classified as "*B. bissettii*" on limited sequence data came from central and eastern United States (Picken et al. 1996; Picken and Picken 2000).

A subsequent multilocus sequence-based analysis of a larger set of isolates of this group established the distinctiveness of the cluster represented by 25015 from the cluster represented by strain DN127 and that eventually came to be redefined as *B. bissettiae* (Margos et al., 2010). The only isolates from mammals have been from the meadow vole *Microtus pennsylvanicus* and the meadow jumping mouse *Zapus hudsonicus* (Picken and Picken, 2000). There is a report of similar organisms isolated from patients in Slovenia (Strle et al., 1997), but *B. kurtenbachii* has not otherwise been documented in either Europe or Asia. The primary tick vector of this species in North America has not been established.

DNA G + C content (mol %): unknown

Type strain: 25015, ATCC BAA-2495, DSM 26572.

Sequence accession no. (16S rRNA): KF052001 (strain 25015).

Borreliella lanei

Gupta 2019 (basonym: *Borrelia lanei* Margos et al. 2017a[VP])

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la'ne.i. N.L. gen. n. *lanei* of Lane. Named in honor of Robert Lane of the University of California for his contributions to research on vector biology and ecology of Lyme disease.

Identified in and isolated from *I. pacificus* and *I. spinipalpis* ticks in California. The type strain was isolated from a *I. pacificus* tick in Kern County, California (Schwan, Schrumpf et al. 1993). Another reference strain is CA2, which was isolated from *I. spinipalpis*, in Mendocino County, California. Originally categorized "Genomospecies 2" and distinguished from known species on the basis of restriction fragment length polymorphisms (Postic et al., 1998). Little is known about the reservoir hosts, but there is some evidence of an association of this species with rabbits (Margos et al., 2017a; Scott et al., 2017). There is no association with human disease.

DNA G + C content (mol %): not known
Type strain: CA28-91, DSM 17992
Sequence accession no. (16S rRNA): AJ224131 (CA28-91).

Borreliella lusitaniae
Adeolu and Gupta 2014 (basonym: *Borrelia lusitaniae* Le Fleche et al., 1997[VP])

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lu.si.ta'ni.ae. L. gen. n. *lusitaniae* of Lusitania, the Roman name for Portugal, where the organism was first isolated.

Previously knows as "*Borrelia* genomic group PotiB2". Identified in *I. ricinus* ticks, lizards, and small rodents, and from the skin lesion of one patient in Portugal. Most prevalent in areas around the Mediterranean basin, including North Africa. Associated with lizards of the family Lacertidae (Majlathova et al., 2006). Unlike other *Borreliella* species that have been examined, *B. lusitaniae* is largely resistant to the lytic effects of reptile sera, which plausibly explains its success in regions where lizards are common. The type strain was infectious for laboratory mice but with a different distribution of pathology than was observed with *B. burgdorferi* under the same conditions (Zeidner et al., 2001).

DNA G + C content (mol %): not known
Type strain: PotiB2, CIP 105366.
Sequence accession no. (16S rRNA gene): X98228 (strain PotiB2).

Borreliella mayonii
Gupta 2019 (basonym: *Borrelia mayonii* Pritt et al., 2016b[VP])

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ma.yo'ni.i. N.L. gen. n. *mayonii* of Mayo. Named after the Mayo Clinic in Minnesota, one of the states where the species is endemic.

Human cases have been reported only from north-central region of the United States (Kingry et al., 2017). In this area *B. mayonii* is less frequent than *B. burgdorferi* as the etiology of illness. In the first reported human cases *B. mayonii* was in higher concentrations of spirochetes in the blood than is typical for *B. burgdorferi* (Pritt et al., 2016a, b). But higher spirochete numbers in the blood than *B. burgdorferi* were not observed in rodents with experimental infections (Dolan et al., 2017). *I. scapularis* is a competent vector of the species (Dolan et al., 2016). Human cases may present as an undifferentiated febrile illness without a discernible localized skin rash. No evidence of transovarial transmission in *I. scapularis* (Breuner et al., 2018). Identified in *P. leucopus* and red squirrel (*Tamiasciurus hudsonicus*) (Johnson et al., 2017).

Whole-genome sequence accession is available for the type strain (CP015780-CP015795 for chromosome and plasmids).

DNA G + C content (mol %): 27.9
Type strain: MN14-1420, ATCC BAA-2743, DSM 10281.
Sequence accession no. (16S rRNA): KM877342 (MN14-1420).

Borreliella sinica

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia sinica* Masuzawa et al., 2001[VP])

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si'ni.ca. M.L. fem. adj. *sinica* of China, the country from which the organism was isolated.

Morphology as described for the genus, except for the report of a maximum of four flagella at each end (Masuzawa et al., 2001). The original source was the murid *Niviventer confucianus*, the Chinese white-bellied rat, in Sichuan Province, China (Masuzawa et al., 2001). Associated with *I. ovatus* ticks in China and Nepal (Masuzawa, 2004). It has not been found in Japan. Infectivity and pathogenicity for laboratory mice is not reported. No documented human cases of infection.

DNA G + C content (mol %): not known
Type strain: CMN3, DSM 23262, JCM 10505.
Sequence accession no. (16S rRNA): AB022101 (strain CMN3).

Borreliella spielmanii

Adeolu and Gupta 2014, VL163 (basonym: *Borrelia spielmanii* Richter et al., 2006[VP])

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spiel.ma'ni.i. N.L. masc. gen. n. *spielmanii* of Spielman. Named in honor of Andrew Spielman (1930-2006) of Harvard University, who described the life cycle of Lyme disease agents and the vector biology of the tick vectors.

Originally called the "*Borrelia* A14S" for an early isolate (Wang et al., 1999). The tick vector is *I. ricinus*. A common reservoir host is the garden dormouse (*Eliomys quercinu*s) or other species of dormouse. In Germany *B. speilmanii* followed *B. garinii*, *B. afzelii*, *B. burgdorferi*, and *B. valaisiana* in descending order of infection prevalence in collected *I. ricinus* (Fingerle et al., 2008). *B. speilmanii* has been infrequently isolated from patients with erythema migrans in Europe (Maraspin et al., 2006; Fingerle et al., 2008). In an attempt to infect laboratory mice with a large inoculum of low-passage of bacteria, *B. speilmanii* A14S was not recovered from any organs or skin (Krupka et al., 2009).

A draft genome sequence for the type strain is available (ABKB00000000 for the scaffolds of the chromosome and CP001464-CP001471 for 8 plasmids).

DNA G + C content (mol %): 27.7
Type strain: PC-Eq17N5, CIP 108855, DSM 16813
Sequence accession no. (16S rRNA): DQ133523 (strain PC-Eq17N5).

Borreliella tanukii

Adeolu and Gupta 2014 (basonym: *Borrelia tanukii* Fukunaga et al., 1996, VL63)

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ta.nu'ki.i. N.L. gen. n. *tanukii* of or from tanuki, named after *Ixodes tanuki*, from which the organism was isolated.

Originally defined as "group Hk501", a name derived from an isolate from an *Ixodes tanuki* tick taken from a raccoon dog (*Nyctereutes procyonoides*). The tick vector is *I. tanuki*, and the reservoirs in Japan are the large Japanese field mouse *Apodemus speciosus*, and the grey red-backed vole *Myodes rufocans* (Masuzawa et

al., 1996). The distribution appears to be largely limited to Japan. The infectivity and pathogenicity for laboratory mice is not reported. No documented cases of human infection.

DNA G + C content (mol %): not known

Type strain: Hk501

Sequence accession no. (16S rRNA gene): D67023 (strain Hk501).

Borreliella turdi

Adeolu and Gupta 2014 (basonym *Borrelia turdi* Fukunaga et al., 1996, VL63)

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tur'di. N.L. gen. n. *turdi* of or from turdus, named after *Ixodes turdus*, from which the organism was isolated.

The tick vector is *Ixodes turdus* ticks, and passerine birds, especially thrushes (*Turdus* spp.), are a reservoir in Japan and Taiwan (Fukunaga et al., 1996; Kuo et al., 2017). All stages of *I. turdus* feed on birds. The infectivity and pathogenicity for laboratory mice is not reported. No documented cases of human infection.

DNA G + C content (mol %): not known

Type strain: strain Ya501, JCM 9661.

Sequence accession no. (16S rRNA gene): D67022 (Ya501).

Borreliella valaisiana

Adeolu and Gupta 2014, VL182 (basonym: *Borrelia valaisiana* Wang et al., 1997[VP])

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va.lai.si.a'na. N.L. fem. adj. *valaisiana* of or belonging to the Valais canton of Switzerland, where this organism was first isolated.

Previously known as "genomic group VS116" and "genomic group M19". Several ticks are competent as vectors, including *I. ricinus* in Europe (Wang et al., 1997), *Ixodes granulatus* in China (Hou et al., 2015), *I. nipponensis* in Korea, and *Ixodes columnae* in Japan (Masuzawa, 2004). The reservoirs are reported to be rodents (Masuzawa et al., 2000). Although commonly found in *I. ricinus* ticks in Europe, there is little evidence that it causes disease in humans in areas of its distribution (Margos et al., 2017).

Draft genome sequences are available for the type strain (ABCY020000001) and strain Tom4006 (CP009117).

DNA G + C content (mol %): 27.5

Type strain: VS116, CIP 105367, DSM 21467.

Sequence accession no. (16S rRNA gene): X98232 (VS116).

Borreliella yangtzensis

Gupta 2019 (basonym: *Borrelia yangtzensis* Margos et al. 2015[VP]; corrig. "*Borrelia yangtze*" Chu et al., 2008)

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yang.tzen'sis. N.L. fem. adj. *yangtzensis*, referring to the Yangtze River valley in China.

Isolated from ticks and rodents in the Yangtze River valley in southwestern China (Zhang et al., 1997a, b) and subsequently in Japan (Masuzawa et al., 2004; Kawabata et al., 2013). Recognized as a sister taxon to *B. valaisiana* and originally called "*B. valaisiana*-related" organisms. But unlike the latter species, which is associated with birds, *B. yangtzensis* is associated with rodents, such as the rice field mouse (*Mus caroli*) and shrews, such as the lesser Ryukyu shrew (*Crocidura watasei*) and the Asian house shrew (*Suncus*

murinus) (Masuzawa et al., 2004; Chu et al., 2008). The black rat (*Rattus rattus*) may also be a reservoir (Margos et al., 2015). Vectors are *I. granulatus* (Hiraoka et al., 2007) and *I. nipponensis* (Masuzawa et al., 1999). *B. garinii* and *B. afzelii* were more frequent causes of human infection than *B. yangtzensis* in an area of China where they are sympatric (Ni et al., 2014), but the illnesses are not well-documented.

DNA G + C content (mol %): not kown.
Type strain: Okinawa-CW62, DSM 24625, JCM 17189.
Sequence accession no. (16S rRNA): AB526082 (strain Okinawa-CW62).

Other species:

"*Borreliella (Borrelia) andersonii*"
(Marconi et al., 1995)

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an.der.so'ni.i. N.L. masc. gen. n. *andersonii* of Anderson. Named in honor of John Anderson, who with his colleagues at the Connecticut Agricultural Field Station first isolated and characterized this organism.

This species is associated with *Ixodes dentatus* tick as a vector and with cottontail rabbits (*Sylvilagus floridanus*) and several species of birds as vertebrate hosts in northeastern and north-central regions of the United States (Anderson et al., 1989; Hamer et al., 2012). *B. andersoni* has also been found in the southeastern and south-central United States in association with *S. floridanus* and *I. dentatus* (Lin et al., 2004). *I. dentatus* ticks are mainly associated with birds and rabbits and rarely bite humans. There is no documented case of human infection.

DNA G + C content (mol %): not known
Type strain: 21038
Sequence accession no. (16S rRNA gene): L46701 (strain 21038)

"*Borreliella (Borrelia) chilensis*"
(Ivanova et al., 2014)

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chi.len'sis. N.L. fem. adj. *chilensis* of or belonging to Chile, referring to Chile, the country of origin of the first isolate.

Isolated from *Ixodes stilesi* ticks in southern Chile. Reservoir suspected to be long-tailed rice rats (*Oligorysomys longicaudatus*), but a pure culture was not achieved. Infectivity and pathogenicity for humans and other mammals are unknown. The sequence of the chromosome and the cp26 and lp54 plasmids of strain VA1 have been determined (CP009910-CP009912) (Huang et al., 2015)

DNA G + C content (mol %): 28.4% (sequence of chromosome scaffold and two complete plasmids)
Type strain: VA1
Sequence accession no. (16S rRNA gene): JX669129 (strain VA1).

"*Borreliella (Borrelia) finlandensis*" sp. nov.
(Casjens et al., 2011)

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fin.lan.den'sis. N.L. fem. adj. *finlandensis*, of or belonging to Finland, the country of the first isolate.

The two original isolates, SV1 and Ri5, of this proposed species, originally called "*Borrelia* sp. SV1", were *I. ricinus* ticks from Finland. There are no isolates and identifications of this organism in North America.

Its distinctiveness was noted first by multi-locus sequence typing (Qiu et al., 2008) and then by whole genome sequencing (Casjens et al., 2011). As an exception to the overall phylogeographic pattern of this genus (see above), this organism is a sister taxon to *B. burgdorferi* in a monophyletic clade (Jacquot et al., 2014). Little is known about its host and vector associations. No reported human disease association.

DNA G + C content (mol %): 28.3% (sequence of chromosome scaffold and nine plasmids)

Type strain: SV1

Sequence accession no. (16S rRNA gene): locus tag BSV1_0437 of draft genome sequence ABJZ02000005 (strain VA1)

Borreliella (Borrelia) sp. CA690 (Fedorova et al., 2014)

Single isolate (CA690) from an *I. spinipalpis* tick collected in Northern California (Fedorova et al., 2014). Comparative analysis of the CA690 chromosome indicates that it is the most basal *Borreliella* lineage in North America identified thus far (W. Qiu). Reservoir species and infectivity for laboratory mouse are not known. No documented cases of human infection.

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Table 1. List of species

***Borreliella* species**	**Geographic distribution**	**Primary *Ixodes* tick vectors**	**Primary vertebrate reservoirs**	**Documented human disease**	**Major phylogenetic clade**	**Genome sequence (2018)**
B. afzelii	Europe, Asia	*I. ricinus, I. persulcatus*	Rodents	Lyme disease (Lyme borreliosis)	Eurasia	Yes
B. americana	Eastern and western United States (U.S.)	*I. minor, I. pacificus, I. spinipalpis*	Unknown	None	North America	No
"B. andersonii"	Eastern and central U.S.	*I. dentatus*	Rabbits, birds	None	North America	No
B. bavariensis	Europe, Asia, seabird colonies of Atlantic Canada	*I. ricinus, I. persulcatus, I. uriae*	Rodents, seabirds	Lyme disease	Eurasia	Yes
B. bissettiae	Western U.S.	*I. pacificus, I. spinipalpis*	Wood rats, black rats	None	North America	Yes
B. burgdorferi	Eastern, central, and western U.S., Canada, Europe	*I. ricinus, I. pacificus, I. scapularis*	White-footed mouse, other rodents, shrews, birds	Lyme disease	North America	Yes
B. californiensis	Far-western U.S.	*I. jellisoni, I. pacificus*	Kangaroo rats	None	North America	No
B. carolinensis	Southeastern U.S.	*I. minor*	Rodents	None	North America	No
"B. chilensis"	Chile	*I. stilesi*	Rodents	None	South America	Yes
"B. finlandensis"	Europe	*I. ricinus*	Unknown	None	North America	Yes
B. garinii	Europe, Asia, seabird colonies of Atlantic Canada and Maine (U.S.)	*I. ricinus, I. persulcatus, I. uriae*	Birds	Lyme disease	Eurasia	Yes
B. japonica	Japan	*I. ovatus*	Rodents, shrews	None	Eurasia	Yes
B. kurtenbachii	Eastern, central, and western U.S.	Unknown	Rodents	None	North America	No
B. lanei	California (U.S.)	*I. pacificus, I. spinipalpis*	Rabbits	None	North America	Yes
B. lusitaniae	Southern Europe, North Africa	*I. ricinus*	Lizards	One case of skin infection	Eurasia	No
B. mayonii	North-central U.S.	*I. scapularis*	Rodents	Lyme disease	North America	Yes
B. sinica	China	*I. minor*	Rodents	None	Eurasia	No
B. spielmanii	Europe	*I. ricinus*	Dormouse species	Lyme disease	Eurasia	Yes
B. tanukii	Japan	*I. tanuki*	Rodents	None	Eurasia	No
B. turdi	Japan, Taiwan	*I. turdus*	Birds	None	Eurasia	No
B. valaisiana	Europe, Asia	*I. columnae, I. granulatus, I. nipponensis, I.ricinus*	Rodents	None	Eurasia	Yes
B. yangtzensis	China, Japan	*I. minor*	Rodents, shrews	Possibly Lyme disease	Eurasia	No
Genomospecies CA690	California (U.S.)	*I. spinipalpis*	Unknown	None	North America	No

Table 2. Fixed chromosomal ORF differences[a]

Locus	Predicted gene product
Genes present in *Borreliella* but not in *Borrelia*[b]	
BB_0364	Methylglyoxal synthase
BB_0576	Hypothetical protein (DUF1761; pfam08570)
Genes present in *Borrelia* but not in *Borreliella*[c]	
BH0123A	Septum formation protein Maf
BH0241A and B	Glycerol-3-phosphate transporter (GlpT; absent in *B.anserina*) and glycerophosphodiester phosphodiesterase (GlpQ) (Figure 3)
BH0253A	DNA repair protein RecO
BH0309A	DNA repair protein RecN
BH0398A	Hypothetical protein
BH0413A	Trigger Factor, a chaperone for nascent peptide
BH0421A, B and C	Uracil phosphoribosyltransferase, adenylosuccinate synthase, and argininosuccinate lyase
BH0438A	DNA replication/repair protein RecF
BH0445A	Hsp20/IbpA family protein (pfam00011)
BH0536A	Hypothetical protein
BH0541A	Hypothetical protein
BH0570A	Succinyl-diaminopimelate desuccinylase
BH0699A and B	Hypothetical protein and flagellar biosynthetic protein FlhB
BH0733A	Heme biosynthesis-associated TPR protein
BH0790A	RIP metalloprotease RseP/Periplasmic serine peptidase DegS

[a] Fixed genomic differences in absence and presence of chromosomal genes were obtained using the synteny view feature of BorreliaBase (Di et al. 2014)

[b] Locus names based on *Borreliella burgdorferi* B31 genome (accession number AE000783)

[c] Locus names based on *Borrelia hermsii* DAH genome (accession number CP000048)

Table 3. Conserved signature indels distinguishing *Borreliella* from *Borrelia* species [a]

Protein Name	*Borreliella burgdorferi* B31 locus	*Borrelia hermsii* DAH locus	Amino acid (aa) indel	Indel position *B. burgdorferi* [b]
Recombinase A	BB_0131	BH0131	1 aa insertion	250
Ferrous iron transporter A	BB_0347	BH0347	1 aa deletion	109
Chemotaxis protein CheY	BB_0415	BH0415	1 aa deletion	213
DNA polymerase III subunit beta	BB_0438	BH0438	1 aa deletion	114
Trigger factor Tig1	BB_0610	BH0610	2 aa insertion	129-130
Glucose-6-phosphate isomerase	BB_0730	BH0730	1 aa insertion	110
Translation factor Sua5	BB_0734	BH_0734	2 aa insertion	168-169

[a] Adeolu and Gupta, 2014; Gupta, 2019

[b] Indel in *B. burgdorferi* strain B31: at listed position for insertion and right after listed position for deletion