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ORIGINAL PAPER

A phylogenomic and molecular marker based proposal for the division of the genus *Borrelia* into two genera: the emended genus *Borrelia* containing only the members of the relapsing fever *Borrelia*, and the genus *Borreliella* gen. nov. containing the members of the Lyme disease *Borrelia* (*Borrelia burgdorferi* sensu lato complex)

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Abstract The genus *Borrelia* contains two groups of organisms: the causative agents of Lyme disease and their relatives and the causative agents of relapsing fever and their relatives. These two groups are morphologically indistinguishable and are difficult to distinguish biochemically. In this work, we have carried out detailed comparative genomic analyses on protein sequences from 38 *Borrelia* genomes to identify molecular markers in the forms of conserved signature inserts/deletions (CSIs) that are specifically found in the *Borrelia* homologues, and conserved signature proteins (CSPs) which are uniquely present in *Borrelia* species. Our analyses have identified 31 CSIs and 82 CSPs that are uniquely shared by all sequenced *Borrelia* species, providing molecular markers for this group of organisms. In addition, our work has identified 7 CSIs and 21 CSPs which are uniquely found in the Lyme disease *Borrelia* species and eight CSIs and four CSPs that are specific for members of the relapsing fever *Borrelia* group. Additionally, 38 other CSIs, in proteins which are uniquely found in *Borrelia* species, also distinguish these two groups of *Borrelia*. The identified CSIs and CSPs provide novel and highly specific molecular markers for identification and distinguishing between the Lyme disease *Borrelia* and the relapsing fever *Borrelia* species. We also report the results of average nucleotide identity (ANI) analysis on *Borrelia* genomes and phylogenetic analysis for these species based upon 16S rRNA sequences and concatenated sequences for 25 conserved proteins. These analyses also support the distinctness of the two *Borrelia* clades. On the basis of the identified molecular markers, the results from ANI and phylogenetic studies, and the distinct pathogenicity profiles and arthropod vectors used by different *Borrelia* spp. for their transmission, we are proposing a division of the genus *Borrelia* into two separate genera: an emended genus *Borrelia*, containing the causative agents of relapsing fever and a novel genus, *Borreliella* gen. nov., containing the causative agents of Lyme disease.

Keywords *Borrelia* · *Borreliella* · *Borrelia* taxonomy · Lyme disease · Phylogenetic trees · Average nucleotide identity · Conserved signature indels · Conserved signature proteins

Introduction

The genus *Borrelia* is an important pathogenic group of helical shaped, motile organisms that form a highly

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distinct, monophyletic lineage within the phylum Spirochaetes (Paster 2011; Wang and Schwartz 2011). Members of this genus are the causative agents of both Lyme disease, which is currently the most prevalent vector-borne disease in North America and temperate regions of Eurasia, and relapsing fever, which is a disease endemic to many disparate regions of the world (Lindgren and Jaenson 2006; Cutler 2010; Adams et al. 2013). Currently, the genus *Borrelia* contains 37 species which are carried by arthropod vectors and exhibit varying pathogenicity in mammalian and avian hosts (Margos et al. 2011; Wang and Schwartz 2011; Parte 2014). These species can be separated into two main groups based upon their pathogenicity profiles. The first group, containing the causative agents of Lyme disease, is commonly referred to as the *Borrelia burgdorferi* sensu lato complex, whereas the other group contains the causative agents of relapsing fever (Postic et al. 1990; Baranton et al. 1992; Wang et al. 1999; Margos et al. 2011; Wang and Schwartz 2011). Although, these two groups are morphologically indistinguishable from each other, their members can be distinguished from each other based on the arthropod vectors which transmit them and by a limited number of biochemical and genetic tests (Wang et al. 1999; Margos et al. 2011; Wang and Schwartz 2011). Our current understanding of the taxonomy and evolutionary relationships among the *Borrelia* species is based largely on DNA–DNA hybridization studies, 16S rRNA gene sequence analysis and multilocus sequence analysis (MLSA) (Margos et al. 2011; Wang and Schwartz 2011). Although these studies provide evidence suggesting separation of the members of the genus *Borrelia* into two distinguishable groups, due to lack of other reliable molecular, morphological, or biochemical characteristics that can distinguish these groups, no formal recognition of these two distinct groups of *Borrelia* has thus far been made (Wang and Schwartz 2011).

Whole genome sequences for members of the genus *Borrelia* are becoming increasingly available in public databases. There are currently 38 genomes from 18 species of *Borrelia* available in the NCBI database (NCBI 2014). These genomes provide a valuable resource to gain insight into the evolutionary history of this group of organisms and to identify novel shared molecular characteristics that are specific for this group of organisms. One useful comparative genomic approach, pioneered by our lab, involves the identification of conserved signature indels (CSIs), which are insertions/deletions uniquely present in protein sequences of organisms from the group of interest, and conserved signature proteins (CSPs), which are lineage specific proteins found only in the group of interest (Gupta and Griffiths 2006; Gupta 2010; Naushad et al. 2014). Due to the specificity of these markers (viz. CSIs and CSPs) for particular groups of bacteria, they represent molecular synapomorphies (markers of common evolutionary decent) which can be used to identify and demarcate specific bacterial groups in clear molecular terms. Additionally, whole genome sequences are also enabling the use of other computational algorithms to determine the overall genome similarity among different organisms (Richter and Rosselló-Móra 2009).

Our recent comparative analysis of Spirochaetes genomes has identified 38 CSIs that clearly delimit the major groups within the phylum and were used to revise the taxonomy of the phylum as a whole (Gupta et al. 2013b). In this work, we extend these studies by examining, in detail, the evolutionary relationships among the *Borrelia* species employing different phylogenetic and comparative genomic approaches. These analyses have identified 31 CSIs and 82 CSPs that are commonly shared by all sequenced *Borrelia* species. More importantly, these studies have identified of 53 CSIs and 25 CSPs, which serve to clearly distinguish the two main groups of *Borrelia* species and provide novel molecular markers to demarcate them in definitive terms. The distinctness of these two groups of *Borrelia* species is also supported by the results of an average nucleotide identity (ANI) analysis of *Borrelia* genomes and by phylogenetic trees constructed based upon 16S rRNA sequences and concatenated protein sequences. On the basis of the identified molecular markers, phylogenetic studies, and other evidence presented here, it is proposed that the genus *Borrelia* should be divided into two separate genera: an emended genus *Borrelia*, containing the causative agents of relapsing fever and a novel genus, *Borreliella* gen. nov., containing the causative agents of Lyme disease.

Methods

Phylogenetic sequence analysis

Phylogenetic analysis was performed on a concatenated sequence alignment of 25 highly conserved

Table 1 Characteristics of the *Borrelia* genomes used for phylogenetic and comparative analysis

Strain Name	Accession number	Size (Mb)	GC %	Chromosomes	Plasmids	Genome source
Borrelia afzelii ACA-1	ABCU02	0.90	27.86	1	14	(Casjens et al. 2011b)
Borrelia afzelii HLJ01	NC_018887	0.91	28.30	1	–	(Jiang et al. 2012b)
Borrelia afzelii PKo	NC_017238	0.90	27.90	1	17	(Casjens et al. 2011b)
Borrelia anserina BA2	CP005829	0.90	29.50	1	–	Rocky Mountain Laboratories[a]
Borrelia bavariensis PBi[T]	NC_006156	0.90	28.12	1	11	(Glöckner et al. 2004)
Borrelia bissettii DN127	NC_015921	0.90	28.33	1	16	(Schutzer et al. 2012)
Borrelia burgdorferi 118a	ABGI02	0.90	28.21	1	19	(Schutzer et al. 2011)
Borrelia burgdorferi 156a	ABCV02	0.91	28.10	1	19	(Schutzer et al. 2011)
Borrelia burgdorferi 29805	ABJX02	0.89	28.26	1	15	(Schutzer et al. 2011)
Borrelia burgdorferi 64b	ABKA02	0.91	28.39	1	18	(Schutzer et al. 2011)
Borrelia burgdorferi 72a	ABGJ02	0.91	28.16	1	13	(Schutzer et al. 2011)
Borrelia burgdorferi 94a	ABGK02	0.91	28.22	1	13	(Schutzer et al. 2011)
Borrelia burgdorferi B31[T]	NC_001318	0.91	28.18	1	21	(Fraser et al. 1997)
Borrelia burgdorferi Bol26	ABCW02	0.91	28.59	1	10	(Schutzer et al. 2011)
Borrelia burgdorferi CA-11.2A	ABJY02	0.91	28.37	1	12	(Schutzer et al. 2011)
Borrelia burgdorferi CA382	NC_022048	0.91	28.60	1	–	UCI[b]
Borrelia burgdorferi CA8	ADMY01	0.90	28.50	1	–	UCI[b]
Borrelia burgdorferi JD1	NC_017403	0.92	28.30	1	20	(Schutzer et al. 2011)
Borrelia burgdorferi N40	NC_017418	0.90	28.24	1	16	(Schutzer et al. 2011)
Borrelia burgdorferi WI91-23	ABJW02	0.90	28.29	1	20	(Schutzer et al. 2011)
Borrelia burgdorferi ZS7	NC_011728	0.91	28.23	1	14	(Schutzer et al. 2011)
Borrelia crocidurae Achema	NC_017808	0.92	29.06	1	39	(Elbir et al. 2012)
Borrelia duttonii Ly	NC_011229	0.93	28.02	1	16	(Lescot et al. 2008)
Borrelia garinii BgVir	NC_017717	0.91	28.23	1	2	(Brenner et al. 2012)
Borrelia garinii Far04	ABPZ02	0.89	27.83	1	7	(Casjens et al. 2011b)
Borrelia garinii NMJW1	NC_018747	0.90	28.40	1	–	(Jiang et al. 2012a)
Borrelia garinii PBr	ABJV02	0.90	27.83	1	11	(Casjens et al. 2011b)
Borrelia hermsii HS1	NC_010673	0.92	29.81	1	2	(Dai et al. 2006)
Borrelia hispanica CRI	AYOU01	0.94	28.00	1	–	(Elbir et al. 2014b)
Borrelia miyamotoi LB-2001	NC_022079	0.91	28.70	1	–	(Hue et al. 2013)
Borrelia parkeri HR1	CP007022	0.92	28.90	1	–	(Barbour and Miller 2014)
Borrelia parkeri SLO	CP005851	0.92	28.90	1	–	Rocky Mountain Laboratories[a]
Borrelia persica No12	AYOT01	0.92	28.70	1	–	(Elbir et al. 2014a)
Borrelia recurrentis A1	NC_011244	0.93	27.51	1	7	Unité des Rickettsies[c]
Borrelia sp. SV1	ABJZ02	0.95	28.27	1	9	(Casjens et al. 2011a)
Borrelia spielmanii A14S	ABKB02	1.01	27.69	1	8	(Schutzer et al. 2012)
Borrelia turicatae 91E135	NC_008710	0.92	29.10	1	–	Rocky Mountain Laboratories[a]
Borrelia valaisiana VS116[T]	ABCY02	0.91	25.83	1	11	(Schutzer et al. 2012)

Genomic information was collected from: http://www.ncbi.nlm.nih.gov/genomes/

[T] type strain

[a] Rocky Mountain Laboratories: Genome sequenced by the Laboratory of Human Bacterial Pathenogenesis at Rocky Mountain Laboratories

[b] UCI: Genome sequenced by the department of Microbiology and Molecular Genetics at the University of California, Irvine

[c] Unité des Rickettsies: genome sequenced by Unité des Rickettsies at Centre National de Référence

proteins (viz. ArgRS, DnaK, EF-G, EF-Tu, GyrA, GyrB, Hsp60, Hsp70, IleRS, RecA, RpoB, RpoC, SecY, ThrRS, TrpRS, ValRS, and ribosomal proteins L1, L2, L5, L6, S3, S8, S9, S11, and S12) which represent a subset of the core proteins present in all bacteria that are widely used for phylogenetic analysis (Harris et al. 2003; Charlebois and Doolittle 2004; Ciccarelli et al. 2006; Vinuesa 2010; Gao and Gupta 2012b; Gupta et al. 2013b). Sequences for these proteins were obtained from the NCBI database for 38 sequenced *Borrelia* species (Table 1) and *Treponema pallidum* Nichols which was used to root the tree. Multiple sequence alignments for these proteins were created using Clustal_X 1.83 (Jeanmougin et al. 1998) and concatenated into a single alignment file. Poorly aligned regions from this alignment file were removed using Gblocks 0.91b (Castresana 2000). The resulting alignment, which contained 12,129 aligned amino acids, was used for phylogenetic analysis. The maximum likelihood tree based on 1,000 bootstrap replicates of this alignment was constructed using MEGA 6.0 (Tamura et al. 2013) employing the Le and Gascuel (Le and Gascuel 2008) substitution model.

A 16S rRNA gene sequence based phylogenetic tree was also created based on 53 sequences that included representative strains of all cultured *Borrelia* species (Supplemental Table 1). 16S rRNA gene sequences larger than 1,200 bp were obtained for all type strains classified under the genus *Borrelia* in release 115 of the SILVA database (Quast et al. 2013). 16S rRNA gene sequences were also obtained for representative strains from *Borrelia* species without a cultured type and for *T. pallidum* Nichols which was used to root the tree. A maximum likelihood tree based on these sequences was created using 1,000 bootstrap replicates of the 16S rRNA sequence alignments in MEGA 6.0 (Tamura et al. 2013) employing the General Time-Reversible (Tavaré 1986) substitution model.

Average nucleotide analysis

Average nucleotide identity values were calculated in order to assess the relatedness of the sequenced *Borrelia* genomes using the JSpecies v1.2.1 program (Richter and Rosselló-Móra 2009) which utilized an algorithm developed by Goris et al. (2007) to analyze the sequence identity of pairwise genome alignments created using the BLAST v2.2.26 program (Altschul et al. 1997).

Identification of conserved signature indels

To identify CSIs that are commonly shared by the different groups of *Borrelia*, BLAST searches (Altschul et al. 1997) were performed using each protein in the genome of *Borrelia recurrentis* A1 as queries. These searches were performed using the default BLAST parameters against all available sequences in the GenBank non-redundant database. For those proteins for whom high scoring homologues (E values $< 1e^{-20}$) were present in other *Borrelia* species, multiple sequence alignments were created using the Clustal_X 1.83 program (Jeanmougin et al. 1998). These alignments were visually inspected for the presence of insertions or deletions that were flanked on both sides by at least 5-6 conserved amino acid residues in the neighbouring 30–40 amino acids. Indels that were not flanked by conserved regions were not further considered, as they do not provide useful molecular markers (Gupta 2010; Naushad et al. 2014). The specificity of potentially useful indels for subgroups within of the genus *Borrelia* was further evaluated by carrying out detailed BLAST searches on short sequence segments containing the indel and the flanking conserved regions (60-100 amino acids long). To ensure that the identified signatures are only present in *Borrelia* homologues, 250 BLAST hits with the highest similarity to the query sequence were examined for the presence or absence of these CSIs. In this work, we report the results of CSIs that are specific for different groups within the *Borrelia* and where similar CSIs were not observed in any other bacteria in the top 250 BLAST hits. The sequence alignment files presented here contain sequence information for all sequenced species within the genus *Borrelia*. However, due to space constraints, different strains of the sequenced species are not shown, but they all displayed similar sequence characteristics.

Identification of conserved signature proteins

To identify proteins that are uniquely present in various groups of *Borrelia*, BLAST searches (Altschul et al. 1997) were performed using each protein in the genomes of *B. burgdorferi* B31 and *B. recurrentis* A1 as queries. These searches were performed using the default BLAST parameters against all available sequences in the GenBank non-redundant database. Proteins were considered CSPs if either all significant

hits were from well-defined groups of *Borrelia* or which involved a large increase in E values from the last hit belonging to a particular group of *Borrelia* to the first hit from any other bacteria and the E values for the latter hits were $>1e^{-04}$, indicating weak similarity that could occur by chance (Gao and Gupta 2007; Naushad et al. 2014). In most cases, the lengths of various significant hits were very similar to those of the query proteins.

Results

Genomic characteristics of the sequenced *Borrelia*

Genome sequences for 38 *Borrelia* strains comprising 18 different species, which are currently available in the NCBI genome database, were used in these analyses. Some characteristics of these *Borrelia* genomes are summarized in Table 1. The genomes of most *Borrelia* species/strains, in addition to containing a linear chromosome, harboured large numbers of linear and circular plasmids, which is very unique among the prokaryotes (Chaconas 2005; Chaconas and Kobryn 2010). The chromosome sizes of the sequenced *Borrelia* fell within a narrow range between 0.89 and 1.01 Mb, with G+C content ranging between 25.83 and 29.81 %.

Phylogenetic sequence analysis

The current understanding of the phylogeny of the genus *Borrelia* is largely based on phylogenetic trees constructed using 16S rRNA, flagellin or housekeeping gene sequences (Fukunaga et al. 1996; Margos et al. 2009; Wang and Schwartz 2011). In this work, we have constructed a phylogenetic tree of the sequenced *Borrelia* species using concatenated sequences for 25 conserved housekeeping and ribosomal proteins (Fig. 1). Members of the genus *Borrelia* have shown some competence for the lateral transfer of tRNA synthetases (Ibba et al. 1997). However, phylogenetic trees based on concatenated sequences for a large number of unlinked and conserved loci minimize the effect of any instances of lateral gene transfer and provide greater resolving power than trees based on any single gene or protein (Rokas et al. 2003; Wu et al. 2009). In the concatenated protein tree, the sequenced *Borrelia* species clustered into two distinct monophyletic and strongly supported clades, which were separated by long branches. One of these clades consisted of the Lyme disease causing *B. burgdorferi* species (*B. burgdorferi* sensu stricto) and its relatives (*B. burgdorferi* sensu lato), while the other clade was comprised of the relapsing fever *Borrelia* (*B. recurrentis*) and its relatives (Fig. 1). These two clades of *Borrelia* are also clearly distinguished in a phylogenetic tree for 3,737 genome sequenced prokaryotes, which was constructed based upon >400 proteins (Segata et al. 2013).

A phylogenetic tree was also constructed based on the 16S rRNA gene sequences, which included representatives from all cultured *Borrelia* species (Fig. 2). Except for *Borrelia turcica*, all *Borrelia* species were grouped into two distinct clades similar to those seen in the concatenated protein tree. However, an earlier study showed that *B. turcica* clusters with several unnamed *Borrelia* isolates in a monophyletic clade related to the relapsing fever *Borrelia* (Takano et al. 2010). The members of the genus *Borrelia* have also been observed to branch into two distinct clades in a number of earlier phylogenetic studies based on 16S rRNA and other individual genes/protein sequences (Takano et al. 2010; Margos et al. 2011; Wang and Schwartz 2011).

Conserved signature indels that distinguish the two clades of Borrelia

CSIs and CSPs that are restricted to a given group of related species provide useful molecular characteristics for evolutionary studies (Gupta 1998; Rokas and Holland 2000; Gao and Gupta 2012a). Recently, CSIs have been used to define novel taxonomic groups and to propose important taxonomic changes for groups of bacteria (viz. Aquificae, *Bacillus*, Chloroflexi, *Neisseriales,* Spirochaetes, Synergistetes and Thermotoga) at different taxonomic ranks (Bhandari and Gupta 2012; Adeolu and Gupta 2013; Bhandari et al. 2013; Gupta et al. 2013a, b; Gupta and Lali 2013; Bhandari and Gupta 2014). In this work we have carried out comprehensive comparative analyses of *Borrelia* genomes in order to identify CSIs that clarify the relationship between the *Borrelia*. These studies have identified 31 CSIs that are specifically found in protein

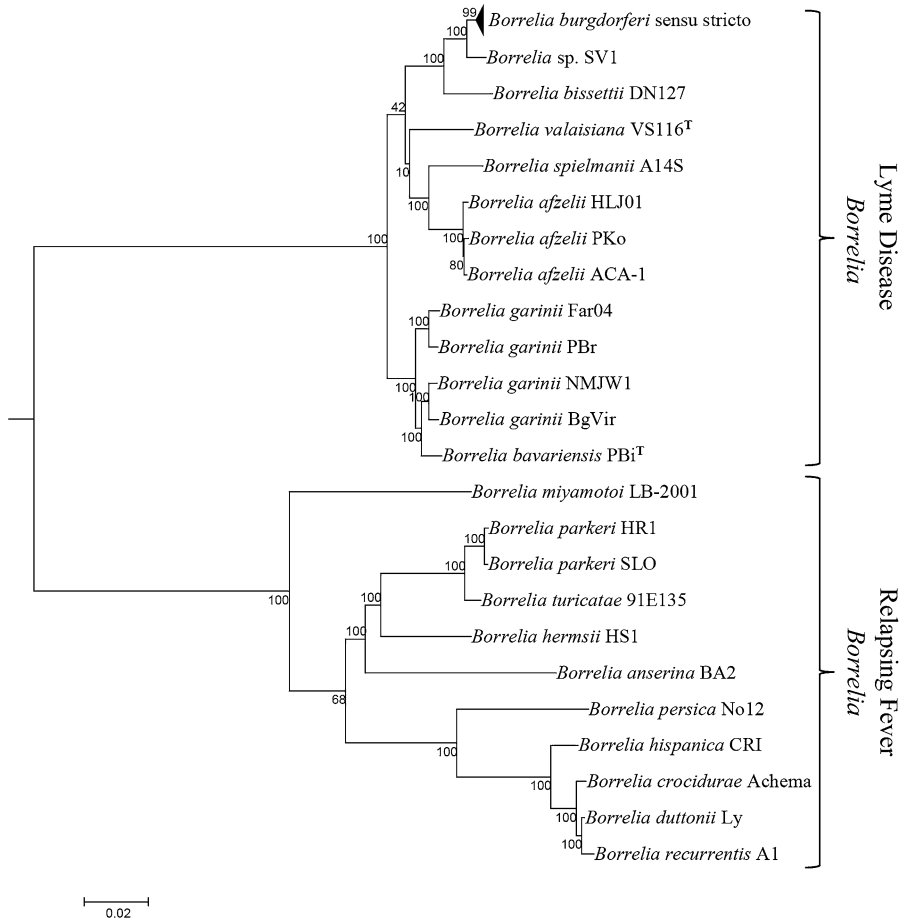


Fig. 1 A maximum likelihood phylogenetic tree of 38 sequenced members of the genus *Borrelia* based on the concatenated amino acid sequences of 25 conserved proteins. Bootstrap values are shown at *branch nodes*. The Lyme disease and relapsing fever clades of *Borrelia* are marked. The *letter*^{T} refers to the type strain of the species

homologues from members of the genus *Borrelia* as currently defined and absent in homologues from all other sequenced bacterial groups. Fifteen of these 31 CSIs are identified for the first time in this work, whereas the remaining 16 CSIs were identified in our earlier analysis of the phylum Spirochaetes (Gupta et al. 2013b). One example of a novel CSI that is uniquely found in all of the sequenced species from the genus *Borrelia* is shown in Fig. 3. In the example shown, a 3 aa insert in a conserved region of the bacterial rod-shaped determining protein MreB is uniquely present in all sequenced *Borrelia* species, but it is not found in sequences from any other Spirochaetes or other phyla of bacteria (Fig. 3). Sequence information for the 14 other novel CSIs that are also specific for the genus *Borrelia* is presented in Supp. Fig. 1–14 and a summary of all 31 *Borrelia* specific CSIs is presented in Table 2.

Our analyses have also identified 53 CSIs that are specific for or distinguish between the two main clades of *Borrelia* species, which are observed in the phylogenetic trees. Of these, seven CSIs are specific for the Lyme disease *Borrelia* clade, whereas another eight novel CSIs are uniquely found in the *Borrelia* species that are part of the relapsing fever clade. Examples of a CSI specific for the Lyme disease *Borrelia* clade and a CSI specific for the relapsing fever *Borrelia* clade are shown in Fig. 4. Figure 4a shows a 1 aa insert in a conserved region of Recombinase A that is uniquely found in all eight sequenced species from the Lyme disease *Borrelia* clade, whereas Fig. 4b shows a 1 aa deletion in the nicotinamide-nucleotide adenylyltransferase protein that is specific for members of the relapsing fever *Borrelia* clade. Sequence information for other CSIs that are specific for these two clades of *Borrelia*

Fig. 2 A maximum likelihood tree based on the 16S rRNA gene sequences of representative strains of *Borrelia*. Bootstrap values are shown at *branch nodes*. The Lyme disease and relapsing fever clades of *Borrelia* are marked. The *letter* ^{T} refers to the type strain of the species. The accession numbers of the 16S rRNA gene sequences used in this analysis are provided in Supplemental Table 1

species are presented in Supp. Fig. 15–27 and Table 3. In addition to these 15 CSIs found in widely distributed proteins, 38 other CSIs in proteins that are mainly found in *Borrelia* species also serve to distinguish the Lyme disease *Borrelia* clade from the relapsing fever *Borrelia* clade. Because homologues for these proteins, or the conserved regions where these CSIs are present in these proteins, are not found in other bacteria, it is difficult to infer whether these CSIs represent insertions or deletions in the two groups. However, these CSIs still serve to distinguish between the two groups of *Borrelia*. One example of a 3 aa

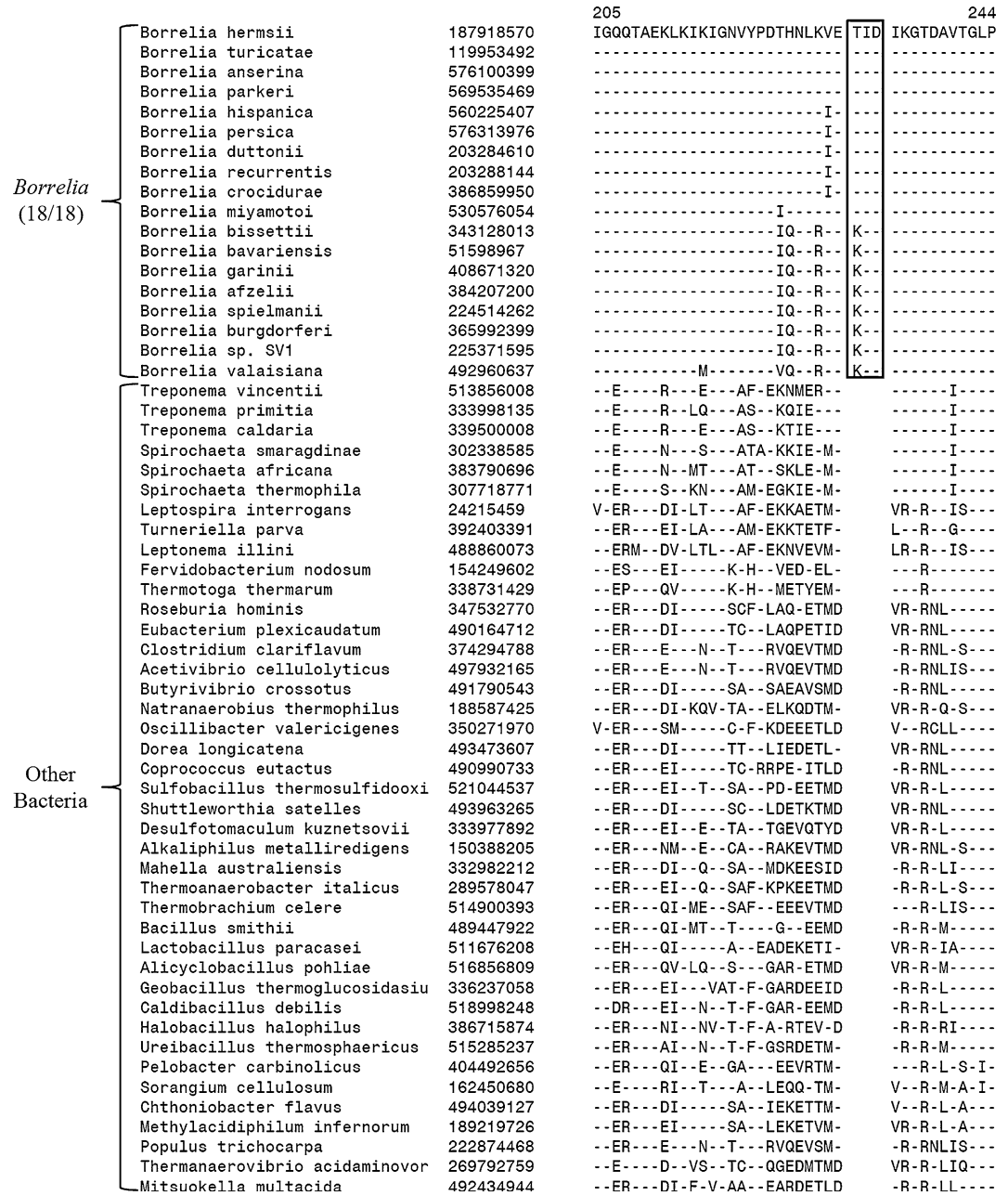


Fig. 3 A partial sequence alignment of the rod shape-determining protein *MreB*, showing a CSI (*boxed*) that is uniquely present in all members of the genus *Borrelia.* Sequence information for a single *Borrelia* strain from each of the 18 sequenced *Borrelia* species and a limited number other bacteria is shown here, but unless otherwise indicated similar CSIs were detected in all members of the indicated group and not detected in any other bacterial species in the top 250 BLAST hits. The *dashes* in the alignments indicate identity with the residue in the top sequence. GenBank identification (GI) numbers for each sequence are indicated in the *second column*. Sequence information for 30 other CSIs that are specific for all sequenced *Borrelia* species is provided in Supplemental figures 1–14 and Table 2

indel in a *Borrelia* specific protein of unknown function that distinguishes the Lyme disease *Borrelia* clade from the relapsing fever *Borrelia* clade is shown in Fig. 5. Sequence information for 37 other CSIs in different proteins that are of a similar kind is presented in Supp. Fig. 28–64 and Table 4.

Table 2 Conserved signature indels that are specific for all sequenced *Borrelia* species (both the Lyme disease *Borrelia* (*Borreliella*) and the relapsing fever *Borrelia*)

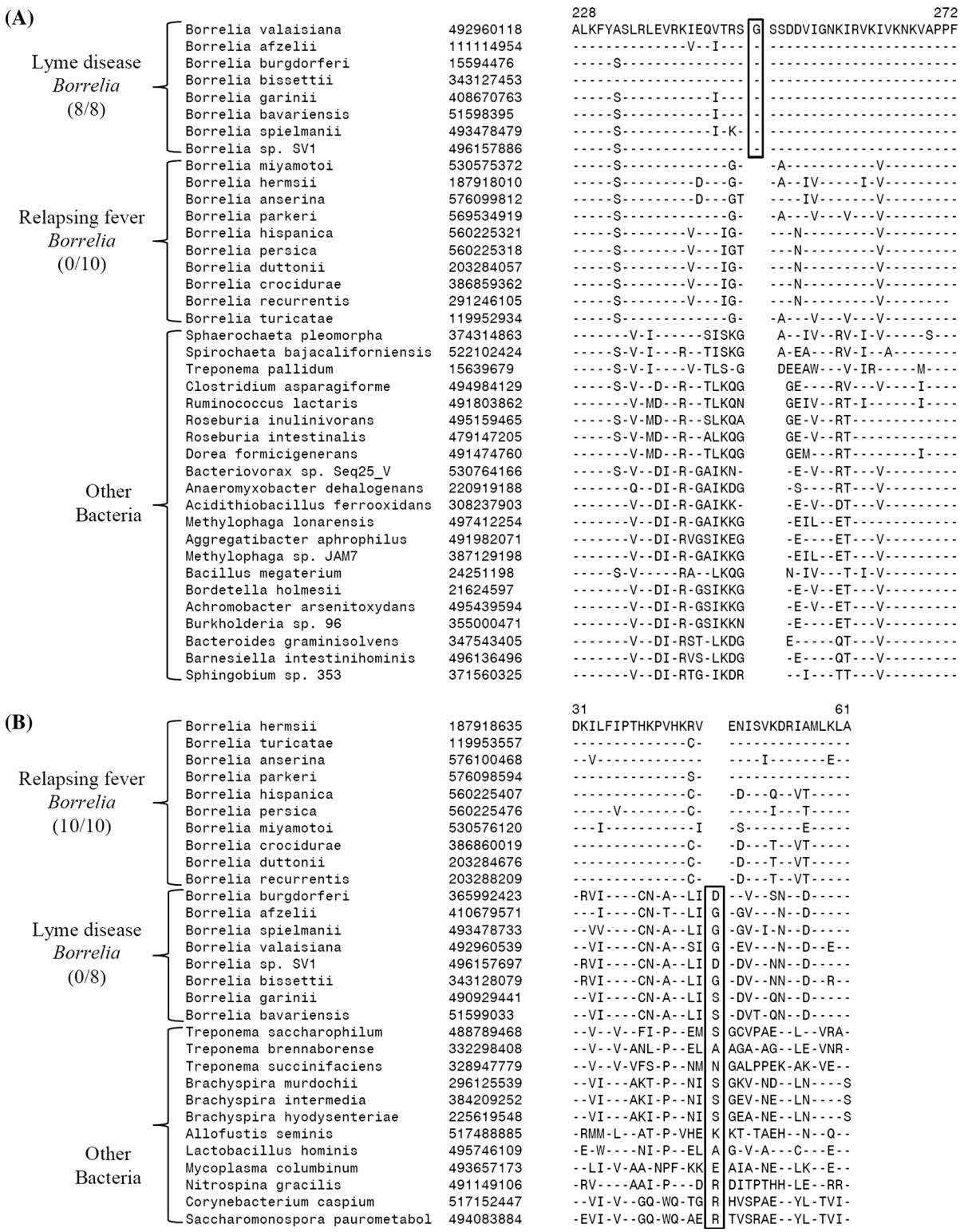
Protein Name	GI number	Figure number	Indel size	Indel position
Rod shape-determining protein MreB	187918570	Figure 3	3 aa ins	205–244
Flagellar motor switch protein FliM	119953077	Sup. Fig. 1	7 aa ins	8–65
ATP-dependent protease peptidase subunit	119953095	Sup. Fig. 2	3 aa ins	60–91
Mg^{2+} transporter MgtE	119953171	Sup. Fig. 3	1 aa ins	163–230
Mg^{2+} transporter MgtE	119953171	Sup. Fig. 4	4 aa ins	347–412
Cobyric acid synthase CobQ[a]	187918297	Sup. Fig. 5	1 aa del	147–184
Jag protein	119953232	Sup. Fig. 6	2 aa ins	121–180
CTP synthetase	119953361	Sup. Fig. 7	6 aa ins[b]	388–411
Chaperonin GroEL	187918505	Sup. Fig. 8	1 aa del	310–379
Ribose ABC transporter ATP-binding protein	15595022	Sup. Fig. 9	30 aa ins[b]	356–427
Phosphopantetheine adenylyltransferase	51598955	Sup. Fig. 10	2 aa ins	31–90
Asparaginyl-tRNA synthetase	365992288	Sup. Fig. 11	1 aa del	186–236
Chemotaxis protein CheY	119953336	Sup. Fig. 12	4 aa ins	66–121
Zn-ribbon protein	187918568	Sup. Fig. 13	1 aa ins	204–236
Chemotaxis protein CheW	15594910	Sup. Fig. 14	10 aa ins	51–119
Phosphofructokinase	219685531	(Gupta et al. 2013b)	6 aa ins	275–319
50S ribosomal protein L4	224534698	(Gupta et al. 2013b)	1 aa ins	103–136
tRNA pseudouridine 55 synthase	203284699	(Gupta et al. 2013b)	2 aa ins	143–178
Translation elongation factor Tu	203284386	(Gupta et al. 2013b)	1 aa del	330–369
Histidyl-tRNA synthetase	187918014	(Gupta et al. 2013b)	1 aa del	273–301
Seryl-tRNA synthetase	187918098	(Gupta et al. 2013b)	1 aa del	231–264
Spoiiij-associtated protein	219684344	(Gupta et al. 2013b)	3 aa ins	114–154
Nicotinate phosphoribosyltransferase	187918492	(Gupta et al. 2013b)	1 aa del	134–159
Ribose 5-phosphate isomerase	119953435	(Gupta et al. 2013b)	1 aa ins	86–110
Ribonuclease Z	195941574	(Gupta et al. 2013b)	2 aa ins	64–94
Hypothetical protein BGAFAR04_0762	386859948	(Gupta et al. 2013b)	1 aa ins	206–236
Signal recognition particle, subunit FFH/SRP54	119953471	(Gupta et al. 2013b)	1 aa ins	374–412
Hypothetical protein BSV1_0075	15594416	(Gupta et al. 2013b)	1 aa del	52–97
Aspartyl/glutamyl-tRNA amidotransferase subunit A	119953137	(Gupta et al. 2013b)	1 aa ins	364–402
Ribosomal RNA methyltransferase	203284234	(Gupta et al. 2013b)	1 aa ins	15–48
LysM domain/M23/M37 peptidase domain protein	224534310	(Gupta et al. 2013b)	1 aa ins	320–365

[a] Protein or indel containing region of the protein missing in two members of the *Borrelia*

[b] Indel was of different size in Lyme disease and Relapsing fever *Borrelia*

Conserved signature proteins which are specific for *Borrelia* or distinguish its two clades

Another useful category of molecular markers whose discovery has been enabled by comparative genomic analysis are conserved signature proteins (CSPs) that are uniquely present in different lineages of prokaryotes. Due to the specific presence of these genes/proteins in particular lineages of bacteria, they again provide useful molecular markers of common evolutionary decent for identifying and demarcating different bacterial groups in clear molecular terms. Our analyses of *Borrelia* genomes in this regard have led to identification of 107 proteins which are uniquely found either in all (or most) sequenced *Borrelia* species or are specific for only the Lyme disease *Borrelia* clade or the relapsing fever *Borrelia* clade. The results of BLAST searches for three CSPs that are specific to either all sequenced *Borrelia*, members of the Lyme disease *Borrelia*, or members of the


(A)
Lyme disease Borrelia (8/8)
Relapsing fever Borrelia (0/10)
Other Bacteria
228
272
Borrelia valaisiana 492960118 ALKFYASLRLEVRKIEQVTRS G SSDDVIGNKIRVKIVKNKVAPPF
Borrelia afzelii 111114954 ----------V--I--- - ----------
Borrelia burgdorferi 15594476 -----S---------- - ----------
Borrelia bissettii 343127453 ---------- - ----------
Borrelia garinii 408670763 -----S----------I--- - ----------
Borrelia bavariensis 51598395 -----S----------I--- - ----------
Borrelia spielmanii 493478479 -----S----------I-K- - ----------
Borrelia sp. SV1 496157886 -----S---------- - ----------
Borrelia miyamotoi 530575372 -----S----------G- -A----------V---------
Borrelia hermsii 187918010 -----S---------D---G- -A--IV-----I-V---------
Borrelia anserina 576099812 -----S---------D---GT ----IV-------V---------
Borrelia parkeri 569534919 -----S----------G- -A---V---V---V---------
Borrelia hispanica 560225321 -----S--------V---IG- ---N---------V---------
Borrelia persica 560225318 -----S--------V---IGT ---N---------V---------
Borrelia duttonii 203284057 -----S--------V---IG- ---N---------V---------
Borrelia crocidurae 386859362 -----S--------V---IG- ---N---------V---------
Borrelia recurrentis 291246105 -----S--------V---IG- ---N---------V--------
Borrelia turicatae 119952934 -----S----------G- -A---V---V---V---------
Sphaerochaeta pleomorpha 374314863 -------V-I------SISKG A--IV--RV-I-V-----S---
Spirochaeta bajacaliforniensis 522102424 -----S-V-I---R--TISKG A-EA---RV-I--A--------
Treponema pallidum 15639679 -----S-V-I----V-TLS-G DEEAW---V-IR-----M----
Clostridium asparagiforme 494984129 -----S-V--D--R--TLKQG GE----RV---V----I----
Ruminococcus lactaris 491803862 -------V-MD--R--TLKQN GEIV--RT-I------I----
Roseburia inulinivorans 495159465 -----S-V-MD--R--SLKQA GE-V--RT----------
Roseburia intestinalis 479147205 -----S-V-MD--R--ALKQG GE-V--RT----------
Dorea formicigenerans 491474760 -------V-MD--R--TLKQG GEM---RT--------I----
Bacteriovorax sp. Seq25_V 530764166 -----S-V--DI-R-GAIKN- -E-V--RT---V---------
Anaeromyxobacter dehalogenans 220919188 -------Q--DI-R-GAIKDG -S----RT---V---------
Acidithiobacillus ferrooxidans 308237903 -------V--DI-R-GAIKK- -E-V--DT---V---------
Methylophaga lonarensis 497412254 -------V--DI-R-GAIKKG -EIL--ET----------
Aggregatibacter aphrophilus 491982071 -------V--DI-RVGSIKEG -E----ET---V---------
Methylophaga sp. JAM7 387129198 -------V--DI-R-GAIKKG -EIL--ET---V---------
Bacillus megaterium 24251198 -----S-V-----RA--LKQG N-IV---T-I-V---------
Bordetella holmesii 21624597 -------V--DI-R-GSIKKG -E-V--ET---V---------
Achromobacter arsenitoxydans 495439594 -------V--DI-R-GSIKKG -E-V--ET---V---------
Burkholderia sp. 96 355000471 -------V--DI-R-GSIKKN -E----ET---V---------
Bacteroides graminisolvens 347543405 -------V--DI-RST-LKDG E-----QT---V---------
Barnesiella intestinihominis 496136496 -------V--DI-RVS-LKDG -E----QT---V---------
Sphingobium sp. 353 371560325 -------V--DI-RTG-IKDR --I---TT---V---------
(B)
Relapsing fever Borrelia (10/10)
Lyme disease Borrelia (0/8)
Other Bacteria
31
61
Borrelia hermsii 187918635 DKILFIPTHKPVHKRV ENISVKDRIAMLKLA
Borrelia turicatae 119953557 ----------C- ----------
Borrelia anserina 576100468 --V---------- ----I-------E--
Borrelia parkeri 576098594 ----------S- ----------
Borrelia hispanica 560225407 ----------C- -D---Q--VT-----
Borrelia persica 560225476 -----V--------C- -----I---T-----
Borrelia miyamotoi 530576120 ---I----------I -S-------E-----
Borrelia crocidurae 386860019 ----------C- -D---T--VT-----
Borrelia duttonii 203284676 ----------C- -D---T--VT-----
Borrelia recurrentis 203288209 ----------C- -D---T--VT-----
Borrelia burgdorferi 365992423 -RVI----CN-A--LI D --V--SN--D-----
Borrelia afzelii 410679571 ---I----CN-T--LI G -GV---N--D-----
Borrelia spielmanii 493478733 --VV----CN-A--LI G -GV-I-N--D-----
Borrelia valaisiana 492960539 --VI----CN-A--SI G -EV---N--D--E--
Borrelia sp. SV1 496157697 -RVI----CN-A--LI D -DV--NN--D-----
Borrelia bissettii 343128079 -RVI----CN-A--LI G -DV--NN--D--R--
Borrelia garinii 490929441 --VI----CN-A--LI S -DV--QN--D-----
Borrelia bavariensis 51599033 --VI----CN-A--LI S -DVT-QN--D-----
Treponema saccharophilum 488789468 --V--V--FI-P--EM S GCVPAE--L--VRA-
Treponema brennaborense 332298408 --V--V-ANL-P--EL A AGA-AG--LE-VNR-
Treponema succinifaciens 328947779 --V--V-VFS-P--NM N GALPPEK-AK-VE--
Brachyspira murdochii 296125539 --VI---AKT-P--NI S GKV-ND--LN----S
Brachyspira intermedia 384209252 --VI---AKI-P--NI S GEV-NE--LN----S
Brachyspira hyodysenteriae 225619548 --VI---AKI-P--NI S GEA-NE--LN----S
Allofustis seminis 517488885 -RMM-L--AT-P-VHE K KT-TAEH--N--Q--
Lactobacillus hominis 495746109 -E-W----NI-P--EL A G-V-A---C---E--
Mycoplasma columbinum 493657173 --LI-V-AA-NPF-KK E AIA-NE--LK--E--
Nitrospina gracilis 491149106 -RV----AAI-P---D R DITPTHH-LE--RR-
Corynebacterium caspium 517152447 --VI-V--GQ-WQ-TG R HVSPAE--YL-TVI-
Saccharomonospora paurometabol 494083884 -EVI-V--GQ-WQ-AE R TVSRAE--YL-TVI-

◀ **Fig. 4** Partial sequence alignments of (**a**) the protein Recombinase A showing a one amino acid insertion (*boxed*) identified in members of the Lyme disease *Borrelia* (**b**) the protein Nicotinamide-nucleotide adenylyltransferase showing a one amino acid deletion identified in members of the relapsing fever *Borrelia*. These CSIs were not found in the sequence homologues from any other sequenced bacteria. Sequence information for other Lyme disease or relapsing fever *Borrelia* specific CSIs is presented in Supplemental figures 15–27 and summarized in Table 3

relapsing fever *Borrelia* are shown in Table 5. As seen from this Table, high scoring homologues for these proteins are only found in different *Borrelia* species belonging to their specified clades, but not in any other bacterial organism. Thus, similar to the CSIs, these CSPs again are distinctive characteristics of the species from these clades and provide valuable molecular markers for their identification and demarcation. Of the CSPs that we have identified, 82 proteins are uniquely present in all or most of the sequenced *Borrelia* species and they are likely distinguishing characteristics of all members of the recently described family *Borreliaceae* (Table 6; Gupta et al. 2013b). In some cases, the homologues of these proteins were not detected in a few isolated strains of *Borrelia*. However, in every case, the proteins were not present in any other bacterial group, suggesting that the strains lacking these homologues have either undergone gene loss or that they are earlier branching lineages within these clades. In addition to the CSPs that are specific for all *Borrelia* (or the family *Borreliaceae*), we have also identified 21 CSPs whose homologues are only found in the Lyme disease *Borrelia* (Table 7) and four other CSPs, which are restricted to members of the relapsing fever *Borrelia* (Table 7). Some characteristics of the different CSPs are summarized in Tables 6 and 7. The cellular functions of most of these CSPs are unknown, but they may be related to some of the distinguishing properties exhibited by their specified clades.

Average nucleotide analysis

DNA–DNA hybridization is a commonly used method to determine the relatedness of different organisms and for assignment of species to either the same or different genera (Thompson et al. 2013). However, concerns have been raised about the scalability and reproducibility of these studies (Rosselló-Mora 2006). The availability of genome sequences have now made it possible to calculate pairwise ANI values between different genomes, which are analogous to DNA homology values (Richter and Rosselló-Móra 2009). We have compared the ANI values for all available genome sequenced *Borrelia* species (Fig. 6). The ANI values for different members within the genus *Borrelia* range between 73.03 and 99.34 % identity. However, based upon the comparisons of the ANI values, the *Borrelia* species can again be divided into two distinct clusters. One cluster, consisting of the members of the Lyme disease *Borrelia*, had intercluster ANI values which ranged between 91.33 and 98.06 % identity. The other cluster, which consisted of the members of the relapsing fever *Borrelia*, had intercluster ANI values which ranged between 82.51 and 99.34 % identity (Fig. 6). In contrast to the high ANI values for species within the two clusters, the ANI values of *Borrelia* species between the members of the two clusters were significantly lower, ranging between 73.03 and 74.85 % identity, indicating that the members of these clusters are distinct from each other.

Discussion

Genetic differences between the Lyme disease and relapsing fever *Borrelia* have been observed in a number of earlier studies (Postic et al. 1990; Fukunaga et al. 1996; Ras et al. 1996; Valsangiacomo et al. 1997; Margos et al. 2009). However, due to lack of distinct characteristics that can clearly distinguish the Lyme disease *Borrelia* from the relapsing fever *Borrelia*, it has proven difficult to reliably distinguish species from these two groups. This is responsible for the failure to diagnose or misdiagnosis of Lyme disease *Borrelia* in many individuals and also an underreporting of the overall incidence of this disease in the population (Wright et al. 2012; Ljostad and Mygland 2013). Detailed comparative analyses on genome sequences from *Borrelia* species that is reported here have identified numerous discrete molecular characteristics that are specifically shared by either members of the Lyme disease *Borrelia* clade or the relapsing fever *Borrelia* clade. The molecular markers described in this work provide novel and highly specific means for identification of members of the Lyme disease *Borrelia* group by either molecular sequence based (e.g. PCR, pyrosequencing, etc.) methods (Ahmod et al. 2011; Dunaj et al. 2013) or immunological methods (Wright et al. 2012; Ljostad and Mygland 2013).

Table 3 Conserved signature indels found in widely distributed proteins that are specific for either members of the Lyme disease *Borrelia* (*Borreliella*) or the relapsing fever *Borrelia*

Protein name	GI number	Figure number	Indel size	Indel position	Specificity
Recombinase A	492960118	Figure 4A	1 aa ins	228–272	Lyme disease *Borrelia*
Trigger factor Tig[a]	386854012	Sup. Fig. 15	2 aa ins	106–142	Lyme disease *Borrelia*
Chemotaxis protein CheY	15594760	Sup. Fig. 16	1 aa del	197–231	Lyme disease *Borrelia*
DNA polymerase III subunit beta	410679212	Sup. Fig. 17	1 aa del	135–176	Lyme disease *Borrelia*
Translation factor Sua5	15595079	Sup. Fig. 18	2 aa ins	149–182	Lyme disease *Borrelia*
Ferrous iron transporter A	51598605	Sup. Fig. 19	1 aa del	88–126	Lyme disease *Borrelia*
Glucose-6-phosphate isomerase	493478887	Sup. Fig. 20	1 aa ins	81–134	Lyme disease *Borrelia*
Nicotinamide-nucleotide adenylyltransferase	187918635	Figure 4B	1 aa del	31–61	Relapsing fever *Borrelia*
Hypothetical protein BRE16	203287484	Sup. Fig. 21	3 aa ins	64–98	Relapsing fever *Borrelia*
Hypothetical protein BDU327	203284245	Sup. Fig. 22	6 aa ins	866–907	Relapsing fever *Borrelia*
Hypothetical protein BT0471[b]	119953261	Sup. Fig. 23	1 aa del	216–261	Relapsing fever *Borrelia*
L-lactate permease	386859838	Sup. Fig. 24	1 aa ins	195–239	Relapsing fever *Borrelia*
1-phosphofructokinase	203288064	Sup. Fig. 25	1 aa del	101–139	Relapsing fever *Borrelia*
GTP-binding protein	203288075	Sup. Fig. 26	2 aa ins	42–87	Relapsing fever *Borrelia*
Sodium/panthothenate symporter	119953591	Sup. Fig. 27	1 aa ins	421–454	Relapsing fever *Borrelia*

[a] Indel also identified in one member of the relapsing fever *Borrelia*

[b] Protein or indel containing region of the protein missing in a member of the Lyme disease *Borrelia*

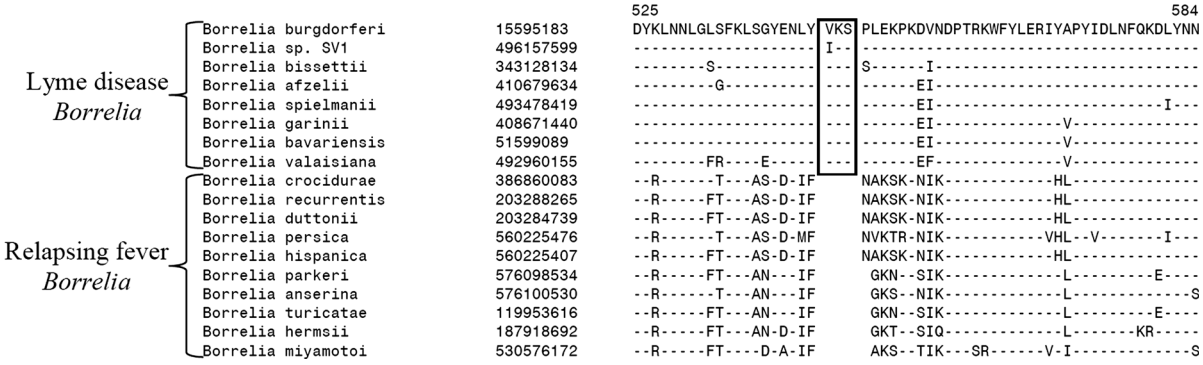


Fig. 5 A partial sequence alignment of a *Borrelia* lineage specific protein with currently unknown function (Hypothetical protein BB0838) showing a three amino acid insertion (*boxed*) which distinguishes the Lyme disease and relapsing fever *Borrelia*. Sequence information for other CSIs present in *Borrelia* lineage specific proteins is presented in Supplemental figures 28–64 and summarized in Table 4

The results reported here from multiple lines of investigations provide compelling evidence that the known *Borrelia* species are comprised of at least two evolutionary distinct groups of organisms corresponding to the Lyme disease *Borrelia* clade and the relapsing fever *Borrelia* clade. The different lines of investigation that support the distinctness of these two clades can be briefly summarized as follows:

1. In phylogenetic trees based on the 16S rRNA gene or concatenated sequences for 25 conserved proteins, the species from these two groups formed distinct and strongly supported clades that are separated from each other by long branches.
2. This work has identified 7 CSIs and 21 CSPs that are uniquely present in all of the genome

Table 4 Conserved signature indels in *Borrelia*-specific proteins or protein regions that distinguish members of the Lyme disease *Borrelia* (*Borreliella*) from the relapsing fever *Borrelia*

Protein name	GI number	Figure number	Indel size	Indel position
Hypothetical protein BB0838	15595183	Figure 5	3 aa	525–584
Hypothetical protein BRE32	203287500	Sup. Fig. 28	2 aa	170–226
Hypothetical protein Q7M33	386859258	Sup. Fig. 29	1 aa	261–317
Hypothetical protein BRE47	203287515	Sup. Fig. 30	5 aa	60–124
L-proline transport system ATP-binding protein	203287610	Sup. Fig. 31	1 aa	276–344
Penicillin-binding protein	203284062	Sup. Fig. 32	1 aa	573–618
Hypothetical protein Q7M131	386859356	Sup. Fig. 33	1 aa	163–213
Hypothetical protein BT0110	119952912	Sup. Fig. 34	2 aa	136–176
Hypothetical protein BT0110	15594456	Sup. Fig. 35	2 aa	269–308
Glutamate racemase	15594446	Sup. Fig. 36	6 aa	189–252
RNA methyltransferase RsmE	187917941	Sup. Fig. 37	1 aa	132–170
DNA mismatch repair protein mutL	386859437	Sup. Fig. 38	4 aa	299–346
Putative lipoprotein	203287684	Sup. Fig. 39	3 aa	160–214
Membrane protein	492960813	Sup. Fig. 40	1 aa	204–250
Hypothetical protein BRE314	203287766	Sup. Fig. 41	1 aa	56–94
Methylgalactoside ABC transporter ATP-binding protein	496157995	Sup. Fig. 42	1 aa	349–397
Hypothetical protein BRE355	203287806	Sup. Fig. 43	1 aa	345–400
Sensory transduction histidine kinase	15594765	Sup. Fig. 44	1 aa	88–149
DNA polymerase III subunit delta	15594800	Sup. Fig. 45	2 aa	11–58
Hypothetical protein Q7M860	203288267	Sup. Fig. 46	2 aa	166–225
Hypothetical protein KK90081	492960371	Sup. Fig. 47	1 aa	39–88
Hypothetical protein Q7M140	203284060	Sup. Fig. 48	2 aa	346–378
Hypothetical protein BG0159	365992302	Sup. Fig. 49	1 aa	32–70
Outer membrane protein	496158025	Sup. Fig. 50	1 aa	145–194
Transglycosylase SLT domain-containing protein	365992320	Sup. Fig. 51	1 aa	253–301
Cell division protein FtsZ	111115124	Sup. Fig. 52	1 aa	338–385
Excinuclease ABC subunit C	365992353	Sup. Fig. 53	1 aa	302–340
Hypothetical protein BG0519	365992363	Sup. Fig. 54	1 aa	75–122
Hypothetical protein BBIDN1270545	343127844	Sup. Fig. 55	4 aa	32–81
Hypothetical protein BBUN400354	365992340	Sup. Fig. 56	3 aa	6–67
Hypothetical protein BBUZS70553	365992365	Sup. Fig. 57	1 aa	82–145
Hypothetical protein BB0554	365992367	Sup. Fig. 58	1 aa	71–130
Hypothetical protein BB0554	365992367	Sup. Fig. 59	2 aa	512–579
Hypothetical protein BBUCA803285	365992388	Sup. Fig. 60	1 aa	29–77
Methyl-accepting chemotaxis protein	203288113	Sup. Fig. 61	2 aa	70–129
Chemotaxis protein	365992392	Sup. Fig. 62	1 aa	116–179
Chemotaxis protein	365992392	Sup. Fig. 63	1 aa	252–315
Hypothetical protein L14403475	496157774	Sup. Fig. 64	1 aa	119–186

sequenced species from the Lyme disease *Borrelia* clade and eight CSIs and four CSPs that are specific for the relapsing fever *Borrelia* clade. The unique and mutually exclusive presence of these molecular characteristics in these two groups of species provides compelling evidence that they are derived from distinct ancestors. The identified molecular markers also provide reliable means for

Table 5 Species specificity of selected conserved signature proteins

Protein specificity (GI number) function	All *Borrelia* (15594428) hypothetical		Lyme disease *Borrelia* (*Borreliella*) (365992370) hypothetical		Relapsing fever *Borrelia* (203288331) inclusion protein	
Organism	E value[a]	Length	E value[a]	Length	E value[a]	Length
Borrelia burgdorferi B31	0	432	$7.43e^{-111}$	174	–	–
Borrelia sp. SV1	0	432	$2.45e^{-103}$	174	–	–
Borrelia bissettii DN127	0	418	$4.98e^{-93}$	174	–	–
Borrelia spielmanii A14S	0	431	$1.95e^{-92}$	174	–	–
Borrelia garinii NMJW1	0	432	$4.27e^{-90}$	174	–	–
Borrelia afzelii HLJ01	0	431	$1.38e^{-88}$	174	–	–
Borrelia valaisiana VS116	0	431	$2.65e^{-94}$	174	–	–
Borrelia bavariensis PBi	0	432	$3.00e^{-89}$	174	–	–
Borrelia duttonii Ly	$9.83e^{-154}$	428	–	–	$3.00e^{-56}$	471
Borrelia crocidurae str. Achema	$1.39e^{-153}$	428	–	–	0	600
Borrelia recurrentis A1	$6.58e^{-153}$	428	–	–	0	622
Borrelia hispanica CRI	$1.00e^{-141}$	427	–	–	0	622
Borrelia persica No12	$4.00e^{-137}$	427	–	–	$1.00e^{-24}$	543
Borrelia turicatae 91E135	$1.63e^{-154}$	427	–	–	$4.00e^{-03}$	347
Borrelia hermsii HS1	$1.95e^{-145}$	427	–	–	$3.00e^{-11}$	575
Borrelia parkeri HR1	$4.00e^{-157}$	427	–	–	$3.00e^{-03}$	329
Borrelia anserina BA2	$1.00e^{-153}$	427	–	–	$2.00e^{-05}$	577
Borrelia miyamotoi LB-2001	$8.45e^{-145}$	428	–	–	$1.40e^{-02}$	146
Next Best BLAST Hit[b]	$7.09e^{00}$	1071	$4.00e^{00}$	1463	$1.07e^{02}$	1998

[a] E values smaller than $1.00e^{-180}$ are reported as 0

[b] Next best BLAST hits for protein 15594428, 365992370, and 203288331 are from *Leeuwenhoekiella blandensis*, *Trichomonas vaginalis*, and *Sulfolobus islandicus*, respectively

the demarcation of these two clades in molecular terms.

3. Whole genome ANI analyses of *Borrelia* genomes show that species from within either the Lyme disease *Borrelia* group or the relapsing fever *Borrelia* group had much higher ANI values when compared to other members of their group (range 82.51–99.34 %) than with members of the opposing *Borrelia* group (range 73.03–74.85 %).
4. The species from these two groups differ in terms of their pathogenicity profiles and the characteristics of the arthropod vectors which are involved in their transmission. The species which are part of the Lyme disease clade are transmitted via arthropod vectors that are hard tick species related to the *Ixodes ricinus* complex, while a majority of the members of the relapsing fever *Borrelia* clade are transferred by soft-bodied ticks within the family *Argasidae* (Table 8).

Taxonomic implications

The evidence obtained from different lines of investigations summarized above provides compelling evidence that the known *Borrelia* species are comprised of two main clades corresponding to the "Lyme disease *Borrelia* and its relatives" and the "relapsing fever *Borrelia* and its relatives". Of these two main groups, the Lyme disease *Borrelia* clade, based upon branching in the 16S rRNA gene tree and concatenated protein tree is comprised of the following 14 validly named species: *B. afzelii, B. americana, B. bavariensis, B. burgdorferi, B. carolinensis, B. garinii, B. japonica, B. kurtenbachii, B. lusitaniae, B. sinica, B. spielmanii, B. tanukii, B. turdi,* and *B. valaisiana*. All other currently validly named *Borrelia* species are part of the relapsing fever *Borrelia* clade. The observations presented in this work make a strong case for division of the existing genus *Borrelia* into two different

Table 6 Conserved signature proteins that are specific for all sequenced *Borrelia* species (both the Lyme disease *Borrelia* (*Borreliella*) and the relapsing fever *Borrelia*)

GI number	Function	Length	GI number	Function	Length
11496678[a]	Hypothetical	277	15594922[a]	Hypothetical	195
11496904	Membrane protein	281	15594962	Hypothetical	122
11497011[a]	Hypothetical	165	15594973	Hypothetical	241
11497031	Hypothetical	183	15594999	Hypothetical	380
11497034[a]	Hypothetical	168	15595012	Hypothetical	183
15594347	Hypothetical	190	15595018	Hypothetical	171
15594374	Hypothetical	349	15595019	Hypothetical	348
15594390	Hypothetical	133	15595020	Hypothetical	287
15594412	Hypothetical	229	15595053	Hypothetical	107
15594419	Hypothetical	186	15595062	Hypothetical	160
15594421	Hypothetical	469	15595118	Hypothetical	144
15594428	Hypothetical	432	15595168	Hypothetical	123
15594448	Hypothetical	173	15595171	Hypothetical	171
15594456	Hypothetical	454	15595177	Hypothetical	274
15594469	Hypothetical	92	15595185[a]	Hypothetical	538
15594470	Hypothetical	240	203287492	Hypothetical	168
15594501	Hypothetical	144	203287514	Hypothetical	349
15594508	Hypothetical	582	203287540	Hypothetical	488
15594525	Flagellar protein	164	203287546	Hypothetical	351
15594538	Hypothetical	246	203287657	Hypothetical	747
15594557	Hypothetical	344	203287666	Hypothetical	571
15594558	Hypothetical	217	203287785	Hypothetical	557
15594572	Hypothetical	233	203287970	Hypothetical	429
15594579	Hypothetical	275	203288080	Serine/threonine kinase	564
15594605	Hypothetical	337	364556647[a]	Hypothetical	272
15594632	Flagellar protein	143	364556751[a]	Hypothetical	212
15594652	Hypothetical	278	364556796[a]	Hypothetical	164
15594653	Hypothetical	358	365992285	Hypothetical	106
15594667	Hypothetical	352	365992310[a]	Hypothetical	217
15594697	Hypothetical	377	365992317[a]	Hypothetical	256
15594698	Hypothetical	599	365992340	Hypothetical	280
15594705[a]	Hypothetical	141	365992358	Lipoprotein	129
15594718	Hypothetical	255	365992367	Hypothetical	622
15594754	Hypothetical	209	365992388	Lipoprotein	222
15594757	Hypothetical	259	365992397	Hypothetical	590
15594805	Hypothetical	237	365992403	Hypothetical	473
15594870	Hypothetical	140	365992414	Hypothetical	424
15594871	Hypothetical	607	365992415	Hypothetical	337
15594880	Hypothetical	257	365992417	Hypothetical	219
15594894	Hypothetical	132	365992425	Hypothetical	493
15594919[a]	Hypothetical	283	365992432	Hypothetical	181

[a] Protein missing in some members of *Borrelia*

Table 7 Conserved signature proteins that are specific for either members of the Lyme disease *Borrelia* (*Borreliella*) or the relapsing fever *Borrelia*

GI number	Function	Length
CSPs that are specific for Lyme disease *Borrelia*		
11496594	Lipoprotein	192
11496595	Hypothetical	227
11496690[a]	Hypothetical	142
11496704	Hypothetical	155
11496896	S1 Antigen	417
11496905	Hypothetical	79
11496906	Lipoprotein	277
11496908	Lipoprotein	68
11496925	Membrane protein	257
11496937	Hypothetical	414
11496964	Lipoprotein	179
11496966	Hypothetical	201
11497026	Hypothetical	345
11497073[b]	Hemolysin	67
15594723	Hypothetical	220
15594749[a]	Hypothetical	138
15594801	Hypothetical	201
15594976[a]	Hypothetical	104
364556745[a]	Hypothetical	241
364556746[a]	Hypothetical	321
365992370	Hypothetical	174
CSPs that are specific for relapsing fever *Borrelia*		
203288331	Inclusion protein	622
203288332	Lipoprotein	619
203288333[a]	Lipoprotein	477
203288334[a]	Hypothetical	765

[a] Protein missing in some members of the specified clade

[b] Multiple copies of this CSP are present in the genome

genera corresponding to the Lyme disease *Borrelia* clade and the relapsing fever *Borrelia* clade. Ideally, the genus name *Borrelia* should be retained for the Lyme disease *Borrelia* clade, which includes the best known species from this genus, *B. burgdorferi*, the first identified causative agent of Lyme disease (Barbour 1984). However, the type species of the genus *Borrelia*, *Borrelia anserina*, is a part of the relapsing fever clade. Hence, the genus name *Borrelia* must be retained for the relapsing fever clade (Bergey 1925; Lapage et al. 1992; Wang and Schwartz 2011). Therefore, species from the Lyme disease clade must be transferred to a new genus indicating their distinctness from the relapsing fever clade (viz. the emended genus *Borrelia*). To minimize confusion among scientists and other health care professionals, we are proposing that the species that are part of the Lyme disease clade should be transferred to a new genus, *Borreliella* gen. nov. The proposed name retains much of the original name of the genus *Borrelia*, thus it is unlikely that the species with the new names (e.g. *B. burgdorferi*) could be confused with any other unrelated species. The emended description of the genus *Borrelia* and a description of the newly proposed genus, *Borreliella* gen. nov, containing 14 new combinations, are provided below.

Emended description of the genus *Borrelia* (Swellengrebel 1907) (approved lists 1980)

Organisms are helical, 0.2–3 μm in diameter and 3–180 μm in length. Cells do not have hooked ends. Periplasmic flagella overlap in the central region of the cell. Cells are motile, host-associated and microaerophilic. The diamino acid component of the peptidoglycan is L-ornithine. Organisms are chemoorganotrophic and utilize carbohydrates or amino acids as carbon and energy sources. Members of this genus are the causative agents of relapsing fever. The G+C content of the genomic DNA is 27–32 (mol%). The type species is *B. anserina* (Bergey 1925) (Approved Lists 1980) (Skerman et al. 1980).

Organisms from this genus are distinguished from all other bacteria examined to date by the CSIs and conserved signature proteins described in this report (Tables 3, 4, 7).

Description of *Borreliella* gen. nov.

Borreliella (Bor.re'li.el'la. N.L. fem. dim. n. *Borreliella*, named after Amédée Borrel, a French bacteriologist)

Organisms are helical, 0.2–0.3 μm in diameter and 20–30 μm in length. Cells do not have hooked ends. Periplasmic flagella overlap in the central region of the cell. Cells are motile, host-associated and microaerophilic. The diamino acid component of the peptidoglycan is L-ornithine. Organisms are chemoorganotrophic and utilize carbohydrates or amino acids as carbon and energy sources. Members of this genus are the causative agents of Lyme disease. The

		Lyme Disease *Borrelia*								Relapsing Fever *Borrelia*									
		B. afzelii	*B. baverensis*	*B. bissettii*	*B. burgdorferi*	*B. garinii*	*B. sp. SV1*	*B. spielmanii*	*B. valaisiana*	*B. anserina*	*B. crocidurae*	*B. duttonii*	*B. hermsii*	*B. hispanica*	*B. miyamotoi*	*B. parkeri*	*B. persica*	*B. recurrentis*	*B. turicatae*
Lyme Disease *Borrelia*	*B. afzelii*	---	93.3	92.2	92.4	93.3	92.4	94.4	93.0	74.1	74.3	74.4	74.6	74.3	74.4	74.7	74.0	74.3	74.7
	B. baverensis	93.3	---	92.1	92.3	98.0	92.3	92.6	93.0	74.0	74.2	74.3	74.5	74.2	74.3	74.6	73.9	74.3	74.6
	B. bissettii	92.2	92.1	---	94.7	92.1	94.6	91.6	92.0	73.9	74.1	74.2	74.4	74.1	74.3	74.5	73.8	74.2	74.5
	B. burgdorferi	92.5	92.4	94.7	---	92.4	98.1	91.8	92.2	73.9	74.1	74.2	74.4	73.9	74.3	74.5	73.7	74.2	74.5
	B. garinii	93.3	98.0	92.1	92.4	---	92.4	92.6	93.0	74.0	74.2	74.3	74.5	74.2	74.3	74.6	73.9	74.3	74.6
	B. sp. SV1	92.4	92.4	94.6	97.8	92.4	---	91.3	92.2	73.9	74.1	74.2	74.4	73.0	74.3	74.5	73.4	74.2	74.5
	B. spielmanii	94.4	92.6	91.6	91.7	92.6	91.6	---	92.4	74.1	74.3	74.4	74.5	73.1	74.4	74.7	73.4	74.4	74.7
	B. valaisiana	93.0	92.9	92.0	92.2	93.0	92.2	92.4	---	74.2	74.3	74.4	74.6	74.3	74.5	74.7	74.1	74.4	74.7
Relapsing Fever *Borrelia*	*B. anserina*	74.2	74.1	74.1	74.0	74.2	74.0	74.3	74.2	---	83.4	83.5	87.9	83.4	85.0	87.8	83.3	83.3	87.8
	B. crocidurae	74.4	74.2	74.2	74.2	74.3	74.2	74.4	74.4	83.4	---	99.0	84.7	96.3	82.7	84.8	88.3	98.8	84.8
	B. duttonii	74.4	74.2	74.3	74.2	74.3	74.2	74.4	74.5	83.4	99.0	---	84.8	95.1	82.7	84.8	87.7	99.3	84.8
	B. hermsii	74.6	74.4	74.5	74.4	74.6	74.3	74.6	74.7	87.9	84.7	84.7	---	84.8	86.7	90.6	84.6	84.7	90.7
	B. hispanica	74.4	74.2	74.2	74.0	74.3	74.0	74.0	74.4	83.5	96.4	96.4	84.9	---	82.7	85.0	84.5	96.3	84.9
	B. miyamotoi	74.5	74.4	74.4	74.3	74.5	74.2	74.6	74.6	85.0	82.6	82.8	86.7	82.7	---	86.6	82.5	82.7	86.6
	B. parkeri	74.8	74.6	74.6	74.6	74.7	74.5	74.8	74.8	87.8	84.7	84.8	90.6	84.8	86.6	---	84.7	84.8	97.7
	B. persica	74.2	74.0	74.1	73.9	74.1	73.8	73.9	74.3	83.4	88.4	88.4	84.8	84.4	82.6	84.8	---	88.4	84.8
	B. recurrentis	74.4	74.2	74.2	74.2	74.3	74.1	74.4	74.4	83.3	98.8	99.3	84.7	95.0	82.7	84.8	87.6	---	84.7
	B. turicatae	74.7	74.6	74.6	74.6	74.7	74.5	74.8	74.8	87.8	84.7	84.8	90.7	84.8	86.6	97.7	84.6	84.8	---

Fig. 6 A summary of the results of average nucleotide identity analysis performed on the sequenced members of the genus *Borrelia*. Cells with higher ANI values are *highlighted*. ANI results for multiple strains of the same species have been averaged

G+C content of the genomic DNA is 26–29 (mol%). The type species is *B. burgdorferi* comb. nov.

Organisms from this genus are distinguished from all other bacteria examined to date by the CSIs and conserved signature proteins described in this report (Tables 3, 4, 7).

Description of *Borreliella afzelii* comb. nov.

Basonym: *Borrelia afzelii* (Canica et al. 1994)

The description of the species is the same as the description given for *B. afzelii* by Canica et al. (1994). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Type Strain: VS461^T (=ATCC 51567^T = CIP 103469^T = DSM 10508^T)

Description of *Borreliella americana* comb. nov.

Basonym: *Borrelia americana* (Rudenko et al. 2010)

The description of the species is the same as the description given for *B. americana* by Rudenko et al. (2010). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: SCW-41^T (=ATCC BAA-1877^T = DSM 22541^T)

Description of *Borreliella bavariensis* comb. nov.

Basonym: *Borrelia bavariensis* (Margos et al. 2013b)

The description of the species is the same as the description given for *B. bavariensis* by Margos et al. (2013a, b). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Table 8 Distinguishing characteristics of the Lyme disease *Borrelia* (*Borreliella*) and the relapsing fever *Borrelia*

Species	DNA homology with[a]		ANI with		Vector[a,b,c]	Disease[a,b,c]
	B. hermsii	*B. burgdorferi*	*B. hermsii*	*B. burgdorferi*		
Lyme disease *Borrelia*						
B. afzelii	16	46	74.6	92.4	*Ixodes ricinus* *I. persulcatus*	Lyme disease
B. americana	–	–	–	–	*I. pacificus* *I. minor*	Possible cause of Lyme disease
"B. andersonii"	–	–	–	–	*I. dentatus*	Possible cause of Lyme disease
B. bavariensis	–	–	74.5	92.3	*I. ricinus*	Lyme disease
"B. bissettii"	–	–	74.4	94.7	*I. scapularis* *I. minor* *I. ricinus* *I. pacificus*	Possible cause of Lyme disease
B. burgdorferi	30–44	100	74.4	100	*I. scapularis* *I. pacificus* *I. ricinus* *I. persulcatus*	Lyme disease
"*B. cliforniensis*"	–	–	–	–	*I. pacificus* *I. jellisonii* *I. spinipalpis*	Possible cause of Lyme disease
B. carolinensis	–	–	–	–	*I. minor*	Possible cause of Lyme disease
B. garinii	27	55	74.5	92.4	*I. ricinus* *I. persulcatus* *I. hexagonus* *I. nipponensis*	Lyme disease
B. japonica	17	50–53	–	–	*I. ovatus*	Possible cause of Lyme disease
B. kurtenbachii	–	–	–	–	*I. scapularis*	Possible cause of Lyme disease
B. lusitaniae	–	–	–	–	*I. ricinus*	Possible cause of Lyme disease
B. sinica	–	58	–	–	*I. ovatus*	Possible cause of Lyme disease
B. spielmanii	–	–	74.5	91.7	*I. ricinus*	Possible cause of Lyme disease
B. tanukii	–	50	–	–	*I. tanukii I. ovatus*	Possible cause of Lyme disease
B. turdi	–	58	–	–	*I. turdus*	Possible cause of Lyme disease
B. valaisiana	–	51–65	74.6	92.2	*I. ricinus* *I. columnae* *I. granulatus*	Possible cause of Lyme disease
"B. yangtze"	–	–	–	–	*Haemaphysalis longicornis* *I. granulates*	Possible cause of Lyme disease

Table 8 continued

Species	DNA homology with[a]		ANI with		Vector[a,b,c]	Disease[a,b,c]
	B. hermsii	*B. burgdorferi*	*B. hermsii*	*B. burgdorferi*		
Relapsing fever *Borrelia*						
B. anserina	53–63	–	87.9	74.0	*Argas miniatus A. Persica*	Avian borreliosis
					A. reflexus	
B. baltazardii	–	–	–	–	–	Relapsing fever
B. brasiliensis	–	–	–	–	*Ornithodoros brasiliensis*	–
B. caucasica	–	–	–	–	*O. verrucosus*	Relapsing fever
B. coriaceae	44–50	–	–	–	*O. coriaceus*	Possible cause of Epizootic bovine abortion
B. crocidurae	32–35	–	84.7	74.2	*O. sonrai*	Relapsing fever
B. dugesii	–	–	–	–	*O. dugesi*	Relapsing fever
B. duttonii	17	–	84.6	74.2	*O. moubata*	Relapsing fever
B. graingeri	–	–	–	–	*O. graingeri*	Relapsing fever
B. harveyi	–	–	–	–	–	Relapsing fever
B. hermsii	100	30–44	100	74.4	*O. hermsi*	Relapsing fever
B. hispanica	–	–	84.9	74.0	*O. erraticus*	Relapsing fever
B. latyschewii	–	–	–	–	*O. tartakov*	Relapsing fever
					O. tartakowskyi	
"B. lonestari"	–	–	–	–	*Amblyomma americanum*	Possible cause of Southern tick-associated rash illness (STARI)
B. mazzottii	–	–	–	–	*O. talaje*	Relapsing fever
B. merionesi	–	–	–	–	*O. erraticus*	Relapsing fever
B. microti	–	–	–	–	*O. erraticus*	Relapsing fever
B. miyamotoi sensu lato	45	13–14	86.7	74.3	*I. persulcatus*	Acute febrile illness
					I. scapularis	
B. parkeri	77	–	90.6	74.6	*O. parkeri*	Relapsing fever
B. persica	–	–	84.8	73.9	*O. tholozani*	Relapsing fever
B. recurrentis	–	–	84.7	74.2	*Pediculus humanus*	Relapsing fever
B. theileri	–	–	–	–	*Rhipicephalus decoloratus*	Bovine borreliosis
					R. evertsi	
					Boophilus micropus	
B. tillae	–	–	–	–	*O. zumpti*	Avian borreliosis
B. turicatae	86	–	90.7	74.6	*O. turicatae*	Relapsing fever
B. venezuelensis	–	–	–	–	*O. rudis*	Relapsing fever
B. turcica	<20	<20	–	–	*Hyalomma aegyptium*	–

– Not determined

[a] Adapted from (Wang and Schwartz 2011)

[b] Adapted from (Margos et al. 2011)

[c] Adapted from (Barbour 2005)

Type Strain: PBi^T (=DSM 23469^T = BAA-2496^T)

Description of *Borreliella burgdorferi* comb. nov.

Basonym: *B. burgdorferi* (Johnson et al. 1984)

The description of the species is the same as the description given for *B. burgdorferi* by Johnson et al. (1984). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Type Strain: B31^T (=ATCC 35210^T = CIP 102532^T = DSM 4680^T)

Description of *Borreliella carolinensis* comb. nov.

Basonym: *Borrelia carolinensis* (Rudenko et al. 2011)

The description of the species is the same as the description given for *B. carolinensis* by Rudenko et al. (2011). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: SCW-22^T (=ATCC BAA-1773^T = DSM 22119^T)

Description of *Borreliella garinii* comb. nov.

Basonym: *Borrelia garinii* (Baranton et al. 1992)

The description of the species is the same as the description given for *B. garinii* by Baranton et al. (1992). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Type Strain: 20047^T (=ATCC 51383^T = CIP 103362^T = DSM 10534^T)

Description of *Borreliella japonica* comb. nov.

Basonym: *Borrelia japonica* (Kawabata et al. 1994)

The description of the species is the same as the description given for *B. japonica* by Kawabata et al. (1994). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: HO14^T (=ATCC 51557^T = JCM 8951^T)

Description of *Borreliella kurtenbachii* comb. nov.

Basonym: *Borrelia kurtenbachii* (Margos et al. 2013a)

The description of the species is the same as the description given for *B. kurtenbachii* by Margos et al. (2013a, b). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: 25015^T (=ATCC BAA-2495^T = DSM 26572^T)

Description of *Borreliella lusitaniae* comb. nov.

Basonym: *Borrelia lusitaniae* (Le Fleche et al. 1997)

The description of the species is the same as the description given for *B. lusitaniae* by Le Fleche et al. (1997). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: PotiB2^T (=CIP 105366^T)

Description of *Borreliella sinica* comb. nov.

Basonym: *Borrelia sinica* (Masuzawa et al. 2001)

The description of the species is the same as the description given for *B. sinica* by Masuzawa et al. (2001). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: CMN3^T (=DSM 23262^T = JCM 10505^T)

Description of *Borreliella spielmanii* comb. nov.

Basonym: *Borrelia spielmanii* (Richter et al. 2006)

The description of the species is the same as the description given for *B. spielmanii* by Richter et al. (2006). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Type Strain: PC-Eq17N5^T (=CIP 108855^T = DSM 16813^T)

Description of *Borreliella tanukii* comb. nov.

Basonym: *Borrelia tanukii* (Fukunaga et al. 1997a)

The description of the species is the same as the description given for *B. tanukii* by Canica et al. (1994). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: Hk501^T (=ATCC BAA-127^T = JCM 9662^T)

Description of *Borreliella turdi* comb. nov.

Basonym: *Borrelia turdi* (Fukunaga et al. 1997b)

The description of the species is the same as the description given for *B. turdi* by Fukunaga et al.

(1997). The species exhibits the genus properties indicated in the description of *Borreliella*.

Type Strain: Ya501^T (=ATCC BAA-126^T = JCM 9661^T)

Description of *Borreliella valaisiana* comb. nov.

Basonym: *Borrelia valaisiana* (Wang et al. 1997)

The description of the species is the same as the description given for *B. valaisiana* by Wang et al. (1997). The species exhibits the genus properties and contains the CSIs and CSPs indicated in the description of *Borreliella*.

Type Strain: VS116^T (=CIP 105367^T)

References

Adams DA, Gallagher KM, Jajosky RA, Kriseman J, Sharp P, Anderson WJ, Aranas AE, Mayes M, Wodajo MS, Onweh DH et al (2013) Summary of notifiable diseases—United States, 2011. MMWR Morb Mortal Wkly Rep 60(53): 1–117

Adeolu M, Gupta RS (2013) Phylogenomics and molecular signatures for the order Neisseriales: proposal for division of the order Neisseriales into the emended family Neisseriaceae and Chromobacteriaceae fam nov. Antonie Van Leeuwenhoek Int J G 104(1):1–24

Ahmod NZ, Gupta RS, Shah HN (2011) Identification of a *Bacillus anthracis* specific indel in the *yeaC* gene and development of a rapid pyrosequencing assay for distinguishing *B. anthracis* from the *B. cereus* group. J Microbiol Methods 87(3):278–285

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402

Baranton G, Postic D, Saint Girons I, Boerlin P, Piffaretti J-C, Assous M, Grimont PAD (1992) Delineation of *Borrelia burgdorferi* Sensu Stricto, *Borrelia garinii* sp. nov., and Group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol 42(3):378–383

Barbour AG (1984) Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 57(4):521

Barbour AG (2005) Relapsing fever. In: Goodman JL, Dennis DT, Sonenshine DE (eds) Tick-borne diseases of humans. ASM Press, Washington, pp 268–291

Barbour AG, Miller SC (2014) Genome sequence of *Borrelia parkeri*, an agent of enzootic relapsing fever in Western North America. Genome Announc 2(1):e00018

Bergey DH (1925) Bergey's manual of determinative bacteriology, 2nd edn. The Williams and Wilkins Co, Baltimore

Bhandari V, Gupta RS (2012) Molecular signatures for the phylum Synergistetes and some of its subclades. Antonie Van Leeuwenhoek 102(4):517–540

Bhandari V, Gupta RS (2014) Molecular signatures for the phylum (class) *Thermotogae* and a proposal for its division into three orders (*Thermotogales*, *Kosmotogales* ord. nov. and *Petrotogales* ord. nov.) containing four families (*Thermotogaceae*, *Fervidobacteriaceae* fam. nov., *Kosmotogaceae* fam. nov. and *Petrotogaceae* fam. nov.) and a new genus *Pseudothermotoga* gen. nov. with five new combinations. Antonie Van Leeuwenhoek 105(1):143–168

Bhandari V, Ahmod NZ, Shah HN, Gupta RS (2013) Molecular signatures for *Bacillus* species: demarcation of the *Bacillus subtilis* and *Bacillus cereus* clades in molecular terms and proposal to limit the placement of new species into the genus *Bacillus*. Int J Syst Evol Microbiol 63:2712–2726

Brenner EV, Kurilshikov AM, Stronin OV, Fomenko NV (2012) Whole-genome sequencing of *Borrelia garinii* BgVir, isolated from Taiga ticks (*Ixodes persulcatus*). J Bacteriol 194(20):5713

Canica MM, Du Merle L, Mazie JC, Baranton G, Postic D (1994) *Borrelia afzelii* sp. nov. Validation of the publication of new names and new combinations previously effectively published outside the IJSB, list no 48. Int J Syst Bacteriol 44:182–183

Casjens SR, Fraser-Liggett CM, Mongodin EF, Qiu WG, Dunn JJ, Luft BJ, Schutzer SE (2011a) Whole genome sequence of an unusual *Borrelia burgdorferi* sensu lato isolate. J Bacteriol 193(6):1489–1490

Casjens SR, Mongodin EF, Qiu WG, Dunn JJ, Luft BJ, Fraser-Liggett CM, Schutzer SE (2011b) Whole-genome sequences of two *Borrelia afzelii* and two *Borrelia garinii* Lyme disease agent isolates. J Bacteriol 193(24): 6995–6996

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552

Chaconas G (2005) Hairpin telomeres and genome plasticity in *Borrelia*: all mixed up in the end. Mol Microbiol 58(3): 625–635

Chaconas G, Kobryn K (2010) Structure, function, and evolution of linear replicons in *Borrelia*. Annu Rev Microbiol 64:185–202

Charlebois RL, Doolittle WF (2004) Computing prokaryotic gene ubiquity: rescuing the core from extinction. Genome Res 14(12):2469–2477

Ciccarelli FD, Doerks T, Von Mering C, Creevey CJ, Snel B, Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. Science 311(5765):1283–1287

Cutler SJ (2010) Relapsing fever: a forgotten disease revealed. J Appl Microbiol 108(4):1115–1122

Dai Q, Restrepo BI, Porcella SF, Raffel SJ, Schwan TG, Barbour AG (2006) Antigenic variation by *Borrelia hermsii* occurs through recombination between extragenic repetitive elements on linear plasmids. Mol Microbiol 60(6):1329–1343

Dunaj J, Moniuszko A, Zajkowska J, Pancewicz S (2013) The role of PCR in diagnostics of Lyme borreliosis. Przegl Epidemiol 67(1): 35–39, 119–123

Elbir H, Gimenez G, Robert C, Bergström S, Cutler S, Raoult D, Drancourt M (2012) Complete genome sequence of *Borrelia crocidurae*. J Bacteriol 194(14):3723–3724

Elbir H, Larsson P, Normark J, Upreti M, Korenberg E, Larsson C, Bergstrom S (2014a) Genome sequence of the Asiatic species *Borrelia persica*. Genome Announc 2(1):e01127

Elbir H, Larsson P, Upreti M, Normark J, Bergstrom S (2014b) Genome sequence of the relapsing fever borreliosis species *Borrelia hispanica*. Genome Announc 2(1):e01171

Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK et al (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 390(6660):580–586

Fukunaga M, Okada K, Nakao M, Konishi T, Sato Y (1996) Phylogenetic analysis of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. Int J Syst Bacteriol 46(4):898–905

Fukunaga M, Hamase A, Okada K, Nakao M (1997a) *Borrelia tanukii* sp. nov. Validation of the publication of new names and new combinations previously effectively published outside the IJSB, list no 63. Int J Syst Bacteriol 47:1274

Fukunaga M, Hamase A, Okada K, Nakao M (1997b) *Borrelia turdi* sp. nov. Validation of the publication of new names and new combinations previously effectively published outside the IJSB, list no 63. Int J Syst Bacteriol 47:1274

Gao B, Gupta R (2007) Phylogenomic analysis of proteins that are distinctive of Archaea and its main subgroups and the origin of methanogenesis. BMC Genom 8(1):86

Gao B, Gupta RS (2012a) Microbial systematics in the post-genomics era. Antonie Van Leeuwenhoek 101(1):45–54

Gao B, Gupta RS (2012b) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76(1):66–112

Glöckner G, Lehmann R, Romualdi A, Pradella S, Schulte-Spechtel U, Schilhabel M, Wilske B, Sühnel J, Platzer M (2004) Comparative analysis of the *Borrelia garinii* genome. Nucleic Acids Res 32(20):6038–6046

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57(1):81–91

Gupta RS (1998) Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol Mol Biol Rev 62(4):1435

Gupta RS (2010) Applications of conserved indels for understanding microbial phylogeny. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 135–150

Gupta RS, Griffiths E (2006) *Chlamydiae*-specific proteins and indels: novel tools for studies. Trends Microbiol 14(12):527–535

Gupta RS, Lali R (2013) Molecular signatures for the phylum Aquificae and its different clades: proposal for division of the phylum Aquificae into the emended order *Aquificales*, containing the families *Aquificaceae* and *Hydrogenothermaceae*, and a new order *Desulfurobacteriales* ord. nov., containing the family *Desulfurobacteriaceae*. Antonie Van Leeuwenhoek 104(3):349–368

Gupta RS, Chander P, George S (2013a) Phylogenetic framework and molecular signatures for the class *Chloroflexi* and its different clades; proposal for division of the class *Chloroflexia* class. nov. [corrected] into the suborder *Chloroflexineae* subord. nov., consisting of the emended family *Oscillochloridaceae* and the family *Chloroflexaceae* fam. nov., and the suborder *Roseiflexineae* subord. nov., containing the family *Roseiflexaceae* fam. nov. Antonie Van Leeuwenhoek 103(1):99–119

Gupta RS, Mahmood S, Adeolu M (2013b) A phylogenomic and molecular signature based approach for characterization of the phylum Spirochaetes and its major clades: proposal for a taxonomic revision of the phylum. Front Microbiol 4:217

Harris JK, Kelley ST, Spiegelman GB, Pace NR (2003) The genetic core of the universal ancestor. Genome Res 13(3): 407–412

Hue F, Ghalyanchi Langeroudi A, Barbour AG (2013) Chromosome sequence of *Borrelia miyamotoi*, an uncultivable tick-borne agent of human infection. Genome Announc 1(5):e00713

Ibba M, Bono JL, Rosa PA, Soll D (1997) Archaeal-type lysyl-tRNA synthetase in the Lyme disease spirochete *Borrelia burgdorferi*. Proc Natl Acad Sci USA 94(26):14383–14388

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23(10):403

Jiang B, Yao H, Tong Y, Yang X, Huang Y, Jiang J, Cao W (2012a) Genome sequence of *Borrelia garinii* strain NMJW1, isolated from China. J Bacteriol 194(23): 6660–6661

Jiang BG, Zheng YC, Tong YG, Jia N, Huo QB, Fan H, Ni XB, Ma L, Yang XF, Jiang JF et al (2012b) Genome sequence of *Borrelia afzelii* Strain HLJ01, isolated from a patient in China. J Bacteriol 194(24):7014–7015

Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ (1984) *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int J Syst Bacteriol 34(4):496–497

Kawabata H, Masuzawa T, Yanagihara Y (1994) *Borrelia japonica* sp. nov. Validation of the publication of new names and new combinations previously effectively published outside the IJSB, list no 50. Int J Syst Bacteriol 44:595

Lapage SP, Sneath PHA, Lessel EF, Skerman VBD, Seeliger HPR, Clark WA (1992) International code of nomenclature of bacteria: bacteriological code, 1990 revision. ASM Press International Union of Microbiological Societies, Washington

Le Fleche A, Postic D, Girardet K, Peter O, Baranton G (1997) Characterization of *Borrelia lusitaniae* sp. nov. by 16S ribosomal DNA sequence analysis. Int J Syst Bacteriol 47(4):921–925

Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. Mol Biol Evol 25(7):1307–1320

Lescot M, Audic S, Robert C, Nguyen TT, Blanc G, Cutler SJ, Wincker P, Couloux A, Claverie JM, Raoult D (2008) The genome of *Borrelia recurrentis,* the agent of deadly louse-borne relapsing fever, is a degraded subset of tick-borne *Borrelia duttonii*. PLoS Genet 4(9):e1000185

Lindgren E, Jaenson TG (2006) Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. WHO Regional Office for Europe, Copenhagen

Ljostad U, Mygland A (2013) Chronic Lyme; diagnostic and therapeutic challenges. Acta Neurol Scand 127 Suppl(196): 38–47

Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, Bormane A, Vitorino L, Collares-Pereira M, Drancourt M et al (2009) A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 75(16):5410–5416

Margos G, Vollmer SA, Ogden NH, Fish D (2011) Population genetics, taxonomy, phylogeny and evolution of *Borrelia burgdorferi* sensu lato. Infect Genet Evol 11(7):1545–1563

Margos G, Piesman J, Lane RS, Ogden NH, Sing A, Straubinger RK, Fingerle V (2013a). *Borrelia kurtenbachii* sp. nov.: a widely distributed member of the *Borrelia burgdorferi* sensu lato species complex in North America. Int J Syst Evol Microbiol 64(Pt 1):128–30. doi:10.1099/ijs.0.054593-0

Margos G, Wilske B, Sing A, Hizo-Teufel C, Cao WC, Chu C, Scholz H, Straubinger RK, Fingerle V (2013b) *Borrelia bavariensis* sp. nov. is widely distributed in Europe and Asia. Int J Syst Evol Microbiol 63(Pt 11):4284–4288

Masuzawa T, Takada N, Kudeken M, Fukui T, Yano Y, Ishiguro F, Kawamura Y, Imai Y, Ezaki T (2001) *Borrelia sinica* sp. nov., a lyme disease-related *Borrelia* species isolated in China. Int J Syst Evol Microbiol 51(Pt 5):1817–1824

Naushad HS, Lee B, Gupta RS (2014) Conserved signature indels and signature proteins as novel tools for understanding microbial phylogeny and systematics: identification of molecular signatures that are specific for the phytopathogenic genera *Dickeya*, *Pectobacterium* and *Brenneria*. Int J Syst Evol Microbiol 64(2):366–383

NCBI (2014) NCBI genome database. http://www.ncbi.nlm.nih.gov/genome/

Parte AC (2014) LPSN—list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 42(D1):D613–D616

Paster BJ (2011) Phylum XV. Spirochaetes Garrity and Holt 2001. In Brenner DJ, Krieg NR, Garrity GM, Staley JT (eds) Bergey's Manual of Systematic Bacteriology, 2nd edn, vol 3. Springer, New York, pp 471–471 (reprinted from: not in File)

Postic D, Edlinger C, Richaud C, Grimont F, Dufresne Y, Perolat P, Baranton G, Grimont PAD (1990) Two genomic species in *Borrelia burgdorferi*. Res Microbiol 141(4): 465–475

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(D1):D590–D596

Ras NM, Lascola B, Postic D, Cutler SJ, Rodhain F, Baranton G, Raoult D (1996) Phylogenesis of relapsing fever *Borrelia* spp. Int J Syst Bacteriol 46(4):859–865

Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci 106(45):19126–19131

Richter D, Postic D, Sertour N, Livey I, Matuschka FR, Baranton G (2006) Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. Int J Syst Evol Microbiol 56(Pt 4):873–881

Rokas A, Holland PWH (2000) Rare genomic changes as a tool for phylogenetics. Trends Ecol Evol 15(11):454–459

Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425(6960):798–804

Rosselló-Mora R (2006) DNA–DNA reassociation methods applied to microbial taxonomy and their critical evaluation. In: Stackebrandt E (ed) Molecular identification, systematics, and population structure of prokaryotes. Springer, Berlin, pp 23–50

Rudenko N, Golovchenko M, Lin T, Gao L, Grubhoffer L, Oliver JH Jr (2010) *Borrelia americana* sp. nov. List of new names and new combinations previously effectively, but not validly, published, list no 135. Int J Syst Evol Microbiol 60:1985–1986

Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH Jr (2011) *Borrelia carolinensis* sp. nov., a novel species of the *Borrelia burgdorferi* sensu lato complex isolated from rodents and a tick from the south-eastern USA. Int J Syst Evol Microbiol 61(Pt 2):381–383

Schutzer SE, Fraser-Liggett CM, Casjens SR, Qiu WG, Dunn JJ, Mongodin EF, Luft BJ (2011) Whole-genome sequences of thirteen isolates of *Borrelia burgdorferi*. J Bacteriol 193(4):1018–1020

Schutzer SE, Fraser-Liggett CM, Qiu WG, Kraiczy P, Mongodin EF, Dunn JJ, Luft BJ, Casjens SR (2012) Whole-genome sequences of *Borrelia bissettii, Borrelia valaisiana,* and *Borrelia spielmanii*. J Bacteriol 194(2):545–546

Segata N, Bornigen D, Morgan XC, Huttenhower C (2013) PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. Nat Commun 4:2304

Skerman VBD, McGowan V, Sneath PHA (1980) Approved lists of bacterial names. Int J Syst Bacteriol 30(1):225–420

Swellengrebel NH (1907) Sur la cytologie comparée des spirochètes et des spirilles. Ann Inst Pasteur (Paris) 21:562–586

Takano A, Goka K, Une Y, Shimada Y, Fujita H, Shiino T, Watabane H, Kawabata H (2010) Isolation and characterization of a novel *Borrelia* group of tick-borne borreliae from imported reptiles and their associated ticks. Environ Microbiol 12(1):134–146

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30(12):2725–2729

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In Miura RM (ed) Lectures on mathematics in the life sciences, 17th edn. American Mathematical Society, Providence, pp 57–86 (reprinted from: not in file)

Thompson CC, Chimetto L, Edwards RA, Swings J, Stackebrandt E, Thompson FL (2013) Microbial genomic taxonomy. BMC Genom 14(1):913

Valsangiacomo C, Balmelli T, Piffaretti JC (1997) A phylogenetic analysis of *Borrelia burgdorferi* sensu lato based on sequence information from the hbb gene, coding for a histone-like protein. Int J Syst Bacteriol 47(1):1–10

Vinuesa P (2010) Multilocus sequence analysis and bacterial species phylogeny estimation. In: Oren A, Papke RT (eds) Molecular phylogeny of microorganisms. Caister Academic Press, Norfolk, pp 41–64

Wang G, Schwartz I (2011) Genus II. *Borrelia Swellengrebel* 1907, 582^{AL}. In: Brenner DJ, Krieg NR, Garrity GM, Staley JT (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 484–498

Wang G, van Dam AP, Le Fleche A, Postic D, Peter O, Baranton G, de Boer R, Spanjaard L, Dankert J (1997) Genetic and phenotypic analysis of *Borrelia valaisiana* sp. nov. (*Borrelia* genomic groups VS116 and M19). Int J Syst Bacteriol 47(4):926–932

Wang G, van Dam AP, Schwartz I, Dankert J (1999) Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic,

epidemiological, and clinical implications. Clin Microbiol Rev 12(4):633–653

Wright WF, Riedel DJ, Talwani R, Gilliam BL (2012) Diagnosis and management of Lyme disease. Am Fam Physician 85(11):1086–1093

Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ (2009) A phylogeny-driven genomic encyclopaedia of bacteria and archaea. Nature 462(7276):1056–1060