Title

Borreliaceae

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Publication Date

2023-12-07

DOI

10.1002/9781118960608.fbm00308

Family

Spirochaetes/Spirochaetia/Spirochaetales

Borreliaceae

Gupta, Mahmood, and Adeolu 2014, 693[VP] (Effective publication: Gupta, Mahmood and Adeolu 2015, 15), emend. Adeolu and Gupta 2014, 1064

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Bor.rel.i.a'ce.ae. N.L. fem. n. *Borrelia* type genus of the family; suff. *-aceae* ending to denote a family; N.L. fem. pl. n. *Borreliaceae*, the family of *Borrelia*.

Cells are helical with regular or irregular coils. 0.2-0.3 μm in diameter and 10-40 μm in length. Cells do not have hooked ends. Motile. Inner and outer membrane with periplasmic flagella with 7 to 20 subterminal insertion points. Aniline-stain-positive. Microaerophilic. Most members of the family cultivable in complex media that includes N-acetylglucosamine. Optimum growth between 33 and 38° C. Diamino acid of peptidoglycan is ornithine. Lacks a lipopolysaccharide. Linear chromosome and plasmids with hairpin telomeres. The family currently accommodates the genera *Borrelia* and *Borreliella*. Members of the family are host-associated organisms that are transmitted between vertebrate reservoirs by a hematophagous arthropod, in all but one case, a tick. Members include the agents of relapsing fever, Lyme disease, and avian spirochetosis.

DNA G+C content (mol%): 26-30

Type genus: Borrelia Swellengrebel 1907, 562[AL]

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Cells are helical, 0.2–0.3 μm in diameter and 10–40 μm in length. The coils, which usually are observed as flat waves, vary in amplitude and are either regular or irregular in spacing, depending on phase of growth and environment. The wavelengths of regularly-spaced coils typically are 2 to 3 μm. Cells do not have hooked ends. The cells are actively motile in liquid or semi-solid media with frequent reversal of the direction of translational movement. An outer membrane (formerly called outer sheath), periplasmic flagella (axial filaments or fibrils), a thin peptidoglycan layer (cell wall), and inner membrane confines the cytoplasm. The peptidoglycan and inner membrane constitute the protoplasmic cylinder. There are no cytoplasmic tubules. The outer membrane is loosely associated with the protoplasmic cylinder and may detach under adverse conditions. Seven to twenty unsheathed flagella are inserted subterminally in the protoplasmic cylinder and overlap centrally as a periplasmic bundle. The diamino acid component of the peptidoglycan is L-ornithine. Cells stain with Giemsa and other aniline dyes.

Borreliaceae organisms are chemo-organotrophic. Grow under microaerophilic conditions in a complex medium, which includes N-acetylglucosamine and long-chain fatty acids as requirements. Ferment glucose and some other monosaccharides. Contain a superoxide dismutase but not catalase. Inherently resistant to rifampin. Optimum growth between 33 and 38° C. Genome comprises a single linear chromosome, one or more linear plasmids of about 10 to 200 kilobases in size, and, in most species, one or more circular plasmids. The linear replicons' telomeres are covalently-closed hairpins, which are produced by a plasmid-borne telomere resolvase unique to *Borreliaceae*. The multiple genomes of these polyploid cells are tandemly-arrayed as nucleoids over the cell's length.

All species are host-associated. They depend on an hematophagous arthropod, usually a tick, for transmission between vertebrate hosts, which serve as natural reservoirs. The range of arthropods that are competent vectors is restricted for each species and is unique for some species. Some species can be maintained in tick populations by transovarial (vertical) transmission for a limited number of generations in the absence of a vertebrate host.

The family currently accommodates the multispecies genera *Borrelia* and *Borreliella*. The type genus *Borrelia* contains all the known agents of relapsing fever, as well as the agents of avian spirochetosis and bovine borreliosis, and the genus *Borreliella* contains all the known agents of Lyme disease.

DNA G+C content (mol%): 26-30

Type genus: **Borrelia** Swellengrebel 1907, 562[AL]

Further description

Although *Borreliaceae* cells are tinctorially gram-negative, they are distinguished from most other gram-negative bacteria, such as *Proteobacteria* by the absence of lipopolysaccharide (LPS) (Takayama et al., 1987). Another characteristic of *Borreliaceae* is a comparatively high content of lipoproteins of the bacterial type (Bergström and Zückert, 2010). The signalling pathways of innate immunity and inflammation in vertebrates are triggered by pattern recognition of bacterial lipoproteins (Weis et al., 1994).

Spirochetes are also characterized by numbers of periplasmic flagella per cell, preferably with counts of insertion points at the ends of cells, as visualized by negative-stain electron microscopy or analogous technique, such as cryo-electron tomography (Hovind-Hougen, 1976, Barbour and Hayes, 1986, Kudryashev et al., 2009). The periplasmic flagella are contribute to morphology of the cells as well as motility (Motaleb et al., 2000).

With the exception of the louse-borne *Borrelia recurrentis*, all *Borreliaceae* species include a tick species in their life cycles (Barbour and Hayes, 1986). Represented among the tick vectors are members of both major families of ticks: soft (argasid) ticks and hard (ixodid) ticks of prostriate and metastriate types (Piesman and Schwan, 2010). Transmission occurs through the tick's saliva as it feeds, generally within an hour in the case of argasid ticks and over few days for ixodid ticks. The wide variations in tick physiology and behavior have implications for the adaptations of the spirochetes that depend on these blood-feeding arthropods for transmission. These correspond to recognized associations between species of spirochete and species of ticks (Davis, 1952). These associations may be strict, with absolute one-to-one restriction, or less stringent with two or more species of tick competent to transmit the same *Borreliaceae* species. Documented spirochete-tick associations of these sorts were used to define species (Murray and Davis, 1948).

Adeolu and Gupta (2014) identified 82 protein coding sequences, called Conserved Signature Proteins (Gupta, 2014), that are unique to the family *Borreliaceae*. Twenty-six of the chromosome-borne sequences would encode a protein of at least 300 amino acids in length. The members of the family *Borreliaceae* are also distinguished from other bacteria, including species of the genera *Treponema* and *Spirochaeta* by unique insertions or deletions (indels) in conserved proteins, and which are called Conserved Signature Indels (Adeolu and Gupta, 2014). Table 1 lists 9 of the 26 core proteins with a family-specific insertion or deletion that were identified by Adeolu and Gupta (2014).

Taxonomic comments

The family *Borreliaceae* is composed of two genera: *Borrelia* and *Borreliella*, each comprising multiple species (Gupta et al., 2013, Adeolu and Gupta 2014). The type genus *Borrelia* was established by Sakharoff (1891) for the description of the agent of avian spirochetosis, *Borrelia anserina*. The genus was named for Amédeé Borrel of the Institut Pasteur (Borrel and Marchoux, 1905). The genus name was subsequently applied to several different relapsing fever agents (Bergey et al., 1925), some of which had previously been named as *Treponema* or *Spirochaeta* species. Through the last edition of

Bergey's Manual the genera of *Borrelia*, *Treponema*, and *Spirocheta*, as well as the monospecific genus, *Cristispira* were under the family *Spirochetaceae* (fbm00241) (Wang and Schwartz ,2011).

The number of species in the genus *Borrelia* expanded beginning with the discovery in the 1980's in North America and Eurasia of spirochetes cause the tick-borne infection Lyme disease (Burgdorfer et al., 1982). These spirochetes were assigned to the genus *Borrelia* and not *Treponema*, which was the alternative choice at the time (Johnson et al., 1984a, Johnson et al., 1984b). The basis for this placement in the genus *Borrelia* was their associations with ticks, morphology, and cultivation requirements, as well as DNA-DNA hybridization findings. The type species of this group of disease agents has been *Borrelia burgdorferi* (Johnson et al., 1984b). Several other spirochetes with tick-vertebrate life cycles were subsequently discovered in North America, Eurasia, and South America and found to be related to *B. burgdorferi*. Some, such as *B. afzelii* and *B. garini*, cause Lyme disease, but most of the newly-described species have not been implicated in human infections. The Lyme disease agents and related species were recognized as a monophyletic clade, distinctive from the relapsing fever group of species. By convention, this group of species was called "*Borrelia burgdorferi sensu lato*" (Wang and Schwartz, 2011). But a comparably-inclusive designation was not available for the second major clade, which is more diverse and contains not only the agents of relapsing fever, avian spirochetosis and bovine borreliosis, but also other species of more recent discovery, such as *Borrelia miyamotoi* and *Borrelia turcica*. Informally, the two clades have been distinguished as the "the Lyme disease group" and "the relapsing fever group", or by similar descriptors.

Gupta et al. (2013) proposed the creation of the family *Borreliaceae* out of the family *Spirochetaceae*, which retained *Treponema* and *Spirochaeta*. In a subsequent paper, Adeolu and Gupta (2014) made the case for the formal re-classification of those species that have been under the rubric of "*Borrelia burgdorferi sensu lato*" as a new genus, *Borreliella* with retention of "Borrelia" for the coherent cluster that includes the relapsing fever agents. Since the type species for the genus is *Borrelia anserina*, a member of the "relapsing fever group", this name had priority.

A proposal to change the scientific name of an important human pathogen is not taken lightly and seldom without controversy. Margos et al. (2017) argued that the division was not justified on several grounds. The two that carry most weight are these: (1) It is conceivable that other species will be discovered in the future and that some may occupy phylogenetic positions in the diversity gap between the two clades, thus weakening claims of distinctiveness. To support this prediction, Margos et al. cited the discovery of species of reptile-associated spirochetes, including *Borrelia turcica* (Güner et al., 2004), which in trees of the 16S ribosomal RNA genes or some other sequences are basal to the other species in the relapsing fever group (Figure 1). (2) Changing the genus name for the Lyme disease agents would be disruptive. In a rebuttal, Barbour et al. (2017) addressed these concerns and presented further data, including both genotypic and phenotypic traits, in support of the reclassification. As Lawson et al. (2016) pointed out with regard to the reclassification of another medically-important pathogen from *Clostridium difficile* to *Clostridioides difficile*, a similarity in genus names, as well as the retention of the genus initial for both professional and lay use, ameliorates the impact of name change and that adoption occurs over time as routine updates in printed materials are made.

Phylogeny

There is a single 16S rRNA (*rrs*) gene in the genome (Schwartz et al., 1992). The 16S rRNA gene sequence-based phylogenetic tree in Figure 1 shows the positions of members of the family *Borreliaceae* among closest related genera *Cristispira*, *Treponema* and *Spirochaeta* of *Spirochetaceae* of the order *Spirochaetales* with two species from the order of *Leptospiriales* (*Leptospiraceae*) of the same order as the out-group. The same relationships with comparable support were observed in trees constructed by maximum likelihood or maximum parsimony methods.

At a species-level resolution, Figure 1 shows the positions of selected species of the two genera, *Borrelia* and *Borreliaceae*. For species for which there has been complete and annotated chromosome sequences available, similar relationships between taxa of both groups were observed by Barbour (2014) with alignments of sequences of the syntenic chromosome, Elbir et al. (2014) with a set of 788 core chromosome genes, Adeolu and Gupta (2014) with a set of of 25 conserved proteins, and

Barbour et al. (2017) with a set of 762 conserved proteins. For 762 conserved core proteins, pairwise average amino acid identities ranged from 89% to 98% for 11 species of *Borreliella* (including *Borreliella chilensis*), 78% to 97% for 11 species of *Borrelia* (including *B. miyamotoi*), and 69% to 73% for the 121 inter-generic pairs (Barbour et al., 2017).

Table 2 gives the patterns of 16S rDNA gene signature nucleotides that define the two genera in a set of sequences of at least 1200 nucleotides and which includes *B. turcica* and two other reptile-associated organisms. Other genotypic characteristics of the two genera are summarized in Table 3. These genetic distinctions between genera have biological and medical significance as well. The large linear plasmids (megaplasmids) of 90-200 kb contain not only genes for nucleotide metabolism but also several coding sequences for proteins that are unique, among all bacteria, to the genus *Borrelia* (Lescot et al., 2008, Lopez et al., 2013, Wilder et al., 2016). The *glpQ* gene found in the genus *Borrelia* but not *Borreliella* produces the glycerophosphodiester phosphodiesterase protein that is the basis for the serologic assay for diagnosis of relapsing fever, including infection with *Borrelia miyamotoi* (Schwan et al., 1996, Krause et al., 2015).

Table 4 summarizes phenotypic differences between the two genera. A standout is disease association. All the agents of Lyme disease, which is characterized by persistent infections with higher spirochete densities in tissues other than blood, are *Borreliella* species. All the agents of relapsing fever, as well as other infections, like avian spirochetosis and from *B. miyamotoi*, that feature high densities of spirochetes in the blood, are *Borrelia* species. Two other major differences between the genera relate to life in the tick environment. All *Borrelia* species that have been studied in this respect are already present in the salivary glands of unfed ticks when they first attach to a new host (Schwan and Piesman 2002, Barbour et al., 2017). This includes those transmitted by slower-feeding ixodid ticks, as well as those transmitted by rapid feeding argasid ticks. In contrast, at the start of the tick's feeding, *Borreliella* species are predominantly in their midgut and not salivary glands. The other tick-related trait that serves to differentiate the two genera is transovarial transmission. While this is frequently (but not invariably) observed among *Borrelia* species (Burgdorfer and Varma, 1967, Barbour, 2004), there has been no documented transovarial transmission of a *Borreliella* species (Rollend et al., 2013).

Differences in morphology and appearances in culture medium between representatives of the Lyme disease and relapsing fever groups were noted in early descriptions (Barbour 1984, Johnson et al., 1984a, Hovind-Hougen et al., 1986). While the tightness and regularity of coiling can vary with growth phase and environmental conditions for all species, cells of *Borrelia* species generally display in culture regularly-spaced coils or waves, even as growth wanes and the stationary phase is reached. In comparison, *Borreliella* cells are looser, more relaxed in coiling, and may even become straight in sections, especially in late growth phases. Correlated with this trait is the greater tendency of *Borreliella* cells to form aggregates in liquid medium (Barbour, 1984).

Two *Borrelia* species, *B. anserina* and *B. recurrentis*, feature exceptions for some traits that otherwise apply across their genus. *B. anserina* has fewer subterminal flagella insertions than other species in the genus (Hovind-Hougen, 1995) and has only one *vsp*/*ospC*-type gene (Elbir et al., 2017). While there is salivary gland localization of *B. anserina* in the unfed *Argas* sp. vector (Diab and Soliman, 1977), this has not been observed for *B. recurrentis* in the louse. The transmission of *B. recurrentis* from the louse occurs not through a bite but when the infected louse is crushed with fingers, allowing spirochetes in the body cavity to be released and enter through the skin. *B. anserina* and *B. recurrentis* are particularly restricted in their vector and vertebrate host associations, as detailed in the *Borrelia* genus article, and have reduced genomes with a comparatively high prevalence of pseudogenes (Lescot et al., 2008, Elbir et al., 2017).

The chromosome-wide analyses of DNA and protein sequences cited above did not include a reptile-associated species, such as *B. turcica* (Güner et al., 2004), or *Candidatus* Borrelia tachyglossi, which was isolated from hard ticks of echidnas in Australia (Loh et al., 2017). At the time of this writing (July 2017), a genome sequence for one of these species has not been published. The phylogenetic evidence from a limited number of genes, including 16S rDNA (Figure 1), to date is consistent with assignment of both the reptile-associated and the echidna tick-associated species to the genus *Borrelia* (Takano et al., 2010, Loh et al., 2017). The reptile-associated organisms described by

Takano l.l. (2010) had a *glpQ* gene, a megaplasmid, and were located in the salivary glands of unfed tick vectors.

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Table 1. Conserved signature insertions and deletions (indels) in core proteins that distinguish *Borreliaceae* from other spirochete groups [a]

Protein name	Gene name	*Borrelia anserina* protein accession	Indel type	Indel size (amino acids)	Amino acid position(s)
1-phosphofructokinase	*pfk*	APR65150	Insertion	6	302-307
Elongation factor Tu (EF-Tu)	*tuf*	APR64929	Deletion	1	312
Nicotinate phosphoribosyltransferase	*pncB*	APR65067	Deletion	1	142
Ribonuclease Z	*rnz*	APR65176	Insertion	2	83-84
Rod shape-determining protein MreB	*mreB*	APR65138	Insertion	3	231-233
Flagellar motor switch protein FliM	*fliM*	AHH08243	Insertion	7	30-36
Mg^{2+} transporter MgtE	*mgtE*	AHH08349	Insertion	4	76-79
Pantetheine-phosphate adenyltransferase	*coaD*	AHH08669	Insertion	2	50-51
Chemotaxis protein CheY	*cheY*	AHH08527	Insertion	4	89-92

[a] 9 of the 26 Conserved Signature Indels identified by Adeolu and Gupta (2014)

Table 2. Patterns of 16S rRNA gene signature nucleotides that define the genera *Borrelia* and *Borreliella*		
Positions (*E. coli* number) [a]	*Borrelia*	*Borreliella*
116	A	G
180	G	U
642 : 661	C-G	U-A
717	A	G
1164/1165 [b]	C	U
1296/1297 [b]	A	G

[a] Number according to sequence positions of 16S rRNA genes of *Escherichia coli* K-12 substrain MG1655 (U00096).

[b] Insertion in all *Borrelia* spp. and some *Borreliella* spp. in alignment at position 840

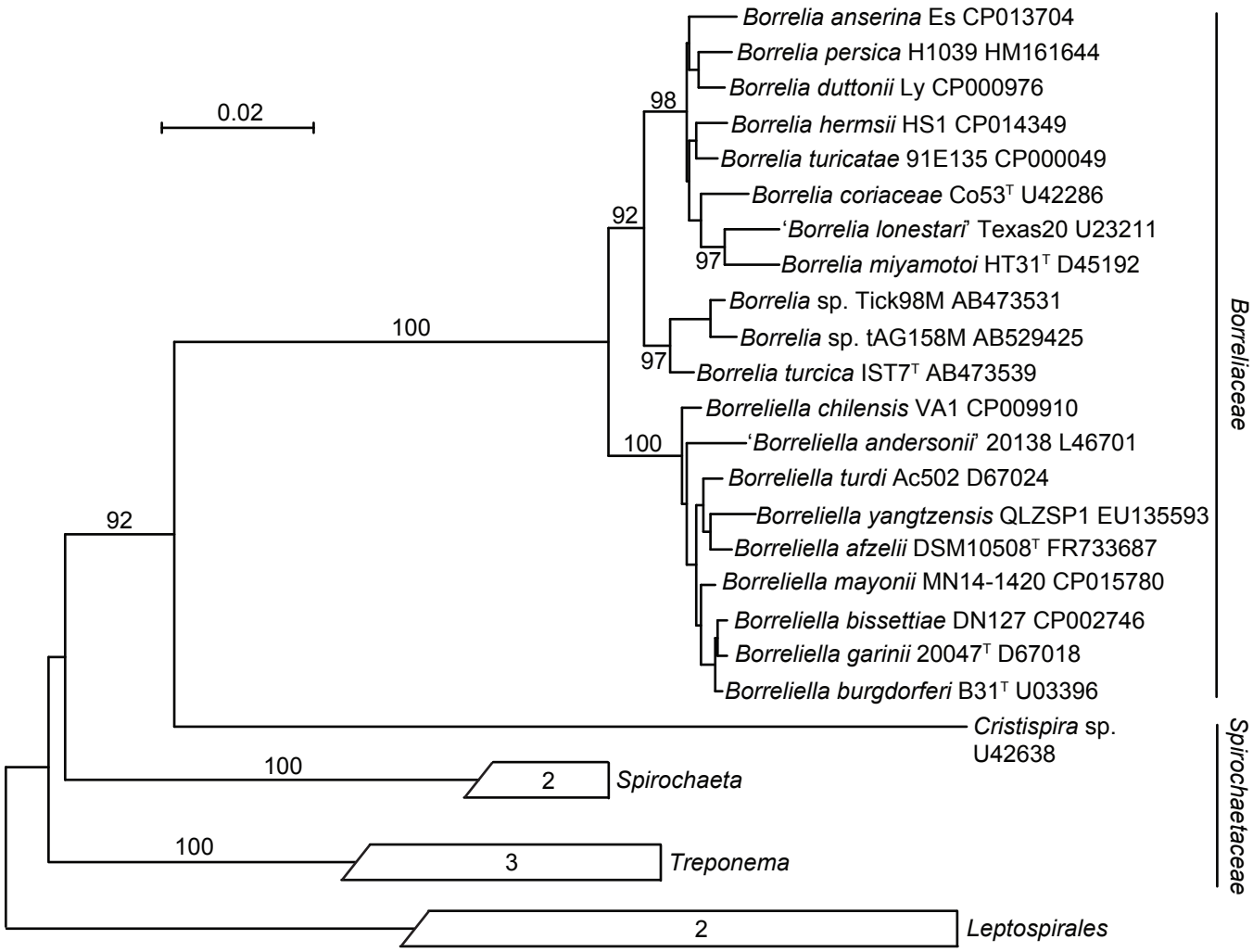
Table 3. Genotypic characteristics of *Borreliaceae* genera *Borrelia* and *Borreliella*		
Characteristic	*Borrelia*	*Borreliella*
Chromosome G+C content (mol%)	27.5-29.8	25.8-28.6
Linear megaplasmid (>90 kilobases) [a]	Yes	No
Number of 23S ribosomal RNA genes (*rrl*) [b]	1	2
glpQ gene (chromosome) [c]	Yes	No
purAB genes (16S-23S spacer) [d]	Yes	No
Firmicute-like *nrdEFI* genes (megaplasmid) [e]	Yes	No
Number of *vsp/ospC*-type genes per genome [f]	Several [f]	One
Arginine to cysteine substitution in active site of flavin-dependent thymidylate synthase [e]	No	Yes
Conserved signature indels specific for genus [h]	8	7

[a] (Casjens et al., 2010; Miller et al., 2013; Elbir, Sitlani et al., 2017)
[b] (Schwartz, J. J. et al., 1992).
[c] (Schwan et al., 2003)
[d] (Barbour et al., 2005; Pettersson et al., 2007)
[e] (Zhong et al., 2006)
[f] (Barbour 2003; Barbour and Travinsky 2010; Barbour 2016)
[g] *Borrelia anserina* is an exception and has only one *vsp* gene
[h] (Adeolu and Gupta 2014; Barbour, Adeolu et al., 2017)

Table 4. Phenotypic characteristics of *Borreliaceae* genera *Borrelia* and *Borreliella*		
Characteristic	*Borrelia*	*Borreliella*
Associated disease(s)	Tick-borne relapsing fever; louse-borne relapsing fever; avian spirochetosis; bovine borreliosis	Lyme disease (Lyme borreliosis)
High cell density in the blood	Yes	No
Arthropod vectors	Argasid ticks, prostriate ixodid ticks, metastriate ixodid ticks, and human body louse (insect)	Prostriate ixodid ticks
Presence in salivary glands in unfed tick	Yes [a]	No [b]
Transovarial transmission in ticks [c]	Most tick-borne species	No
Number of sub-terminal insertions of flagella at each end [d]	15-20 [d, e]	7-11 [f]
Coils or flat waves of cells	Regularly spaced	Regularly or irregularly spaced
Aggregates of cells in liquid medium	No	Yes [g]

[a] (Burgdorfer 1951, Diab and Soliman 1977, Smith et al., 1978, Gaber et al., 1984, Schwan and Hinnebusch 1998, Takano, Goka et al., 2010, Lopez, Wilder et al., 2013, Bockenstedt 2017)
[b] (Burgdorfer et al., 1991, Piesman 1995, Schwan and Piesman 2002, Piesman and Schwan 2010)
[c] (Burgdorfer and Varma 1967, Smith, Brener et al., 1978, Scoles et al., 2001, Barbour 2004, Rollend, Fish et al., 2013, Killmaster et al., 2014)
[d] (Hovind-Hougen 1974, Karimi et al., 1978, Barbour et al., 1982, Hovind-Hougen 1995, Naddaf et al., 2012, Guyard et al., 2013)
[e] Exceptions with fewer insertion points are *Borrelia anserina* (Hovind-Hougen 1995) and possibly *Borrelia recurrentis* (Cutler et al., 1997)
[f] (Hovind-Hougen 1974, Barbour and Hayes 1986, Hovind-Hougen, Åsbrink et al., 1986, Hovind-Hougen 1995, Yano et al., 1997, Masuzawa et al., 2001, Kudryashev, Cyrklaff et al., 2009)
[g] (Barbour 1984)

Figure 1. Neighbor-joining distance tree based on nearly full-length 16S rRNA gene sequences showing the monophyletic cluster formed by members of the family *Borreliaceae* among the closest related family *Spirochaetaceae* and the out-group *Leptospiraceae* within the order Spirochaetales. 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. Numbers at nodes represent bootstrap values ≥ 90% (1000 replications) by the distance protocol with the observed-differences criterion of SeaView v. 4 (Gouy et al., 2010). The same levels of support for the nodes between genera and families were obtained with the LogDet and Jukes Cantor models for distance and maximum liklihood under the General Time Reversible model. GenBank/EMBL/DDBJ accession numbers follow the strain name of individually identified species of *Borreliaceae* and for the *Cristispira* sp. sequence. The type strains are indicated by superscript "T". The *Spirochaeta* representatives in the family *Spirochaetaceae* were *Spirochaeta aurantia* M1 (AY599019) and *Spirochaeta* sp. MWH-HuW24 (AJ565434). The *Treponema* representatives were *T. refringes* CIP 51.64 (AF426101), *T. denticola* ATCC 35405 (AE017226), and *T. pallidum* Nichols (CP010422). The *Leptospirales* representatives were *Leptospira interrogans* serovar Li (NC_004342) and *Leptonema illini* DB52 (JQ988853). Numbers in boxes represent the number of sequences included in a genus or family cluster. The bar indicates distance.


0.02
Borrelia anserina Es CP013704
Borrelia persica H1039 HM161644
Borrelia duttonii Ly CP000976
Borrelia hermsii HS1 CP014349
Borrelia turicatae 91E135 CP000049
Borrelia coriaceae Co53T U42286
'Borrelia lonestari' Texas20 U23211
Borrelia miyamotoi HT31T D45192
Borrelia sp. Tick98M AB473531
Borrelia sp. tAG158M AB529425
Borrelia turcica IST7T AB473539
Borreliella chilensis VA1 CP009910
'Borreliella andersonii' 20138 L46701
Borreliella turdi Ac502 D67024
Borreliella yangtzensis QLZSP1 EU135593
Borreliella afzelii DSM10508T FR733687
Borreliella mayonii MN14-1420 CP015780
Borreliella bissettiae DN127 CP002746
Borreliella garinii 20047T D67018
Borreliella burgdorferi B31T U03396
Cristispira sp. U42638
Spirochaeta
Treponema
Leptospirales
98
92
97
97
100
100
92
100
100
2
3
2
Borreliaceae
Spirochaetaceae