Borrelia kurtenbachii sp. nov., a widely distributed member of the Borrelia burgdorferi sensu lato species complex in North America

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Lyme borreliosis group spirochaetes are parasitic bacteria transmitted by vector ticks of the genus Ixodes and distributed mainly between 40° and 60° northern latitudes. Since Borrelia burgdorferi sensu stricto (hereinafter, B. burgdorferi) was described in the north-eastern USA during the early 1980s, an increasing diversity has been noted within the species complex. Here, we describe a novel genomic species, Borrelia kurtenbachii sp. nov. (type strain 25015T = ATCC BAA-2495T = DSM 26572T), that is prevalent in transmission cycles among vector ticks and reservoir hosts in North America. Confirmation of the presence of this species in Europe awaits further investigation.

Borrelia burgdorferi sensu lato (also termed the Lyme borreliosis group of spirochaetes) is a species complex of spirochaetal bacteria comprising pathogenic (Lyme-borreliosis-causing) and non-pathogenic species and other species of uncertain pathogenicity (Margos et al., 2011). This group of bacteria is maintained in natural transmission cycles between ixodid vector ticks and vertebrate reservoir hosts (Piesman & Gern, 2004). In North America, B. burgdorferi has been recognized as the main aetiological agent of Lyme disease (Dennis & Hayes, 2002). However, the species richness on this continent comprises eight named and unnamed genomic species at the time of writing (Margos et al., 2010; Postic et al., 1998, 2007; Rudenko et al., 2009b, 2011). Some of these species are maintained by vectors that have strong host preference such as Ixodes dentatus, which prefers rabbits as hosts, or Ixodes spinipalpis, which prefers woodrats and, consequently, they rarely if ever attach to or bite humans. Nonetheless, the transmission cycles of spirochaetes maintained by host-specialist vectors may overlap those of generalist vectors, such as Ixodes scapularis, and, depending upon the breadth of their vector competence, may spill over into transmission cycles that directly affect humans (Hamer et al., 2010). Hence, unambiguous genotyping systems are critical for epidemiological and clinical investigations. In this context, as well as for bacterial taxonomy, multilocus sequence analysis (MLSA) has proven to be an invaluable tool (Bishop et al., 2009; Postic et al., 2007; Richter et al., 2006; Rudenko et al., 2011).

In 1987, an atypical strain representing a member of the genus Borrelia, designated 25015T, was isolated from blood-fed I. scapularis larvae that had been removed from Peromyscus in New York State (Anderson et al., 1990). A decade later, the species ‘Borrelia bissettii’ was described (Postic et al., 1998) from borrelial isolates from California. Based on the restriction pattern of the 5S–23S intergenic spacer and sequence similarities of the 16S rRNA locus, strain 25015T initially was integrated into the species ‘B. bissettii’ (Postic et al., 1998). Subsequently, strains with genomic

Abbreviation: MLSA, multilocus sequence analysis.

Sequences of the housekeeping loci used in multilocus sequence typing (MLST) have been deposited on the Borrelia MLST website (borrelia.mlst.net) at Imperial College London, UK, and in GenBank/EMBL/DDBJ with the accession numbers KF052001–KF052032.
restiction patterns and RFLP patterns for the 5S–23S intergenic spacer similar to that of strain 25015 have been described from Illinois, Florida, Georgia and South Carolina (Lin et al., 2001; Picken & Picken, 2000), and most recently a closely related strain has been identified in Canada on the basis of MLSA (Ogden et al., 2010).

To gain deeper insight into the phylogenetic relationship of 25015T and related strains we performed MLSA based on eight housekeeping genes (clpA, clpX, nifS, pepX, pyrG, recG, rplB and uvrA) as described previously (Margos et al., 2009). Sequences were compared with sequences available in the MLST database at Imperial College London (http://borrelia.mlst.net) and allelic profiles and ST numbers were determined (25015T = ST280; IL96-255 = ST278, IL97-236U = ST279, NS07-121 = ST281). This sequence comparison and the allelic profiles suggested that 25015T-like strains were dissimilar to all strains present in the MLST database.

This finding prompted us to perform a genetic-distance analysis using concatenated sequences of the eight MLST loci in MEGA 5.0 (Kumar et al., 2004). The results revealed that the distance to ‘B. bissettii’ and all other species of the genus Borrelia (>0.03) exceeded the previously determined threshold for species determination (0.017). This threshold is consistent with DNA–DNA hybridization for the MLSA system in question (Margos et al., 2009; Postic et al., 2007).

Taken together, the analyses of these strains, i.e. 25015T, IL96-255, IL97-236U and NS07-121, revealed a distinct species that was named Borrelia kurtenbachii sp. nov. in honour of Klaus Kurtenbach for his outstanding contributions to Lyme borreliosis research (Margos et al., 2010). Although the novel species was proposed on the basis of only four available strains, recent published and unpublished data have provided evidence indicating that B. kurtenbachii is abundant in certain areas of the USA [(Margos et al., 2010) and references cited therein]. Firstly, most strains described by Picken & Picken (2000) from the greater Chicago area, Illinois, had a genomic restriction pattern similar to that of strain 25015T. Secondly, Lin et al. (2001) likewise reported strains from the south-eastern USA with an RFLP pattern similar to that of strain 25015T. Additional investigations conducted by researchers from The Czech Academy of Sciences, Prague, Czech Republic, and the James H. Oliver, Jr. Institute of Arthropodology and Parasitology (IAP), Georgia, USA, suggest that this species is abundant in the south-eastern USA (N. Rudenko, J. Oliver, personal communications). In that region, potential vector ticks include Ixodes affinis, Ixodes minor and the southern clades of I. scapularis. Which species constitute competent vectors in other regions of North America remains to be determined. Although two of the samples reported here were found in I. scapularis (Anderson et al. 1988; Ogden et al. 2011), the isolation of spirochaetes from blood-fed larvae or demonstration of DNA in questing ticks indicates ingestion of the bacteria but is not evidence for vector competence. As B. kurtenbachii sp. nov. is found infrequently in I. scapularis (e.g. Tsao et al., 2004), we speculate that a different tick species may be the main vector. However, this hypothesis needs vigorous testing. Ecologically, B. kurtenbachii sp. nov. appears to be associated with rodents (Picken & Picken, 2000, Lin et al. 2001) but its full host spectrum needs to be characterized.

Whether or not B. kurtenbachii sp. nov. causes clinical illness in humans is not known. In their initial description, Anderson et al. (1990) claimed that strain 25015T was non-pathogenic in mice. However, later reports indicated mild pathology in mice following repeated passages (Fikrig et al., 1992). Furthermore, clinical samples with genetic similarity to B. kurtenbachii sp. nov. have been described from Slovenia (Picken et al., 1996) but in recent years, this species has not been described in European patients. We conclude that the geographical distribution of B. kurtenbachii in both the Old and New Worlds, its eco-epidemiology and its pathogenicity for humans require clarification.

**Description of Borrelia kurtenbachii sp. nov.**

*Borrelia kurtenbachii* (kur.ten.bach’i.i. N.L. gen. n. kurten-bachii in honour of Klaus Kurtenbach and his contributions to research on the Lyme borreliosis group of spirochaetes).

The morphology matches that of previously described species of the genus Borrelia (Barbour & Hayes, 1986). *In vitro* culture properties are as described previously (Johnson et al., 1984). B. kurtenbachii can be distinguished from all other Lyme borreliosis-group spirochaetes by MLSA of eight housekeeping loci [clpA, clpX, nifS, pepX, pyrG, recG, rplB and uvrA, (Margos et al., 2010)] and via the restriction pattern of the rrf–rrl intergenic spacer and the rrs–rrl intergenic spacer.

The type strain, 25015T, was isolated from *Ixodes scapularis* collected in upstate New York (Anderson et al., 1990); it has been deposited in two microbial culture collections: ATCC, deposit number ATCC BAA-2495T; DSMZ, deposit number DSM 26572T.

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**References**


