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

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A group of 16 isolates with genotypic characteristics different from those of known species of the *Borrelia burgdorferi sensu lato* complex were cultured from ear biopsies of the rodents *Peromyscus gossypinus* and *Neotoma floridana* trapped at five localities in South Carolina, USA, and from the tick *Ixodes minor* feeding on *N. floridana*. Multilocus sequence analysis of members of the novel species, involving the 16S rRNA gene, the 5S–23S (*rrf–rrl*) intergenic spacer region and the flagellin, *ospA* and *p66* genes, was conducted and published previously and was used to clarify the taxonomic status of the novel group of *B. burgdorferi sensu lato* isolates. Phylogenetic analysis based on concatenated sequences of the five analysed genomic loci showed that the 16 isolates clustered together but separately from other species in the *B. burgdorferi sensu lato* complex. The analysed group therefore represents a novel species, formally described here as *Borrelia carolinensis* sp. nov., with the type strain SCW-22^T (=ATCC BAA-1773^T =DSM 22119^T).

Borrelia burgdorferi was first isolated from the tick *Ixodes scapularis* (formerly known as *Ixodes dammini*) by Burgdorfer *et al.* (1982). Later, Johnson *et al.* (1984) identified this spirochaete as a novel species belonging to the genus *Borrelia*. Since this first discovery, a large number of *Borrelia* isolates, in general referred to as *Borrelia burgdorferi sensu lato*, has been obtained from various vertebrate species, including humans. Seventeen species of spirochaetes from this complex are recognized globally today: *Borrelia burgdorferi sensu stricto* (Johnson *et al.*, 1984), *B. afzelii* (Canica *et al.*, 1993), '*B. andersonii*' (Marconi *et al.*, 1995), '*B. bissettii*' (Postic *et al.*, 1998), '*B. californiensis*' (Postic *et al.*, 2007), *B. garinii* (Baranton *et al.*, 1992), *B. japonica* (Kawabata *et al.*, 1993), *B. lusitaniae* (Le Fleche *et al.*, 1997), *B. sinica* (Masuzawa *et al.*, 2001), *B. spielmanii* (Richter *et al.*, 2006), *B. tanukii* (Fukunaga *et al.*, 1996a), *B. turdi* (Fukunaga *et al.*, 1996a) and *B. valaisiana* (Wang *et al.*, 1997) and the recently described '*Borrelia yangtze*' (Chu *et al.*, 2008), '*B. bavariensis*' (Margos *et al.*, 2009), *B. americana* (Rudenko *et al.*, 2009b) and '*B. carolinensis*' (Rudenko *et al.*, 2009a).

In a recent study, we reported the isolation of 16 strains belonging to a novel group of the *B. burgdorferi sensu lato* complex that we named '*Borrelia carolinensis*' (Rudenko *et al.*, 2009a). Nine strains were isolated from ear biopsies of the cotton mouse *Peromyscus gossypinus* (strains SCCH-6, SCCH-10, SCW-13, SCW-14, SCW-19, SCW-21, SCGT-6, SCGT-21 and SCSC-1), six strains were isolated from ear biopsies of the eastern woodrat *Neotoma floridana* (strains SCCH-11, SCCH-12, SCJ-1, SCJ-5, SCJ-6 and SCGT-18) and one strain, SCW-22^T, was cultured from the hard tick *Ixodes minor*, that was feeding on *N. floridana*.

Borreliae from ear tissues were cultured using standard procedures in BSK-H medium that contained 0.15 % agarose, rifampicin, phosphomycin and amphotericin B (Oliver *et al.*, 2000). The cultures were incubated in 5 % CO_2 at 33–34 °C and stored at −80 °C after cell densities reached 2×10^6 spirochaetes ml^{-1}. The presence of a single *Borrelia* species in each culture was confirmed by repeated amplification of selected loci and bidirectional sequencing of PCR products. Multilocus sequence analysis (MLSA) was conducted using standard procedures and previously described primers (Guy & Stanek, 1991; Postic *et al.*, 1994; Fukunaga *et al.*, 1996b; Thompson *et al.*, 1997; Posada &

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Abbreviation: MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the sequences of the 16S rRNA gene, 5S–23S intergenic spacer region and flagellin, *p66* and *ospA* genes of strain SCW-22^T are respectively EU085407, EU072436, EU076496, EU076512 and EU085398.

Crandall, 1998; Le Fleche *et al.*, 1997; Guindon & Gascuel, 2003; Güner *et al.*, 2003; Clark *et al.*, 2005). Results of genetic and phylogenetic analyses have been presented in detail previously (Rudenko *et al.*, 2009a). Briefly, MLSA revealed that the newly described isolates were highly homogeneous between themselves but distant from known spirochaete species. Unique RFLP patterns were detected for the 5S–23S intergenic spacer region and flagellin gene and unique, phylogenetically significant signature nucleotides (Marconi *et al.*, 1992) were identified in the 16S rRNA gene sequences of '*B. carolinensis*' strains. Sequences from five genomic loci of all 16 strains of '*B. carolinensis*' have been deposited in GenBank under the accession numbers EU085403–EU085418 for the 16S rRNA gene, EU072425–EU072440 for the 5S–23S (*rrf–rrl*) intergenic spacer region, EU076485–EU076500 for the *fla* gene, EU076501–EU076516 for *p66* and EU085387–EU085402 for *ospA*. Reference sequences of known *Borrelia* species were downloaded from GenBank.

The results of MLSA and phylogenetic analysis clearly showed that the '*B. carolinensis*' strains constituted a novel taxon in the *B. burgdorferi sensu lato* complex (Rudenko *et al.*, 2009a). *P. gossypinus* and *N. floridana* are shown to be the primary reservoir hosts of '*B. carolinensis*'. The geographical distribution of the two rodent species may be used as indirect evidence of the possible distribution of '*B. carolinensis*' in the USA (Oliver, 1996). The geographical range of *P. gossypinus* extends northward from the Gulf of Mexico to south-eastern Virginia and southern Illinois, and westward from the Atlantic Ocean to eastern Texas and south-eastern Oklahoma. The species appears to be absent from the southern Appalachians. *N. floridana* occurs throughout Mississippi; its geographical range includes South Dakota and Colorado, eastern Texas, east and central Florida, north to the western and Piedmont areas of Maryland and then west following the Appalachian Mountains (Guilliams & Francl, 2008). Identification of well-established populations of '*B. carolinensis*' was perhaps predetermined by natural factors that exist in the south-eastern United States.

Description of *Borrelia carolinensis* sp. nov.

Borrelia carolinensis (ca.ro.li.nen′sis. N.L. fem. adj. *carolinensis* of or belonging to Carolina, referring to South Carolina, USA, where the organism was first isolated).

Morphology is as described previously for the genus (Barbour & Hayes, 1986). Cultural properties are as described for *B. burgdorferi sensu lato* (Johnson *et al.*, 1984). Can be differentiated from other species of the *B. burgdorferi sensu lato* complex by MLSA of five genomic loci and phylogenetic analysis. The RFLP pattern of the 5S–23S intergenic spacer region consists of four fragments after digestion by *Mse*I (107, 67, 52 and 27 bp) and three fragments after digestion by *Dra*I (173, 53 and 27 bp). Strains exhibit the following unique signature nucleotides in the 16S rRNA gene: A_{171}, T_{203}, C_{323}, C_{1028} and G_{1111}. Strain SCJ-1 also has a unique signature nucleotide: G_{990}. The RFLP pattern of the partial *fla* gene consists of five fragments after digestion by *Dde*I (221, 117, 78, 45 and 27 bp) and two fragments after digestion by *Cel*II (365 and 123 bp); strain SCGT-18 lacks the *Cel*II restriction site and has only four fragments after digestion by *Dde*I (221, 117, 78 and 72 bp). Strains have been isolated from the rodents *Peromyscus gossypinus* and *Neotoma floridana* trapped at five localities in South Carolina, USA, as well as a single strain isolated from a hard tick.

The type strain, SCW-22^T (=ATCC BAA-1773^T =DSM 22119^T), was isolated from a male *Ixodes minor* tick fed on *N. floridana.*

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