

Brevinema andersonii gen. nov., sp. nov., an Infectious Spirochete Isolated from the Short-Tailed Shrew (*Blarina brevicauda*) and the White-Footed Mouse (*Peromyscus leucopus*)

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A spirochete which infects short-tailed shrews (*Blarina brevicauda*) and white-footed mice (*Peromyscus leucopus*) has been shown previously to be ultrastructurally and serologically distinct from other spirochetes. Two of the original isolates from Connecticut and Minnesota and 16 new isolates obtained from shrews captured in Minnesota were characterized phenotypically and genetically in this study. A comparative analysis of the 16S rRNA sequences of two shrew isolates and one mouse isolate and the 16S rRNA sequences of 16 other spirochetes and *Escherichia coli* revealed that these organisms exhibited low levels of similarity (range of similarity values, 73.9 to 77.8%; average level of similarity, 74.7%). The shrew and mouse isolates which we examined formed a deeply branching subgroup that was clearly distinct from the other genera of spirochetes examined. These and other results indicated that the new spirochetes represent a unique taxon in the order *Spirochaetales*. Accordingly, we propose that they should be classified as members of a new genus, *Brevinema*. The three strains of *Brevinema* which we examined had 16S rRNA sequences that were nearly identical. We also compared these isolates by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, fatty acid and enzyme analyses, restriction endonuclease analysis, and Southern hybridization and found that the levels of genetic and phenotypic homogeneity among the strains were very high. We concluded that the isolates which we examined were members of a single species, for which we propose the name *Brevinema andersonii*. The type strain of *Brevinema andersonii* is CT11616 (= ATCC 43811). The guanine-plus-cytosine content of the DNA of this species was determined to be 34 to 36 mol% by the thermal denaturation method.

In 1987 Anderson et al. described the isolation of morphologically unique spirochetes from the blood and other tissues of the short-tailed shrew (*Blarina brevicauda*) and the white-footed mouse (*Peromyscus leucopus*) in Connecticut and Minnesota (1). Each cell of these spirochetes had single sheathed flagella that extended subterminally from its ends and overlapped near the center. The cells were shorter in length (4 to 5 μ m) and longer in wavelength (1.5 to 2.5 μ m) than the cells of most spirochetes. Rabbit and murine antisera to representative *Leptospira*, *Borrelia*, *Treponema*, and *Spirochaeta* species were not reactive with these spirochetes (1, 5). In addition, antisera to four of the shrew-mouse spirochete strains were not reactive with antigens from species belonging to these other spirochete genera. The distinct ultrastructural and serological properties of the spirochetes isolated from shrews and mice suggested that they represent a unique taxon. Partial sequences of the 16S rRNA of a single isolate showed that this spirochete branched separately from other genera (17).

In this paper we describe additional studies performed to classify the spirochetes isolated from shrews and mice. The results of 16S rRNA sequence analysis, DNA restriction enzyme analysis, DNA hybridization analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, DNA base composition analysis, and enzyme and fatty acid analyses, as well as previously published data, support the conclusion that a single species in a new genus should be created for these organisms. We propose the genus name

Brevinema for the single species that has been identified so far, *Brevinema andersonii*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The strains of *Brevinema andersonii* which we used are shown in Table 1. The spirochetes were cultured in a modified BSK medium (2) which is referred to below as shrew-mouse spirochete medium. Shrew-mouse spirochete medium was prepared as follows. To 900 ml of distilled water we added 3 g of *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) (Sigma Chemical Co., St. Louis, Mo.), 5 g of dextrose, 3 g of neopeptone (Difco Laboratories, Detroit, Mich.), 1 g of tryptone (Difco), 3 g of TC yeastolate (Difco), 0.8 g of sodium citrate, 2.2 g of sodium bicarbonate, 0.2 g of catalase (Sigma), and 0.4 g of cysteine (Sigma). After these components were fully dissolved, 100 ml of 10 \times 1066 CMRL tissue culture medium (Gibco, Grand Island, N.Y.) was added, and the pH was adjusted to 7.6. The medium was then filter sterilized, and 35 ml of sterile fetal calf serum (Gibco) and 35 ml of bovine embryonic fluid (Sigma) were added. Finally, 200 ml of sterile 7.5% gelatin (Difco) was added before dispensing. The final pH was 7.4. This medium was dispensed into 7-ml tubes; each tube was filled to 95% of its capacity and tightly capped to provide a microaerophilic environment. Cultures were incubated at 30°C. The numbers of cells were determined by using a Petroff-Hausser counting chamber.

Electron microscopy. For transmission microscopy, spirochetes were harvested by centrifugation at 10,000 \times g, washed twice in phosphate-buffered saline (PBS) (pH 7.4) at 4°C, and resuspended in a small volume of PBS. Specimens were placed on Formvar-coated grids and stained for 1 min with 1% phosphotungstic acid (pH 7.4). Specimens for scanning microscopy were prepared by pretreating 10-mm glass coverslips with 1% bovine serum albumin and coating the preparations with cells for 20 min at 37°C. The cell preparations on coverslips were then immersed in 0.2% glutaraldehyde, washed in Hanks balanced salt solution, immersed in 1% glutaraldehyde, washed, and finally treated with 1% osmium tetroxide; in each fixation step the preparation was incubated at 4°C for 1 h. The cells were then dehydrated by using 15-min incubations in serially increasing concentrations of ethanol from 70% to absolute. The specimens were removed from ethanol and immediately subjected to critical point drying. Samples were then sputter coated and examined by conventional methods.

Gas chromatographic analysis of cellular fatty acids. Cells were harvested at the mid-exponential growth phase by centrifugation at 10,000 \times g and were washed once in PBS (pH 7.4). Fatty acids were extracted, separated, and analyzed as described previously (19) by workers at Microbial ID, Newark, Del.

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TABLE 1. *Brevinema andersonii* strains used

Strain	Source	Location	Reference(s)
CT11616 ^T	<i>Blarina brevicauda</i>	West Haven, Conn.	1, 5
FS47 (= MN47)	<i>Peromyscus leucopus</i>	Minneapolis, Minn.	1, 5
MV104	<i>Blarina brevicauda</i>	Minneapolis, Minn.	5
MV116	<i>Blarina brevicauda</i>	Minneapolis, Minn.	5
715	<i>Blarina brevicauda</i>	Columbus, Minn.	5
7281	<i>Blarina brevicauda</i>	Lakeville, Minn.	5
7282	<i>Blarina brevicauda</i>	Lakeville, Minn.	5
812	<i>Blarina brevicauda</i>	Minnetonka, Minn.	5
8132	<i>Blarina brevicauda</i>	Columbus, Minn.	5
8261	<i>Blarina brevicauda</i>	Andover, Minn.	5
8274	<i>Blarina brevicauda</i>	Linwood, Minn.	5
901	<i>Blarina brevicauda</i>	Columbus, Minn.	5
9031	<i>Blarina brevicauda</i>	Chaska, Minn.	5
9033	<i>Blarina brevicauda</i>	Columbus, Minn.	5
9035	<i>Blarina brevicauda</i>	Hollywood, Minn.	5
9102	<i>Blarina brevicauda</i>	Champlin, Minn.	5
9292	<i>Blarina brevicauda</i>	Forest Lake, Minn.	5
1071	<i>Blarina brevicauda</i>	Chaska, Minn.	5

SDS-PAGE. Proteins were separated by using the SDS-PAGE procedure described by Guilian et al. (7). Cells in the early- to mid-log stage of growth were harvested by centrifugation at $10,000 \times g$ for 20 min, washed twice in PBS (pH 7.4), and resuspended in buffer. The protein concentration in the cells was determined by using a modified Bradford assay (Bio-Rad, Mellville, N.Y.). Samples were boiled in buffer containing 2% (vol/vol) SDS and 15 mM dithiothreitol, and 2 to 5 μ g of protein per lane was subjected to electrophoresis

on a 12% polyacrylamide gel. Protein was then visualized by silver staining as described previously (7).

Western blot (immunoblot) analysis. Proteins were transferred from SDS-PAGE gels to polyvinylidene difluoride membranes (Immobilon P; Millipore, Bedford, Mass.) by using the buffer system of Towbin et al. (20) and a Transphor cell (Hoefer Scientific Instruments, San Francisco, Calif.) at 1.0 A for 30 min. Membranes were processed as previously described (3) by using a 1:5,000 dilution of immune rabbit sera.

Isolation of DNA. DNA was isolated and purified by using the method described by Lefebvre et al. (13). Cultures (50 ml) of exponentially growing cells were harvested by centrifugation at $10,000 \times g$ and then washed once in PBS (pH 7.4). DNA was extracted and purified exactly as described previously (13).

Restriction endonuclease analysis. DNA was digested with 1 U of restriction endonuclease per μ g of DNA in the appropriate buffers (Boehringer Mannheim Corp., Indianapolis, Ind.) for 5 h at 37°C. Fragments were separated in 1.2% agarose by electrophoresis, using Tris-acetate buffer (pH 7.2). Lambda phage DNA digested with *Hind*III was used as a molecular weight standard. DNA was also separated by clamped homogeneous electric field electrophoresis, using a model LKB 2015 Pulsaphor system (Pharmacia LKB Biotechnology, Uppsala, Sweden). Cells and DNA were prepared as previously described (8). Field geometry varied, and the conditions necessary for optimal separation of DNA were determined.

Southern hybridization. DNA was isolated, purified, and separated as described above. After electrophoresis and depurination, DNA was transferred to membranes (GeneScreen; NEN Research, Boston, Mass.). Genomic probe DNA was digested with *Eco*RI and labeled for detection by chemiluminescence (Boehringer Mannheim). DNA labeling with digoxigenin and hybridization were carried out according to the instructions of the manufacturer. Hybridization was performed at 60°C by using 500 ng of labeled DNA. Following hybridization, the membranes were washed twice in $2\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS at room temperature, twice for 30 min in $0.5\times$ SSC–0.1% SDS at 65°C, and once in $0.1\times$ SSC for 15 min at 65°C. Hybridized probe DNA was detected by using the procedure recommended by the manufacturer.

DNA base composition. The base composition of spirochetal DNA was determined by the method of Marmur and Doty (14). The thermal denaturation

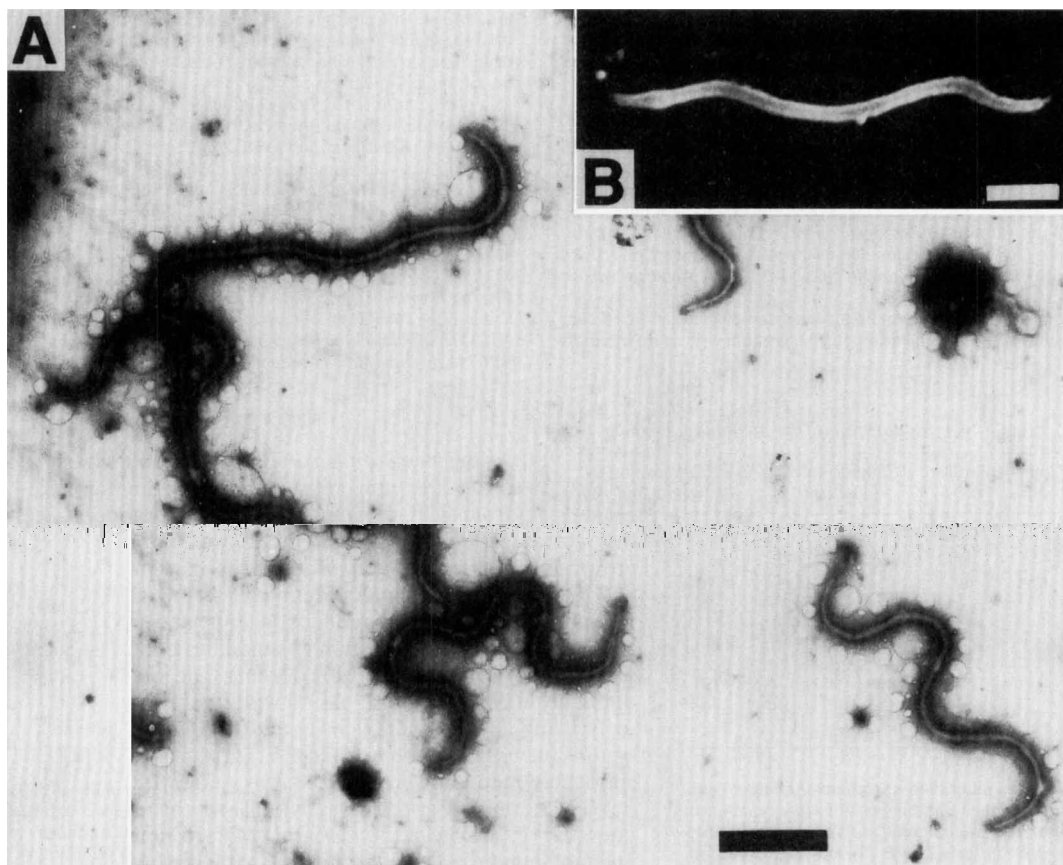


FIG. 1. *Brevinema andersonii* CT11616^T. (A) Transmission electron micrograph of negatively stained preparation. (B) Scanning electron micrograph. Bars = 1.0 μ m.

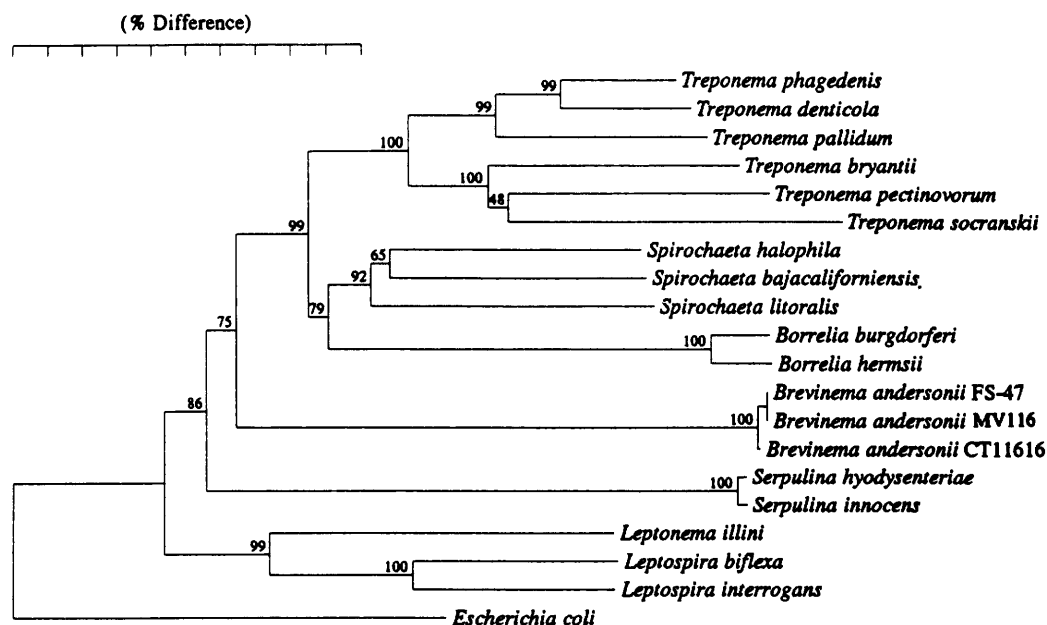


FIG. 2. Dendrogram showing the phylogenetic relationship between *Brevinema andersonii* and other spirochetes, with *E. coli* as the outgroup. Horizontal distance is proportional to the difference between sequences, expressed as a percentage. Bar = 10% difference in nucleotide sequences. Values at branch points represent bootstrap confidence intervals.

kinetics of DNA samples were determined by using a Gilford Response spectrophotometer and thermal programming software (Ciba Corning Diagnostics Corp., Medford, Mass.). Samples (10 µg) of DNA were suspended in 200 µl of 1× SSC in a thermocuvette, and the temperature was increased at a rate of 0.1°C/min. The melting temperature (T_m) was determined from the midpoint of the increase in absorbance. The guanine-plus-cytosine (G+C) content was calculated by using the following equation: $G+C \text{ content} = (T_m - 69.3) \times 2.44$ (14). The values given below are the means of the values from at least three separate experiments in which the standard deviations were less than 10% of the means. DNA samples from *Borrelia burgdorferi*, *Leptospira interrogans*, and *Treponema phagedenis* were analyzed simultaneously as controls.

16S rRNA isolation and sequencing. rRNA was isolated and partially purified by the method described by Pace et al. (15), as modified by these authors (15). Reverse transcriptase was used to elongate eight primers complementary to conserved eubacterial 16S rRNA regions. Sequences were determined directly by a modified Sanger dideoxy chain termination method (11). The primers which we used have been described elsewhere (6).

Sequences were aligned, similarity matrices were constructed, and tree configurations were determined by using a computer algorithm written in Microsoft

Quick Basic (17). The sequences were aligned as previously described (16). Only unambiguously aligned sequences were used in the comparisons which served as the basis for calculations of levels of similarity. Similarity matrices were corrected for multiple base changes at single locations by the method of Jukes and Cantor (9). The neighbor-joining method of Saitou and Nei was used to construct dendrograms (18). The reliability of tree nodes was analyzed by using the bootstrapping program MEGA (10). A total of 300 bootstrap trees were generated, and the bootstrap confidence levels were determined.

Nucleotide accession numbers. Nucleotide sequence data are available from the EMBL, GenBank, and DDBJ nucleotide sequence databases under the following accession numbers: *Brevinema andersonii* CT11616^T (T = type strain), M59179; *Brevinema andersonii* FS47, L31543; *Brevinema andersonii* MV116, L31544; *Treponema pallidum* Nichols, M34266; *Treponema bryantii* RUS-1^T, M57737; *Treponema denticola* ATCC 33520, M71236; *Treponema succinifaciens* 6091^T, M57738; *Spirochaeta aurantia* J1^T, M57740; *Spirochaeta halophila* RS1^T, M34262; *Spirochaeta littoralis* R1^T, M34263; *Borrelia burgdorferi* B31^T, M59293; *Borrelia hermsii* M1001, M72398; *Leptonema illini* 3055, M34118; *Leptospira* sp. strain A183, M34261; *Leptospira interrogans* serovar canicola strain Moulton, X17547; *Serpulina hyodysenteriae* B78^T, M57741; and *Serpulina innocens* B256^T, M57744. *Escherichia coli* sequences have been published previously (4).

RESULTS AND DISCUSSION

Brevinema andersonii grew optimally in shrew-mouse spirochete medium. This culture medium differed considerably from BSK medium as it did not contain *N*-acetylglucosamine or pyruvate. Bovine serum albumin was not required for growth of *Brevinema andersonii*. In addition, tryptone, catalase, and cysteine were added, and fetal calf serum was substituted for rabbit serum. Serum, reducing agents, and peptones were required for growth. Growth in this medium occurred when we used a small inoculum (10 cells per ml). The spirochetes grew optimally at 30 to 34°C and pH 7.4 with a generation time of 11 to 14 h and a typical final density of 4×10^8 cells per ml. Cells of *Brevinema andersonii* CT11616^T grown in shrew-mouse spirochete medium were 0.2 to 0.3 by 4 to 5 µm (Fig. 1).

We determined approximately 95% of the 16S rRNA sequences of *Brevinema andersonii* MV116 and FS47. The aligned sequences were compared with the previously published sequences of strain CT11616^T, 16 other spirochetes that are representatives of known spirochete genera, and *E. coli*

TABLE 2. Single-base signatures of *Brevinema andersonii*

Position(s) of base(s) ^a	Composition in eubacteria ^b	Composition in spirochetes ^c	Composition in <i>Brevinema andersonii</i>
28 · 555	G · C	A · U	A · U
47	C	U	U
50	A	U	U
52 · 359	C · G or U · A	A · U	G · C
53 · 358	A · U	G · C	G · C
783 · 799	G · C	G · C	A · U
361	G	A	A
893	C	U	C
1415 · 1485	G · Y	C · G	C · G

^a Position(s) in *E. coli* rRNA (4).

^b Composition in 250 to 350 eubacteria other than spirochetes. Different bases occur in the sequences of a few strains at the following positions (the numbers in parentheses are the numbers of taxa that have different bases): positions 28 · 555 (2), 47 (2), 50 (4), 52 · 359 (1), 53 · 358 (3), 361 (2), 893 (3), and 1415 · 1485 (4).

^c Composition in the spirochetes shown in Table 3. The only exception is at positions 53 · 358; at these positions the composition is U · A in *Leptospira* spp. and *Leptonema illini*.

TABLE 3. Similarity matrix^a

Bacterial species	% similarity and % difference with:																			
	B.a1	B.a2	B.a3	B.b	B.h	S.h	S.l	S.b	T.pa	T.ph	T.d	T.b	T.pe	T.s	S.h	S.i	L.i	L.b	L.in	E.c
<i>Brevinema andersonii</i> FS-47		100.0	99.7	75.3	75.0	77.5	77.0	77.8	76.2	76.7	75.7	74.3	75.5	74.5	74.3	74.2	75.1	75.6	75.5	73.3
<i>Brevinema andersonii</i> MV116	0.0		99.7	75.3	75.0	77.5	77.0	77.8	76.2	76.7	75.7	74.3	75.5	74.5	74.3	74.2	75.1	75.6	75.5	73.3
<i>Brevinema andersonii</i> CT11616	0.3	0.3		75.5	75.4	78.1	77.5	77.8	76.4	76.9	76.0	74.5	75.5	74.4	73.9	74.1	74.9	75.7	75.5	73.7
<i>Borrelia burgdorferi</i>	29.9	29.9	29.6		96.6	80.9	81.4	80.6	79.2	78.4	79.3	78.6	78.2	75.7	75.0	74.6	74.6	77.2	76.2	72.3
<i>Borrelia hermsii</i>	30.3	30.3	29.9	3.4		81.3	81.3	81.8	79.4	77.9	78.5	79.0	78.6	75.9	74.6	74.3	74.5	76.2	75.7	72.3
<i>Spirochaeta halophila</i>	26.7	26.7	26.0	22.1	21.5		85.9	86.9	81.7	82.4	82.1	81.6	79.9	79.3	77.8	77.8	76.9	77.6	77.8	74.4
<i>Spirochaeta litoralis</i>	27.5	27.5	26.8	21.4	21.5	15.6		85.6	81.9	82.3	81.5	80.8	81.3	77.5	75.3	75.4	77.3	77.7	77.1	74.3
<i>Spirochaeta bajacaliforniensis</i>	26.3	26.3	26.3	22.4	20.8	14.4	16.0		83.0	82.1	82.1	81.1	79.2	79.6	76.0	76.6	78.6	78.8	78.9	75.2
<i>Treponema pallidum</i>	28.7	28.7	28.3	24.4	24.1	21.0	20.7	19.2		90.1	88.7	82.5	83.3	81.9	76.1	76.3	76.3	75.8	76.5	74.3
<i>Treponema phagedenis</i>	27.9	27.9	27.6	25.5	26.2	20.1	20.2	20.4	10.6		93.2	84.2	85.5	83.7	76.8	76.9	76.4	75.7	76.0	75.4
<i>Treponema denticola</i>	29.3	29.3	28.9	24.3	25.3	20.5	21.2	20.4	12.3	7.1		84.9	85.4	83.7	76.5	76.5	75.9	76.0	75.6	74.3
<i>Treponema bryantii</i>	31.5	31.5	31.2	25.2	24.7	21.2	22.2	21.8	20.0	17.7	16.8		86.9	83.8	76.1	76.2	76.6	74.9	75.2	73.7
<i>Treponema pectinovorum</i>	29.6	29.6	29.7	25.7	25.1	23.4	21.5	24.4	18.9	16.1	16.2	14.3		84.7	74.2	74.3	75.2	74.5	73.8	72.2
<i>Treponema socranskii</i>	31.1	31.1	31.4	29.4	29.1	24.2	26.8	23.8	20.7	18.4	18.3	18.3	17.1		73.4	73.4	74.1	74.0	74.2	71.2
<i>Serpulina hyodysenteriae</i>	31.4	31.4	32.1	30.4	31.0	26.4	29.9	28.9	28.8	27.8	28.2	28.8	31.6	32.9		99.5	75.6	75.5	75.1	73.5
<i>Serpulina innocens</i>	31.6	31.6	31.7	31.1	31.5	26.3	29.9	28.1	28.5	27.6	28.2	28.7	31.5	32.9	0.5		75.6	75.3	74.9	73.2
<i>Leptonema illini</i>	30.3	30.3	30.6	31.0	31.1	27.6	27.1	25.2	28.5	28.3	29.0	28.0	30.1	31.8	29.6	29.5		82.0	83.6	75.8
<i>Leptospira biflexa</i>	29.5	29.5	29.4	27.2	28.6	26.6	26.4	25.0	29.3	29.4	28.9	30.5	31.1	32.0	29.7	29.9	20.6		89.3	75.1
<i>Leptospira interrogans</i>	29.7	29.7	29.7	28.6	29.3	26.4	27.3	24.8	28.2	28.9	29.4	30.1	32.3	31.7	30.3	30.5	18.5	11.6		75.7
<i>Escherichia coli</i>	33.0	33.0	32.4	34.6	34.6	31.3	31.4	30.0	31.5	29.8	31.5	32.4	34.7	36.3	32.7	33.1	29.3	30.2	29.4	

^a The values on the upper right are uncorrected percentages of similarity, and the values on the lower left are percentages of difference corrected for multiple base changes. Abbreviations: B.a1, *Brevinema andersonii* FS47; B.a2, *Brevinema andersonii* MV116; B.a3, *Brevinema andersonii* CT11616^T; B.b, *Borrelia burgdorferi*; B.h, *Borrelia hermsii*; S.h, *Spirochaeta halophila*; S.l, *Spirochaeta litoralis*; S.b, *Spirochaeta bajacaliforniensis*; T.pa, *Treponema pallidum*; T.ph, *Treponema phagedensis*; T.d, *Treponema denticola*; T.b, *Treponema bryantii*; T.pe, *Treponema pectinovorum*; T.s, *Treponema socranskii*; S.h, *Serpulina hyodysenteriae*; S.i, *Serpulina innocens*; L.i, *Leptonema illini*; L.b, *Leptospira biflexa*; L.in, *Leptospira interrogans*; E.c, *Escherichia coli*.

(17). A comparative analysis of the sequences revealed similarities between other genera of spirochetes, and the sequence data were consistent with classification of the genus *Brevinema* as a separate genus (17, 22). The topology of the dendrogram created from 16S rRNA sequences (Fig. 2) revealed that the genus *Brevinema* is a separate and deeply branching subgroup of the order *Spirochaetales*. Designation of a separate genus for

Brevinema strains was also supported by the results of the bootstrap analysis as the confidence level for each of the spirochete subgroups was 100%.

The results of a single-base signature analysis revealed that the genus *Brevinema* is related to other spirochetes and provided evidence that this genus should be included in the order *Spirochaetales* (Table 2) (17). The unique base compo-

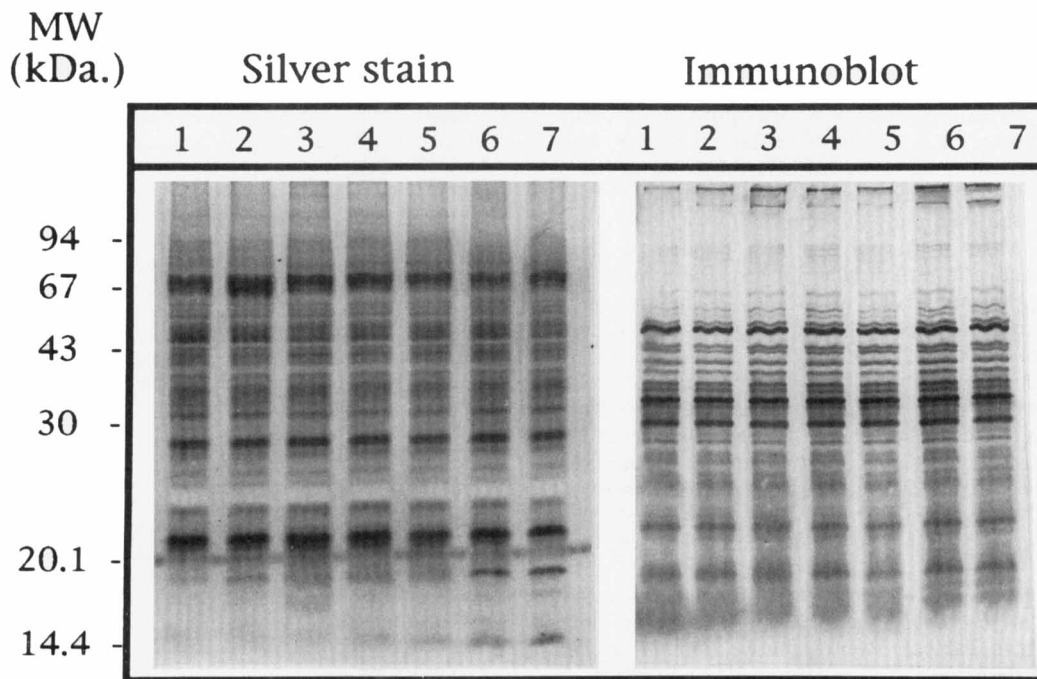


FIG. 3. Silver-stained electrophoretic profiles of various *Brevinema andersonii* strains and Western blots of identical samples probed with rabbit antisera against strain CT11616^T. Lanes 1 through 7, strains CT11616^T, FS47, MV104, MV116, 715, 7281, and 7282, respectively. Molecular masses (in kilodaltons) are indicated on the left. MW, molecular weight.

TABLE 4. Fatty acid compositions of *Brevinema andersonii* strains

Strain	Relative peak area (%)																
	12:0 ^a	13:0	14:0	14:0A ^b	15:0	16:0	16:0 3OH	16:1	18:0	18:1	18:2	18:3	19:0	19:1	19:2	20:3	20:4
CT11616 ^T (= ATCC 43811 ^T)	0.4	0.4	47.5	0.5	2.0	20.7	ND ^c	ND	5.3	8.2	5.6	1.4	0.6	ND	ND	3.2	2.5
PS47 (= ATCC 43810)	0.5	0.3	46.6	0.4	1.7	21.8	0.4	ND	5.3	10.2	6.1	1.1	ND	ND	ND	3.2	2.5
MV104	0.5	0.4	42.6	0.4	1.8	22.2	0.5	0.5	5.4	13.1	5.7	1.1	ND	ND	ND	3.5	2.5
MV116	0.5	ND	40.3	ND	1.9	20.8	ND	ND	7.4	9.3	5.8	1.2	4.4	0.9	1.7	3.3	2.5

^a The number before the colon is the number of carbon atoms in the chain, and the number after the colon is the number of double bonds.

^b Aldehyde.

^c ND, not detected.

sition of members of the genus *Brevinema* at positions 52·359 and 783·799 further distinguishes this genus as a separate and distinct subgroup of spirochetes. Furthermore, the sequences of *Brevinema andersonii* did not possess the usual 20- to 30-base extension at the 5' end which is typical of *Treponema*, *Spirochaeta*, *Leptospira*, and *Leptonema* sequences. We identified a 27-nucleotide signature sequence (5'-GGCAGCUAC CUAUGCUAAGAUUGACGC-3', equivalent to positions 724 to 750 in *E. coli* [4]) that differed from the signature sequences of other spirochetes in 6 to 12 bases. This sequence, which was found in the three strains examined, could potentially serve as a target region for *Brevinema* strains and may be useful for creating species- or genus-specific DNA probes. Such probes would be useful for rapid screening, detection, and identification of new isolates.

The rRNA sequences of strains FS47, MV116, and CT11616^T, which were isolated from a Minnesota mouse, a Connecticut shrew, and a Minnesota shrew, respectively, were nearly identical (Table 3). The few differences in base composition may have been due to sequencing error or strain variation.

The homogeneity of the 16S rRNA sequences of these three strains was reflected in the phenotypic and genotypic characteristics of the isolates. The results of SDS-PAGE obtained for seven *Brevinema* strains (Fig. 3) revealed the homogeneity of the phenotypes of the isolates. Approximately 40 to 50 bands were produced on SDS-PAGE gels. The profiles of other isolates were indistinguishable from those shown in Fig. 3 (data not shown). When proteins were transferred to membranes and probed with rabbit hyperimmune sera raised against strain CT11616^T, we found that many of the proteins were immunogenic in rabbits. Most of the polypeptides revealed by silver staining were reactive in an immunoblot test. Patterns of the reactive polypeptides observed in profiles of heterologous isolates were identical to those of the polypeptides that reacted with homologous strain CT11616^T (Fig. 3).

The fatty acid compositions of four *Brevinema andersonii* isolates are shown in Table 4. We found that the major fatty acid components of the cells were myristic acid (14:0), palmitic acid (16:0), and oleic acid (18:1) and that smaller amounts of stearic acid (18:0) and linoleic acid (18:2) were also present; in addition, several fatty acids were detected at low levels (less than 1%). We found few significant differences among the four strains analyzed.

The API ZYM system was used to examine constitutive enzyme activity in *Brevinema* isolates. The results of these assays are shown in Table 5. Representative *Borrelia*, *Treponema*, and *Leptospira* species were examined concurrently as controls, and our results were always consistent with the previously published profiles.

Isolate CT11616^T from a Connecticut shrew, isolate FS47

from a Minnesota mouse, and isolates 901 and 8274, two Minnesota shrew isolates, had G+C contents of 36, 35, 34, and 34 mol%, respectively.

To determine the levels of genetic relatedness of *Brevinema andersonii* isolates to each other, a restriction enzyme analysis was performed. The results of separation of *Sfi*I-digested *Brevinema andersonii* genomic DNAs by electrophoresis are

TABLE 5. Enzyme activities of *Brevinema andersonii*

Enzyme	% of strains with activity (n = 18)
Aminopeptidases	
L-Tyr.....	0
L-Glu.....	0
L-Phe.....	0
L-Lys.....	0
L-Pro.....	0
L-His.....	0
L-Gly.....	0
L-Asp.....	0
L-Arg.....	0
L-Ala.....	0
L-Leu.....	0
L-Val.....	0
L-Cys.....	0
Esterases	
Butyrate (C ₄).....	100
Valerate (C ₅).....	100
Caproate (C ₆).....	100
Caprylate (C ₈).....	100
Nonanoate (C ₉).....	100
Caprate (C ₁₀).....	83
Laurate (C ₁₂).....	0
Myristate (C ₁₄).....	0
Palmitate (C ₁₆).....	0
Stearate (C ₁₈).....	0
Esterase lipase (C ₄).....	89
Lipase (C ₁₄).....	0
Other enzymes	
Alkaline phosphatase.....	100
Acid phosphatase.....	100
Trypsin.....	0
Chymotrypsin.....	0
Phosphohydrolase.....	0
α-Galactosidase.....	0
β-Galactosidase.....	0
β-Glucuronidase.....	78
α-Glucosidase.....	0
β-Glucosidase.....	0
N-Acetylglucosaminidase.....	0
α-Mannosidase.....	0
α-Fucosidase.....	0

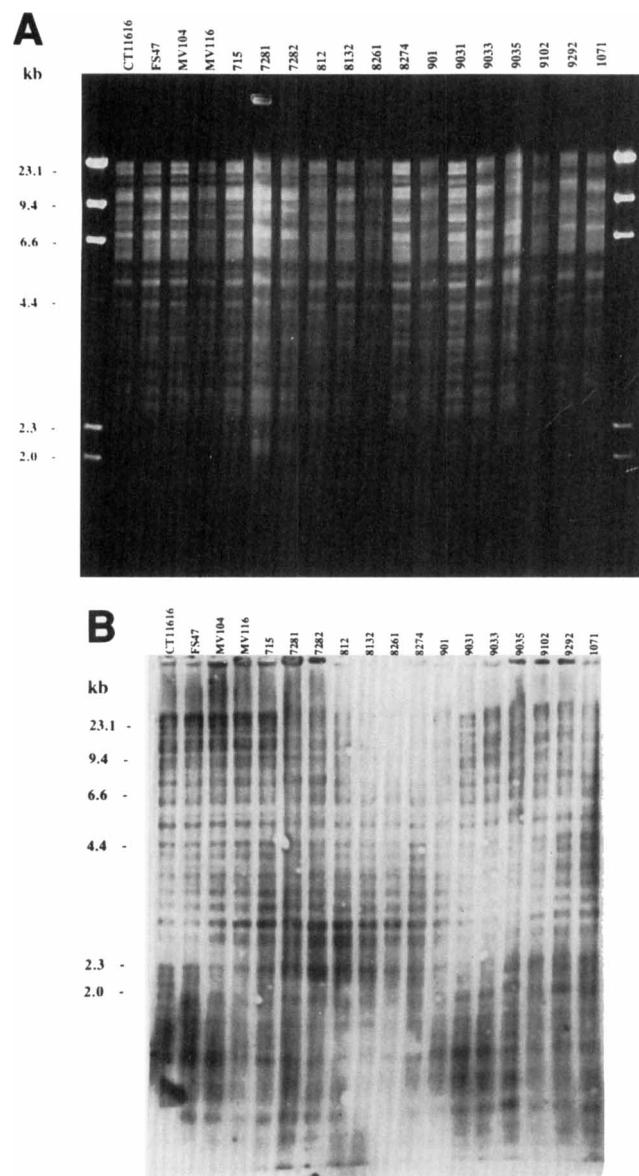


FIG. 4. Restriction enzyme analysis of fragment patterns and Southern hybridization of DNAs from *Brevinema andersonii* isolates. (A) *Sfi*I-digested genomic DNAs separated by using a 1.2% agarose gel. (B) DNAs from the *Sfi*I digests were transferred to a membrane and hybridized with labeled genomic DNA from strain CT11616^T. The strain in each lane is indicated at the top. The positions of molecular size markers (in kilobase pairs) are indicated on the left.

shown in Fig. 4A. The patterns of bands obtained for all 18 isolates under these conditions were virtually identical. The same degree of homogeneity was obtained when we used different restriction enzymes and both constant-field electrophoresis and pulsed-field gel electrophoresis (data not shown). To further assess the genetic relatedness of the isolates, *Sfi*I digests were transferred to membranes and probed with labeled CT11616^T genomic DNA under high-stringency conditions. This probe hybridized strongly to all isolates, and the patterns resulting from DNA-DNA hybridization were essentially identical, demonstrating the close genetic relatedness of the organisms (Fig. 4B).

The results of previous studies of the shrew-mouse spirochete led investigators to suggest that this organism represents

a unique taxon belonging to the order *Spirochaetales* (1, 2, 12, 13, 17). Our results supported and extended this suggestion by providing data for a number of isolates obtained from different hosts and geographic regions. Our results contributed phenotypic, genetic, and phylogenetic evidence which indicated that the strains which we examined are closely related members of a distinct taxon of spirochetes. The phylogenetic distance of this group of organisms from other spirochete genera indicated that it should be classified as a distinct genus.

On the basis of the results of phenotypic analyses of the 18 *Brevinema andersonii* isolates which we studied we were not able to distinguish between strains. The enzyme profiles of the *Brevinema andersonii* strains indicated that they are members of a single biotype. Furthermore, the protein profiles and immunoblot data did not provide distinguishing characteristics or establish the presence of separate serotypes.

Brevinema andersonii can be readily distinguished from other spirochetes on the basis of its unique morphological characteristics (1). In addition, the genus *Brevinema* can be differentiated from other genera by several types of data. *Brevinema* strains are serologically distinct from other spirochetes (1, 5), and *Brevinema andersonii* DNA exhibits no detectable hybridization with DNAs from members of other genera, as determined by Southern analysis (5, 12, 13). In addition, we identified a nucleic acid sequence that corresponds to an rRNA region in *Brevinema andersonii* that differentiates this organism from other spirochetes. This sequence may be useful for constructing DNA probes for identification of *Brevinema andersonii*. At the species level, *Brevinema andersonii* can be readily identified by restriction enzyme analysis, and SDS-PAGE, or fatty acid composition data. As determined by comparative 16S rRNA analysis, restriction enzyme analysis, and SDS-PAGE patterns, *Brevinema andersonii* isolates are members of a genetically homologous group, despite their diverse hosts and diverse geographic origins.

Description of the genus *Brevinema* Defosse, Johnson, Paster, Dewhirst, and Fraser gen. nov. *Brevinema* (Brev. i. ne' ma. L. adj. *brevis*, short; Gr. n. *nema*, thread; N.L. neut. n. *Brevinema*, a short thread.) The following description is based on our data and data obtained from previous studies (1, 5, 12, 13, 17). Cells are flexible and helical; they are 0.2 to 0.3 μ m in diameter and 4 to 5 μ m long. One to two waves occur, and the irregular wavelengths of the spirochete cells range from 2 to 3 μ m. Ultrastructurally, the cells possess traits that are typical of spirochetes. They possess an outer envelope, a protoplasmic cylinder, and sheathed periplasmic flagella in a 1-2-1 arrangement. Cytoplasmic tubules have not been observed. Cells are motile and exhibit flexing, rotational, and translational movements at 30°C. Cells stain negatively by Gram reaction. While cells are visible by dark-field or phase-contrast microscopy, they cannot be seen when bright-field microscopy is used.

Brevinema strains have unique single-base nucleotide signatures at positions 52·359 (G·C) and 783·799 (U·A) which differentiate them from other major spirochete phylogenetic groups. *Brevinema andersonii* CT11616^T, FS47, and MV116 have a distinguishing 16S rRNA sequence corresponding to positions 724 to 750 in *E. coli* (5'-GGCAGCUACCUAUGC UAAGAUUGACGC-3').

Brevinema andersonii is host associated. Strains have been isolated from blood and other tissues of short-tailed shrews (*Blarina brevicauda*) and white-footed mice (*Peromyscus leucopus*) and are infectious for laboratory mice and Syrian hamsters.

The type species is *Brevinema andersonii*.

Description of *Brevinema andersonii* sp. nov. *Brevinema andersonii* (an.der.so'ni.i. N.L. gen. n. *andersonii*, of Anderson, named for John F. Anderson, who first described the organ-

ism). The characteristics of *Brevinema andersonii* are the same as the characteristics described above for the genus. Chemoor-ganotrophic. Microaerophilic and catalase negative. Growth occurs in modified BSK medium at an optimal growth temperature of 30 to 34°C and an optimal pH of 7.4; under these conditions the generation time is 11 to 14 h. Does not grow at 25°C. Exhibits the following enzyme activities: C₄, C₅, C₆, C₈, C₉, and C₁₀ esterases, C₄ esterase lipase, alkaline phosphatase, acid phosphatase, and β -glucuronidase.

Designation of the type strain. The type strain of *Brevinema andersonii* is CT11616 (= ATCC 43811). This strain was isolated from tissues of a short-tailed shrew (*Blarina brevicauda*) captured in West Haven, Conn. (1). The G+C content of this organism is 34 mol%.

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