**Bartonella birtlesii** sp. nov., isolated from small mammals (**Apodemus** spp.)

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Three strains isolated from **Apodemus** spp. were similar to **Bartonella** species on the basis of phenotypic characteristics. Futhermore, genotypic analysis based on sequence analysis of the 16S rRNA and **gltA** genes and on DNA–DNA hybridization showed that the three isolates represented a distinct and new species of **Bartonella**. The name **Bartonella birtlesii** is proposed for the new species. The type strain of **B. birtlesii** sp. nov. is IBS 325T (**= CIP 106294T = CCUG 44360T**).

**Keywords**: **Bartonella birtlesii**, rodents, taxonomy, citrate synthase, 16S rDNA gene

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**INTRODUCTION**

Prior to 1993, **Bartonella bacilliformis** was the only member of the **Bartonella** genus. Now, after only a few years of research and taxonomic study, this genus includes 12 approved species available in the international bacterial collections (Brenner et al., 1993). For two species [**B. bacilliformis** (Weinman, 1968) and **Bartonella quintana** (Vinson & Fuller, 1961)] the human is the natural host, for three others [**Bartonella henselae** (Regnery et al., 1992; Welch et al., 1992), **Bartonella clarridgeiae** (Lawson & Collins, 1996) and **Bartonella koehleri** (Droz et al., 1999)] it is the cat (**Felix domesticus**) and for one other (**Bartonella alpatica**; Heller et al., 1999) it is the rabbit (**Oryctolagus cuniculus**). For five **Bartonella** species, small mammals are the hosts: the rat (**Rattus rattus**) is the host for **Bartonella tribocorum** (Heller et al., 1998), **Microtus pennsylvanicus** is the host for **Bartonella vinsonii** (Baker, 1946), **Apodemus** spp. are the hosts for **Bartonella taylorii** (Birtles et al., 1995), **Clethrionomyys glareolus** is the host for **Bartonella grahamii** (Birtles et al., 1995) and **Microtus agrestis** is the host for **Bartonella doshiae** (Birtles et al., 1995). Furthermore, **B. taylorii**, **B. grahamii** and **B. doshiae** are able to infect numerous species of small woodland mammals and there is no narrow animal host spectrum of infection. The 12th approved species is **Bartonella elizabethae** (Daly et al., 1993), for which only a single isolate, originating from a human case of endocarditis, was described. The 16S rRNA gene sequence of this species is close to that of the rat **Bartonella sp., B. tribocorum** (Heller et al., 1998) and to that of other isolates not yet validly described, but all were isolated from small wild mammals (Birtles & Raoult, 1996; Kosoy et al., 1997). In addition to these 12 species, two others have been described in **Peromyscus** spp. (**Bartonella peromysci** [Birtles et al., 1995; Ristic & Kreier, 1984]) and in the mole (**Bartonella talpae** [Birtles et al., 1995; Ristic & Kreier, 1984]), but type strains for these species are no longer extant. Moreover, **B. vinsonii** (Baker, 1946;
Breitschwerdt et al., 1995), now includes three sub-
species, one of which, B. vinsonii subsp. arupensis, was
isolated from humans and mice (Peromyscus leucopus)
and is therefore presumably considered as patho-
genic for humans (Welch et al., 1999; Hofmeister et al.,
1998).

Thus, several Bartonella species have been isolated
from numerous small mammals. For one species that
is potentially pathogenic for humans (B. elizabethae)
small woodland mammals are suspected as a reservoir;
B. vinsonii subsp. arupensis (Welch et al., 1999),
however, was isolated from mice and humans. B. grahamii
DNA was detected from the anterior chamber fluid of
the eye in an HIV-negative woman who presented with
bilateral eye inflammatory disease. This detection was
performed by using PCR amplification and sequence
analysis of the 16S rRNA gene (Kerkhoff et al., 1999).
Thus, the members of the bacterial genus Bartonella,
particularly species isolated from small woodland
mammals, appear to be a group of emerging human
pathogens. Better knowledge of the bacteria harboured
by wild small mammals is therefore necessary.

The aim of this work was to characterize a new
Bartonella group, members of which were isolated
in France and the UK from the blood of small mammals
belonging to the genus Apodemus.

METHODS

Animals, blood sampling and culture conditions. Small
mammals were caught in February 1997 by a professional
trapper in a wetland conservation area (at point kilometre
PK 275, from the Bodensee in Germany), near the River
Rhine, in Gerstheim, France. Identification of the small
mammals was based on morphology and dentition. Animals
were anaesthetized by diethylether inhalation and then
0.2 ml blood was collected from each animal by intracardiac
puncture. Blood was transferred to a paediatric lysis-
centrifu-
gation tube (Wampole Laboratories). After centrifuga-
tion, the pellet was plated onto Columbia-base agar plates
containing 5% fresh, defibrinated rabbit blood. The plates
were incubated at 35 °C in a moist atmosphere containing
5% CO₂ for 4 weeks.

Bacterial strains. Two isolates obtained from Apodemus spp.
and named IBS 325° and IBS 358 were examined. (We were
unable to identify the Apodemus spp. precisely, because the
animals captured were too young.) In addition, strain N40,
kindly provided by R. Birtles (Bristol, UK) and isolated in
the UK from Apodemus sylvaticus, was included in the study.

Reference strains used for DNA–DNA hybridization. B.
elizabethae ATCC 49927°, B. henselae ATCC 49882° and B.
qinuta ATCC VR-358° were purchased from the American
Type Culture Collection (ATCC; Manassas, VA, USA). B.
aldrichia CIP 105477° and B. tribocorum CIP 105476° were
kindly provided by R. Heller (Strasbourg, France). B.
koehlerae ATCC 700693° was kindly given by B. B. Chomel
(Davis, CA, USA). B. doshiae NCTC 12862°, B. grahamii
NCTC 12860°, B. taylorii NCTC 12861° and B. vinsonii
ATCC VR-152° were kindly provided by R. Birtles (Bristol,
UK). B. claridgeae ATCC 51734° was kindly given by J.
Claridge (Houston, TX, USA).

Electron microscopy. Bacteria were grown on solid medium
and submitted to electron microscopy as described pre-
viously (Fussenegger et al., 1996). Briefly, bacteria were
suspended in PBS (0.145 M NaCl, 0.15 M sodium phos-
phate), spread onto a water surface, absorbed onto Form-
var-coated nickel grids, stained in 1% (w/v) uranyl acetate
and then air-dried. Samples were examined with a JEOL
100CX2 electron microscope.

Biochemical analysis. The following biochemical assays were
performed with diagnostic tablets (Rosco Diagnostica): the
Voges–Proskauer reaction, tributyrine hydrolysis and pyra-
znimidase, proline aminopeptidase and trypsin-like ac-
tivities. Pre-formed bacterial enzyme activity was tested
using the MicroScan Rapid Anaerobe Panel (Dade Inter-
national) according to the manufacturer’s instructions
and as described previously (Welch et al., 1993; Birtles et al.,
1995).

Amplification of 16S rRNA and citrate synthase genes. DNA
was extracted from bacterial suspensions via the Chelex
extraction technique (De Lamballerie, 1992). An approxi-
ately 1500 bp fragment of the 16S rRNA gene was
amplified from the extracted DNA by using the eubacterial
universal primers specific for the 16S rRNA gene, i.e. primer
P8 (5’-AGAGATTTGATCCTGGCTAG-3’) and primer
Pc1544 (5’-AAGAGGAGGTGATCAGCGCA-3’) (Heller et al.,
1998). PCR amplification of the citrate synthase gene
was performed, as described by Birtles & Raoult (1996),
with two primers, i.e. primer CS.140f (5’-TTACTTGATCC-
GGYTTA-3’, where K represents equimolar amounts of G
and T and Y represents equimolar amounts of C and T) and
primer Bhs.1137n (5’-AATGCAAAAAGAACAGTCAA-
ACA-3’). Standard procedures for preventing sample DNA
cross-contamination were undertaken (Kwok & Higuchi,
1989). Each set of reactions also included negative controls
to confirm the absence of cross-contamination between
samples and previously amplified DNA or field samples. The
presence of the desired amplicons was controlled by elec-
rophoresis on a 1:5% agarose gel, followed by ethidium
bromide staining and visualization on a UV transillumin-
ator.

Purification of the amplicons and DNA sequencing. The
amplified fragments were purified via phenol extraction
and 2-propanol precipitation (Brow, 1990). Sequencing of the
complete 16S rRNA gene was performed on the coding and
complementary strands by using the following four primer
couples 5’-labelled with FITC (Eurogentec): P8 and Pc335
(5’-GTATTACCGGGCTGCTGGACC-3’); P.1515 (5’-GGTG-
CAGCACCGGGGTAGAAK-3’) and P.804 (5’-GACTAC-
CAACGGTATCTAAATCCT-3’) and P.1784 (5’-GGATTAGATA-
CCCTGGTATGTC-3’) and P.1998 (5’-ACTTGACGTTAT-
CCCCACCTTCTC-3’) and P.1174 (5’-GAGGAAAGGTTGG-
GAATACGC-3’) and P.1544.

Partial sequencing of the 3’-end of the citrate synthase gene
(glt.A) was performed on coding and complementary strands
by using primer pairs Bhs.1137n and Bhs.781p (5’-
GGGGAGGCAAGTCTATGGTG-3’). All primers were 5’-
labelled with fluorescein isothiocyanate.

The sequencing reaction was performed by using a Thermo
Sequenase Fluorescent Labelled Primer Cycle Sequencing
kit with 7-deaza-dGTP (Amersham), according to the
manufacturer’s instructions. The sequences were obtained
with an ALF DNA Sequencer (Pharmacia Biotech) used
according to the manufacturer’s instructions.

Sequence analysis. The DNA sequences obtained were
compared with those from other Bartonella spp. and other bacteria, contained in the EMBL/GenBank database. The sequences were aligned by using the CLUSTAL method (Higgins & Sharp, 1988) with DNASTAR software.

Nucleotide sequence accession numbers. The EMBL/GenBank accession numbers for the 16S rRNA sequences used for the sequence comparison given are as follows: B. alcatraca, AJ002139; B. bacilliformis, M65249; B. claridgeaei, X8920; B. doshiae, Z31351; B. elizabethae, L01260; B. grahamii, Z31349; B. henselae, AF76237; B. quintana, Fuller, M11927; B. taylorii, Z31350; B. tribocorum, AJ003070; B. vinsonii, L01259; B. vinsonii subsp. berkoffii, L35052; B. vinsonii subsp. arupensis, U71322; B. vinsonii Baker, Z31352; Escherichia coli, Z46753; Afpia felis, M65248, Brucella abortus, AF091354; Agrobacterium tumefaciens, M11223; Rickettsia prowazekii, M21789; Rickettsia rickettsii, M21293; Bartonella strain C7-rat, Z70004; Bartonella strain C5-rat, Z0008; Bartonella strain C4-phy, Z70007; Bartonella strain C1-phy, Z70006; Bartonella strain R-phy2, Z70001; Bartonella strain R-phy1, Z70005; and Bartonella strain N40, Z70002.

The EMBL/GenBank accession numbers for the citrate synthase gene sequences used for sequence comparisons, as given by Birles & Raoult (1996), are as follows: B. bacilliformis LA6.3, Z30021; B. claridgeaei, U84386; B. doshiae, Z70017; B. elizabethae, Z70009; B. grahamii, Z70016; Bartonella henselae, L38987; B. quintana, Fuller, Z70014; B. taylorii, Z70013; B. tribocorum, AJ005494; B. vinsonii, Z70015; B. vinsonii subsp. berkoffii, U28075; Bartonella strain C7-rat, Z70020; Bartonella strain C5-rat, Z70018; Bartonella strain C4-phy, Z70019; Bartonella strain C1-phy, Z70022; Bartonella strain R-phy2, Z70011; Bartonella strain R-phy1, Z70010; and Bartonella strain N40, Z70012.

The EMBL/GenBank accession numbers for the citrate synthase gene sequences of Bartonella spp., as given by Kosoy et al. (1997), are as follows: strain A1, U84372; strain A2, U84373; strain A3, U84374; strain B1, U84375; strain B2, U84376; strain C1, U84377; strain C2, U84378; strain D1, U84379; strain D2, U84380; strain D3, U84381; strain D4, U84382; strain D5, U84383; strain D6, U84384; strain D7, U84385.

The EMBL/GenBank accession number for the citrate synthase gene sequences of B. vinsonii subsp. arupensis (Welch et al., 1999) and B. alcatraca (Heller et al., 1999) are U77057 and AF204273, respectively.

DNA-DNA hybridization. DNA extraction and purification were performed as described previously (Riegel et al., 1994). DNA hybridizations between the labelled DNA and the unlabelled fragmented DNA were performed at 58 °C for 16 h in 0.2 M NaCl by using the nuclease trichloroacetic acid method as described earlier (Grimont et al., 1980; Riegel et al., 1994).

Determination of DNA base composition. The G + C content of DNA was determined by using the capillary electrophoresis method as described previously (Riegel et al., 1995).

RESULTS

Among the 28 Bartonella strains isolated from the 34 small mammals, two (IBS 325T and IBS 358) were isolated from two Apodemus spp. and had exactly the same 16S rRNA gene and gltA sequences (Table 1). These sequences were identical to those of strain N40, which was also isolated from an Apodemus species. The 16S rRNA gene sequences of 26 other strains isolated from the other wild mammals were different and so these strains were not included in this study.

Phenotypic characteristics

The three strains produced homogeneous, rough, round, grey–white colonies, 0.6–1.0 mm in diameter, that were slightly adherent to the surface of the culture medium. Bacterial suspension in PBS was not homogeneous because of bacterial aggregation. Gram-staining revealed short, slender, straight or slightly curved Gram-negative bacilli. Electron microscopy examination with negative staining showed small bacilli that lacked flagella. They were catalase and oxidase-negative, gave a negative Voges–Proskauer reaction, had no urease activity and did not produce acid from trehalose. They hydrolysed bis-p-nitrophenyl phosphate but not N-acetyl β-D-glucosaminide. They had amino acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. The strains had trypsin-like activity and glycyglycylarylaminidase activity but no pyrolidonylarylaminidase activity. These data were identical for the three strains tested; only the results obtained with strain IBS 325T are given in Table 2.

16S rRNA gene sequence analysis

The length of the 16S rRNA gene sequence was 1394 bp (all located within the 16S rRNA gene). We sequenced the amplicon obtained with the eubacterial universal primers P8 and Pc1544.

Strains IBS 325T, IBS 358 and Bartonella isolate N40 had an identical 16S rRNA gene sequence, which differed from that of the 12 other Bartonella species contained in the EMBL/GenBank database. The percentage similarities are presented in Table 1.

The species with the closest 16S rRNA gene sequence was B. taylorii (99.3% similarity). The most distant 16S rRNA gene sequences within the genus Bartonella were those of B. claridgeaei and B. bacilliformis (97.0 and 97.2% similarity, respectively).

When bacteriologically that Bartonella were studied, the 16S rRNA gene sequence of strain IBS 325T was found to be 93.8% similar to that of B. abortus and 91.4% similar to that of A. tumefaciens.

Citrate synthase gene sequence analysis

The partial sequence of 320 bp in the 3′-end of the citrate synthase gene was identical for the three strains, but differed from the corresponding sequences of all
Table 1. DNA–DNA hybridization rate and 16S rRNA gene and gltA similarity between the *Bartonella* species and the strain IBS 325<sup>T</sup>

<table>
<thead>
<tr>
<th><em>Bartonella</em> strain</th>
<th>Mean hybridization (%) with strain IBS 325&lt;sup&gt;T&lt;/sup&gt; of (trial 1 and trial 2)*</th>
<th>Hybridization with strain IBS 325&lt;sup&gt;T&lt;/sup&gt; (%)</th>
<th>Similarity (%)</th>
<th>Actual base differences (16S rRNA gene)</th>
<th>G + C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain IBS 325&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(100/100)</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Strain IBS 358</td>
<td>(92/96)</td>
<td>94</td>
<td>100</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Strain N40</td>
<td>(94/95)</td>
<td>95</td>
<td>100</td>
<td>0</td>
<td>38†</td>
</tr>
<tr>
<td><em>B. alsatica</em> CIP 105477&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(28/27)</td>
<td>27</td>
<td>86.5</td>
<td>97.2</td>
<td>25</td>
</tr>
<tr>
<td><em>B. bacilliformis</em> ATCC 35685&lt;sup&gt;T&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>85.6</td>
<td>97.0</td>
<td>—</td>
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<tr>
<td><em>B. claridgeiae</em> ATCC 51734&lt;sup&gt;T&lt;/sup&gt;</td>
<td>—</td>
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<tr>
<td><em>B. doshiiae</em> NCTC 12862&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(27/32)</td>
<td>28</td>
<td>88.4</td>
<td>98.5</td>
<td>11</td>
</tr>
<tr>
<td><em>B. elizabethae</em> ATCC 49927&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(17/21)</td>
<td>18</td>
<td>87.7</td>
<td>97.9</td>
<td>41§</td>
</tr>
<tr>
<td><em>B. grahamii</em> NCTC 12860&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(22/24)</td>
<td>22</td>
<td>89.6</td>
<td>98.8</td>
<td>6</td>
</tr>
<tr>
<td><em>B. henselae</em> ATCC 49882&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(21/20)</td>
<td>21</td>
<td>90.6</td>
<td>97.5</td>
<td>15</td>
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<tr>
<td><em>B. koehlerae</em> ATCC 700693&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(24/27)</td>
<td>25</td>
<td>90.9</td>
<td>98.3</td>
<td>10</td>
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<tr>
<td><em>B. quintana</em> ATCC VR-358&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(28/31)</td>
<td>29</td>
<td>87.4</td>
<td>98.1</td>
<td>14</td>
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<tr>
<td><em>B. taylorii</em> NCTC 12861&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(23/27)</td>
<td>25</td>
<td>90.6</td>
<td>99.3</td>
<td>3</td>
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<tr>
<td><em>B. tribocorum</em> CIP 105476&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(22/23)</td>
<td>23</td>
<td>90.6</td>
<td>98.5</td>
<td>8</td>
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<tr>
<td><em>B. vinsonii</em> subsp. vinsonii ATCC VR-152&lt;sup&gt;T&lt;/sup&gt;</td>
<td>(27/33)</td>
<td>30</td>
<td>90.9</td>
<td>98.8</td>
<td>6</td>
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<tr>
<td><em>B. vinsonii</em> subsp. arupensis ATCC 700727&lt;sup&gt;T&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>90.8</td>
<td>98.8</td>
<td>6</td>
</tr>
<tr>
<td>Strain C1-phy</td>
<td>—</td>
<td>—</td>
<td>87.1</td>
<td>99.0</td>
<td>5</td>
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<tr>
<td>Strain C4-phy</td>
<td>—</td>
<td>—</td>
<td>87.7</td>
<td>98.8</td>
<td>6</td>
</tr>
<tr>
<td>Strain R-phy1</td>
<td>—</td>
<td>—</td>
<td>88.4</td>
<td>99.1</td>
<td>4</td>
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<tr>
<td>Strain R-phy2</td>
<td>—</td>
<td>—</td>
<td>87.7</td>
<td>99.0</td>
<td>5</td>
</tr>
<tr>
<td>Strain A1</td>
<td>—</td>
<td>—</td>
<td>89.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Strain A2</td>
<td>—</td>
<td>—</td>
<td>88.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Strain A3</td>
<td>—</td>
<td>—</td>
<td>88.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Strain B1</td>
<td>—</td>
<td>—</td>
<td>91.2</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Strain B2</td>
<td>—</td>
<td>—</td>
<td>91.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Strain D7</td>
<td>—</td>
<td>—</td>
<td>90.6</td>
<td>—</td>
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</tr>
</tbody>
</table>

* Mean of data from trial 1/trial 2.
† Birtles & Raoult (1996).
‡ Heller et al. (1999).
§ Birtles et al. (1995).
‖ Daly et al. (1993).
¶ Heller et al. (1998).
* Welch et al. (1999).

The closest gltA sequences were those of *B. alsatica* (92.5% similarity) isolated from wild rabbits (Heller et al., 1999) and *Bartonella* strain B2 (91.8%) isolated from a small rodent (Kosoy et al., 1997). All of the results are presented in Table 1. All other 3’-end citrate synthase gene sequences available in the EMBL/GenBank database shared less than 91.2% similarity.

**DNA–DNA hybridizations**

DNA of the strain IBS 325<sup>T</sup> was labelled and hybridized with unlabelled DNA of isolate N40, isolate IBS 358, isolate 325 and of the type strains of *B. alsatica,*
Bartonella birtlesii sp. nov.

Table 2. Differential phenotypic characteristics of the isolate IBS 325T when compared with those of type strains of the Bartonella species tested

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Taxa:</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Oxidase</td>
<td></td>
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<tr>
<td>Catalase</td>
<td></td>
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<tr>
<td>Haemolysis</td>
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<td>Motility</td>
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<td>Urea</td>
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<td>Voges–Proskauer</td>
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<td>Glycglycine</td>
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<td>Lysine (alkaline)</td>
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<td>Lysine (acidic)</td>
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<td>Methionine</td>
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<tr>
<td>Proline</td>
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<td>Tryptophan</td>
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</table>

B. doshiae, B. elizabethae, B. grahamii, B. henselae, B. koehlerae, B. quintana, B. taylorii, B. tribocorum and B. vinsoni. IBS 325T showed less than 30% relatedness with all type strains of Bartonella species tested, but showed more than 94% relatedness with DNA from IBS 358 and DNA from N40.

DISCUSSION

Strains IBS 325T, IBS 358 and Bartonella isolate N40 exhibited phenotypic characteristics similar to those of other Bartonella species: they grew slowly (10 d for primary isolation and 6 d for subcultures), required CO₂ and produced small colonies on Columbia–blood agar plates. Furthermore, they were negative for oxidase, catalase and the Voges–Proskauer reaction. They did not hydrolyse trehalose. They had amino acid arylamidase activity with arginine, lysine, glycine, leucine, methionine, proline and tryptophan and had a trypsin-like activity.

Additional data were obtained from genetic analysis. The three isolates had exactly the same sequence for the 16S rRNA gene. All of them belonged to the Bartonella genus, since this sequence showed 97.0–99.3% similarity to those of other Bartonella species; this is within the range of similarity found between the type strains of other Bartonella species (97.5–99.3%; Birtles & Raoult, 1996). The closest 16S rRNA gene sequences were those of B. taylorii, B. vinsonii and B. vinsonii subsp. arupensis and those of strains C1-phy, C4-phy, R1-phy and R2-phy (Birtles & Raoult, 1996), all of which had been isolated from small woodland mammals (Fig. 1).

Furthermore, the three isolates IBS 325T, IBS 358 and N40 showed a unique 3'-end citrate synthase sequence. The level of sequence similarity between IBS 325T and the previously described Bartonella species was in the range 84.5–92.5%. This range was similar to those previously described for the type strains of Bartonella species [83.8–93.2% (Birtles & Raoult, 1996) and 88.7–96.4% (Kosoy et al., 1997)]. These data, together with the 16S rRNA gene sequence (Table 1), the culture characteristics (colonial morphology, duration of bacterial growth, microscopic appearance of the bacteria after Gram-staining) and the isolation of the bacteria from the blood of Apodemus spp., all support the classification of these three isolates within the genus Bartonella.

On the basis of DNA–DNA hybridization, the three isolates IBS 325T, IBS 358 and N40 belong to the same species, since they showed more than 94.0% hybridization to each other. Furthermore, the DNA of strains N40 and IBS 325T showed 95 and 97% hybridization, respectively, with the labelled DNA of strain IBS 358 (data not shown). Conversely, the DNA of strains IBS 358 and N40 showed 94 and 95% relatedness, respectively, with the labelled DNA of strain IBS 325T. Moreover, all of the type strains of Bartonella species tested showed DNA relatedness to strain IBS 325T of less than 30% (Table 1). These data indicated that the three isolates belong to a single Bartonella species distinct from all those previously described, according
to previously established criteria (Stackebrandt & Goebel, 1994).

In spite of the fact that the three isolates of the new Bartonella species were isolated from wild Apodemus spp., this animal genus should not be regarded as a specific host, since we used IBS 325<sup>T</sup> to inoculate outbred and inbred laboratory mice and found that they developed long-term bacteraemia (unpublished data). The ability of this new species to infect humans should be assessed, since other rodent-associated Bartonella spp. associated with human diseases have been described, namely B. grahamii (Kerkhoff et al., 1999) and B. vinsonii subsp. arupensis (Hofmeister et al., 1998; Welch et al., 1999).

To detect possible cases of human infection by Bartonella birtlesii, serological and genetic tools specific for B. birtlesii should be established.

**Description of Bartonella birtlesii sp. nov.**

Bartonella birtlesii (birt.les‘i.i. M.L. gen. n. birtlesii of Richard J. Birtles, whose studies have contributed to an improved understanding of the taxonomy of the Bartonella genus).

Colonies grown on blood agar appeared homogeneous, rough, round and grey–white. Electron microscopic examination showed small bacilli without flagella. Primary culture of strain B. birtlesii was obtained after 10 d incubation at 35 °C in a moist atmosphere containing 5% CO<sub>2</sub>. Subculture on agar was obtained after 6 d culture in the same conditions. The three strains tested were all oxidase-negative and catalase-negative, had negative Voges–Proskauer reactions, showed no urease activity and were unable to hydrolyse trehalose or N-acetylβ-D-glucosaminide. They all hydrolysed bis-p-nitrophenyl phosphate and had amino acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. The new species showed a trypsin-like activity and a glycylglycylarylamidase activity but no pyrrolidonyl-arylamidase activity. The new species was distinguished from the other Bartonella species by its 16S rRNA gene sequence and by whole-DNA hybridization. The type strain is IBS 325<sup>T</sup> (isolated from the blood of an Apodemus sp.) and is deposited in the Collection de l’Institut Pasteur, Paris, France (CIP 106294<sup>T</sup> = CCUG 44360<sup>T</sup>).

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