Bartonella chomelii sp. nov., isolated from French domestic cattle (*Bos taurus*)

Renaud Maillard,^{1,3} Philippe Riegel,² Francine Barrat,¹ Corinne Bouillin,¹ Danielle Thibault,¹ Christelle Gandoin,¹ Lnaig Halos,¹ Christine Demanche,¹ Annie Alliot,¹ Jacques Guillot,¹ Yves Pimont,² Henri-Jean Boulouis¹ and Muriel Vayssier-Taussat¹

Correspondence

Henri-Jean Boulouis
hjboulouis@vet-alfort.fr

¹UMR 956 INRA/AFSSA/ENVA/UPVM, Microbiologie, Ecole Nationale Vtrinaire, 7 Avenue du Gnral de Gaulle, 94 704 Maisons-Alfort, France

²Institut de Bactriologie de la Facult de Mdecine, Universit Louis Pasteur, Hpitaux Universitaires de Strasbourg, 3 rue Koeberl, 67 000 Strasbourg, France

³Unit de Pathologie du Btail, Ecole Nationale Vtrinaire, 7 Avenue du Gnral de Gaulle, 94 704 Maisons-Alfort, France

Two strains of bacteria isolated from the blood of French domestic cows were found to be similar to *Bartonella* species on the basis of phenotypic characteristics. Genotypic analysis based on sequence comparison of the 16S rRNA and citrate synthase (*gltA*) genes and on DNA–DNA hybridization showed that the two isolates represent a distinct and new species of *Bartonella*. Moreover, the phylogenetic analysis inferred from comparison of 16S rRNA and *gltA* sequences demonstrated that the new *Bartonella* species is related to other ruminant-derived *Bartonella* species. The name *Bartonella chomelii* is proposed for the new species. The type strain of *Bartonella chomelii* sp. nov. is A828^T (= CIP 107869^T = CCUG47497^T).

INTRODUCTION

In recent years, the genus *Bartonella* has undergone a rapid expansion, from the initially described *Bartonella bacilliformis* to 18 species that are currently recognized (Bermond *et al.*, 2002). *Bartonella* species are small Gram-negative bacteria, often piliated or flagellated. They are intracellular parasites of red blood cells and endothelial cells. In reservoir hosts, *Bartonella* infections usually result in asymptomatic bacteraemia. This bacteraemic phase is required for the long-term perpetuation of the bacterium, allowing its transmission by blood-sucking arthropods (Breitschwerdt & Kordick, 2000; Chomel *et al.*, 1996; Alexander, 1995). Some species of *Bartonella* are able to infect not only reservoir hosts, but also accidental hosts for which the bacterium is not adapted or poorly adapted. In this case, infections are associated with human or animal diseases including cat scratch disease (CSD), bacillary angiomatosis,

peliosis hepatitis and endocarditis, in either immunocompetent or immunocompromised patients (Breitschwerdt & Kordick, 2000).

To date, molecular characterization of *Bartonella* species has included PCR amplification of protein-coding genes (Houpikian & Raoult, 2001a), as well as the 16S rRNA gene (Birtles, 1995) and the 16S–23S rRNA intergenic spacer region (ITS) (Jensen *et al.*, 2000). The degree of identity of these genes among *Bartonella* species has then been assessed both indirectly, by using RFLP analysis (Birtles, 1995; Joblet *et al.*, 1995; Norman *et al.*, 1995; Houpikian & Raoult, 2001b), and directly by base sequence comparisons. While 16S rDNA has been shown to be a powerful tool for appreciating genetic variability among *Bartonella* species, the protein-encoding genes appear to be good indicators of interspecies divergence and RFLP-PCR on the *gltA* gene is often used to identify *Bartonella* species in biological samples (La Scola *et al.*, 2003).

Recently, three *Bartonella* species have been isolated from ruminants. Two of these (*Bartonella schoenbuchensis* and *Bartonella capreoli*) were recovered from wild roe deer (*Capreolus capreolus*) (Dehio *et al.*, 2001; Bermond *et al.*, 2002), and a third one (*Bartonella bovis*) from domestic cattle (Bermond *et al.*, 2002). All of these three species appear to be adapted to ruminants. In view of the potential zoonotic pathogenicity of *Bartonella* and the high exposure

Published online ahead of print on 1 August 2003 as DOI 10.1099/ijs.0.02770-0.

The GenBank accession numbers for the 16S rRNA and 3' end *gltA* sequences of *Bartonella chomelii* are AY254309 and AY254308, respectively.

A tree showing the phylogenetic relationships of *Bartonella* species inferred from 16S rRNA sequences, and a table of DNA–DNA hybridization data, and 16S rRNA and *gltA* gene similarity are available in IJSEM Online.

of humans to ruminants through consumption of bovine-derived products or hunting practices, the risk of zoonotic transmission of ruminant-derived *Bartonella* to humans should not be underestimated.

METHODS

Animals, blood samples and culture conditions. During routine surveys of *Bartonella* bacteraemia in two cattle herds in two regions in France (Loire-Atlantique and Nord departments), blood samples (4 ml) were collected from cows over a 3 year period (2000–2002). Blood samples were collected into EDTA plastic tubes (Greiner Vacuette). All tubes were frozen and stored at -80°C until tested. After thawing, the blood samples were centrifuged at 1800 g for 75 min and then plated onto agar plates containing 5% fresh defibrinated rabbit blood. Plates were incubated at 35°C in a moist 5% CO_2 atmosphere for 6 weeks.

Bacterial strains. One hundred and fifty bacterial strains isolated from 75 cows were identified using PCR amplification followed by analysis of the RFLP-profile of the *gltA* gene (citrate synthase) using *TaqI*, *MseI*, *HhaI* and *DdeI* restriction enzymes as described previously (Birtles & Raoult, 1996; Norman *et al.*, 1995), according to the manufacturer's instructions (New England Biolabs).

Electron microscopy. Bacteria were grown on Columbia base agar (bioMérieux) plates containing 5% fresh defibrinated rabbit blood for 5 days. Bacteria were then suspended in PBS, spread on a water surface and absorbed to Formvar-coated nickel grids, stained with 1% (w/v) uranyl acetate and air-dried (Fussenecker *et al.*, 1996). Samples were examined with a JEOL 100CX2 electron microscope.

Biochemical analysis. *Bartonella* species are biochemically inert in almost all conventional tests. Biochemical assays performed with diagnostic tablets (Rosco; Eurobio) were as follows: Voges–Proskauer reaction, tributyrin hydrolysis, pyrazinamidase, proline aminopeptidase and trypsin-like activity. Commercially available reagents (Rapid ID 32 A; bioMérieux) were used to test the activity of bacterial enzymes according to the manufacturer's instructions.

Reference strains used for DNA–DNA hybridization. *Bartonella grahamii* (V2^T, ATCC 49927^T), *Bartonella clarridgeiae* (Houston-2^T, ATCC 51734^T), *Bartonella elizabethae* (F9251^T, ATCC 49927^T), *Bartonella henselae* (Houston-1 G5436^T, ATCC 49882^T), *Bartonella koehlerae* (C29^T, ATCC 700693^T), *Bartonella quintana* (Fuller^T, ATCC VR-358^T), *Bartonella doshiae* (R18^T, ATCC 700133^T), *Bartonella vinsonii* subsp. *vinsonii* (Baker^T, ATCC VR-152^T), *Bartonella vinsonii* subsp. *berkhoffii* (ATCC 51672^T) and *Bartonella bacilliformis* (ATCC 35685^T) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). *Bartonella taylorii* (M6^T, CIP 107028^T) was purchased from the Collection of the Pasteur Institute (CIP, Paris, France). *Bartonella alsatica* (IBS 382^T, CIP 105477^T), *Bartonella birtlesii* (IBS 325^T, CIP 106294^T), *Bartonella bovis* (91-4^T, CIP 106692^T), *Bartonella capreoli* (IBS 193^T, CIP 106691^T), *Bartonella schoenbuchensis* (R1^T, NCTC 13165^T) and *Bartonella tribocorum* (IBS 506^T, CIP 105476^T) were kindly given by one of the authors.

DNA–DNA hybridization. DNA extraction and purification were performed as described by Riegel *et al.* (1994). DNA hybridization between labelled DNA and unlabelled fragmented DNAs was performed at 58°C for 16 h in 0.42 M NaCl using the nuclease/trichloroacetic method (Grimont *et al.*, 1980; Riegel *et al.*, 1994). When the percentage hybridization was above 30%, experiments were performed in triplicate. Therefore, the given value represents the mean of triplicate values $\pm$ standard deviation.

Amplification and sequencing of the 16S rRNA and citrate synthase genes. A fragment of approximately 1500 bp of the

16S rRNA gene was amplified from the extracted DNA by using eubacterial universal primers specific for 16S rRNA gene: P8 (5'-AGAGTTTGTATCTGGCTCAG-3') and Pc1544 (5'-AAGGAGGT-GATCCAGCCGCA-3') (Heller *et al.*, 1997). PCR amplification of a 350 bp fragment of the citrate synthase gene was performed as described by Norman *et al.* (1995) with primers BhCS.781p (5'-GGGGACCAGCTCATGGTGG-3') and BhCS.1137 (5'-AATG-CAAAAAGAACAGTAAACA-3') (Norman *et al.*, 1995). The presence and size of the amplicons were verified by electrophoresis on a 1.5% agarose gel, followed by ethidium bromide staining and visualization on a UV transilluminator.

Amplified fragments were sent to Genome express (Meylan, France) for sequencing. Sequencing of the almost complete 16S rRNA gene was performed bidirectionally, using three primer pairs: P8 and PC535 (5'-GTATTACCGCGGCTGCTGGCA-3'), P350F (5'-CTCC-TACGGGAGGCAGCAGT-3') and P785R (5'-GGGTATCTAATCC-3'), and P353F (5'-TGCCAGCAGCCGCGTAATAG-3') and Pc1544.

Sequence alignment and tree construction. The 16S rRNA and *gltA* sequences obtained in this study were aligned with sequences from other *Bartonella* species first with CLUSTAL X software (version 1.63b, December 1997; Thompson *et al.*, 1997) and then by visual optimization. The alignments are available upon request. Nucleic acid sequences of *gltA* were then translated into amino acid sequences. The aligned sequences were converted to a distance matrix (percentage differences without weighting). A distance tree was generated using the neighbour-joining method (Saitou & Nei, 1987). Both the branch-and-bound and heuristic search options in the PAUP program version 4.0 (Swofford, 1993) were used for comparison of sequence alignments and generation of parsimonious trees. The statistical support of the internal branches from the resulting trees was statistically tested by bootstrap analysis (Felsenstein, 1985) from 500 bootstrap replications. The list of the *Bartonella* species used for the phylogenetic analysis is indicated in Fig. 2 and the 16S rDNA tree is available as supplementary data in IJSEM Online. The 16S rRNA and the *gltA* sequences of *Rickettsia rickettsii* were used as outgroups.

Nucleotide sequence accession numbers. The strain designation and GenBank accession numbers for the sequences derived from 16S rRNA and *gltA* gene analysis are shown in Fig. 2 and the 16S rDNA tree available as supplementary data in IJSEM Online.

RESULTS AND DISCUSSION

Isolation of a new RFLP-type bovine *Bartonella*

The *gltA* gene was amplified from 150 isolates derived from 75 bovine RFLP profiles and was analysed and compared with other known *Bartonella* species, in particular with those derived from ruminants, i.e. *Bartonella capreoli*, *Bartonella bovis* and *Bartonella schoenbuchensis*. Two distinct profiles were obtained, one corresponding to *Bartonella bovis* (Bermond *et al.*, 2002) and characterized by the presence of restriction sites for *TaqI* and *MseI*, but not *HhaI* and *DdeI*, and a second RFLP profile different from that of other known *Bartonella* species and characterized by the presence of restriction sites for *MseI*, but not *TaqI*, *HhaI* and *DdeI* (Table 1).

Among the 150 isolates, 148 presented an RFLP profile identical to that of *Bartonella bovis*, while two presented a new RFLP profile.

Table 1. PCR/RFLP profiles of the citrate synthase gene of isolates from French cattle with *TaqI*, *MseI*, *DdeI* and *HhaI* compared to known *Bartonella* species

–, Undigested.

Isolate/ <i>Bartonella</i> species	<i>TaqI</i>	<i>MseI</i>	<i>DdeI</i>	<i>HhaI</i>
Isolates corresponding to <i>Bartonella bovis</i>	250 + 100	220 + 150	–	–
Isolates corresponding to new profile				
A828 ^T	–	180 + < 100	–	–
A12.90	–	180 + < 100	–	–
<i>Bartonella alsatica</i>	250 + 100	150 + < 100	–	–
<i>Bartonella schoenbuchensis</i>	–	180	250	–
<i>Bartonella capreoli</i>	–	180 + 120	250 + 100	–
<i>Bartonella clarridgeiae</i>	–	250 + 150	250 + 100	250 + 100
<i>Bartonella henselae</i>	220 + 150	250 + 150	–	200 + 150
<i>Bartonella tribocorum</i>	–	150	–	250 + 120
<i>Bartonella vinsonii</i> subsp. <i>berkhoffii</i>	220 + 150	220 + < 100	–	–

The high prevalence of *Bartonella bovis* bacteraemia in domestic cattle in the absence of any apparent pathological effect strongly suggests that domestic cows are a natural reservoir of *Bartonella bovis* (Chang *et al.*, 2001). In contrast, the low prevalence of this new *Bartonella* species in cattle suggests that this animal species might not be its natural reservoir, but rather an accidental host. If this hypothesis proves to be true, the potential for pathogenicity of A828^T in cows would warrant further investigation. Therefore, these two strains (A828^T and A12.90) sharing the same new RFLP profile and originating from two different herds were selected for further species characterization.

Phenotypic characterization

The A828^T and A12.90 isolates displayed similar morphological characteristics. They both grew slowly (10 days for primary isolation), required CO₂ and produced smooth colonies, 2–3 mm in diameter with straight and slender Gram-negative bacilli. Both of them were able to grow on rabbit blood and sheep blood agar plates, but with a higher efficiency on rabbit blood agar plates. Like other *Bartonella* species, they were oxidase- and catalase-negative, had a negative Voges–Proskauer reaction and a positive reaction for tributyrin. Results of amino acid arylamidase activity, using the rapid ID 32A system, are given in the species description below.

Electron microscopic examination of a subculture of isolate A828^T revealed bacilli with multiple unipolar flagella (Fig. 1).

16S rRNA and citrate synthase gene sequence analysis

The A828^T and A12.90 isolates had an identical 16S rRNA gene sequence. This sequence was most similar to 16S rRNA gene sequences from *Bartonella* species, but was different from that of all other *Bartonella* species or isolates available in the EMBL/GenBank database (a table is available as

supplementary data in IJSEM Online). The 16S rRNA gene of A828^T was most closely related to three ruminant-derived species, i.e. *Bartonella schoenbuchensis* (99.6%), *Bartonella capreoli* (99.0%) and *Bartonella bovis* (98.9%). The 16S rRNA sequence of A828^T displayed approximately 97% identity with the 16S rRNA gene from other *Bartonella* species.

For *gltA* sequences, A828^T and A12.90 had identical 3' end sequences, which differed from those of the 18 other type strains of *Bartonella* and the other unidentified *Bartonella* isolates contained in the EMBL/GenBank database.

As for the 16S rRNA gene, the 3'-end sequence of *gltA* of A828^T was closest to species isolated from ruminants, i.e. *Bartonella schoenbuchensis* (96.2%), *Bartonella capreoli* (94.8%) and *Bartonella bovis* (92.9%). The most distant *gltA* gene sequence was that of *Bartonella bacilliformis* (82.0%). These data, together with the culture characteristics (colonial morphology, duration of bacterial growth,

Fig. 1. Electron micrograph of *Bartonella chomelii*. Bar, 0.2 µM.

Fig. 2. Phylogenetic relationships of *Bartonella* species inferred from citrate synthase sequences. The presented phylogram resulted from bootstrapped datasets (Felsenstein, 1985) using parsimony analysis (heuristic search option in PAUP 4.0; Swofford, 1993). This tree was identical to the tree generated from the branch-and-bound algorithm in PAUP 4.0. The percentages above the branches are the frequencies with which a given branch appeared in 500 bootstrap replications. Bootstrap values below 60% are not displayed. Branch length corresponds to mean amino acid changes assigned to each branch by PAUP 4.0. The sequence from *Rickettsia rickettsii* was chosen as outgroup.

microscopic appearance of the bacteria) and the recovery of the bacteria from the blood of cows, all supported the classification of these two isolates within the genus *Bartonella*.

To investigate whether they defined a new *Bartonella* species, DNA–DNA hybridization was performed.

DNA–DNA hybridization

DNA of isolate A828^T was labelled and hybridized against unlabelled DNA of A12.90 and 16 type/reference strains. The extent of hybridization between A828^T DNA with heterologous DNA was 78 % with isolate A12.90, 56.5 % with *Bartonella schoenbuchensis*, 52 % with *Bartonella capreoli*, 31.5 % with *Bartonella bovis* and less than 20 % with other *Bartonella* species. Considering that when DNA–DNA hybridization exceeds 70 % two isolates belong to the same species, a novel species was identified (Grimont *et al.*, 1980). A828^T and A12.90 were shown to belong to the same species since their degree of hybridization exceeded 70 %. Isolate A828^T, though phylogenetically close to other *Bartonella* ruminant-derived species, was clearly different from them. Among the ruminant-derived *Bartonella* species, the new species appeared to be more closely related to species recovered from wild ruminants (*Bartonella capreoli* and *Bartonella schoenbuchensis*) than to *Bartonella bovis*.

Sequence alignment and tree construction

The comparison of aligned 16S rRNA sequences was carried out on 1318 positions, including gaps, for a total of 20 taxa. Among those positions, 1090 were constant characters and only 38 were parsimony-informative characters. The phylogenetic analysis inferred from the 16S rRNA sequence comparison clearly demonstrated that A828^T clustered with other ruminant-derived *Bartonella* species, *Bartonella capreoli*, *Bartonella schoenbuchensis* and *Bartonella bovis* (Bermond *et al.*, 2002) (available as supplementary data in IJSEM Online). Phylogenetic analyses, neighbour-joining, and heuristic and branch-and-bound searches produced topologically similar trees, although statistical support for the branches varied (data not shown). All analyses distinguished four monophyletic groups; the first comprised A828^T along with the three already described *Bartonella* species from ruminants, the second contained *Bartonella taylorii*, *Bartonella birtlesii*, *Bartonella doshiae* and *Bartonella vinsonii* subsp. *vinsonii*, the third was composed of *Bartonella koehlerae* and *Bartonella henselae* and the fourth contained *Bartonella tribocorum* and *Bartonella elizabethae*. These groups were clearly separated with branches supported by 79, 59, 79 and 82 % bootstrap values, respectively. The comparison of aligned citrate synthase sequences was carried out on 289 characters. Among these, 142 were constant and 81 were parsimony-informative characters. The phylogenetic analyses inferred from *gltA* amino acid sequences were in accordance with those inferred from 16S rRNA sequences (Fig. 2). The new

species clustered with *Bartonella capreoli*, *Bartonella bovis* and *Bartonella schoenbuchensis* in a group supported by a 96 % bootstrap value. Additional groupings could be distinguished and the species *Bartonella bacilliformis* was in a paraphyletic position.

Description of *Bartonella chomelii* sp. nov.

Bartonella chomelii (cho.mel'i.i. N.L. gen. n. *chomelii* in honour of Bruno B. Chomel, who was the first to experimentally demonstrate the transmission of *Bartonella henselae* by fleas in cats, and to isolate *Bartonella bovis* from domestic cattle. His studies on *Bartonella* infection in animals contributed to an improved understanding of epidemiology and vectors of *Bartonella*-associated disease in animals.)

Colonies, grown on rabbit blood agar, appeared homogeneous, smooth, round and grey–white. Electron microscopic examination revealed small bacilli with unipolar flagellae. A primary culture strain of *Bartonella chomelii* was obtained after 10 days incubation at 35 °C in a moist atmosphere containing 5 % CO₂. A subculture on blood agar was obtained after 6 days culture under the same conditions. The two strains tested were oxidase- and catalase-negative, had negative Voges–Proskauer reactions and had a positive reaction to tributyrin. Amino acid arylamidase activity was obtained with the following amino acids: arginine (ArgA), proline (ProA), phenylalanine (PheA), leucine (LeuA), tyrosine (TyrA), alanine (AlaA) and glycine (GlyA). The new species was distinguished from other *Bartonella* species by its 16S rRNA gene and *gltA* gene 3' end sequences as well as by whole-DNA hybridization. The type strain, A828^T (isolated from the blood of *Bos taurus*), has been deposited in the CIP, Paris, France (CIP 107869^T) and in the Culture Collection of the University of Göteborg, Sweden (CCUG 47497).

ACKNOWLEDGEMENTS

We are grateful to Dr Jennifer Richardson (UMR 1161, INRA, Ecole Nationale Vétérinaire d'Alfort, France) for kindly reviewing the manuscript.

REFERENCES

- Alexander, B. (1995). A review of bartonellosis in Ecuador and Colombia. *Am J Trop Med Hyg* **52**, 354–359.
- Bermond, D., Boulouis, H. J., Heller, R., Van Laere, G., Monteil, H., Chomel, B. B., Sander, A., Dehio, C. & Piemont, Y. (2002). *Bartonella bovis* Bermond *et al.* sp. nov. and *Bartonella capreoli* sp. nov., isolated from European ruminants. *Int J Syst Evol Microbiol* **52**, 383–390.
- Birtles, R. J. (1995). Differentiation of *Bartonella* species using restriction endonuclease analysis of PCR-amplified 16S rRNA genes. *FEMS Microbiol Lett* **129**, 261–265.
- Birtles, R. J. & Raoult, D. (1996). Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *Int J Syst Bacteriol* **46**, 891–897.

- Breitschwerdt, E. B. & Kordick, D. L. (2000). *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity and zoonotic potential for human infection. *Clin Microbiol Rev* **13**, 428–438.
- Chang, C. C., Chomel, B. B., Kasten, R. W., Romano, V. & Tietze, N. (2001). Molecular evidence of *Bartonella* spp. in questing adult *Ixodes pacificus* ticks in California. *J Clin Microbiol* **39**, 1221–1226.
- Chang, C. C., Hayashidani, H., Pusterla, N., Kasten, R. W., Madigan, J. E. & Chomel, B. B. (2002). Investigation of *Bartonella* infection in ixodid ticks from California. *Comp Immunol Microbiol Infect Dis* **25**, 229–236.
- Chomel, B. B., Kasten, R. W., Floyd-Hawkins, K. & 7 other authors (1996). Experimental transmission of *Bartonella henselae* by the cat flea. *J Clin Microbiol* **34**, 1952–1956.
- Dehio, C., Lanz, C., Pohl, R., Behrens, P., Bermond, D., Piemont, Y., Pelz, K. & Sander, A. (2001). *Bartonella schoenbuchii* sp. nov., isolated from the blood of wild roe deer (2001). *Int J Syst Evol Microbiol* **51**, 1557–1565.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fussenecker, M., Kahrs, A. F., Facius, D. & Meyer, T. F. (1996). Tetrapac (*tcp*), a novel genotype of *Neisseria gonorrhoeae* affecting epithelial cell invasion, natural transformation competence and cell separation. *Mol Microbiol* **19**, 1357–1372.
- Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynault, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. *Curr Microbiol* **4**, 325–330.
- Heller, R., Artois, M., Xemar, V., De Briel, D., Gehin, H., Jaulhac, B., Monteil, H. & Piemont, Y. (1997). Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in stray cats. *J Clin Microbiol* **35**, 1327–1331.
- Houpikian, P. & Raoult, D. (2001a). 16S/23S rRNA intergenic spacer regions for phylogenetic analysis, identification, and subtyping of *Bartonella* species. *J Clin Microbiol* **39**, 2768–2778.
- Houpikian, P. & Raoult, D. (2001b). Molecular phylogeny of the genus *Bartonella*: what is the current knowledge? *FEMS Microbiol Lett* **12**, 2001–2007.
- Jensen, W. A., Majilindez, Z. F., Rooney, J., Kordick, D. L. & Breitschwerdt, E. B. (2000). Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. *J Clin Microbiol* **38**, 1717–1722.
- Joblet, C., Roux, V., Drancourt, M., Gouvernet, J. & Raoult, D. (1995). Identification of *Bartonella* (*Rochalimaea*) species among fastidious Gram-negative bacteria on the basis of the partial sequence of the citrate-synthase gene. *J Clin Microbiol* **33**, 1879–1883.
- La Scola, B., Zeaiter, Z., Khamis, A. & Raoult, D. (2003). Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. *Trends Microbiol* **11**, 318–321.
- Norman, A. F., Regnery, R., Jameson, P., Greene, C. & Krause, D. C. (1995). Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J Clin Microbiol* **33**, 1797–1803.
- Riegel, P., de Briel, D., Prevost, G., Jehl, F. & Monteil, H. (1994). Genomic diversity among *Corynebacterium jeikeium* strains and comparison with biochemical characteristics and antimicrobial susceptibilities. *J Clin Microbiol* **32**, 1860–1865.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Swofford, D. L. (1993). PAUP: phylogenetic analysis using parsimony. Illinois Natural History Survey, Champaign, IL, USA.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmoulin, F. & Higgins, D. G. (1997). The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.