Bartonella rattaaustraliansi sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats

Vijay A. K. B. Gundi,¹ Carmel Taylor,² Didier Raoult¹
and Bernard La Scola¹

Correspondence

Bernard La Scola

bernard.lascola@medecine.univ-mrs.fr

¹URMITE, CNRS IRD UMR 6236, Faculté de Médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France

²Public Health Virology, Queensland Health Forensic and Scientific Services, Coopers Plains, QLD 4108, Australia

A total of 11 *Bartonella* isolates were recovered from the blood of *Melomys*, *Uromys* and *Rattus* species in Australia and were characterized using phenotypic and genotypic methods. Comparison of 16S rRNA gene, *ftsZ*, *gltA* and 16S–23S rRNA internal transcribed spacer region sequences from the isolates indicated that they formed three sequence similarity groups that were distinct from one another and from the currently recognized *Bartonella* species. Phylogenetic analysis based on alignment of concatenated sequences inferred distinct evolutionary lineages for each of the three groups within the genus *Bartonella*. On the basis of these data, we propose the isolates be accommodated in three novel *Bartonella* species, namely *Bartonella rattaaustraliansi* sp. nov. (type strain AUST/NH4^T = CIP 109051^T = CCUG 52161^T = CSUR B609^T), *Bartonella queenslandensis* sp. nov. (type strain AUST/NH12^T = CIP 109057^T = CCUG 52167^T = CSUR B617^T) and *Bartonella coopersplainsensis* sp. nov. (type strain AUST/NH20^T = CIP 109064^T = CCUG 52174^T = CSUR B619^T).

Bacteria of the genus *Bartonella* (a member of the *Alphaproteobacteria*) are fastidious, Gram-negative and aerobic bacilli. The genus currently consists of 19 species, including three subspecies of *Bartonella vinsonii*, and some subtypes of *Bartonella quintana* and *Bartonella henselae*. Of these, among species with validly published names, *Bartonella elizabethae*, *B. bacilliformis*, *B. quintana*, *B. koehlerae*, *B. henselae*, *B. grahamii*, *B. alatica*, *B. clarridgeiae* and the three subspecies of *B. vinsonii* have been recognized as potential human pathogens, in addition to currently unnamed isolates (Breitschwerdt & Kordick, 2000; Chomel *et al.*, 2006a; Minnick & Anderson, 2006; Ereemeeva *et al.*, 2007; Kosoy *et al.*, 2008). Numerous reports have been made of the worldwide occurrence of *Bartonella* organisms in ruminants, rodents and pets (Chomel *et al.*, 2006b). There has been increased interest

in the isolation and description of novel species of the genus (Castle *et al.*, 2004). Recent studies have shown that bartonellae were widely distributed among rodents in southern China (Ying *et al.*, 2002), Thailand (Castle *et al.*, 2004) and Bangladesh (Bai *et al.*, 2007). To our knowledge, no isolates have previously been recovered from rats in Australia. The purpose of the present study was to characterize a group of *Bartonella* strains isolated from rodents in Australia.

Animal blood was collected during two separate mammal surveys carried out in Queensland, Australia, in 1999. The first was performed by the Australian Army and involved collection of tissues and blood from mammals trapped in central Queensland. The second survey was carried out by Queensland Health and involved trapping small mammals in north Queensland. Blood was collected from each animal and transferred to the laboratory where, upon arrival, the samples were placed at –80 °C for long-term storage.

Each vial of blood was thawed for isolation of *Bartonella* strains, which was carried out as described previously (Heller *et al.*, 1998). Putative bartonella colonies were subcultured by streaking onto heart infusion agar plates

Abbreviation: ITS, 16S–23S rRNA internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, ITS, *ftsZ*, *rpoB* and *gltA* sequences determined in this study are EU111749–EU111803, as detailed in Table 1.

Neighbour-joining dendrograms based on individual analysis of ITS, *ftsZ*, *rpoB* and *gltA* sequences and a table of accession numbers of reference sequences are available as supplementary material with the online version of this paper.

supplemented with 5% defibrinated sheep blood (Microdiagnostics). Cultures were then stored frozen in brain heart infusion broth containing glycerol. Cultures were retrieved from frozen storage and subjected to DNA extraction using Qiagen blood kits. PCR was then performed as described previously (La Scola *et al.*, 2002). Cultures that were positive by PCR were shipped frozen on dry ice to the Unité des Rickettsies of the Faculté de Médecine at Marseille, France, for further studies.

Electron microscopy was performed for size determination and flagella study. Bacterial isolates were grown in solid and liquid media and submitted to electron microscopy as described previously (Maillard *et al.*, 2004). Briefly, the bacteria were suspended in PBS (0.145 M NaCl, 0.15 M sodium phosphate), spread on a water surface and adsorbed to Formvar-coated nickel grids, stained with 1% (w/v) uranyl acetate and air-dried. Samples were examined with a Philips transmission electron microscope.

Molecular characterization was performed by sequence analysis of the 16S rRNA gene, the 16S–23S rRNA internal transcribed spacer (ITS) and the *ftsZ*, *rpoB* and *gltA* genes according to methods described previously (Birtles *et al.*, 1995; Birtles & Raoult, 1996; Renesto *et al.*, 2001; Zeaiter *et al.*, 2002; Houpijian & Raoult, 2001). For *gltA*, a 964 bp fragment encompassing the 326 bp hypervariable region was sequenced. The hypervariable region was used for similarity comparisons for consistency with our earlier study (La Scola *et al.*, 2003), whereas the large fragment was used for phylogeny as it was expected to be more informative in phylogenetic tree reconstruction.

Eleven *Bartonella* isolates from Australian rats were studied: two from *Rattus tunneyi*, one from *Rattus fuscipes*, one from *Uromys caudimaculatus*, two from *Rattus conatus*, three from *Rattus leucopus* and two from *Melomys* sp. Based on phenotypic characteristics (small, Gram-negative bacilli better observed by Gimenez staining, slow growth on blood agar but not on nutrient agar), the organisms were initially suspected to be *Bartonella* strains.

The ranges of sequence similarity of the isolates to the closest members of *Bartonella* for the 16S rRNA gene, ITS, *ftsZ*, *rpoB* and *gltA* sequence were respectively 98.9–99.7, 80.5–94.2, 89.1–98.3, 89.5–95.9 and 89.0–95.4% (Table 1). The 11 isolates were scattered into two different clusters in the neighbour-joining phylogenetic tree constructed with concatenated 16S rRNA gene, ITS, *ftsZ*, *rpoB* and *gltA* sequences (cluster 1: AUST/NH4^T, AUST/NH9, AUST/NH10, AUST/NH14 and AUST/NH18; cluster 2a: AUST/NH5, AUST/NH8, AUST/NH11, AUST/NH12^T and AUST/NH15; cluster 2b: AUST/NH20^T) (Fig. 1; accession numbers for individual reference sequences are detailed in Supplementary Table S1, available in IJSEM Online. This suggested that these isolates could represent three novel species. In the concatenated phylogenetic tree, cluster 1 isolates shared a branch with *Bartonella tribocorum*, *B. grahamii* and *B. elizabethae*. The branching pattern and distribution of the new isolates in trees constructed with

individual genes were the same as in the concatenated tree (Supplementary Fig. S1). The branching of members of cluster 2 is more difficult to appreciate, as it is not supported by high bootstrap values in the concatenated tree or in the individual trees. We proposed to classify these isolates in three novel species, *Bartonella rattaaustraliansi* sp. nov. (isolates AUST/NH4^T, AUST/NH9, AUST/NH10, AUST/NH14 and AUST/NH18), *Bartonella queenslandensis* sp. nov. (isolates AUST/NH5, AUST/NH8, AUST/NH11, AUST/NH12^T and AUST/NH15) and *Bartonella coopers-plainsensis* sp. nov. (isolate AUST/NH20^T). The ranges of similarity of these novel species to their closest relatives and levels of intraspecies similarity are summarized in Table 2. The latter isolate AUST/NH20^T was closely related to but distinct from isolates of cluster 2a, as its closest relatives were the novel strain AUST/NH4^T (*B. rattaaustraliansi* sp. nov.) for the *gltA*, *rpoB* and *ftsZ* sequences, with 92.3, 93.8 and 92.9% similarity, respectively, and *B. grahamii* V2^T for the 16S rRNA gene and ITS sequences, with 98.7 and 81.9% similarity, respectively.

The ad hoc committee for the re-evaluation of the species definition in bacteriology has previously proposed that descriptions of novel species could be based on house-keeping gene sequence analysis with a comparison of at least five genes, provided there is a sufficient degree of congruence between the technique used and DNA–DNA reassociation (Stackebrandt *et al.*, 2002). For *Bartonella* species, it was added that there should be congruence of multilocus gene analysis with results of phylogenetic analysis based on gene sequence analysis that give high bootstrap values (Pitulle *et al.*, 2002). In a study by La Scola *et al.* (2003), we compared similarities of several coding sequences between known species of *Bartonella* and identified that several genes such as *ribC* could not provide firm evidence that a new isolate of *Bartonella* represented a novel species. For this gene, in nearly all cases, similarity between species did not exceed 93%; however, for two species, it was as high as 99.5%, suggesting horizontal gene transfer (La Scola *et al.*, 2003). Of the genes studied, only partial *rpoB* and *gltA* sequences, with respective interspecies similarities of 95.4 and 96%, allowed clear discrimination of all species. From these data, we proposed that newly encountered *Bartonella* isolates should be considered to represent novel species if the hypervariable 327 bp *gltA* fragment shares less than 96% sequence similarity and if a 825 bp *rpoB* fragment shares less than 95.4% sequence similarity with sequences of strains of species with validly published names (La Scola *et al.*, 2003). In the present case, through analysis of 825 bp *rpoB* sequences, five isolates, AUST/NH5, AUST/NH8, AUST/NH11, AUST/NH12^T and AUST/NH15, showed 95.5–96.0% similarity with members of the closest species. These values are above the proposed cut-off value of 95.4%. However, these five isolates show 92.3–95.4% similarity to strains of known *Bartonella* species for the 327 bp *gltA* fragment, for which the determined cut-off value is 96.0% (La Scola *et al.*, 2003). Thus, in the present study, as the *rpoB* gene sequence

Table 1. Sequence similarity to their closest relatives for the *Bartonella* isolates from the present study

Details of the sequences of the closest relatives are given in Supplementary Table S1.

Strain	Sequence	GenBank accession no.	Closest relative(s) (similarity, %)
<i>Bartonella rattaaustraliani</i> sp. nov.			
AUST/NH4 ^T	16S rRNA gene	EU111749	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.4)
	ITS	EU111760	<i>B. grahamii</i> (80.5)
	<i>ftsZ</i>	EU111771	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (95.9)
	<i>rpoB</i>	EU111782	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> / <i>B. vinsonii</i> subsp. <i>vinsonii</i> (90.3)
	<i>gltA</i>	EU111793	<i>B. vinsonii</i> subsp. <i>arupensis</i> (89.9)
AUST/NH9	16S rRNA gene	EU111750	<i>B. doshiae</i> (99.3)
	ITS	EU111761	<i>B. grahamii</i> (84.1)
	<i>ftsZ</i>	EU111772	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (96.5)
	<i>rpoB</i>	EU111783	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (89.5)
	<i>gltA</i>	EU111794	<i>B. grahamii</i> (90.2)
AUST/NH10	16S rRNA gene	EU111751	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (99.3)
	ITS	EU111762	<i>B. grahamii</i> (81.3)
	<i>ftsZ</i>	EU111773	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (94.8)
	<i>rpoB</i>	EU111784	<i>B. vinsonii</i> subsp. <i>arupensis</i> (89.8)
	<i>gltA</i>	EU111795	<i>B. vinsonii</i> subsp. <i>arupensis</i> (89.9)
AUST/NH14	16S rRNA gene	EU111752	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (99.3)
	ITS	EU111763	<i>B. grahamii</i> (81.1)
	<i>ftsZ</i>	EU111774	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (95.4)
	<i>rpoB</i>	EU111785	<i>B. vinsonii</i> subsp. <i>arupensis</i> (90.3)
	<i>gltA</i>	EU111796	<i>B. vinsonii</i> subsp. <i>arupensis</i> (90.5)
AUST/NH18	16S rRNA gene	EU111753	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.3)
	ITS	EU111764	<i>B. grahamii</i> (84.1)
	<i>ftsZ</i>	EU111775	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (96.4)
	<i>rpoB</i>	EU111786	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (89.7)
	<i>gltA</i>	EU111797	<i>B. grahamii</i> (90.2)
<i>Bartonella queenslandensis</i> sp. nov.			
AUST/NH5	16S rRNA gene	EU111755	<i>B. grahamii</i> (99.6)
	ITS	EU111766	<i>B. tribocorum</i> (93.5)
	<i>ftsZ</i>	EU111777	<i>B. elizabethae</i> (98.0)
	<i>rpoB</i>	EU111788	<i>B. tribocorum</i> (95.9)
	<i>gltA</i>	EU111799	<i>B. grahamii</i> (95.1)
AUST/NH8	16S rRNA gene	EU111756	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.7)
	ITS	EU111767	<i>B. tribocorum</i> (94.0)
	<i>ftsZ</i>	EU111778	<i>B. elizabethae</i> (97.9)
	<i>rpoB</i>	EU111789	<i>B. tribocorum</i> (95.5)
	<i>gltA</i>	EU111800	<i>B. grahamii</i> (94.5)
AUST/NH11	16S rRNA gene	EU111757	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.7)
	ITS	EU111768	<i>B. tribocorum</i> (93.6)
	<i>ftsZ</i>	EU111779	<i>B. elizabethae</i> (98.3)
	<i>rpoB</i>	EU111790	<i>B. tribocorum</i> (95.8)
	<i>gltA</i>	EU111801	<i>B. grahamii</i> (95.1)
AUST/NH12 ^T	16S rRNA gene	EU111754	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.6)
	ITS	EU111765	<i>B. tribocorum</i> (93.7)
	<i>ftsZ</i>	EU111776	<i>B. vinsonii</i> subsp. <i>berkhoffii</i> (95.9)
	<i>rpoB</i>	EU111787	<i>B. tribocorum</i> (95.6)
	<i>gltA</i>	EU111798	<i>B. grahamii</i> (94.8)
AUST/NH15	16S rRNA gene	EU111758	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (99.7)
	ITS	EU111769	<i>B. tribocorum</i> (94.2)
	<i>ftsZ</i>	EU111780	<i>B. elizabethae</i> (98.0)
	<i>rpoB</i>	EU111791	<i>B. tribocorum</i> (96.0)
	<i>gltA</i>	EU111802	<i>B. grahamii</i> (95.4)
<i>Bartonella coopersplainsensis</i> sp. nov.			
AUST/NH20 ^T	16S rRNA gene	EU111759	<i>B. grahamii</i> (98.7)
	ITS	EU111770	<i>B. grahamii</i> (81.9)

Table 1. cont.

Strain	Sequence	GenBank accession no.	Closest relative(s) (similarity, %)
	<i>ftsZ</i>	EU111781	<i>B. vinsonii</i> subsp. <i>vinsonii</i> (89.1) (AUST/NH4 ^T , 92.9)
	<i>rpoB</i>	EU111792	<i>B. alsatica</i> (91.9) (AUST/NH4 ^T , 93.8)
	<i>gltA</i>	EU111803	<i>B. vinsonii</i> subsp. <i>arupensis</i> (89.0) (AUST/NH4 ^T , 92.3)

similarities exceeded the proposed cut-off, analysis of the sequences of more genes had to be performed. However, it should be noticed that *gltA* and *rpoB* fragments still give the highest confidence for definition of novel species as, in all cases, the intraspecies similarity of sequences from *B. rattaustraliani* and *B. coopersplainsensis* strains is greater than the interspecies similarity.

In the concatenated phylogenetic tree, strains of the newly proposed species *B. queenslandensis* branched strictly with *B. tribocorum*, *B. elizabethae* and *B. grahamii*. *B. rattaustraliani* and *B. coopersplainsensis* branched consistently with *Bartonella doshiae* and *B. taylorii* (Fig. 1), but the relationships with other species are not well-defined.

In conclusion, for the first time, *Bartonella* species have been recovered from rats in Australia. The sequences of five genomic regions, *gltA*, *rpoB*, the 16S rRNA gene, *ftsZ* and the ITS region, reveal similarity values in the range that allows the isolates to be afforded distinct species status. To fulfil the rules of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), we provide the following

descriptions of the novel species identified in this investigation.

Description of *Bartonella rattaustraliani* sp. nov.

Bartonella rattaustraliani (ratt.aus.tra.li.a'ni. N.L. n. *Rattus* zoological name of a genus of rat; N.L. adj. *australianus* Australian; N.L. gen. n. *rattaustraliani* of an Australian rat, from which the first strains were isolated).

All five known isolates assigned to the species exhibit all of the characteristics of the genus (Birtles *et al.*, 1995). Good growth is observed on Columbia agar with 5 % sheep blood in a 5 % CO₂ atmosphere at 37 °C, where colonies appear small, white, smooth and irregular (about 0.5 mm in diameter) after 7 days. Electron microscope examination reveals small bacilli without flagella, approx. 1–2 µm long by 0.5 µm wide. Gram-negative and Gimenez-positive. All known strains are susceptible to antibiotics such as aminoglycosides, macrolides, doxycycline and rifampicin, but are resistant to vancomycin. Moreover, imipenem is more effective against all known strains except AUST/

Fig. 1. Dendrogram representing the phylogenetic relationships between *Bartonella* species, derived from concatenated sequences of the 16S rRNA gene, the ITS region and the *rpoB*, *ftsZ* and *gltA* sequences. MEGA4 software (<http://www.megasoftware.net>) was used to infer the tree by using the neighbour-joining method with Kimura's two-parameter model. Support for each branch, as determined from 1000 bootstrap samples, is indicated by percentages at nodes. Accession numbers for individual reference sequences are detailed in Supplementary Table S1. Concatenated sequences from *Brucella melitensis* 16M^T were used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 2. Ranges of similarity (%) with their closest relatives and intraspecies similarity (%) of the three novel species

'Intra' refers to intraspecies sequence similarity; *B. coopersplainsensis* sp. nov. accommodates only a single strain.

Species	ITS		<i>ftsZ</i>		<i>rpoB</i>		<i>gltA</i>	
	Range	Intra	Range	Intra	Range	Intra	Range	Intra
<i>B. rattaaustraliani</i>	81.1–84.1	81–99.6	94–96.5	95.8–99.6	89.5–90.3	93.2–99.3	89.9–90.5	94.8–100
<i>B. queenslandensis</i>	93.5–94.2	95.1–97.9	95.9–98.3	98.5–99.7	95.5–96	97.9–99.4	94.5–95.4	96.3–100
<i>B. coopersplainsensis</i>	81.9	NA	92.9	NA	93.8	NA	92.3	NA

NH4^T. The type strain of the species is non-motile. It is differentiated genetically from other *Bartonella* species on the basis of sequences of the 16S rRNA, *ftsZ*, *gltA* and *rpoB* genes and the ITS region.

The type strain is AUST/NH4^T (=CIP 109051^T =CCUG 52161^T =CSUR B609^T), isolated from blood of a wild rat of the species *Rattus tunneyi*. Other isolates belonging to the same species, isolated from the blood of rats, are AUST/NH9 (=CIP 109055 =CCUG 52164 =CSUR B610) from *Uromys caudimaculatus*, AUST/NH10 (=CIP 109054 =CCUG 52165 =CSUR B611) from *Rattus leucopus*, AUST/NH14 (=CIP 109059 =CCUG 52169 =CSUR B612) from *Rattus conatus* and AUST/NH18 (=CIP 109063 =CCUG 52173 =CSUR B613) from *Melomys* sp.

Description of *Bartonella queenslandensis* sp. nov.

Bartonella queenslandensis (queens.lan.den'sis. N.L. fem. adj. *queenslandensis* referring to Queensland, the Australian state where the rats were trapped from which the first strains were isolated).

All six known isolates assigned to the species exhibit all of the characteristics of the genus (Birtles *et al.*, 1995). Good growth is observed on Columbia agar with 5 % sheep blood in a 5 % CO₂ atmosphere at 37 °C, where colonies appear small, white, smooth and irregular (about 0.5 mm in diameter) after 7 days. Electron microscope examination reveals small bacilli without flagella, approximately 1–2 µm long by 0.5 µm wide. Gram-negative, Gimenez-positive. All known strains show susceptibility to aminoglycosides, macrolides, doxycycline and rifampicin, but are resistant to vancomycin. Furthermore, imipenem is more effective against all strains. The type strain of the species is non-motile. It is differentiated genetically from other *Bartonella* species on the basis of sequences of the 16S rRNA, *ftsZ*, *gltA* and *rpoB* genes and the ITS region.

The type strain is AUST/NH12^T (=CIP 109057^T =CCUG 52167^T =CSUR B617^T), isolated from blood of a wild rat belonging to the genus *Melomys*. Other isolates belonging to the species, isolated from the blood of rats, are AUST/NH5 (=CIP 109052 =CCUG 52162 =CSUR B614) from *Rattus tunneyi*, AUST/NH8 (=CIP 109053 =CCUG 52163 =CSUR B615) from *Rattus fuscipes*, AUST/NH11 (=CIP 109056 =CCUG 52166 =CSUR B616) from *Rattus conatus*

and AUST/NH15 (=CIP 109060 =CCUG 52170 =CSUR B618) from *Rattus leucopus*.

Description of *Bartonella coopersplainsensis* sp. nov.

Bartonella coopersplainsensis (coo.pers.plain.sen'sis. N.L. fem. adj. *coopersplainsensis* pertaining to Coopers Plains, Queensland, the name of the area where the rat was trapped from which the type strain was isolated).

The single known isolate assigned to this species exhibits all of the characteristics of the genus (Birtles *et al.*, 1995). Good growth is observed on Columbia agar with 5 % sheep blood in a 5 % CO₂ atmosphere at 37 °C, where colonies appear small, white, smooth and irregular (about 0.5 mm in diameter) after 20 days. Electron microscope examination reveals small bacilli without flagella, approximately 1–2 µm long by 0.5 µm wide. Gram-negative and Gimenez-positive, and non-motile. Susceptible to aminoglycosides, macrolides, doxycycline and rifampicin; resistant to vancomycin, and imipenem is more effective against the type strain. Differentiated genetically from other *Bartonella* species on the basis of sequences of the 16S rRNA, *ftsZ*, *gltA* and *rpoB* genes and the ITS region.

The type strain is AUST/NH20^T (=CIP 109064^T =CCUG 52174^T =CSUR B619^T), isolated from blood of a wild rat of the species *Rattus leucopus*.

Acknowledgements

The authors wish to thank the following Queensland Health staff who were involved in animal trapping: Greg Smith, Ina Smith, Lee Smythe, Michael Dohnt and Meegan Symonds.

References

- Bai, Y., Montgomery, S. P., Sheff, K. W., Chowdhury, M. A., Breiman, R. F., Kabeya, H. & Kosoy, M. Y. (2007). *Bartonella* strains in small mammals from Dhaka, Bangladesh, related to *Bartonella* in America and Europe. *Am J Trop Med Hyg* 77, 567–570.
- 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.
- Birtles, R. J., Harrison, T. G., Saunders, N. A. & Molyneux, D. H. (1995). Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov., *Bartonella peromysci*

comb. nov., and three new species, *Bartonella grahamii* sp. nov., *Bartonella taylorii* sp. nov., and *Bartonella doshiae* sp. nov. *Int J Syst Bacteriol* 45, 1–8.

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.

Castle, K. T., Kosoy, M., Lerdtusnee, K., Phelan, L., Bai, Y., Gage, K. L., Leepitakrat, W., Monkanna, T., Khlaimeanee, N. & other authors (2004). Prevalence and diversity of *Bartonella* in rodents of northern Thailand: a comparison with *Bartonella* in rodents from southern China. *Am J Trop Med Hyg* 70, 429–433.

Chomel, B. B., Boulouis, H. J., Maruyama, S. & Breitschwerdt, E. B. (2006a). *Bartonella* spp. in pets and effect on human health. *Emerg Infect Dis* 12, 389–394.

Chomel, B. B., Kasten, R. W., Henn, J. B. & Molia, S. (2006b). *Bartonella* infection in domestic cats and wild felids. *Ann N Y Acad Sci* 1078, 410–415.

Eremeeva, M. E., Gerns, H. L., Lydy, S. L., Goo, J. S., Ryan, E. T., Mathew, S. S., Ferraro, M. J., Holden, J. M., Nicholson, W. L. & other authors (2007). Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. *N Engl J Med* 356, 2381–2387.

Heller, R., Riegel, P., Hansmann, Y., Delacour, G., Bermond, D., Dehio, C., Lamarque, F., Monteil, H., Chomel, B. & Piémont, Y. (1998). *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. *Int J Syst Bacteriol* 48, 1333–1339.

Houpikian, P. & Raoult, D. (2001). 16S/23S rRNA intergenic spacer regions for phylogenetic analysis, identification, and subtyping of *Bartonella* species. *J Clin Microbiol* 39, 2768–2778.

Kosoy, M., Morway, C., Sheff, K. W., Bai, Y., Colborn, J., Chalcraft, L., Dowell, S. F., Peruski, L. F., Maloney, S. A. & other authors (2008). *Bartonella tamiae* sp. nov., a newly recognized pathogen isolated from three human patients from Thailand. *J Clin Microbiol* 46, 772–775.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code*

of Nomenclature of Bacteria (1990 Revision). *Bacteriological Code*. Washington, DC: American Society for Microbiology.

La Scola, B., Davoust, B., Boni, M. & Raoult, D. (2002). Lack of correlation between *Bartonella* DNA detection within fleas, serological results, and results of blood culture in a *Bartonella*-infected stray cat population. *Clin Microbiol Infect* 8, 345–351.

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.

Maillard, R., Riegel, P., Barrat, F., Bouillin, C., Thibault, D., Gandoin, C., Halos, L., Demanche, C., Alliot, A. & other authors (2004). *Bartonella chomelii* sp. nov., isolated from French domestic cattle (*Bos taurus*). *Int J Syst Evol Microbiol* 54, 215–220.

Minnick, M. F. & Anderson, B. E. (2006). The genus *Bartonella*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 5, pp. 467–492. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Pitulle, C., Strehse, C., Brown, J. W. & Breitschwerdt, E. B. (2002). Investigation of the phylogenetic relationships within the genus *Bartonella* based on comparative sequence analysis of the *rnpB* gene, 16S rDNA and 23S rDNA. *Int J Syst Evol Microbiol* 52, 2075–2080.

Renesto, P., Gouvernet, J., Drancourt, M., Roux, V. & Raoult, D. (2001). Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. *J Clin Microbiol* 39, 430–437.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 52, 1043–1047.

Ying, B., Kosoy, M. Y., Maupin, G. O., Tsuchiya, K. R. & Gage, K. L. (2002). Genetic and ecologic characteristics of *Bartonella* communities in rodents in southern China. *Am J Trop Med Hyg* 66, 622–627.

Zeaiter, Z., Liang, Z. & Raoult, D. (2002). Genetic classification and differentiation of *Bartonella* species based on comparison of partial *ftsZ* gene sequences. *J Clin Microbiol* 40, 3641–3647.