

Brucella papionis sp. nov., isolated from baboons (*Papio* spp.)

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Two Gram-negative, non-motile, non-spore-forming coccoid bacteria (strains F8/08-60^T and F8/08-61) isolated from clinical specimens obtained from baboons (*Papio* spp.) that had delivered stillborn offspring were subjected to a polyphasic taxonomic study. On the basis of 16S rRNA gene sequence similarities, both strains, which possessed identical sequences, were assigned to the genus *Brucella*. This placement was confirmed by extended multilocus sequence analysis (MLSA), where both strains possessed identical sequences, and whole-genome sequencing of a representative isolate. All of the above analyses suggested that the two strains represent a novel lineage within the genus *Brucella*. The strains also possessed a unique profile when subjected to the phenotyping approach classically used to separate species of the genus *Brucella*, reacting

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Abbreviations: MLSA, multilocus sequence analysis; MLVA, multilocus variable number of tandem repeat analysis; RTD, routine test dilution.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *omp2a* and *omp2b* gene sequences of strains F8/08-60^T and F8/08-61 are HG932316 and HG932317 (16S rRNA gene), KJ493822 and KJ493823 (*omp2a*) and KJ510540 and KJ510541 (*omp2b*), respectively.

A supplementary table and a supplementary figure are available with the online Supplementary Material.

only with *Brucella* A monospecific antiserum, being sensitive to the dyes thionin and fuchsin, being lysed by bacteriophage Wb, Bk₂ and Fi phage at routine test dilution (RTD) but only partially sensitive to bacteriophage Tb, and with no requirement for CO₂ and no production of H₂S but strong urease activity. Biochemical profiling revealed a pattern of enzyme activity and metabolic capabilities distinct from existing species of the genus *Brucella*. Molecular analysis of the *omp2* locus genes showed that both strains had a novel combination of two highly similar *omp2b* gene copies. The two strains shared a unique fingerprint profile of the multiple-copy *Brucella*-specific element IS711. Like MLSA, a multilocus variable number of tandem repeat analysis (MLVA) showed that the isolates clustered together very closely, but represent a distinct group within the genus *Brucella*. Isolates F8/08-60^T and F8/08-61 could be distinguished clearly from all known species of the genus *Brucella* and their biovars by both phenotypic and molecular properties. Therefore, by applying the species concept for the genus *Brucella* suggested by the ICSP Subcommittee on the Taxonomy of *Brucella*, they represent a novel species within the genus *Brucella*, for which the name *Brucella papionis* sp. nov. is proposed, with the type strain F8/08-60^T (=NCTC 13660^T=CIRMBP 0958^T).

The genus *Brucella* currently comprises ten species, *Brucella melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. neotomae*, *B. ovis*, *B. pinnipedialis*, *B. ceti*, *B. microti* and *B. inopinata* (Whatmore, 2009). The latter four species have been described in the last decade after a long period of stability in the taxonomy of the genus *Brucella* and reflect an ongoing widening of the understanding of the host range of *Brucella* (Godfroid *et al.*, 2011). Because of the high DNA–DNA relatedness of the six longstanding species (relatedness >70%), it had been suggested that the genus should be monospecific and consist of *B. melitensis* only, with six biovars, Melitensis, Abortus, Suis, Ovis, Neotomae and Canis (Verger *et al.*, 1985). However, in 2003, the ICSP Subcommittee on the Taxonomy of *Brucella* agreed unanimously on a return to the pre-1986 taxonomy of six species, with recognized biovars of *B. suis*, *B. abortus* and *B. melitensis* (Osterman & Moriyón, 2006). Three of the newly described species, *B. ceti*, *B. pinnipedialis* and *B. microti* (Foster *et al.*, 2007; Scholz *et al.*, 2008), conform to the existing pattern of high genetic homogeneity, for example all sharing identical 16S rRNA gene sequences. However, *B. inopinata*, although clearly much more closely related to *Brucella* than to the nearest neighbour genus *Ochrobactrum* (De *et al.*, 2008), substantially extends the described genetic diversity within the group (Scholz *et al.*, 2010; Wattam *et al.*, 2012, 2014). This has led some authors to divide the genus informally into ‘atypical’ *Brucella*, including *B. inopinata* and other groups recently isolated from rodents, amphibians and humans but yet to be formally described taxonomically (Tiller *et al.*, 2010a, b; Eisenberg *et al.*, 2012; Fischer *et al.*, 2012), and ‘core’ *Brucella*, representing the nine remaining genetically conserved species that cluster with the longstanding ‘classical’ species of the genus *Brucella*.

Historically, classification of species of the genus *Brucella* has been based on a combination of host species preference and phenotypic biotyping, which examines a range of characteristics including CO₂ requirement, H₂S production, dye-sensitivity, lysis by *Brucella*-specific bacteriophage

and agglutination with monospecific sera. Phenotyping is still used widely, at least in reference laboratories, although its limitations are increasingly widely recognized, and the emergence of novel species is further eroding its value. Recent descriptions have relied heavily on additional data from emerging molecular approaches, and a process of updating the minimal standards to describe novel species of the genus *Brucella*, written almost 40 years ago (Corbel & Brinley-Morgan, 1975), to reflect these approaches is ongoing within the current ICSP Subcommittee on the Taxonomy of *Brucella* (<http://icsp.org/subcommittee/brucella/>).

Here, we describe the classification of two strains (AHVLA F8/08-60^T=Baboon 15719=NVSL 07-0026-1 and AHVLA F8/08-61=Baboon 15331=NVSL 07-0224-1, referred to hereafter as F8/08-60^T and F8/08-61) that were isolated in 2006 and 2007 from two cases of stillbirth and retained placenta in baboons at a primate research centre in Texas, USA (Schlabritz-Loutsevitch *et al.*, 2009). The index case was a 13-year-old baboon captured in Tanzania with a history of three previous pregnancies, including one abortion/stillbirth. The suspect *Brucella* isolate (F8/08-60^T) was isolated from a cervical swab obtained following a stillbirth in August 2006. Banked sera from the animal were serologically positive for *Brucella* using testing validated for *B. abortus* in cattle (Brewers’ Diagnostic kits; Hynson, Westcott and Dunning Inc.). A second isolate (F8/08-61) was obtained from a swab of uterine content in January 2007, 1 month after a stillbirth in an 8-year-old colony-born baboon with no previous contact with the index case. This animal was serologically negative for *Brucella*.

Phenotypic analysis was performed by characterizing growth on different media, CO₂ requirement, H₂S production, growth in the presence of dyes (thionin and basic fuchsin), reaction with *Brucella* unabsorbed and monospecific A and M antisera and microscopy. Metabolic activity in comparison with other strains of *Brucella* was assessed using API 20E, API 20NE and API ZYM (bioMérieux), with some confirmatory standalone biochemical

testing and application of the Micronaut BfR *Brucella* assay (Al Dahouk *et al.*, 2010). Molecular analysis comprised multiplex PCR, DNA–DNA hybridization studies (Ziemke *et al.*, 1998), 16S rRNA gene sequence analysis, *omp2a* and *omp2b* sequencing, multilocus sequence analysis (MLSA), multilocus variable number of tandem repeat analysis (MLVA with 16 loci) and IS711 fingerprinting, all of which have been used in recent descriptions of novel species of the genus *Brucella*, with recently completed whole-genome sequencing supporting the findings.

Phenotypic analysis

Both isolates grew slowly on sheep blood agar (SBA) after incubation both aerobically and in CO₂, with small, raised, circular, convex, non-haemolytic, greyish colonies, ~0.5 mm in diameter, appearing after 3 days of incubation at 37 °C, increasing to ~1 mm in diameter after 6 days. Growth was poor at 28 °C. On Farrell's agar, growth also occurred without additional CO₂, with both isolates producing pinpoint colonies after 3 days of incubation at 37 °C, becoming cream in colour, circular, entire, convex and ~0.5–1 mm in diameter after 6 days of incubation. On serum dextrose agar (SDA), growth was apparent after 24 h at 37 °C without additional CO₂, with colonies of around 1 mm diameter increasing to 3–4 mm in diameter after 72 h of incubation. Colonies were smooth, entire and circular and showed characteristic blue iridescence using obliquely transmitted light (Henry, 1933). Growth was poor at 28 °C. On nutrient agar, there was poor growth at 37 °C and no growth at 28 °C. Both isolates were sensitive to doxycycline (MIC <0.016 µg ml⁻¹), rifampicin (MIC 0.032 µg ml⁻¹), ciprofloxacin [MIC 0.094 µg ml⁻¹ (F8/08-60) and 0.064 µg ml⁻¹ (F8/08-61)] and streptomycin [MIC 0.125 µg ml⁻¹ (F8/08-60) and 0.094 µg ml⁻¹ (F8/08-61)] on SDA and failed to grow in nutrient both with 6% NaCl.

Cells of both isolates were Gram-negative cocci, coccobacilli or short rods, approximately 0.5–0.7 µm in diameter and 0.5–1.5 µm long, arranged singly or, occasionally, in pairs, small groups and chains. Both isolates were consistent with *Brucella* using the modified Ziehl–Neelsen stain (Brinley-Morgan *et al.*, 1978), being resistant to decolorization with 0.5% acetic acid. Flagellation was determined by transmission electron microscopy. Both isolates were dried onto Formvar/carbon-coated support grids that had been subjected to plasma glow discharge, negatively stained with 2% phosphotungstic acid (pH 6.6) and examined immediately in a Phillips CM10 transmission electron microscope at 80 kV. Cells were unflagellated coccobacilli, approximately 0.5–1.0 µm long, arranged as individual cells or irregular clusters (Fig. 1). Both isolates were examined by slide agglutination test using unabsorbed *Brucella* antiserum, negative control serum and sterile normal saline, and agglutinated with *Brucella* antiserum but not with either the negative control or saline.

Using classical phenotyping approaches that are traditionally used to divide the genus *Brucella* into species and

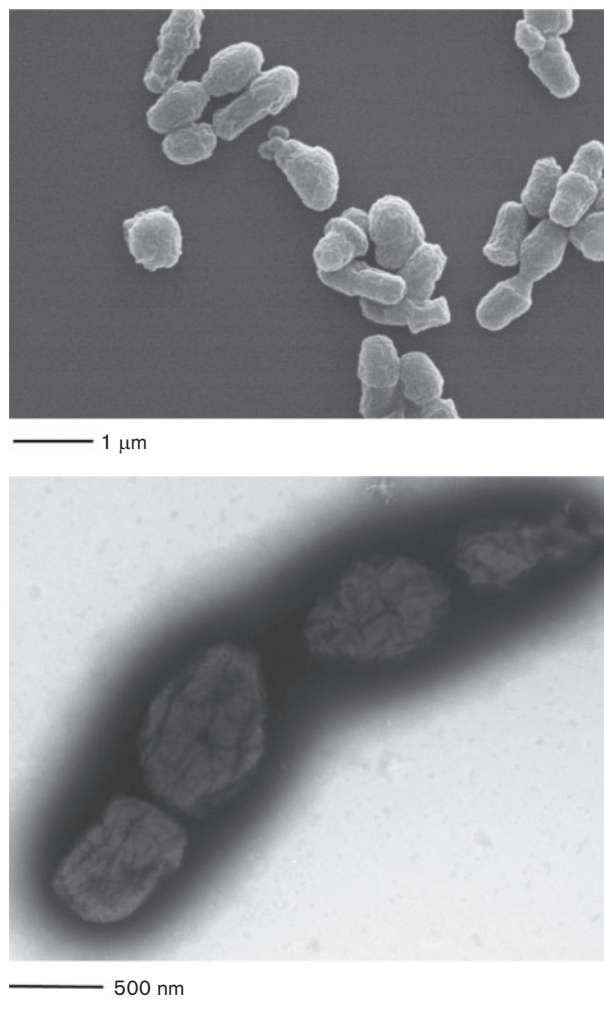


Fig. 1. Electron micrographs showing non-flagellated cells of strain F8/08-61. The appearance of cells of strain F8/08-60^T was identical.

biovars (Alton *et al.*, 1988), the organism did not conform to the characteristics of any recognized species of *Brucella* (Table 1). The unusual profile includes no requirement for CO₂, no production of H₂S, strong urease activity, sensitivity to the dyes thionin and basic fuchsin at 1:50 000, agglutination only with monospecific anti-A serum, and lysis with bacteriophages Wb, Bk₂ and Fi. With regard to the strong urease activity, F8/08-60^T has been reported previously to share mutations with the urease-negative *B. ovis* in one of two urease clusters (Wattam *et al.*, 2014), such as changes in the *ure2* cluster that make *ureF* a pseudogene. However, analysis of *ureC* of the *ure1* cluster shows that *B. ovis* has a 30 bp deletion that is not shared by F8/08-60^T. This deletion is probably the significant factor in the different urease activity of *B. ovis* and the novel strains, as it has been shown that urease activity can be restored in *B. ovis* by complementation with *ureC* from *B. melitensis* (Tsolis *et al.*, 2009).

Table 1. Characteristics that differentiate the novel strains from other species and biovars of the genus *Brucella* based on classical biotyping approaches

Biovars are listed for *B. abortus*, *B. suis* and *B. melitensis*. The status of *B. abortus* biovar 7 is under investigation (Garin-Bastuji *et al.*, 2014). Other reference species: 1, *B. ovis*; 2, *B. canis*; 3, *B. neotomae*; 4, *B. ceti*; 5, *B. pinnipedialis*; 6, *B. microti*; 7, *B. inopinata*. (+), Most strains positive; (-), most strains negative; RTD, routine test dilution; ND, no data available.

Characteristic	F8/08-60 ^T and F8/08-61	<i>B. abortus</i>									<i>B. suis</i>					<i>B. melitensis</i>			1	2	3	4	5	6	7
		1	2	3	4	5	6	7	9	1	2	3	4	5	1	2	3								
Urease	+	(+)*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	
Requirement for CO ₂	-	(+)	(+)	(+)	(+)	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	(-)	(+)	-	-	
Production of H ₂ S	-	+	+	+	+	-	(+)	(+)	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	
Growth on media containing:																									
Thionin†	-	-	-	+‡	-	+	+‡	+	+	+	+	+	+	+	+	+	+	+	+	-	(+)	+	+	+	
Fuchsin†	-	+	-	+§	+	+	+	+	+	-	-	+	(-)	-	+	+	+	(+)	-	-	(+)	+	+	+	
Agglutination with monospecific antisera																									
A	+	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	(+)	-	-	-	
M	-	-	-	+	+	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-	(-)	(-)	+	+¶	
R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	ND	ND	
Lysis by phage at RTD#																									
Tb	PL/NL	L	L	L	L	L	L	L	L	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL/PL	NL ^z	NL ^z	NL	NL	
Wb	L	L	L	L	L	L	L	L	L	L	L	L	L	L	NL	NL	NL	NL	NL	L	L ^β	L ^β	L	ND	
Bk ₂	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	NL	NL	L	L ^β	L ^β	ND	ND		
Fi	L	L	L	L	L	L	L	L	L	PL	PL	PL	PL/L	PL	NL	NL	NL	NL	L	NL/PL	NL/PL	NL	NL		
R/C	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	NL	L	L	NL	NL	NL	ND	ND	
Tb × 10 ⁴	PL	L	L	L	L	L	L	L	L	L	L	L	L	L	NL	NL	NL	NL	NL	L	ND	ND	L	PL	

*Reference strain is negative but most field strains are positive.

†Concentration 1/50 000 (w/v).

‡For more certain differentiation of biotypes 3 and 6, thionin is used at 1/25 000 (w/v) in addition; biovar 3 is positive and biovar 6 is negative.

§Some strains of this biotype are inhibited by basic fuchsin.

||Some isolates may be resistant to fuchsin.

¶Weak agglutination.

#Tb, Tbilisi; Wb, Weybridge; Bk₂, Berkeley; Fi, Firenze; R/C, rough strains; Tb × 10⁴, Tb at RTD × 10⁴. Results scored as follows: L, confluent lysis; PL, partial lysis; NL, no lysis; NL^z, most strains no lysis; L^β, most strains lysis.

Both isolates were run through API 20E and API 20NE, incubated aerobically at 37 and 30 °C, respectively, and API ZYM (bioMérieux). As observed for other classical species of the genus *Brucella*, and in contrast to the recently described *B. inopinata* and *B. microti*, the isolates showed limited metabolic reactivity. After 24 h of incubation at 37 °C, API 20E strips for both strains showed positive reactions only for urea hydrolysis, the Voges–Proskauer test and fermentation of D-glucose and L-arabinose. Both strips were reincubated for a further 24 h (not normally done under the manufacturer's instructions), and this did produce a slight colour change in the sorbitol cupule for isolate F8/08-61, perhaps indicating slow fermentation. After 24 h of incubation at 30 °C, the API 20NE strips for both isolates were read: reactions occurred only for urea hydrolysis; there was no reduction of nitrates or indole production. Reincubation of the API 20NE strips for a further 24 h according to the manufacturer's instructions did not produce any changes to the profile of either isolate. API ZYM strips were inoculated and incubated for 4.5 h according to the manufacturer's instructions. The two isolates produced identical results: strongly positive for esterase (C4), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, positive for esterase lipase (C8), valine arylamidase and cystine arylamidase and negative for alkaline phosphatase, lipase (C14), α -chymotrypsin, α - and β -galactosidase, β -glucuronidase, α - and β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Oxidase and catalase production were examined. Both isolates produced catalase but were negative for oxidase production by using both pyotest strips (Medical Wire and Equipment) and freshly made oxidase reagent. Urea slopes were also inoculated and both isolates hydrolysed urea rapidly, within 30 min, consistent with the positive urea results recorded on API 20E and API 20NE. Nitrate broths were inoculated with both isolates and incubated for 24 h and 3 days at 37 °C, together with a positive control (*Serratia marcescens*), negative control (*Acinetobacter lwoffii*) and an uninoculated broth. Neither isolate reduced nitrate.

In addition to classical biochemical reactions, extended metabolic activity was examined using the Micronaut BfR *Brucella* assay (Merlin Diagnostika), which assesses reactions with 93 different metabolites (Al Dahouk *et al.*, 2010). Once again, and in contrast to *B. inopinata* and *B. microti*, the isolates displayed limited metabolic reactivity, especially in sugar metabolism, consistent with the 'classical' species group of the genus *Brucella* (Table S1, available in the online Supplementary Material). Interestingly, the enzyme reaction using H-hydroxyproline- β NA, hitherto defining the genus *Brucella* (Al Dahouk *et al.*, 2010, 2012), revealed a negative result for the first time. Hence, the novel isolates differ metabolically from existing species of the genus *Brucella*.

Molecular analysis

The virtually complete 16S rRNA gene sequence was determined from both strains using an approach described

previously (Hunt *et al.*, 2013). Sequences of 1407 bp obtained from the two isolates were identical and confirmed the identity of the isolates as members of the genus *Brucella*. The sequences show two nucleotide differences from the sequences of the nine 'core' species of the genus *Brucella* shown previously to share identical 16S rRNA gene sequences (Gee *et al.*, 2004; Al Dahouk *et al.*, 2012) and seven nucleotide differences from the sequence of *B. inopinata*, the only species of the genus *Brucella* shown previously to have a divergent 16S rRNA gene sequence (Scholz *et al.*, 2010).

Results from DNA–DNA hybridizations using labelled DNA of *B. melitensis* 16M^T showed 79.1 ± 0.7% DNA relatedness with strain F8/08-60^T. This result is in agreement with previous reports (Verger *et al.*, 1985) that showed that, if the 70% DNA relatedness threshold is applied, species of the genus cannot be separated based on results from DNA–DNA hybridizations. The DNA relatedness to the phylogenetically closest neighbour *Ochrobactrum intermedium* LMG 3301^T was 45.7%.

Both strains produce a strong band of identical size to the control from *B. melitensis* 16M^T in a PCR performed to determine the presence of the *Brucella* genus-specific *bscp31* gene (Baily *et al.*, 1992). Fingerprinting using probes against the *Brucella*-specific IS711 (Halling *et al.*, 1993) has been shown to be a useful tool for differentiating members of the genus *Brucella* at the species and/or subspecies level (Ouahrani *et al.*, 1993; Bricker *et al.*, 2000; Dawson *et al.*, 2008). By Southern blot, the two baboon isolates could be seen to share an identical high-copy-number IS711 profile, distinct from any profile seen previously (data not shown). Some of these IS711 copies have been located in the genome sequence of one of the baboon isolates (Audic *et al.*, 2011) and, while some IS711 chromosomal locations are common to other species of the genus *Brucella*, at least seven may be specific to the baboon isolates.

Two frequently applied multiplex PCRs were applied to the isolates. AMOS (*B. abortus*, *B. melitensis*, *B. ovis* and *B. suis*) PCR (Bricker & Halling, 1994; Ewalt & Bricker, 2000; Bricker *et al.*, 2003) with additional species-specific primers confirmed that the isolates are members of the genus *Brucella* because of the generation of the specific 180 bp amplicon. However, both isolates failed to produce any of the expected PCR products that would identify them as members of a known species (Schlabritz-Loutsevitch *et al.*, 2009). The strains were examined by Bruceladder PCR, a multiplex PCR that can differentiate all currently recognized species of the genus *Brucella* (López-Goñi *et al.*, 2008). The profile of bands obtained was identical to the profile of *B. melitensis* (data not shown); the existing Bruceladder protocol would therefore need to be adapted to distinguish the species represented by the new isolates. The novel IS711 localities described above give a straightforward means to do this.

Studies of DNA polymorphism at the *omp2* locus have also been used extensively to characterize the genus *Brucella*.

The baboon strains were shown uniquely to possess two almost-identical copies of *omp2b*, as observed previously for *B. ceti*, but distinct from the pattern of *B. ceti* in being more *omp2b*-like at the 3' end of both *omp2* gene copies (data not shown).

Both isolates were examined using an MLSA scheme that characterizes the sequences of 21 independent genomic fragments equating to >10.2 kbp (Whatmore *et al.*, 2007; Al Dahouk *et al.*, 2012). The two baboon isolates shared identical sequences at all loci, but possessed unique alleles at 11 of 21 loci not seen in characterization of over 700 isolates of the genus *Brucella* representing all known species and biovars. Phylogenetic analysis comparing the baboon isolates with type strains representing all extant species of the genus *Brucella* and strains of their biovars confirmed that they represent a well-separated lineage related most closely to *B. ovis* (Fig. 2).

Both isolates were typed using the MLVA assay described by Le Flèche *et al.* (2006) and Al Dahouk *et al.* (2007) comprising 16 loci (Scholz & Vergnaud, 2013) with an allele-coding convention following version 3.6 of the table for allele assignment at <http://mlva.u-psud.fr/brucella/spip.php?rubrique29>. All loci could be amplified with the exception of Bruce11. The MLVA genotype is 2–5–0–14–2–2–5–3 for the eight panel 1 loci (0 reflects lack of amplification at locus Bruce11), 6–36–9 for panel 2A and 2–2–3–4–10 or 2–2–3–5–11 for the most variable panel, 2B. The two strains differ only at loci Bruce16 and Bruce30, typical of the minor variation seen in closely related strains (García-Yoldi *et al.*, 2007; Maquart *et al.*, 2009). Fig. S1 shows the relative position of the two strains compared to approximately 1900 different MLVA16 genotypes. In this minimum spanning tree (MST), connecting lines longer than 6 are masked. Seven unconnected groups are

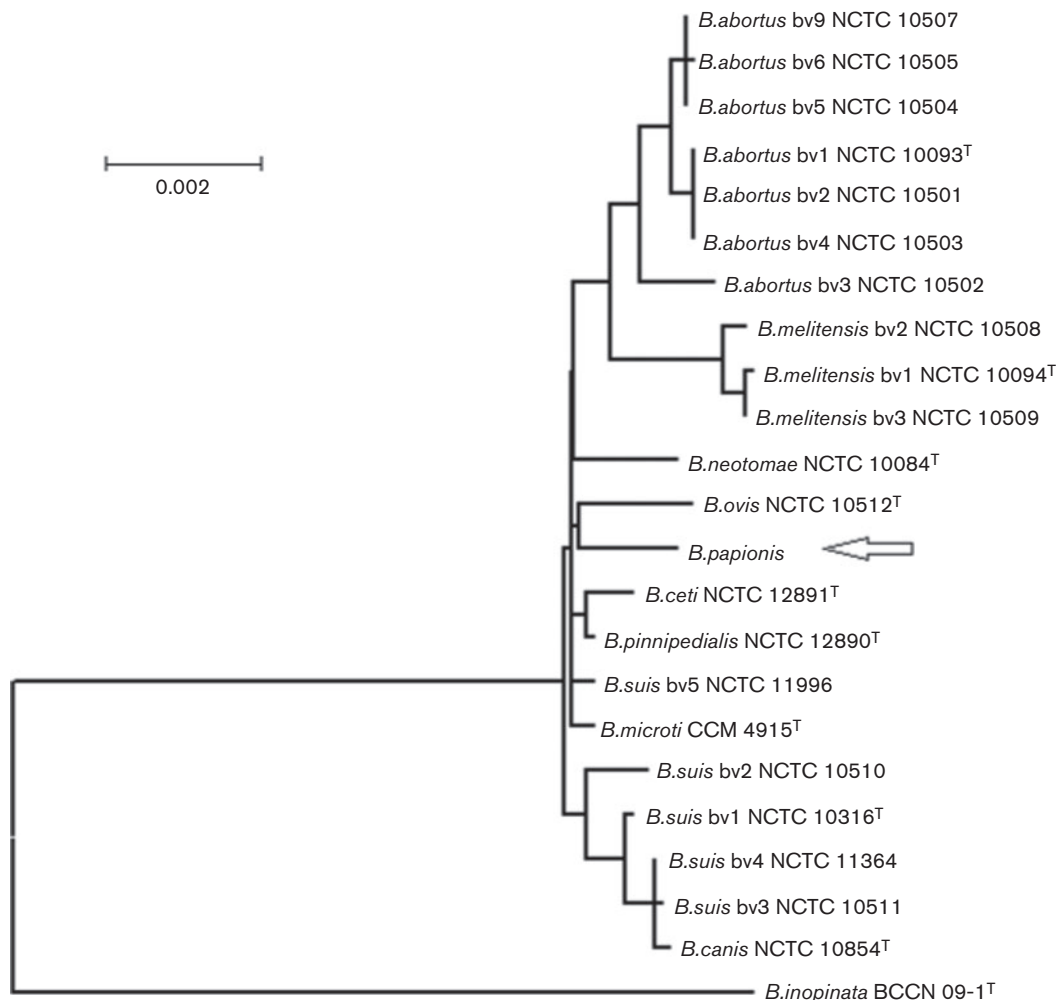


Fig. 2. Phylogenetic analysis of the baboon isolates (indicated by an arrow) in comparison with the type strains (see <http://icsp.org/subcommittee/brucella/taxa.html> for details of strains) of all species and biovars of the genus *Brucella* inferred by MLSA. Bar, 0.002 substitutions per nucleotide position. Analysis was performed with the MEGA5 package using the neighbour-joining approach.

identified. The largest one contains *B. melitensis*, *B. abortus*, *B. pinnipedialis*, *B. ceti*, *B. suis* biovar 5 and *B. microti*. The second largest is made up by *B. suis* biovar 2. The third group aggregates *B. canis* and *B. suis* biovars 1, 3 and 4. *B. inopinata*, *B. ovis*, *B. neotomae* and the novel strains constitute the last four groups.

In addition, one of the isolates (F8/08-60^T) was subjected to whole-genome sequencing. Two independent comparative genomic analyses have been published based on this sequence (Audic *et al.*, 2011; Wattam *et al.*, 2014), comparing this strain with the type strains of all other species of the genus *Brucella*. Both analyses confirmed the phylogenetic position suggested by MLSA, showing the baboon isolate and *B. ovis* united by an extremely shallow branch, indicating a shared common ancestor, but with the long branch length indicative of significant divergence since that time (data not shown). Further, the *in silico* MLVA genotyping tool accessible at <http://mlva.u-psud.fr> was applied to the whole-genome sequence (WGS). The deduced code was exactly as determined by PCR and electrophoresis size measurement, illustrating that tandem repeats in *Brucella* have been correctly assembled from the initial 454 WGS sequence data. Investigation of the WGS data showed that one of the two flanking sequences of the Bruce11 VNTR contains an approximately 141 bp deletion (101 bp of flanking sequence, 40 bp of 63 bp of the first repeat unit, plus potentially an unknown number of full repeat units). This explains the lack of amplification of Bruce11 in the novel strains, since one of the usual Bruce11 PCR primers is within this deletion. If primer 5'-GTAGCGATTGACACCTTGCCTG is used instead of 5'-CTGTTGATCTGACCTTGCAACC, an identical 396 bp PCR product is obtained from the two strains, whereas the Bruce11 PCR product is 93 bp longer in the other strains of *Brucella* (data not shown).

Conclusions

In summary, extensive phenotypic and genotypic analyses demonstrate that isolates F8/08-60^T and F8/08-61 are members of the genus *Brucella*, but can be differentiated clearly from all established species of this genus, including their biovars. Hence, by applying the species concept for the genus *Brucella* suggested by the ICSP Subcommittee on the Taxonomy of *Brucella* (Osterman & Moriyón, 2006), the two strains represent a novel species of the genus *Brucella*, for which we propose the name *Brucella papionis* sp. nov.

Description of *Brucella papionis* sp. nov.

Brucella papionis (pa.pi.o'nis. N.L. gen. n. *papionis* of the baboon, from which the first strains of this species were isolated).

Cocci, coccobacilli or short rods, ~0.5–0.7 µm in diameter and ~0.5–1.5 µm long, arranged singly and occasionally in pairs, small groups and chains. Gram-negative and resistant

to decolourization with 0.5% acetic acid. Non-motile and non-spore-forming. Aerobic. Does not require supplementary CO₂ for growth. Growth occurs at 30–37 °C. Colonies on SBA and Farrell's agar are visible at 3–4 days and are small, raised, circular, entire and convex, ~0.5–1 mm in diameter. Non-haemolytic and greyish in colour (on SBA) or honey-coloured (on Farrell's agar). No growth occurs on MacConkey agar. No growth in broth with 6.5% NaCl. Isolates do not grow in the presence of thionin or basic fuchsin at 1/50 000. Both known strains agglutinate with monospecific anti-A serum but not anti-M or anti-R serum. Cells are lysed by Wb, Bk₂ and Fi phage at routine test dilution (RTD). Cells are sensitive to doxycycline, rifampicin, ciprofloxacin and streptomycin. Catalase-positive. Oxidase-negative. Strongly urease-positive. Negative for indole hydrolysis. No production of H₂S. Acetoin is produced (Voges-Proskauer test). Nitrates are not reduced. No production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β-galactosidase, β-glucosidase or gelatinase. Positive (API 20E) for fermentation of L-arabinose and D-glucose (weak at 37 °C but not 30 °C) and variable, but weak, fermentation of D-sorbitol. No fermentation or oxidation of D-mannitol, inositol, L-rhamnose, sucrose, melibiose or amygdalin at 37 °C. No assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate or phenylacetic acid at 30 °C. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are produced. Alkaline phosphatase, lipase (C14), α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase and tryptophan deaminase are not produced. Differentiating physiological reactions (Micronaut BfR *Brucella* assay) of strains F8/08-60^T and F8/08-61 with respect to other species of the genus *Brucella* are given in Table S1.

The type strain is F8/08-60^T (=NCTC 13660^T=CIRMBP 0958^T), isolated in 2006 from a cervical swab taken from a baboon (*Papio* sp.) following stillbirth in an animal handling facility in Texas, USA.

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