The genomic structure of strains isolated from marine mammals gives clues to evolutionary history within the genus

Gisèle Bourg, David O'Callaghan, Maria Laura Boschioli

► To cite this version:

Gisèle Bourg, David O'Callaghan, Maria Laura Boschioli. The genomic structure of strains isolated from marine mammals gives clues to evolutionary history within the genus. *Veterinary Microbiology*, 2007, 125 (3-4), pp.375. 10.1016/j.vetmic.2007.06.002 . hal-00532274

HAL Id: hal-00532274

<https://hal.science/hal-00532274>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: The genomic structure of *Brucella* strains isolated from marine mammals gives clues to evolutionary history within the genus

Authors: Gisèle Bourg, David O'Callaghan, Maria Laura Boschioli

PII: S0378-1135(07)00303-3
DOI: doi:10.1016/j.vetmic.2007.06.002
Reference: VETMIC 3726

To appear in: *VETMIC*

Received date: 25-4-2007
Revised date: 30-5-2007
Accepted date: 4-6-2007

Please cite this article as: Bourg, G., O'Callaghan, D., Boschioli, M.L., The genomic structure of *Brucella* strains isolated from marine mammals gives clues to evolutionary history within the genus, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.06.002

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The genomic structure of *Brucella* strains isolated from marine mammals gives clues to evolutionary history within the genus.

Gisèle Bourg , David O'Callaghan ¹ and Maria Laura Boschioli²

Institut National de la Santé et de la Recherche Médicale, ESPRI 26, UFR de Médecine, CS 83021, Avenue J.F. Kennedy, 30908 NIMES Cedex 02, France

¹ Corresponding author: Institut National de la Santé et de la Recherche Médicale, ESPRI 26, UFR de Médecine, CS 83021, Avenue J.F. Kennedy, 30908 NIMES Cedex 02, France. Tel : 33 4 66 02 81 46 ; Fax : 33 4 66 02 81 48 ;
E mail: david.ocallaghan@univ-montp1.fr

² Current address: Unité Zoonoses Bactériennes, Agence Française de Sécurité Sanitaire des Aliments, 23 av. du Général de Gaulle, 94706 Maisons-Alfort cedex, France

1 **Abstract**

2 The genomic structure and the restriction maps were studied in 24 *Brucella* strains isolated
 3 from marine mammals. From *SpeI* restriction profiles, the strains could be ascribed to three
 4 clonal groups, each corresponding to a specific host. Cross contamination between
 5 exclusively terrestrial and exclusively marine hosts is unlikely suggesting the divergence of
 6 the different species of the genus *Brucella* may have taken place 60 million years ago,
 7 concomitant with the radiation of their mammalian hosts (Artiodactyla) from other
 8 mammalian orders.

9
 10 **Key words:** *Brucella*; genomic organisation; physical map; evolution; marine mammal;
 11 PFGE

12 13 **1. Introduction**

14 *Brucella* is a small Gram negative bacterium usually isolated from ruminants, pigs and
 15 rodents. Classically the genus is divided into six species which are further subdivided into
 16 several biovars, each infecting a preferential but not exclusive host (Boschiroli *et al.*, 2001).
 17 From the mid 1990s *Brucella*-like bacteria were isolated from carcasses of marine mammals,
 18 such as seals, dolphins, porpoises and whales. These bacteria have been found in a wide
 19 range of tissues and have been shown to cause both abortion and meningoencephalitis (Foster
 20 *et al.*, 2002; Ohishi *et al.*, 2003; Miller *et al.*, 1999; Gonzalez *et al.*, 2002) however very few
 21 human infections have been reported (Sohn *et al.*, 2003; Brew *et al.*, 1999; McDonald *et al.*,
 22 2006). These strains isolated from marine mammals could not be ascribed to the known six
 23 species based on classical typing methods but could, however, be subdivided into three
 24 groups based on CO₂ dependence, galactose oxidation, dominant antigen and animal host
 25 (Jahans *et al.*, 1997).

Over the past few years, molecular analysis using DNA/DNA hybridization and ribotyping (Verger *et al.*, 2000), genomic fingerprinting of *Xba*I profiles using PFGE (Jensen *et al.*, 1999), analysis of insertion sequence profiles (Cloeckaert *et al.*, 2000) and DNA polymorphism in outer membrane protein genes (Cloeckaert *et al.*, 2001a; Vizcaino *et al.*, 2004), has confirmed that the marine strains are genetically distinct from the terrestrial strains and, therefore, two new species, *Brucella pinnipediae* (group I strains) and *Brucella cetaceae* (group II and III strains) have been proposed (Cloeckaert *et al.*, 2001b). However, these names have not yet been validly published and there remains some debate about the genetic relationships within this group. Here we present the physical maps of the genomes of three representative strains of marine *Brucella* which suggest that the group II and III strains represent two distinct species.

2 Materials and Methods

2.1 Bacterial Strains

The *Brucella* strains used in this study and their hosts are listed in Table 1. Isolates were from seals, dolphins, porpoises, an otter and a minke whale (Jahans *et al.*, 1997; Clavareau *et al.*, 1998).

2.2 Pulsed Field Gel Electrophoresis

PFGE was performed as previously described (Jumas-Bilak *et al.*, 1995; Michaux-Charachon *et al.*, 1997). Briefly, intact genomic DNA was prepared from bacterial embedded in agarose plugs and subjected to PFGE (BioRad CHEF DRII), either undigested or after digestion with *Pac*I or *Spe*I. Pulse conditions are described in figure legends.

49 **2.3 Phylogenetic Analysis**

50 Similarity between strains was calculated as the Dice coefficient as described by Grothues
51 and Tummler (Grothues and Tummler, 1991) and clustered using the Phylip programme.

52

53 **3. Results and Discussion**

54 **3.1 Restriction fragment length polymorphism (RFLP) within marine *Brucella* strains.**

55 The genomes of *Brucella* marine strains contain two chromosomes with sizes of about 2.1
56 Mb and 1.15 Mb, similar to that of the reference strain of the genus, *B. melitensis* 16M (data
57 not shown). The macrorestriction profiles obtained for genomic DNA digested by *SpeI* are
58 shown in Fig. 1. The restriction patterns fell into three groups that were identical to the
59 groups defined by Jahans *et al* (Jahans *et al.*, 1997) based on classical typing methods. The
60 group I pattern was found in strains isolated from common, grey and hooded seals, and an
61 otter, group II pattern in strains isolated from common and striped dolphins and group III
62 pattern in strains isolated from porpoises, a white-sided dolphin and a minke whale. One 182
63 kb fragment was missing from two group I strains (39-94 and 44-94; isolated from common
64 seals). Strain 56-94 (isolated from a hooded seal) shows the most divergence from the group
65 as it lacks the 182 kb fragment, a 62 kb and has other minor differences in the 20-30 kb
66 region. The 62 kb fragment missing from strain 56-94 is specific to group I strains, since it
67 failed to hybridize with genomic DNA from any of the classical *Brucella* species or other
68 marine mammal strains (not shown). Comparison of the published *Brucella* genome
69 sequences (Paulsen *et al.*, 2002; Halling *et al.*, 2005; DelVecchio *et al.*, 2002) suggests that
70 such species specific regions are rare in the genus, however, we have previously reported a
71 34kb *SpeI* fragment specific to *B. ovis* (Michaux-Charachon *et al.*, 1997). Digestion of the
72 genomic DNA of the same strains by *XbaI* gives a similar strain grouping (Jensen *et al.*,

1999). In group II strains, an inversion in the large chromosome (see below) has increased the size of the 330kb fragment to 390 kb in strain 5-94.

75

3.2 Restriction mapping of the *Brucella* marine isolates. The *PacI* and *SpeI* restriction maps and localization of *rrn* loci and insertion sequences were determined as described previously (Michaux-Charachon *et al.*, 1997; Jumas-Bilak *et al.*, 1998). The maps are shown in Fig. 2, together with the previously published map of *B. melitensis* 16M (Michaux-Charachon *et al.*, 1997). The location of the restriction sites on the large chromosome of the marine strain shows few modifications when compared to that of *B. melitensis*. In a dolphin isolate, strain 5-94, there is a large inversion of at least 550 kb in the large chromosome. This rearrangement was first suggested by our observation that the 305 kb and 420 kb *SpeI* fragments from *B. melitensis* both hybridized with the 390 kb and 260 kb *SpeI* fragments of strain 5-94. The two fragments flanking the inversion contain copies of IS650/IS711, suggesting a possible role for the insertion sequence in the rearrangement. On the contrary, the maps of the small chromosome differed greatly from one strain to another, with numerous indels (including the 62 kb *SpeI* fragment unique to the seal isolates) and/or with creation or loss of restriction sites. The reason for this observation is unknown. All the strains contained three *rrn* loci, two in the large chromosome and one in the small.

91

3.3 Phylogenetic analysis of the marine *Brucella* isolates. A phylogenetic tree (Fig. 3) was obtained from the restriction data to complete the tree proposed previously (Michaux-Charachon *et al.*, 1997). The tree is unrooted, since the macrorestriction patterns are very specific for *Brucella* and definition of a related extra group to place the root is artificial. The marine isolates form a new group, which originates very near the *B. ovis* branch. Within this group, the three clones are separated from each other by branches that are deeper than those

98 between members of the *melitensis-abortionus* group or the *suis* group. This suggest a probable
 99 ancient divergence of the three restriction groups described within the marine isolates.

100

101 **3.4 Evolutionary history of the *Brucella* genus** The genus *Brucella* was proposed to be
 102 monospecific based on the high levels of homology revealed by DNA-DNA hybridization
 103 (Verger *et al.*, 1985). This, however, does not reflect the 'classical' classification based on
 104 phenotypic characteristics and host specificity and recently the 'classical' system has been
 105 readopted. Since *Brucella* grow poorly in the environment and since each species infects a
 106 preferential host, we and others proposed that each species is an evolutionary line adapted to
 107 a particular mammalian host (Michaux-Charachon *et al.*, 1997; Moreno *et al.*, 2002;
 108 Michaux-Charachon *et al.*, 2002). It has been proposed that *B. suis* is the closest to the
 109 common ancestor of the genus (Moreno *et al.*, 2002; Jumas-Bilak *et al.*, 1998). Recent
 110 investigation of the distribution of a 18.3 kb genomic island inserted downstream of *guaA*
 111 supports this theory, being present in the genomes of *B. suis*, *B. neotomae* and the marine
 112 strains, but not *B. ovis*, *B. abortus* and *B. melitensis* (Lavigne *et al.*, 2005). Interestingly,
 113 three point mutations in the 23S *rrn* gene group the marine strains with *B. melitensis*, *B. ovis*
 114 and *B. neotomae* (Halling and Jensen, 2006), giving rise to the question of whether the loss of
 115 the incP island occurred once or at different times during the evolution of the genus. The
 116 divergence could be concomitant either with the radiation of mammalian species, since each
 117 natural host harbors a particular clone, or with the emergence of domestication, allowing
 118 cross-contamination by close contact of different animal species. The phylogeny of the
 119 marine strains supports arguments for a divergence of the *Brucella* strains concomitant with
 120 the radiation of mammals, since each of the different hosts is infected by a specific clone and
 121 cross contamination between exclusively terrestrial and exclusively marine hosts is unlikely.
 122 Moreno et al (Moreno *et al.*, 2002) have suggested that *B. abortus* and *B. melitensis* became

isolated about 20 million years ago with the radiation of the artiodactyls. This isolation could be more ancient, since seals and otters belong to the order of Carnivora, which diverged from the Artiodactyla more than 60 million years ago, and since wild rodents, another anciently isolated order, are natural hosts for *B. suis*. However, a cross-contamination cannot be excluded in this last case, rodents and wild pigs having similar territories. Finally, our results further highlight how the results of the classical typing methods reflect the groups defined by genome organization. From the tree in Fig 3 (where the length of a branch represents evolutionary distance), we divergence between the three marine groups is far greater than that between, for example, *B. melitensis* and *B. abortus*, suggesting that the three groups could each be classified as a separate species. Further, recent data from both VNTR and MLST typing also suggest that the strains can be divided into three groups (A Whatmore, personal communication). These data are compatible with the proposition in the latest Bergey's Manual of three new species; *B. phocae* (seals), *B. phocoenae* (porpoises) and *B. delphini* (dolphins) (Corbel and Banai, 2005). Determination of the complete genome sequences of these, and more *Brucella* strains is the next step in deciphering their evolutionary history.

4. Acknowledgments

We thank Alistair Macmillan, Bruno Garin-Bastuji and Jacques Godfroid for providing *Brucella* strains. We thank Michel Ramuz for guidance and encouragement throughout this work. Maria Laura Boschioli was supported by fellowships from, CONICET and the UNU-BIOLAC. Work in our laboratory is supported by INSERM, the European Community (QLK2-CT-2001-01200), Université de Montpellier 1 (BQR), La Region Languedoc Roussillon and La Ville de Nîmes.

References

- Boschiroli, M.L., Foulongne, V., and O'Callaghan, D. (2001) Brucellosis: a worldwide zoonosis. *Curr Opin Microbiol* **4**: 58-64.
- Brew, S.D., Perrett, L.L., Stack, J.A., MacMillan, A.P., and Staunton, N.J. (1999) Human exposure to *Brucella* recovered from a sea mammal. *Vet Rec* **144**: 483.
- Clavareau, C., Wellemans, V., Walravens, K., Tryland, M., Verger, J.M., Grayon, M. *et al.* (1998) Phenotypic and molecular characterization of a *Brucella* strain isolated from a minke whale (*Balaenoptera acutorostrata*). *Microbiology* **144** (Pt 12): 3267-3273.
- Cloeckaert, A., Grayon, M., and Grepinet, O. (2000) An IS711 element downstream of the bp26 gene is a specific marker of *Brucella* spp. isolated from marine mammals. *Clin Diagn Lab Immunol* **7**: 835-839.
- Cloeckaert, A., Verger, J.M., Grayon, M., Paquet, J.Y., Garin-Bastuji, B., Foster, G., and Godfroid, J. (2001b) Classification of *Brucella* spp. isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes Infect* **3**: 729-738.
- Cloeckaert, A., Verger, J.M., Grayon, M., Paquet, J.Y., Garin-Bastuji, B., Foster, G., and Godfroid, J. (2001a) Classification of *Brucella* spp. isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes Infect* **3**: 729-738.
- Corbel, M.J., and Banai, M. (2005) *Genus I. Brucella Meyer and Shaw 1920, 173AL*. In Bergey's Manual of Systematic Bacteriology. Vol 2. Brenner D.J, Krieg N.R, and Staley J.T (eds). Springer, pp. 370-386.
- DelVecchio, V.G., Kapatral, V., Redkar, R.J., Patra, G., Mujer, C., Los, T. *et al.* (2002) The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc Natl Acad Sci U S A* **99**: 443-448.
- Foster, G., MacMillan, A.P., Godfroid, J., Howie, F., Ross, H.M., Cloeckaert, A. *et al.* (2002) A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. *Vet Microbiol* **90**: 563-580.
- Gonzalez, L., Patterson, I.A., Reid, R.J., Foster, G., Barberan, M., Blasco, J.M. *et al.* (2002) Chronic meningoencephalitis associated with *Brucella* sp. infection in live-stranded striped dolphins (*Stenella coeruleoalba*). *J Comp Pathol* **126**: 147-152.
- Grothues, D., and Tummler, B. (1991) New approaches in genome analysis by pulsed-field gel electrophoresis: application to the analysis of *Pseudomonas* species. *Mol Microbiol* **5**: 2763-2776.
- Halling, S.M., and Jensen, A.E. (2006) Intrinsic and selected resistance to antibiotics binding the ribosome: analyses of *Brucella* 23S rrn, L4, L22, EF-Tu1, EF-Tu2, efflux and phylogenetic implications. *BMC Microbiol* **6**: 84.
- Halling, S.M., Peterson-Burch, B.D., Bricker, B.J., Zuerner, R.L., Qing, Z., Li, L.L. *et al.* (2005) Completion of the genome sequence of *Brucella abortus* and comparison to

the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *J Bacteriol* **187**: 2715-2726.

Jahans, K.L., Foster, G., and Broughton, E.S. (1997) The characterisation of *Brucella* strains isolated from marine mammals. *Vet Microbiol* **57**: 373-382.

Jensen, A.E., Cheville, N.F., Thoen, C.O., MacMillan, A.P., and Miller, W.G. (1999) Genomic fingerprinting and development of a dendrogram for *Brucella* spp. isolated from seals, porpoises, and dolphins. *J Vet Diagn Invest* **11**: 152-157.

Jumas-Bilak, E., Maugard, C., Michaux-Charachon, S., Allardet-Servent, A., Perrin, A., O'Callaghan, D., and Ramuz, M. (1995) Study of the organization of the genomes of *Escherichia coli*, *Brucella melitensis* and *Agrobacterium tumefaciens* by insertion of a unique restriction site. *Microbiology* **141** (Pt 10): 2425-2432.

Jumas-Bilak, E., Michaux-Charachon, S., Bourg, G., O'Callaghan, D., and Ramuz, M. (1998) Differences in chromosome number and genome rearrangements in the genus *Brucella*. *Mol Microbiol* **27**: 99-106.

Lavigne, J.P., Vergunst, A.C., Bourg, G., and O'Callaghan, D. The 'incP island' in the genome of *Brucella suis* 1330 was acquired by site specific integration. *Infect.Immun.* 2005.

Ref Type: Generic

McDonald, W.L., Jamaludin, R., Mackereth, G., Hansen, M., Humphrey, S., Short, P. *et al.* (2006) Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *J Clin Microbiol* **44**: 4363-4370.

Michaux-Charachon, S., Bourg, G., Jumas-Bilak, E., Guigue-Talet, P., Allardet-Servent, A., O'Callaghan, D., and Ramuz, M. (1997) Genome structure and phylogeny in the genus *Brucella*. *J Bacteriol* **179**: 3244-3249.

Michaux-Charachon, S., Jumas-Bilak, E., lardet-Servent, A., Bourg, G., Boschioli, M.L., Ramuz, M., and O'Callaghan, D. (2002) The *Brucella* genome at the beginning of the post-genomic era. *Vet Microbiol* **90**: 581-585.

Miller, W.G., Adams, L.G., Ficht, T.A., Cheville, N.F., Payeur, J.P., Harley, D.R. *et al.* (1999) *Brucella*-induced abortions and infection in bottlenose dolphins (*Tursiops truncatus*). *J Zoo Wildl Med* **30**: 100-110.

Moreno, E., Cloeckaert, A., and Moriyon, I. (2002) *Brucella* evolution and taxonomy. *Vet Microbiol* **90**: 209-227.

Ohishi, K., Zenitani, R., Bando, T., Goto, Y., Uchida, K., Maruyama, T. *et al.* (2003) Pathological and serological evidence of *Brucella*-infection in baleen whales (*Mysticeti*) in the western North Pacific. *Comp Immunol Microbiol Infect Dis* **26**: 125-136.

Paulsen, I.T., Seshadri, R., Nelson, K.E., Eisen, J.A., Heidelberg, J.F., Read, T.D. *et al.* (2002) The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. *Proc Natl Acad Sci U S A* **99**: 13148-13153.

Sohn, A.H., Probert, W.S., Glaser, C.A., Gupta, N., Bollen, A.W., Wong, J.D. *et al.* (2003) Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerg Infect Dis* **9**: 485-488.

Verger, J.M., Grayon, M., Cloeckaert, A., Lefevre, M., Ageron, E., and Grimont, F. (2000) Classification of *Brucella* strains isolated from marine mammals using DNA-DNA hybridization and ribotyping. *Res Microbiol* **151**: 797-799.

Verger, J.M., Grimont, F., Grimont, P.A., and Grayon, M. *Brucella*, a monospecific genus as shown by deoxyribonucleic acid hybridization. *Int J Syst Bacteriol* **35**, 292-295. 1985.

Ref Type: Generic

Vizcaino, N., Caro-Hernandez, P., Cloeckaert, A., and Fernandez-Lago, L. (2004) DNA polymorphism in the omp25/omp31 family of *Brucella* spp.: identification of a 1.7-kb inversion in *Brucella cetaceae* and of a 15.1-kb genomic island, absent from *Brucella ovis*, related to the synthesis of smooth lipopolysaccharide. *Microbes Infect* **6**: 821-834.

Table 1. *Brucella* strains included in this study.

<i>Brucella</i> strain	Host Vernacular name	Scientific name
Group I*		
2-94	Common seal	<i>Phoca vitulina</i>
40-94	Common seal	<i>Phoca vitulina</i>
41-94	Common seal	<i>Phoca vitulina</i>
48-94	Common seal	<i>Phoca vitulina</i>
46-94	Common seal	<i>Phoca vitulina</i>
54-94	Common seal	<i>Phoca vitulina</i>
61-94	Grey seal	<i>Halicherus grypus</i>
55-94	Otter	<i>Lutra lutra</i>
39-94	Common seal	<i>Phoca vitulina</i>
44-94	Common seal	<i>Phoca vitulina</i>
56-94	Hooded seal**	<i>Cystophora cristata</i>
Group II		
47-94	Common dolphin	<i>Delphinus delphis</i>
14-94	Common dolphin	<i>Delphinus delphis</i>
59-94	Striped dolphin	<i>Stenella coeruleoalba</i>
5-94	Striped dolphin	<i>Stenella coeruleoalba</i>
Group III		
202-R	Mink whale	<i>Balaenoptera acutorostrata</i>
49-94	White-sided dolphin	<i>Lagenorhynchus actus</i>
1-94	Harbour porpoise	<i>Phocoena phocoena</i>
34-94	Harbour porpoise	<i>Phocoena phocoena</i>
35-94	Harbour porpoise	<i>Phocoena phocoena</i>
36-94	Harbour porpoise	<i>Phocoena phocoena</i>
45-94	Harbour porpoise	<i>Phocoena phocoena</i>
52-94	Harbour porpoise	<i>Phocoena phocoena</i>
57-94	Harbour porpoise	<i>Phocoena phocoena</i>

* Groups I, II and III contain strains with a similar *SpeI*-restriction pattern .

** The *SpeI* profile of this isolate differs from the other strains included in this group by the absence of a 62 kb fragment.

Figure Legends

Figure 1. Pulsed field gel electrophoresis of *SpeI*-digested DNA from *Brucella* marine isolates. *SpeI* fragments were separated in a contour clamped electric field apparatus (CHEF-DRII Biorad) with pulse ramps of 35 s - 4 s for 40 h at 180 V. The strains are organized as described in Table 1. λ concatamer size markers are on the left of the figure. Patterns of *SpeI*-digests of *B. melitensis* 16M, *B. abortus* 544 and *B. suis* 1330 DNA are shown at the right. Arrowheads indicate bands migrating differently or absent.

Figure 2. *SpeI-PacI* restriction maps of the chromosomes of *B. melitensis* 16M, strains 59-94 and 5-94 (isolated from dolphins), 34-94 (isolated from a porpoise) and 2-94 (isolated from a seal). The two circular chromosomes are represented in linear form, each one starting from a conserved *SpeI* fragment. A: large chromosomes; B: small chromosomes. For each chromosome map, *SpeI* sites are located above, and *PacI* sites below. Sizes are given in kilobases for all the restriction fragments of the *B. melitensis* chromosomes, and only for those different from *B. melitensis* for the other species. Fragments carrying *rrn* loci are represented by open squares, and fragments carrying IS6501/IS711 copies by stars. The inversion in the large chromosome of strain 5-94 is shown by two broken lines.

Figure 3. Phylogenetic tree of the marine isolates and the different species and biovars of the genus *Brucella* (modified from (Boschiroli *et al.*, 2001). The terrestrial strains are represented by the reference strains for each species and/or biovar using data from (Michaux-Charachon *et al.*, 1997). *B. abortus* contains two major groups, represented by Bv 1 and 5, due to the 600kb inversion in the small chromosome in certain strains.