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# Novel IS*711* Chromosomal Location Useful for Identification of Marine Mammal *Brucella* Genotype ST27, Which Is Associated with Zoonotic Infection[▽]

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**We report a novel IS*711* chromosomal location that is specific for the *Brucella* genotype ST27 previously associated with Pacific marine mammals and human zoonotic infection in New Zealand and Peru. Our data support the previous observation that this peculiar genotype is distinct from those commonly isolated from the Atlantic and currently classified within the species *B. ceti* and *B. pinnipedialis*.**

Brucellae are Gram-negative, facultative, intracellular bacteria that can infect humans and many species of animals. Six species were initially recognized within the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* (13, 19, 29). This classification is based mainly on differences in pathogenicity, host preference, and phenotypic characteristics. Four additional species have been included since 2007 in the genus *Brucella*. These comprise the species *B. ceti* and *B. pinnipedialis* isolated from marine mammals, with cetaceans (dolphin, porpoise, and whale species) and pinnipeds (various seal species) as the preferred hosts, respectively (11, 12). *Brucella microti*, first described in 2008, was isolated initially from the common vole but later also from the red fox and from soil (21–23). The latest species is *B. inopinata*, which was isolated from a human breast implant infection and represents the most distant *Brucella* species at the phenotypic and molecular levels relative to the others (10, 24). The animal or environmental reservoir of the latter species is not known. New *Brucella* species will likely be described in the future, including isolates from baboons (20), from wild rodents in Australia (26), and for strain BO2 isolated from a patient with chronic destructive pneumonia (27). Strain BO2 and strains from wild Australian rodents have been proposed as novel lineages of the *B. inopinata* species (26, 27).

Molecular and phenotypic typing of marine mammal *Brucella* strains has led to their classification into two species for strains originating mostly from the Atlantic, i.e., *B. ceti* and *B. pinnipedialis* (12). However, several subgroups within each species have been identified by molecular typing methods, such as multilocus sequence analysis (MLSA), multilocus variable number of tandem repeats (VNTR) analysis (MLVA), or *omp2a* and *omp2b* porin gene diversity analysis (2, 4, 6, 8, 9, 14, 15, 16, 18, 28, 32). Among them, one subgroup within *B. ceti*, which is composed exclusively of strains isolated from various dolphin species, has been proposed to constitute a separate species with the name *B. delphini* (2, 14, 29). The isolates from cetaceans from the Pacific may also constitute a separate species (16). Three human cases with naturally acquired infection by *Brucella* strains presumably of marine origin have been reported, one case of spinal osteomyelitis from a patient in New Zealand (18), and two neurobrucellosis cases from Peruvian patients (25). Interestingly, these human isolates presented the same genotype as a strain from a cetacean from the Pacific (30).

The presence of the mobile genetic element IS*711* (GenBank accession no. M94960) has been a useful target for molecular characterization of classical terrestrial mammal *Brucella* species and biovars based on the number and distribution of IS*711* copies within the bacterial genomes. Among classical *Brucella* species, IS*711*-based fingerprints are stable, species specific (except *B. canis*), and to some extent biovar specific (3). Marine mammal *Brucella* strains have been shown to carry a higher number of the IS*711* element in their genome than terrestrial mammal *Brucella* species and biovars, with the exception of *B. ovis* (3, 4, 9, 17). Several IS*711*-based molecular typing method studies have confirmed the classification into two marine mammal *Brucella* species with subgroups in each and correlating closely with classifications made using other molecular typing methods, such as MLSA and MLVA (32). It must be mentioned that this classification into *B. ceti* and *B. pinnipedialis* refers in particular to marine mammal strains isolated mostly from the Atlantic (around 300 strains isolated to date) (11, 16). Thus, for each of these marine mammal *Brucella* species and their subgroups, specific chromosomal IS*711* locations have been identified and PCR detection methods developed (5, 6, 9, 15, 32). However, for marine mammal isolates from the Pacific and the three human cases indicated above which are of the MLSA ST27 genotype, found to be specifically associated with these strains (30), no specific IS*711* location has been identified so far.

Recently, the complete genome sequence of *B. pinnipedialis* reference strain B2/94 has revealed the presence of 31 IS*711*

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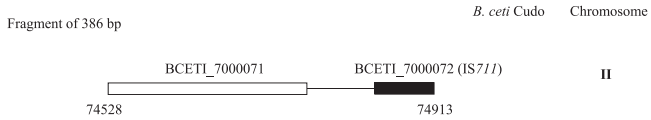


FIG. 1. Schematic representation of the IS*711*-containing DNA fragment used for the ST27-specific PCR in this study. The nucleotide positions relative to the genome sequence of *B. ceti* Cudo chromosome II (GenBank accession no. NZ_ACJD01000007) are indicated. Locus tag BCETI_7000071 codes for endonuclease V. Locus tag BCETI_7000072 corresponds to IS*711*.

TABLE 1. Primers used in this study

| Procedure and target region | Fragment size (bp) | Primer name | Nucleotide sequence (5′–3′) |
|---|---|---|---|
| PCR | | | |
| BCETI_1000667 to BCETI_1000668 (IS*711*) | 535 | ST27I-F | GACTATATGGTCGGCACGAT |
| | | IS3 | GGCTGTTCTCCTTCAACAAC |
| BCETI_2000198 to BCETI_1000199 (IS*711*) | 222 | ST27II-F | GAGGCTTGTCAGTGCAGAAA |
| | | IS3 | GGCTGTTCTCCTTCAACAAC |
| BCETI_7000071 to BCETI_7000072 (IS*711*) | 386 | ST27IV-F | GATAGCCAAGTCGGGCATAA |
| | | IS2 | GCTCACGGCTGTTCTCCTTT |
| BCETI_7000553 to BCETI_7000555 (IS*711*) | 283 | ST27V-F1 | AGAGCCATAGCGATGACCAC |
| | | IS2 | GCTCACGGCTGTTCTCCTTT |
| Southern blot | | | |
| IS*711* | 730 | ISA | TGTCTGCATTCAACGCAACC |
| | | ISB | GGATCGAAGCATATCTTCCG |
| BCETI_7000071 | 296 | ST27IV-F1 | GGTTGATGGCTATTGCTTCG |
| | | ST27IV-R1 | GGTTGCCGAATATCGTGAGT |

copies with specific or common chromosomal locations to other *Brucella* species or biovars (1). A phylogenetic analysis based on whole-genome sequences confirmed the MLSA- or MLVA-16-based molecular classification of clusters of marine mammal isolates. Interestingly, in this analysis, *B. ceti* strain Cudo, for which a high number of IS*711* elements were also located was found to be closely related to *Brucella* sp. strain F5/99 isolated from a common dolphin from the Pacific, which may be considered the marine mammal type strain of genotype ST27. The aim of this study was to identify IS*711* locations that would be specific of this ST27 genotype, taking advantage of other published complete *Brucella* genome sequences. Therefore, after genomic comparisons, several potential specific IS*711* locations were selected from the genome sequence of *B. ceti* strain Cudo for PCR screening. These locations corresponded to locus tags BCETI_1000668, BCETI_2000199, BCETI_7000072, and BCETI_7000555 (GenBank accession no. NZ_ACJD01000001, NZ_ACJD01000002, NZ_ACJD01000007, and NZ_ACJD01000007, respectively). A first set of PCRs were performed using a forward primer in the open reading frame upstream of IS*711* at these locations and the reverse IS*711*-specific primers IS2 or IS3 (Table 1). According to the PCR results for the four ST27 strains (common dolphin isolate F5/99 and the three human isolates) and representative strains of the other marine mammal ST genotypes described so far, only locus tag BCETI_7000072 appeared of interest (data not shown). This location is schematized in Fig. 1. It was further confirmed by sequencing of the PCR fragment of the human ST27 isolate 02/611 from New Zealand (GenBank accession no. JN383971). Consequently, a large panel of *Brucella* strains of marine mammal origin (Table 2) were used to screen this IS*711* location by PCR. They were from diverse marine mammal species (seal, otter, dolphin, porpoise, whale species) and of diverse geographic origins (France, Norway, Scotland). Bacterial culture and DNA extraction were performed as described previously (7). PCR was performed using forward primer ST27IV-F and reverse IS*711*-specific primer IS2 (Table 1). This PCR was found positive with a fragment of the expected size (386 bp) only for the four ST27 strains in this study. Reference strains for all terrestrial mammal *Brucella* species and biovars were also negative in this PCR (data not shown). The IS*711* ST27-specific location was further confirmed by Southern blotting on EcoRI-digested DNAs using an IS*711*-specific probe and a probe specific to the region upstream of this IS*711* element (primers shown in Table 1), and the latter probe detected an EcoRI fragment with a shift in size in ST27

TABLE 2. *Brucella* strains of marine mammal origin used in this study

| Species and strain | Host or source | Latin name | Geographic origin | MLVA-16 cluster (panel 1 genotype)[a] | MLSA ST[b] | *omp2b-omp2a* RFLP pattern[c] | IS*711* RFLP pattern[d] (cluster)[e] | IS*711* copy no.[d] | IS*711*-anchored PCR[f] | | | | New PCR specific to ST27 locus tag BCETI_7000072 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | A | B | C | D | |
| *B. pinnipedialis* | | | | | | | | | | | | | |
| 9a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 17a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 22a-2 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 23a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (75) | | P-I | C | 19 | + | − | − | − | − |
| 24a-2 | Hooded seal | *Cystophora cristata* | Norway | C3 (75) | | P-I | C | 19 | + | − | − | − | − |
| 25a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 30a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (74) | | P-I | C | 19 | + | − | − | − | − |
| 37a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 38g-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 39a-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| 53c-1 | Hooded seal | *Cystophora cristata* | Norway | C3 (76) | | P-I | C | 19 | + | − | − | − | − |
| M163/99/10 | Hooded seal | *Cystophora cristata* | Scotland | C3 (76) | ST25 | P-I | C (4) | 19 | + | − | − | − | − |
| M603/99/7 | Hooded seal | *Cystophora cristata* | Scotland | C3 (76) | ST25 | P-I | C (4) | 19 | + | − | − | − | − |
| M2006/94/6 | Hooded seal | *Cystophora cristata* | Scotland | C3 (76) | ST25 | P-I | C (4) | 19 | + | − | − | − | − |
| B2/94 (NCTC 12890; BCCN 94-73)[g] | Common seal | *Phoca vitulina* | Scotland | C2 (25) | | L-I | D (1) | 23 | + | + | − | − | − |
| M13/01/1 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | ST25 | L-I | D (1) | 23 | + | + | − | − | − |
| M336/94/1 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | | L-I | D (1) | 23 | + | + | − | − | − |
| M339/94/1 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | | L-I | D (1) | 23 | + | + | − | − | − |
| M445/99/2 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | ST25 | L-I | D (1) | 23 | + | + | − | − | − |
| M514/96/4 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | ST25 | L-I | D (1) | 23 | + | + | − | − | − |
| M972/94/1 | Common seal | *Phoca vitulina* | Scotland | C2 (25) | | L-I | D (1) | 23 | + | + | − | − | − |
| M621/99/2 | Gray seal | *Halichoerus grypus* | Scotland | C2 (25) | ST25 | L-I | D (1) | 23 | + | + | − | − | − |
| M1771/94/1 | Otter | *Lutra lutra* | Scotland | C2 (25) | ST25 | L-I | D (1) | 23 | + | + | − | − | − |
| M292/94/1 | Common seal | *Phoca vitulina* | Scotland | C1 (71) | ST24 | O-I | E (1) | 24 | + | + | − | − | − |
| M449/02/2 | Common seal | *Phoca vitulina* | Scotland | C1 (71) | | O-I | E (1) | 24 | + | + | − | − | − |
| M2466/93/4 | Common seal | *Phoca vitulina* | Scotland | C2 (72) | | L-I | E (1) | 24 | + | + | − | − | − |
| M2533/93/1 | Common seal | *Phoca vitulina* | Scotland | C1 (71) | ST24 | O-I | E (1) | 24 | + | + | − | − | − |
| M2375/94/3 | Gray seal | *Halichoerus grypus* | Scotland | C2 (25) | ST25 | L-I | E (1) | 24 | + | + | − | − | − |
| M194/00/1 | Gray seal | *Halichoerus grypus* | Scotland | C2 (25) | | L-I | E (1) | 24 | + | + | − | − | − |
| M192/00/1 | Minke whale | *Balaenoptera acutorostrata* | Scotland | C1 (71) | ST24 | O-I | E (1) | 24 | + | + | − | − | − |
| *B. ceti* | | | | | | | | | | | | | |
| M490/95/1 | Common seal | *Phoca vitulina* | Scotland | B (23) | ST23 | M-J | F (2) | 30 | + | − | + | − | − |
| 05-0684-1144 | Porpoise | *Phocoena phocoena* | France | B (23) | | M-J | F | 30 | + | − | + | − | − |
| M23/03/4 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M39/94/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | F (2) | 30 | + | − | + | − | − |
| M51/04/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M58/05/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F | 30 | + | − | + | − | − |
| M78/05/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |
| M93/04/3 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M117/01/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M165/03/6 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M195/03/10 | Porpoise | *Phocoena phocoena* | Scotland | B (70) | | M-J | F (2) | 30 | + | − | + | − | − |
| M234/05/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |
| M291/03/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M499/99/10 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M515/96/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |
| M615/99/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M854/98/8 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M1570/94/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7763/2 | Bottlenose dolphin | *Tursiops truncatus* | France | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |
| M452/97/2 | Common dolphin | *Delphinus delphis* | Scotland | B (23) | ST23 | M-J | F (2) | 30 | + | − | + | − | − |
| M2/00/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M52/01/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | | M-J | F (2) | 30 | + | − | + | − | − |
| M181/97/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | ST23 | M-J | F | 30 | + | − | + | − | − |
| M187/00/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | ST23 | M-J | F (2) | 30 | + | − | + | − | − |
| M2438/95/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | | M-J | F | 30 | + | − | + | − | − |
| B202R | Minke whale | *Balaenoptera acutorostrata* | Norway | B (23) | ST23 | M-J | F (2) | 30 | + | − | + | − | − |
| B1/94 (NCTC 12891; BCCN 94-74)[g] | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| M12/00/3 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| M38/04/3 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| M103/99/1 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| M199/04/2 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | ST23 | M-J | G | 29 | + | − | + | − | − |
| M1747/98/3 | Porpoise | *Phocoena phocoena* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| M870/97/1 | White-beaked dolphin | *Lagenorhyncus albirostris* | Scotland | B (23) | | M-J | G | 29 | + | − | + | − | − |
| M997/94/2 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (69) | | M-J | G (2) | 29 | + | − | + | − | − |
| M2788/97/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | B (23) | | M-J | G (2) | 29 | + | − | + | − | − |
| B14/94 | Common dolphin | *Delphinus delphis* | Scotland | A1 (24) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M13/05/1 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A1 (24) | ST26 | N-K | H | 26 | + | − | − | + | − |
| M40/95/1 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A1 (24) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M642/99/2 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A1 (24) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M654/99/1 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A2 (77) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M656/99/1 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A1 (24) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M2194/94/1 | Striped dolphin | *Stenella coeruleoalba* | Scotland | A1 (24) | ST26 | N-K | H (3) | 26 | + | − | − | + | − |
| M18/96/1 | White-sided dolphin | *Lagenorhyncus acutus* | Scotland | A1 (24) | | N-K | H | 26 | + | − | − | + | − |
| *Brucella* spp. | | | | | | | | | | | | | |
| F5/99 | Bottlenose dolphin | *Tursiops truncatus* | United States (West Coast) | | ST27 | Q-I | | | + | − | − | − | + |
| 02/611 | Human | *Homo sapiens* | New Zealand | (78) | ST27 | Q-I | I | 23 | + | − | − | − | + |
| 01A09163 | Human | *Homo sapiens* | Peru | | ST27 | | | | + | − | − | − | + |
| 85A05748 | Human | *Homo sapiens* | Peru | | ST27 | | | | + | − | − | − | + |

[a] According to Maquart et al. (16).
[b] According to Groussaud et al. (14).
[c] According to Cloeckaert et al. (8), Dawson et al. (9), and Zygmunt et al. (32).
[d] According to Maquart et al. (17) and Zygmunt et al. (32).
[e] According to Dawson et al. (9) for the strains indicated.
[f] PCRs A to D are a summary of data published in references 6, 9, 15, 18, 25, and 32. PCR ST27 is the novel IS*711*-anchored PCR of this study; the locus tag of the IS*711* position is indicated.
[g] Reference strains are given in parentheses.

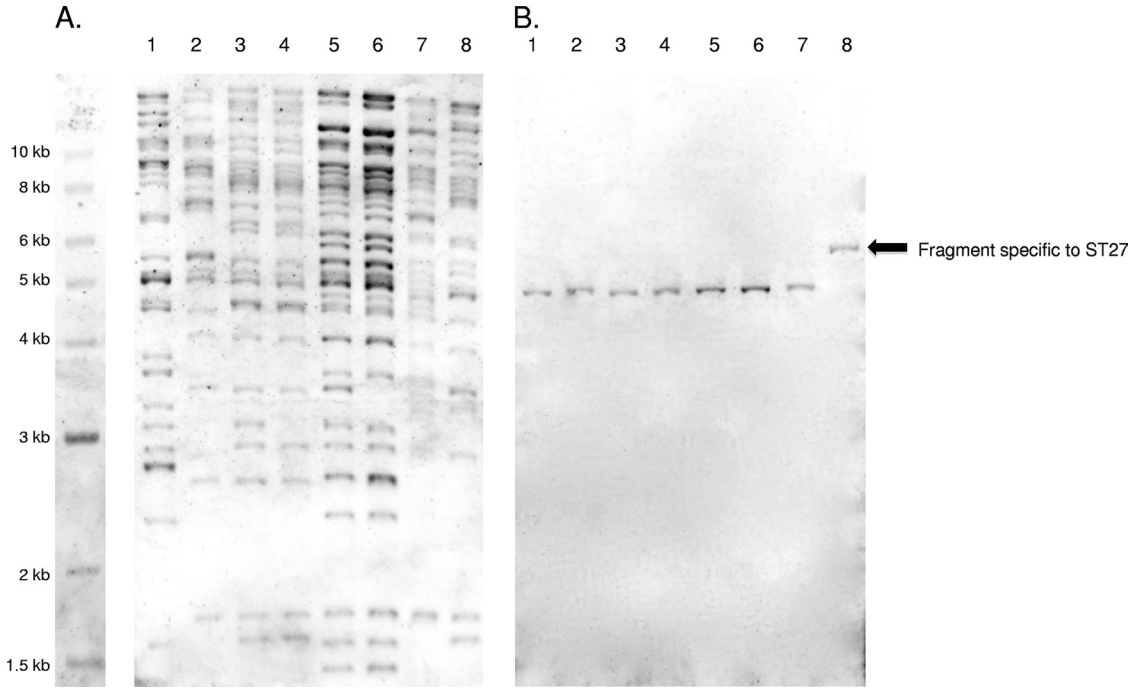


FIG. 2. Southern blot of EcoRI-digested DNAs using an IS*711*-specific probe (A) or a probe specific for region BCETI_7000071 (B) (Table 1). Strains studied were representatives of those carrying a high IS*711* copy number (Table 2) and are *B. ovis* 63/290 (lanes 1), *B. pinnipedialis* M163/99/10 (lanes 2), *B. pinnipedialis* B2/94 (lanes 3), *B. pinnipedialis* M292/94/1 (lanes 4), *B. ceti* M39/94/1 (lanes 5), *B. ceti* B1/94 (lanes 6), *B. ceti* B14/94 (lanes 7), and *Brucella* sp. 02/611 of genotype ST27 (lanes 8).

relative to the others as expected due to the presence of IS*711* (Fig. 2).

This novel IS*711* location, the distinct IS*711* locations in group of strains consisting only of dolphin isolates (proposed as *B. delphini*), and other molecular data support the suggestion of additional *Brucella* species in marine mammal *Brucella* isolates besides *B. ceti* and *B. pinnipedialis* (29). The specific IS*711* location of ST27 identified in this study, together with others previously reported (5, 6, 31, 32), may be of further use for the molecular identification of *Brucella* isolates of marine origin.

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