Small Gram-negative cocco-bacilli resembling *Brucella* strains have been reported from marine mammals since the mid-1990s. Their placement in the genus *Brucella* has been supported by the following characteristics: they are aerobic, non-motile and catalase-positive, do not produce acid from carbohydrates and have a DNA–DNA relatedness value of >77% with the six established members of the genus. Twenty-eight European isolates of the genus *Brucella* from marine mammals were distinguished from the six recognized species by their pattern of utilization of eleven substrates in oxidative metabolism tests and phage lysis. The 28 strains could be further separated into two groups with cetaceans and seals as their respective preferred hosts on the basis of molecular methods and on differences in the metabolism of L-arabinose, D-galactose and D-xylose. The names *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. are proposed for the isolates from cetaceans and seals, respectively. The type strain of *Brucella ceti* sp. nov. is NCTC 12891T (=BCCN 94-74T) and the type strain of *Brucella pinnipedialis* sp. nov. is NCTC 12890T (=BCCN 94-73T).
there was a unanimous verdict to return to the six species taxonomy for the genus *Brucella*. This lead to a recommendation from the *Brucella* Subcommittee that there should be a return to the pre-1986 taxonomy of the genus *Brucella* (Osterman & Moriyón, 2006). This Subcommittee recommendation implies the reapproval of the six classical *Brucella* nomenspecies with their recognized biovars; the classical names related to these six species having been validly published in the Approved List of Bacterial Names (Skerman et al., 1980, 1989). In this context, reference should also be made to the *Bacteriological Code* (1990 Revision) (Lapage et al., 1992) and an updated Taxonomic Note (Tindall et al., 2006).

*Brucella* infections of terrestrial mammals have long been recognized and have been researched extensively; however, it was only during the last few years of the twentieth century that the first reports of *Brucella* species from animals living in the marine environment were made. The first marine mammal isolations of *Brucella* strains came from common seals (*Phoca vitulina*), a porpoise (*Phocea phocea*) and a common dolphin (*Delphinus delphis*) in Scotland (Ross et al., 1994) and a captive bottlenose dolphin (*Tursiops truncatus*) in the USA (Ewalt et al., 1994). Since these first reports, there have been many more isolations and the range of hosts has expanded significantly (Foster et al., 2002). Additional species from which bacteriological cultures have proved positive include the Atlantic white-sided dolphin (*Lagenorhynchus acutus*), striped dolphin (*Stenella coeruleoalba*) (Foster et al., 1996), minke whale (*Balaenoptera acutorostrata*) (Clavareau et al., 1998; Foster et al., 2002), hooded seal (*Cystophora cristata*), grey seal (*Halichoerus grypus*) (Foster et al., 1996), Pacific harbour seal (*Phoca vitulina richardsi*) (Garner et al., 1997), ringed seal (*Phoca hispida*), harp seal (*Phoca groenlandica*) (Forbes et al., 2000; Maratea et al., 2003) and a European otter (*Lutra lutra*) (Foster et al., 1996). In addition, there have also been reports of human infections with strains from marine mammals (Brew et al., 1999; Sohn et al., 2003; McDonald et al., 2006).

Since their discovery, *Brucella* strains from marine mammals have been subjected to a range of characterization tests which have compared them with each other and also with the terrestrial *Brucella* species. Representative *Brucella* strains of marine mammals, which include the designated type strains of *B. ceti* sp. nov. and *B. pinnipedialis* sp. nov., have been shown by DNA–DNA hybridization to be related to the six classical *Brucella* nomenspecies at a level of >77 % DNA–DNA relatedness. The type strains of all these species form a homogeneous DNA–DNA relatedness group (Verger et al., 2000; Wayne et al., 1987). These results may be interpreted as evidence for the two proposed novel marine mammal *Brucella* species being affiliated to the genus *Brucella*. The appearance of these novel *Brucella* strains fits with previous descriptions of members of the genus *Brucella* made by Corbel & Brinley-Morgan (1975, 1984) and by Corbel & Banai (2005). The *Brucella* strains from marine mammals are small Gram-negative cocci, cocobacilli or short rods, 0.5–0.7 μm in diameter and 0.6–1.5 μm in length, non-motile, aerobic, catalase-positive, oxidase-positive, able to reduce nitrates, urease-positive, indole-negative, negative for gelatin liquefaction, do not produce acid from carbohydrates in conventional media and possess *Brucella* antigens, as demonstrated by reactions with *Brucella*-specific antisera.

Jahans et al. (1997) managed to distinguish the marine mammal strains from the terrestrial species of the genus *Brucella* on the basis of a substrate-specific oxidative metabolism test and suggested that they may belong to a separate species for which the name ‘*Brucella maris*’ was suggested but not formally proposed. Jahans further demonstrated that galactose metabolism served as a useful test to separate cetacean-derived species from those derived from seals. Ribotyping further suggested that marine strains of the genus *Brucella* may represent a separate group on the basis of their rRNA gene HindIII restriction pattern (Verger et al., 2000). Other distinctive characteristics at the molecular and genomic level have been provided by IS711 DNA fingerprinting which showed that a higher number of IS711 copies occur in the genomes of *Brucella* isolates from marine mammals than in strains isolated from land mammals (Clavareau et al., 1998; Bricker et al., 2000). A specific marker of marine mammal isolates has been identified, consisting of an IS711 element downstream of the bp26 gene (Cloeckaert et al., 2000). Most recently, a study of DNA polymorphism at the omp2 locus showed that the isolates from marine mammals can be classified into two major groups, one comprising isolates from seals that carry one copy of both the *omp2a* and *omp2b* genes and another group comprising the cetacean isolates carrying two copies of the *omp2b* gene (Cloeckaert et al., 2001). Both copies of the *omp2* gene were shown to contain motifs typical of marine mammal isolates. 16S rRNA gene sequence data, as well as recA gene sequences, identify the strains from marine mammals as belonging to the genus *Brucella* (Scholz et al., 2006).

With respect to the classification of species of the genus *Brucella* according to their preferential host, two new species names have been suggested, but not validly published: ‘*B. pinnipedialis*’ (for isolates from seals) and ‘*B. cetaceae*’ (for isolates from cetaceans) (Cloeckaert et al., 2001). Furthermore, development of an infrequent restriction site-PCR (IRS-PCR) method, taking into account the higher number of IS711 elements in the genome of isolates from marine mammals compared with *Brucella* species from terrestrial mammals, allowed the classification of the marine mammal isolates into two distinct clusters of strains. These two clusters were also distinct from the clusters representing the recognized *Brucella* species from terrestrial mammals (Cloeckaert et al., 2003). These two clusters of strains correlated well with the hosts from which they had been isolated, i.e. a cluster of isolates from cetaceans and a cluster of isolates from pinnipeds. Therefore, IRS-PCR confirmed the previous classification
of isolates from marine mammals, based on DNA polymorphism at the omp2 locus and their preferential hosts, into two novel species of the genus Brucella: 'Brucella cetaceae' for cetacean isolates and 'Brucella pinnipediae' for pinniped isolates (Cloeckaert et al., 2001).

Studies by Vizcaíno et al. identified an additional specific marker for 'B. cetaceae' found in most cetacean isolates consisting of a 1.7 kb inversion including a gene coding for one of the Omp25/Omp31 family of proteins (Vizcaíno et al., 2004). More recently, Jacques et al. (2007) used oxidative metabolism to confirm the findings of Jahans and provide further support for these two novel species of the genus Brucella. The approval of the taxonomic rank of species for each of these two groups of Brucella strains from marine mammals is in line with the recommendations made by the ad hoc Committees on approaches to taxonomy (Wayne et al., 1987; Murray et al., 1990; Stackebrandt et al., 2002) and with our current understanding of the evolution of the genus and of the relevant criteria for inclusion in this group (Moreno et al., 2002).

In view of the data presented above and in the descriptions of the novel isolates from marine mammals (given below), the characteristics of the two novel species meet the criteria for inclusion in the genus Brucella and conform to the minimal standards for the description of novel Brucella species as laid down by the Brucella Taxonomy Subcommittee (Corbel & Brinley-Morgan, 1975). The recommended investigation procedures have been used and have provided results that concur with the requirements for members of the genus Brucella as regards particular morphological features observable by electron microscopy, cultural characteristics and colony morphologies. Strains of the two novel species are obligate aerobes with an oxidative metabolism and have oxidative metabolic profiles that enable inter-species differentiation. The Brucella antigenic profiles and results from Brucella phage sensitivity tests, as well as results from classical Brucella biochemical tests (Table 1), also confirm their assignment to the genus Brucella. The facultatively intracellular behaviour of the proposed novel species is also in congruence with that of recognized members of the genus

Table 1. Differential characteristics of Brucella ceti sp. nov., Brucella pinnipedialis sp. nov. and other members of the genus Brucella

Data from Alton et al. (1975, 1988); Corbel & Banai (2005); Jacques et al. (2007); Meyer & Cameron (1961); Verger & Grayon (1977). Results are expressed as $QO_2$(N) values corresponding to microlitres of oxygen uptake per milligram of bacterial nitrogen per hour. +, $QO_2$(N) over 100 for all strains; -, $QO_2$(N) below 100 for all strains; D, doubtful, some strains showing $QO_2$(N) over 100, some strains showing $QO_2$(N) below 100; NL, no lysis; L, lysis; RTD, routine test dilution.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>B. ceti (17 strains)</th>
<th>B. pinnipedialis (11 strains)</th>
<th>B. melitensis</th>
<th>B. abortus</th>
<th>B. suis biovars</th>
<th>B. neotomae</th>
<th>B. ovis</th>
<th>B. canis</th>
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</thead>
<tbody>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+*</td>
<td>+</td>
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<td>+</td>
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<td>Lysis by phage at RTD:</td>
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<td>L-Alanine</td>
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<td>+</td>
<td>+</td>
<td>D</td>
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<td>L-Arginine</td>
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<td>+</td>
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<td>L-Asparagine</td>
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<td>+</td>
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<td>meso-Erythitol</td>
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<td>L-Glutamic acid</td>
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<td>L-Lysine</td>
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<td>DL-Ornithine</td>
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<td>D-Ribose</td>
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<td>D-Xylose</td>
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<tr>
<td>Preferred host</td>
<td>Cetaceans</td>
<td>Seals</td>
<td>Sheep, goats</td>
<td>Cattle</td>
<td>Swine</td>
<td>Swine, hares</td>
<td>Reindeer</td>
<td>Rodents</td>
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<td>Desert</td>
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</tbody>
</table>

*Except for reference strain 544 and occasional field strains which are negative.
†Lysis occurs with a few strains.
**Brucella**, as are the special characteristics of specific host preferences and virulence-pathogenicity.

The proposals of these two groups of marine mammal **Brucella** strains as being of the taxonomic rank of species of the genus *Brucella* are based on investigations according to the Subcommittee recommendation (Corbel & Brinley-Morgan, 1975) 'that strains should be classified into species on the basis of (i) preferred natural host, (ii) sensitivity to **Brucella** phages, and (iii) oxidative metabolic profiles'. The results of molecular investigations are also included. The proposal is in line with decisions (focusing on new methods of pheno-genotypic analysis) taken by the Subcommittee at the 2003 Pamplona meeting (Osterman & Moriyón, 2006), where the earlier acknowledged importance of host-preference and virulence-pathogenicity for the delineation of species of the genus *Brucella* was re-emphasized. The results are based on phenotypic and molecular tests on over 30 isolates collected from marine mammals in several European countries, thereby providing clear evidence of host preference for cetacean- and pinniped-derived strains, respectively.

Following the return to the six-species taxonomy based on the unanimous verdict of the International Subcommittee on the Taxonomy of *Brucella* (Osterman & Moriyón, 2006) and the reasons presented above, we formally propose two novel species of the genus *Brucella* for strains from marine mammals (with corrected etymology). The name *Brucella ceti* sp. nov. is proposed for *Brucella* strains with cetaceans as their preferred host and the name *Brucella pinnipedialis* sp. nov. is proposed for *Brucella* strains with pinnipeds as their preferred host.

**Description of Brucella ceti** sp. nov.

*Brucella ceti* [ce.ti. L. n. cetus large sea animal (whale, porpoise, dolphin); L. gen. n. ceti of a large sea animal].

Cocci, cocacobacilli or short rods, 0.5–0.7 μm in diameter and 0.6–1.5 μm in length. Arranged singly and, less frequently, in pairs, short chains or small groups. Gram-negative. Non-motile; does not produce flagella. Aerobic. Colonies on Columbia sheep blood agar and Farrell’s medium are normally visible after 3–4 days and are raised, convex, circular, entire and non-haemolytic. Nitrate reductase is produced. Most strains require supplementary CO₂ for growth. Growth is improved by the addition of serum or blood. Colonies on serum-glucose agar are transparent, raised, convex, with an entire edge and a smooth shiny surface and appear as a pale honey colour by transmitted light. Optimum temperature is 37°C. Growth occurs between 20 and 40°C. Optimum pH is between 6.6 and 7.4. Catalase-, oxidase- and urease-positive. H₂S negative. In oxidative metabolism tests, L-glutamic acid, D-ribose and meso-erythritol are oxidized, but L-alanine, L-arginine, L-asparagine, DL-ornithine, L-lysine, L-arabinose, D-galactose and D-xylose are not oxidized. Cultures are lysed by Iz and Wb phages, but no lysis occurs with R/C. A small number of strains exhibit lysis with the Tb phage. An antigen dominant. The preferred hosts are porpoises, dolphins and whales in which a range of pathologies occur.

The type strain, NCTC 12891T (=BCCN 94-74T), was isolated from a skin lesion on a harbour porpoise.

**Description of Brucella pinnipedialis** sp. nov.

*Brucella pinnipedialis* (pin.ni.ped.i’a’lis. N.L. fem. adj. pinnipedialis pertaining to pinnipeds). Cocci, cocacobacilli or short rods, 0.5–0.7 μm in diameter and 0.6–1.5 μm in length. Arranged singly and, less frequently, in pairs, short chains or small groups. Gram-negative. Non-motile; does not produce flagella. Aerobic. Colonies on Columbia sheep blood agar are normally visible after 3–4 days and are raised, convex, circular, entire and non-haemolytic with a diameter of 0.5–1.0 mm. Growth on Farrell’s medium is typically slower (7–10 days) or absent. Nitrate reductase is produced. Most strains require supplementary CO₂ for growth. Growth is improved by the addition of serum or blood. Colonies on serum-glucose agar are transparent, raised, convex, with an entire edge and a smooth shiny surface and appear as a pale honey colour by transmitted light. Optimum temperature is 37°C. Growth occurs between 20 and 40°C. Optimum pH is between 6.6 and 7.4. Catalase-, oxidase- and urease-positive. H₂S negative. In oxidative metabolism tests, L-glutamic acid, D-ribose and meso-erythritol are oxidized, but L-alanine, L-arginine, L-asparagine, DL-ornithine, L-lysine, L-arabinose, D-galactose and D-xylose are not oxidized.

The type strain, NCTC 12890T (=BCCN 94-73T), was isolated from the spleen of a common seal (*Phoca vitulina*).

**Acknowledgements**

We want to express our gratitude to Alastair MacMillan for the successful resurrection of the ICSP Subcommittee on the Taxonomy of *Brucella*, in this way making possible the *Brucella* Taxonomy Subcommittee recommendation for a return to pre-1986, classical *Brucella* taxonomy, thereby paving the way for each of the two groups of marine mammal *Brucella* strains to be approved as being of the taxonomic rank of species. We also wish to express our gratitude to Ignacio Moriñon and Mike Corbel for helpful discussions concerning this manuscript. The Scottish Strandings Scheme receives funding from the UK Department of Environment, Farming and Rural Affairs.

**References**


