Brucella ceti and Brucella pinnipedialis genome characterization unveils genetic features that highlight their zoonotic potential

Massimiliano Orsini1 | Andrea Ianni2 | Luca Zinzula3,4

1Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Microbial Ecology and Genomics, Legnaro, Italy
2Research Unit in Hygiene, Statistics and Public Health, Campus Bio-Medico di Roma University, Rome, Italy
3Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, Germany
4Centro di Educazione Ambientale e alla Sostenibilità (CEAS) Laguna di Nora, Pula, Italy

Correspondence
Massimiliano Orsini, Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Microbial Ecology and Genomics, Legnaro 35020, Italy.
Email: MOrsini@izsvenezie.it
Luca Zinzula, Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried 82152, Germany.
Email: zinzula@biochem.mpg.de

Abstract
The Gram-negative bacteria Brucella ceti and Brucella pinnipedialis circulate in marine environments primarily infecting marine mammals, where they cause an often-fatal disease named brucellosis. The increase of brucellosis among several species of cetaceans and pinnipeds, together with the report of sporadic human infections, raises concerns about the zoonotic potential of these pathogens on a large scale and may pose a threat to coastal communities worldwide. Therefore, the characterization of the B. ceti and B. pinnipedialis genetic features is a priority to better understand the pathological factors that may impact global health. Moreover, an in-depth functional analysis of the B. ceti and B. pinnipedialis genome in the context of virulence and pathogenesis was not undertaken so far. Within this picture, here we present the comparative whole-genome characterization of all B. ceti and B. pinnipedialis genomes available in public resources, uncovering a collection of genetic tools possessed by these aquatic bacterial species compared to their zoonotic terrestrial relatives. We show that B. ceti and B. pinnipedialis genomes display a wide host-range infection capability and a polyphyletic phylogeny within the genus, showing a genomic structure that fits the canonical definition of closeness. Functional genome annotation led to identifying genes related to several pathways involved in mechanisms of infection, others conferring pan-susceptibility to antimicrobials and a set of virulence genes that highlight the similarity of B. ceti and B. pinnipedialis genotypes to those of Brucella spp. displaying human-infecting phenotypes.

KEYWORDS
Brucella ceti, Brucella pinnipedialis, brucellosis, genome annotation, marine mammals, virulence factors

1 INTRODUCTION

Brucella ceti and Brucella pinnipedialis are Gram-negative bacteria known to infect cetacean and pinniped populations, respectively, and to cause brucellosis—a severe chronic disease—with major clinical signs such as meningoencephalitis, discospondylitis, sub-cutaneous abscesses, endometritis, and myocarditis, often resulting in affected animals stranding ashore and in fatal outcome (Foster et al., 2007; Guzmán-Verri et al., 2012; Nymo et al., 2011). As members of the Brucella genus within the Brucellaceae family, B. ceti and B. pinnipedialis are closely related to other species that cause brucellosis in terrestrial mammals and, occasionally, in humans (Cloeckaert et al., 2021). However, together with other marine unclassified lineages found in sea otters (Burgess et al., 2017; Miller et al., 2017), they...
constitute a subgroup that is unique in its ability to live in and spread through the aquatic environment (Guzmán-Verri et al., 2012; Nymo et al., 2011). Since the first described case in an aborted Bottle-nose dolphin (Tursiops truncatus) fetus in 1994 (Ewalt et al., 1994), B. ceti has been detected among a variety of species from both odontocetes (toothed whales) and mysticetes (baleen whales) groups, including the very recent first isolation from a Risso’s dolphin (Grampus griseus) and a Killer whale (Orcinus Orca) (Davison, Dagleish, Dale, et al., 2021), from a Minke whale (Balaenoptera acutorostrata) (Davison et al., 2017) and three Sowerby’s Beaked whales (Mesoplodon bidens) (Davison, Brownlow, et al., 2021). Nevertheless, human cases of B. ceti infection have been reported, either as a result of exposure to the pathogen in laboratory settings or naturally acquired and putatively related to raw shellfish consumption (Brew et al., 1999; McDonald et al., 2006; Sohn et al., 2003; Whatmore et al., 2008). Likewise, since its putative first detection in harbor seals (Phoca vitulina) in 1994 (Ross et al., 1994), B. pinnipedialis was reported to infect a variety of marine mammal species, including both true-seals (Foster et al., 2018; Hirvälä-Koski et al., 2017; Kroese et al., 2018; Lambourn et al., 2013) and eared-ones (Nymo et al., 2018) among pinnipeds, as well as cetaceans of both odontocetes and mysticetes suborders (Buckle et al., 2017; Davison, Dagleish, Ten Doeschate, et al., 2021; Whatmore et al., 2017). The increase of reported B. ceti and B. pinnipedialis infections in marine mammals, as well as their host range (Foster et al., 2018; Davison, Brownlow, et al., 2021; Davison, Dagleish, Ten Doeschate, et al., 2021; Mauroo et al., 2020) and geographical endemicity expansion (Jensen et al., 2013; Ohishi et al., 2016; Whatmore et al., 2017), are aspects of concern, since they may impact conservation efforts on most vulnerable species already threatened by anthropogenic factors, environmental elements (Van Bressem et al., 2009) or by other pathogens (e.g., morbilliviruses) (Zinzula et al., 2022). Furthermore, the ability of B. ceti, and potentially also of B. pinnipedialis, to spread across the human population by entering the food chain or after direct contact of humans with infected marine mammals represents a new potential zoonotic threat (Larsen et al., 2018; Maquart et al., 2008; Moreno, 2014; Nymo et al., 2016). Within this picture, undertaking a comprehensive characterization of the B. ceti and B. pinnipedialis genome is a preliminary and fundamental step to either understand the genetic features that contributed to the evolutionary success of these organisms infecting their hosts and spread through the marine environment or to identify the genetic basis of brucellosis pathological phenotypes. Although research efforts have recently focused in this direction, so far molecular investigations have mainly concentrated on genomic surveillance (Duvnjak et al., 2017; Maquart et al., 2008; Ueno, Kumagai, et al., 2020; Zygmunt et al., 2021) and molecular epidemiology (Garofolo et al., 2020; Kroese et al., 2018; Lambourn et al., 2013; Tian et al., 2019; Ueno, Yanagisawa, et al., 2020), evolutionary phylogenesis (Audic et al., 2011; Duncan et al., 2014; Suárez-Esquível et al., 2017; Whatmore et al., 2017) and more recently on the differences in pathogenesis between genotypes (Curtiss et al., 2022; Damiano et al., 2015; Ocampo-Sosa & García-Lobo, 2008), whereas functional characterization of B. ceti and B. pinnipedialis genes has gained less attention. Our study provides a comprehensive description of core- and pan-genome elements from all available (as of February 2022) B. ceti and B. pinnipedialis full genomes and raw sequencing data, with a specific focus on putative genetic determinants for virulence and pathogenesis. Our findings highlight the zoonotic potential of these emerging pathogens, whose ecological role at the human-wildlife interface poses a threat to the health of coastal communities worldwide.

2 | MATERIALS AND METHODS

2.1 | Available assemblies

The National Center for Biotechnology Information (NCBI) Assembly database (Kitts et al., 2016) was queried for the “B. ceti” and “B. pinnipedialis” keywords (first access on 23 June 2021, last access on 1 August 2022) and all available assemblies were downloaded (n = 15). When accessible, relevant metadata such as date of collection, country of isolation, host species, and pathology were retained.

2.2 | Raw sequences and assembling

The Sequence Read Archive (SRA) database (Leinonen et al., 2011) was queried with the “B. ceti” and “B. pinnipedialis” keywords and available experiments were further selected using the SRA Run selector imposing the Illumina platform, and metadata reporting at least the year of isolation and the geographic area. After download, the raw reads were trimmed using the fastp software (Chen et al., 2018), checked for contamination by Confindr software (Low et al., 2019), and only experiments showing a theoretical coverage, calculated on trimmed reads, equal or higher than 70X, and no contamination, were retained. Trimmed reads were de novo assembled using the Spades 3.15 software (Bankevich et al., 2012) with default parameters for 2 × 250 or 2 × 150 Illumina paired-end reads. Scaffolds longer than 200 bp were retained and polished by the Pilon software (Walker et al., 2014).

2.3 | Criteria for assembly inclusion into data sets

Both the downloaded genomes and those assembled in this study were evaluated by the Quast software (Gurevich et al., 2013), and only those showing an overall size of between 3.0 and 3.4 megabases, the number of contigs <250 and N50 > 125,000 were retained (n = 56). As an additional check, assemblies were analyzed to evaluate completeness and contamination by using the CheckM software (Parks et al., 2015), imposing at least 98% of completeness and contamination lower than 2%. Genome assemblies were also checked for the correct species assignment using the Minihash database (Ondov et al., 2016). Finally, the sequence type (ST) was extracted using the multi-locus sequence typing (MLST) software.
values were both set to 80% for considering a positive match. Finally, the integrated prophages were searched using the Phage Search Tool Enhanced release (PHASTER) web server (Arndt et al., 2016).

2.6 Graphics

Plots were drawn by Prism v.9.3.1 software (GraphPad). Phylogenetic trees were visualized with FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, last accessed on 10 May 2022). Marine mammal icons were retrieved from PhyloPic (credit: Chris huh; http://phylopic.org).

3 RESULTS

3.1 Characterization of B. ceti and B. pinnipedialis genomic data set

All B. ceti (n = 47) and B. pinnipedialis (n = 9) raw sequencing data available in the NCBI SRA database were downloaded and assembled and, together with already assembled B. ceti and B. pinnipedialis genomes (nine and six entries, respectively), made up the initial genomic data set for this study. Genome assemblies that did not match our inclusion criteria (see methods) were excluded from further analysis (n = 11). Overall, 60 genomic sequences were further characterized, including 50 B. ceti (Table 1) and 10 B. pinnipedialis genomes (Table 2). Metadata of this initial data set showed that samples were acquired over 26 years (1993–2018) and, when indicated, referred to a large sampling campaign from South America (Costa Rica, n = 20), several samplings from Europe (Italy, n = 8; Great Britain, n = 12; Norway, n = 2; Spain, n = 3) and one from Asia (Japan, n = 1). B. ceti samples were collected from specimens belonging to several cetacean species, mostly odontocetes (toothed whales) such as the striped dolphin (Stenella coeruleoalba, n = 31), the common bottlenose dolphin (T. truncatus, n = 3), the short-beaked common dolphin (Delphinus delphis, n = 3), the harbor porpoise (Phocoena phocoena, n = 3), and the Atlantic white-sided dolphin (Lagenorhynchus acutus, n = 1), but also including mysticetes (baleen whales) such as the common minke whale (B. acutorostrata, n = 1). Most B. ceti samples were isolated from the central nervous system (including the brain, vertebral lesions, and cerebrospinal fluid, n = 29) followed by the spleen (n = 4), skin or subcutaneous lesions (n = 3), lungworms (n = 2), lung (n = 1), liver (n = 1), uterus (n = 1), placenta (n = 1), and connective tissue (n = 1). At the time of collection, signs of neurobrucellosis were reported in most individuals (n = 23), one showing signs of focal necrosis in the spleen, the liver, and the lymph nodes, and one displaying osteomyelitis, while in a few others (n = 3), no Brucella-related pathology was observed. Notably, while most of the samples were putatively ascribable to cetacean specimens found either dead or live-stranded, one was taken from one T. truncatus kept in captivity in an aquarium and reported to be affected by osteomyelitis (Table 1). For B. pinnipedialis, samples were collected from specimens belonging to the species P. vitulina (n = 2) and the hooded seal (Cystophora cristata) (n = 1) among pinnipeds, to
| SRA ID     | Year | Country   | Host                  | Source             | Status         |
|------------|------|-----------|-----------------------|--------------------|----------------|
| ERR418023  | 2010 | Costa Rica| *Stenella coeruleoalba* | Cerebrospinal fluid | Neurobrucellosis|
| ERR418024  | 2011 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR418025  | 2012 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471312  | 2007 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471314a | 2014 | NA        | NA                    | NA                 | NA             |
| ERR471315a | 2014 | NA        | NA                    | NA                 | NA             |
| ERR471316  | 2011 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471317  | 2011 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471318  | 2012 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471319  | 2012 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471320  | 2013 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471321  | 2013 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471322  | 2013 | Costa Rica| *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471323  | 2013 | Costa Rica| *S. coeruleoalba*     | Lung worm          | Neurobrucellosis|
| ERR471324  | 2013 | Costa Rica| *S. coeruleoalba*     | Lung               | Neurobrucellosis|
| ERR471325  | 2012 | Spain     | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR471326  | 2012 | Spain     | *Tursiops truncatus*   | Vertebral abscess  | Neurobrucellosis|
| ERR471329  | 1994 | Great Britain| *Delphinus delphis* | Sub-cutaneous lesion | Asymptomatic   |
| ERR471330  | 1994 | Great Britain| *Phocoena phocoena* | Skin lesion        | NA             |
| ERR471333  | 2005 | Great Britain| *S. coeruleoalba*     | Brain              | NA             |
| ERR473728  | 2009 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR473729  | 2011 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR485943b | 2008 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR485944  | 2009 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR485945  | 2009 | Costa Rica | *S. coeruleoalba*     | Lung worm          | Neurobrucellosis|
| ERR485946  | 1994 | Great Britain| *D. delphis*          | Sub-cutaneous lesion | Asymptomatic   |
| ERR485947  | 2000 | Great Britain| *L. acutus*           | Spleen             | Focal necrosis |
| ERR485948  | 2005 | Great Britain| *P. phocoena*         | Vertebral lesion   | NA             |
| ERR485949  | 1995 | Norway     | *Balaenoptera acutorostrata* | Spleen, liver | NA             |
| ERR485951  | 1997 | Great Britain| *D. delphis*          | Spleen             | NA             |
| ERR554829  | 1994 | Great Britain| *P. phocoena*         | Uterus             | Asymptomatic   |
| ERR775242  | NA   | NA        | NA                    | NA                 | NA             |
| ERR775249  | NA   | NA        | NA                    | NA                 | NA             |
| ERR775250  | 2006 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| ERR775251b | 2007 | Costa Rica | *S. coeruleoalba*     | Cerebrospinal fluid | Neurobrucellosis|
| SRR11510450| 2018 | Italy      | *S. coeruleoalba*     | CNS                | NA             |
| SRR11510451| 2017 | Italy      | *S. coeruleoalba*     | CNS                | NA             |
B. acutorostrata \((n = 1)\) among cetaceans, and the Eurasian otter \((Lutra lutra)\) \((n = 1)\) among other aquatic mammals. Samples were isolated from the spleen \((n = 3)\) and lymph nodes \((n = 2)\), with one individual showing signs of brucellosis, one with focal necrosis in the liver, and the other two reported as asymptomatic (Table 2). Overall, the size of the genome assemblies in the data set ranged from 3.27 to 3.39 Mb (GC\%: 56.09–57.29), while the number of protein-coding genes returned in the annotation ranged from 3054 to 3251. Furthermore, for all but one sample, the MLST returned an assignment to an already established ST among the ST23, ST26, ST27, and ST24, ST25 for \(B. ceti\) and \(B. pinnipedialis\) respectively (Jolley & Maiden, 2010). The remaining sample (\(B. pinnipedialis\), GCF_015624465) could not be assigned to any ST due to gaps in one locus (Supporting Information: - Table S1: https://doi.org/10.6084/m9.figshare.21251391.v1).

### 3.2 Phylogenetic placement of \(B. ceti\) and \(B. pinnipedialis\) strains

We performed a phylogenetic placement of our genome data set against all sequences from members of the \(Brucella\) genus available in the NCBI assembly database (last access on 1 August 2022; full list in Supporting Information: Table S1: https://doi.org/10.6084/m9.figshare.21251391.v1), that matched inclusion criteria, based on SNPs. Results confirmed what was reported in other studies (Wattam et al., 2009, 2012), that is all the strains belonging to species related to an aquatic environment (i.e., \(B. ceti\) and \(B. pinnipedialis\)) group as a single monophyletic clade, clustering at the same level of the \(Brucella suis/Brucella canis\) group, and of the \(Brucella ovis\), \(Brucella abortus\), and \(Brucella melitensis\) clades (Figure 1). All other \(Brucella\) spp. sequences, including the only genome assembly available for \(Brucella microti\), position in early divergent separated clusters (Figure 1). Noteworthily, our phylogenetic analysis revealed that 10 NCBI assemblies previously annotated as \(Brucella\) spp. (GCA_000371045, GCA_000158995, GCA_000367125, GCA_000371005, and GCA_000367105) unequivocally cluster within the aquatic \(brucellae\) monophyletic clade, thereby expanding the current knowledge about the landscape of genomic diversity among those species. These were coming from Europe (Great Britain, \(n = 2\)) and North America (the United States, \(n = 1\)) and were sampled from \(P. vitulina\) \((n = 3)\) and \(L. acutus\) \((n = 1)\).

We, therefore, included these samples in the following analysis raising our final data set to 53 \(B. ceti\) and 12 \(B. pinnipedialis\) genomes,

### Table 1 (Continued)

| SRA ID          | Year | Country | Host           | Source     | Status |
|-----------------|------|---------|----------------|------------|--------|
| SRR11510452     | 2017 | Italy   | S. coerulealba | CNS        | NA     |
| SRR11510453     | 2017 | Italy   | S. coerulealba | CNS        | NA     |
| SRR11510454     | 2014 | Italy   | S. coerulealba | CNS        | NA     |
| SRR11510455     | 2013 | Italy   | S. coerulealba | CNS        | NA     |
| SRR13221976     | 2017 | NA      | NA             | Placenta   | NA     |
| SRR4038979      | NA   | NA      | NA             | NA         | NA     |
| SRR4038980      | NA   | NA      | NA             | NA         | NA     |
| SRR4038981      | NA   | NA      | NA             | NA         | NA     |
| SRR4039008      | NA   | NA      | NA             | NA         | NA     |

**B. ceti genomes already assembled (NCBI SRA)**

| Accession       | Year | Country | Host | Source       | Status       |
|-----------------|------|---------|------|--------------|--------------|
| GCA_000157835   | NA   | NA      | NA   | NA           | NA           |
| GCA_000157855   | NA   | NA      | NA   | NA           | NA           |
| GCA_000158755   | NA   | NA      | NA   | NA           | NA           |
| GCA_000158775   | NA   | NA      | T. truncatus | NA           |
| GCA_000182425   | NA   | NA      | S. coerulealba | Brain       |
| GCA_000590795   | NA   | Italy   | S. coerulealba | Brain       |
| GCA_000590815c  | 2012 | Italy   | S. coerulealba | Brain       |
| GCA_000662035c  | 2013 | NA      | NA   | NA           | NA           |
| GCA_014193785   | 2018 | Japan   | T. truncatus | Connective tissue | Osteomyelitis |

Abbreviation: CNS, central nervous system.

*Genomes excluded from the further analysis did not pass the criteria for species assignment.*

*Showed high contamination.*

*Showed a low level of completeness.*
respectively (Table 3). Similar results were observed for several other assemblies generically annotated as *B. spp*, which ended up clustering in well-defined terrestrial species clades (Figure 1). Moreover, in the monophyletic clade of the aquatic brucellae, *B. ceti* samples form a paraphyletic subclade, similarly to what was reported by Suárez-Esquível and colleagues in their multi-locus variant analysis (MLVA)-based analysis (Suárez-Esquível et al., 2017). Indeed, the *B. ceti* subclade shows a bipartite structure, where a large, compact group includes all ST26 samples, whereas a minor group is further divided into two sister *B. ceti* subgroups, gathering all ST23 and ST27 samples. Altogether the ST23 and ST27 *B. ceti* sister subgroups cluster as a sister group of the *B. pinnipedialis* (Figure 1).

Furthermore, ML analysis of *B. ceti* and *B. pinnipedialis* core genome-derived alignments allowed us to better sort out the relationship between samples. Interestingly, the *B. ceti* phylogeny confirmed the ST-related structure, except for the ST26. While this group showed a bipartite structure in the SNP-based analysis, it emerged from the ML analysis as a monophyletic group comprising both European and Costa Rica-related samples (Figure 2a). Likewise, the *B. pinnipedialis* SNP-based phylogeny showed the gathering of samples from different STs to the same clade and subclades (Figure 1). Conversely, the core genome ML-base analysis revealed clustering of the samples according to the ST, grouping ST24 and ST25 in two different subclades (Figure 2b).

### 3.3 | Characterization of *B. ceti* and *B. pinnipedialis* pan-genome

Annotation of the 53 *B. ceti* genomic samples resulted in a pan-genome composition of 3192 loci, of which 2841 (∼89%) are located in the strict-core genome (namely, 100% of the data set as a more conservative criterion instead of the canonical 95% commonly adopted for the core genome definition) (Brockhurst et al., 2019), 319 (∼10%) are in the accessory genome, and 32 (∼1%) are singlet genes annotated only in one sample each (Figure 3a). Since we adopted a conservative approach to defining the core genome, the accessory one was further divided into high-frequency accessory (HFA) and low-frequency accessory (LFA) genes (according to their presence in more or less than 50% of the data set), the two distinct groups accounting for 202 and 117 loci, respectively (Figure 3a).

Among them, three sets of genes characterizing the different *B. ceti* STs emerged. One set of 37 genes in the HFA subcategory, absent from those in ST23 and ST27, is unique to the ST26 samples (Figure 3b). These were mainly identified as uncharacterized proteins, with an exception made for some encoding for known proteins including the flgJ peptidoglycan hydrolase, the elastin, the virulence-associated protein E, and a Toll-interleukin-1 receptor (TIR) domain-containing protein (Supporting Information: Table S2: https://doi.org/10.6084/m9.figshare.21251394.v1). Another set of 20 genes,
observed in the LFA subcategory, is unique to the ST23 and ST27 samples and consists of several uncharacterized proteins and some with known functions, such as ATP-binding cassette (ABC) transporters, extracellular solute-binding proteins and an endoribonuclease (Figure 3b). Finally, a set of 63 genes, observed in the ST27 samples only and in addition to some uncharacterized proteins comprises ABC transporters, transposases, DNA binding and metabolism enzymes, regulators of transcription, amino-acids metabolism enzymes, peptidoglycan binding proteins, one porine and, noteworthy, one type II anti-toxin protein and one multidrug resistance efflux transporter family protein (Figure 3b and Supporting Information: Table S2: https://doi.org/10.6084/m9.figshare.21251394.v1).

The annotation of the 12 B. pinnipedialis genomic samples resulted in a pan-genome composition of 3151 loci, with a core genome of 2998 genes (∼95%), an accessory genome of 139 genes (∼4%), and just 13 singlets (∼0.4%) (Figure 3a). HFA and LFA genes accounted for ∼3.9%
and ~0.5% of the data set, respectively. Furthermore, no specific sets of genes in the accessory genome could be uniquely associated with any of the two ST24 and ST25 samples (Figure 3b). Among singlets of B. ceti (n = 32) and B. pinnipedialis (n = 12), 10 and 4 genes were found encoding for known proteins, respectively, whereas the remaining ones were either associated with hypothetical proteins or did not have any match within the NCBI nr database (Supporting Information: Table S2: https://doi.org/10.6084/m9.figshare.21251394.v1). Singlet genes from both species were excluded from subsequent analysis.

### 3.4 | B. ceti and B. pinnipedialis comparative pan-genome model

Comparative analysis of the total genes and conserved ones as a function of the genome number of aquatic B. ceti and B. pinnipedialis, as well as of terrestrial B. abortus, B. melitensis, and B. suis genomes, clearly showed that all species have a close genome (Figure 4). As shown by the alpha values calculated according to the Heaps law described by Tettelin and colleagues (Tettelin et al., 2008), B. abortus, B. melitensis, and B. suis genomes reach their plateau in the 3016–3067 pan-genome range, thereby featuring the criteria for closeness definition (Keller & Ankenbrand, 2021) (α_{B. abortus} = 1.644; α_{B. melitensis} = 1.253; α_{B. suis} = 2.000). Assignment of closeness to the B. ceti genome, which contains up to 3166 pan-genes, is the result of an alpha value that lies in between those of B. abortus and B. melitensis (α_{B. ceti} = 1.305). Yet, the B. ceti genome displays a clear step-wise trend, opening the possibility that it may be prone to horizontal gene transfer events (Figure 4). Possibly, such difference in the genome profile trend of B. ceti can be ascribed to the contribution brought by samples from the ST23 and ST27 groups of the data set, which are enough genetically divergent from each other and distant from ST26 samples (Figure 3b). B. pinnipedialis, whose genome possesses 3105 pan-genes, displays an alpha value identical to that of B. suis (α_{B. pinnipedialis} = 2.000) and a profile of continuous trend, which together clearly support genome closeness assignment (Figure 4). However, given that the number of available B. ceti and B. pinnipedialis genomic sequences is still limited, a statement about the closeness of their genome could still be susceptible to revision in case more sequencing data become available.

### 3.5 | Functional annotation of B. ceti and B. pinnipedialis pan-genome

Among B. ceti gene products, 2490 out of 2841 (87.6%) from the core genome, and 198 out of 319 (62.1%) from the accessory one, were annotated in the least one category of the COG database (Tatusov et al., 2000) (Figure 5a). Moreover, gene products belonging to multiple COG categories were 170 from the core genome and 22 from the accessory one (17 HFA and 5 LFA genes, respectively). Functional protein domains among B. ceti gene products were most frequently annotated as belonging to the COG category E (amino acid metabolism and transport), accounting for 11.8% of all annotated domains. Other frequently represented COGs were the R (general functional prediction only), M (cell wall/membrane/envelope biogenesis), G (carbohydrate metabolism and transport), J (translation), K (transcription), C (energy production and conversion), P (inorganic ion transport and metabolism), and the S (function unknown) categories. On average, each of these accounted for more than 6% of the core genome and 1.1%–9.0% of the accessory one (Figure 5a). Other COGs followed, each accounting for 0.03%–5.29% of the total annotated domains. Some of them were almost equally distributed in the three genome categories, such as the F (nucleotide transport and metabolism) and the Q (secondary metabolite biosynthesis transport and catabolism), whereas COGs such as the L (replication recombination and repair), X (mobility: prophages, transposons), N (cell motility), and W (extracellular structures) were represented by genes located almost exclusively in the accessory genome (Figure 5a). Annotation of the B. pinnipedialis pan-genome showed a similar distribution among the most frequent COG categories, with 2606 out of 2998 (86.9%) annotated gene products from the core genome, and 73 out of 139 (52.5%) from the accessory one. However, some differences with respect to the B. ceti genome were observed. COG categories T (signal transduction mechanisms), V (defense mechanisms), U (intracellular trafficking, secretion, and vesicular transport), W, D (cell cycle control), and B (chromatin structure and dynamics)
were exclusively represented by gene products from the core genome, the X was represented only by genes located in the accessory genome and the N was represented by genes equally distributed between the core and the accessory genome (Figure 5a).

Furthermore, analysis of the B. ceti pan-genome based on the KEGG database (Kanehisa et al., 2021) returned 1953 annotated proteins (61.2% of the submitted genes products) spread over 238 pathways, grouped in 40 categories and 66 complete modules (Figure 5b and Supporting Information: Table S3: https://doi.org/10.6084/m9.figshare.21251403.v1). Following a knowledge-based approach, the further analysis focused on pathways involved in bacterial virulence and pathogenesis. Among the annotated gene products, 40 were assigned to the quorum sensing pathway (KEGG 02024), 11 to biofilm formation pathways (KEGG 05111, 02025, and 02026), 7 to...

**FIGURE 2** Brucella ceti and Brucella pinnipedialis intra-species phylogenetic analysis. (a) B. ceti clusters into three clades corresponding to analogous STs, of which ST26 shows a monophyletic structure. ST26 genomes from Costa Rica and Europe are highlighted in deep and pale purple, respectively. ST23 and ST27 genomes are highlighted in pale pink and light magenta, respectively. (b) B. pinnipedialis clusters into two clades corresponding to ST24 and ST25, which are highlighted in green laurel and green mint, respectively. ST, sequence type.
FIGURE 3 (See caption on next page)
the chemotaxis pathway (KEGG 02030), and 32 to the flagellar assembly pathway (KEGG 02040). Noteworthily, \textit{B. \textit{ceti}} annotations included products encoded by genes belonging to the type III secretion system (T3SS) FliE-R and Flih operons, the FlgA-L operon that encodes for the rod, the P/L ring, and the hook parts of the flagellar machinery as well as products encoded by genes of the hook filament junction FlgK and FlgL operons, the filament part-coding FlIC gene, the MotA-C operon and the FliY regulator. Overall, 155 gene products were involved in pathways related to the Human Disease KEGG category, of which 22 were specifically linked to bacterial infectious disease pathways (KEGG 05120, 05130, 05131, 05132, 05132, 05133, 05134, 05150, 05152). Other genes, whose annotation points to more generic human pathways (e.g., Cardiovascular Disease or Cancer) were not taken into consideration, since their annotation process can be influenced by higher hierarchical categorization. Moreover, 24 gene products were associated with patterns for antimicrobial drug resistance, including 11 of the beta-lactam (KEGG 015), 5 of the vancomycin-resistance (KEGG 01502), and 8 of the cationic-antimicrobial (KEGG 01503) pathways. Nevertheless, none of the five gene products related to the vancomycin-resistance pathway is encoded by genes belonging to the Van operon (Courvalin, 2006), which may be indicative of a non-fully functioning related pathway. Similarly, while efflux pumps were among the gene products related to the beta-lactam resistance pathway (Gruenheid & Moual, 2012), the lack of products encoded by genes such as BlaI/Z and AmpR/C suggests that the metabolic pathway branch leading to the beta-lactam degradation is somehow interrupted. In addition, no gene products were assigned to any heavy-metal resistance pathway, with the only exception of three gene products putatively involved in conferring resistance to platinum (Supporting Information: Table S3: https://doi.org/10.6084/m9.figshare.21251412.v1). Overall, the KEGG assignment profile was identical to that of \textit{B. \textit{ceti}} genome annotation, with an exception made for the gene products TrbJ and TrbL only-lacking (two Type IV secretion system proteins) which are missing in the \textit{B. pinnipedialis} assignment to the KEGG 02024 quorum sensing pathway (Figure 5b).

### 3.6 \textit{B. \textit{ceti}} and \textit{B. pinnipedialis} virulence factors genes

To further characterize the \textit{B. \textit{ceti}} pan-genome for the presence of determinants of virulence and pathogenesis, our genomic data set was searched against the VFDB (Chen et al., 2005). Results showed 61 entries matching with genes encoding for well-established virulence factors identified within multiple bacterial species including those in the \textit{Brucella} genus (Figure 6a and Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1). Of these, 59 entries correspond to products of genes located in the \textit{B. \textit{ceti}} core genome, whereas two were products of HFA genes of the \textit{B. \textit{ceti}} accessory genome. Moreover, grouping the gene products according to the VFDB classification, showed that 32 of those genes are involved in immune modulation, including 31 core gene products related to the lipopolysaccharide (LPS) biosynthesis and the product of the ndvB/cgs gene, a cyclic beta-1,2-glucano synthase that interferes with the maturation of the phagosomal membrane, thus preventing the \textit{Brucella} containing vacuole (BCV) to fuse with lysosomes (Arellano-Reynoso et al., 2005). Noteworthy, \textit{B. \textit{ceti}} genes involved in LPS biosynthesis (Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1) displayed a 99%–100% sequence similarity with orthologs identified in \textit{B. \textit{melitensis}} bv.1, \textit{B. \textit{suis}} and \textit{B. \textit{abortus}}. Interestingly, the \textit{pmm} gene, conserved in all other \textit{Brucella} species and contributing to the LPS synthesis and its functions was not found in the \textit{B. \textit{ceti}} genome (Figure 6b). Conversely, the loci glmM_1 (manBcore gene) and

---

**FIGURE 3** Characterization of \textit{Brucella \textit{ceti}} and \textit{Brucella \textit{pinnipedialis}} pan-genome. (a) Relative composition of \textit{B. \textit{ceti}} (left panel) and \textit{B. \textit{pinnipedialis}} (right panel) pan-genome into core, HFA, LFA, and singlets gene categories. (b) Distribution of \textit{B. \textit{ceti}} (upper panel) and \textit{B. \textit{pinnipedialis}} (lower panel) gene categories within the genomic data set. HFA, high-frequency accessory; LFA, low-frequency accessory.

**FIGURE 4** \textit{Brucella \textit{ceti}} and \textit{Brucella \textit{pinnipedialis}} pan-genome model. Comparative analysis of the \textit{B. \textit{ceti}} and \textit{B. \textit{pinnipedialis}} pan-genome profile against that of zoonotic species \textit{B. \textit{abortus}}, \textit{B. \textit{melitensis}}, and \textit{Brucella \textit{suis}}; alpha values calculated according to the Heaps law are indicated.
Figure 5: COG categories and KEGG pathways in the *Brucella ceti* and *Brucella pinnipedialis* pan-genome. (a) Relative distribution of *B. ceti* (left panel) and *B. pinnipedialis* (right panel) core and accessory genes within the data set assigned to different COG categories. (b) Relative distribution of *B. ceti* (left panel) and *B. pinnipedialis* (right panel) core and accessory genes within the data set assigned to different KEGG pathways. COG, clusters of orthologous genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.
paralogue glmM_2, both ascribable to the bacterial pmm gene family—which encode for a phospho-mannomutase involved in the fructose-to-mannose conversion for the LPS biosynthesis—were found in all B. ceti genomes. In addition, 17 gene products grouped under the effector delivery system (EDS) category and showed a 95%–100% sequence similarity with B. melitensis and B. suis orthologs. Of these, 16 and 1 are encoded by genes respectively found in the core and in the accessory genome. According to their putative function, 13 encode for proteins involved in the VirB type IV Secretion System (T4SS), which are required at late stages in the Brucella life cycle and govern the interaction of the BCV with the endoplasmic reticulum (Boschirolli et al., 2002). Two gene products were found in all the samples, namely the vceA, and vceC, which are secreted effectors translocated into macrophages by the Brucella T4SS (de Jong et al., 2008). We identified the BtpA and BtpB gene products belonging to the EDS category, both acting as substrates for the VirB T4SS and able to interfere with Toll-like receptors (TLRs) signaling to mitigate the host inflammatory response (Atluri et al., 2011; Salcedo et al., 2013). Moreover, expression of the VirB T4SS itself is controlled by two gene products found in all samples, namely the bvrS and bvrR (Guzmán-Verri et al., 2002), both encoded by genes in the B. ceti core genome (Figure 6b and Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1). Furthermore, the ricA gene product was found, whose interaction with the host Rab2 protein affects the BCV maturation, thus decreasing intracellular replication rates and contributing to the evasion of the innate immune response (de Barsy et al., 2011). Interestingly, 6 genes, all located in the B. ceti core genome are related to the production of brucebactin, a highly-conserved siderophore firstly identified in B. abortus and putatively acting as an iron transporter in iron-limiting conditions at the beginning of the stationary growth phase (González Carreró et al., 2002). Four additional gene products, all but one encoded by genes in the B. ceti core genome, namely the ugpB, bhuA, flhP, and fliQ, are virulence factor candidates according to the VFDB classification. The latter two display significant sequence similarity with orthologs in Bartonella bacilliformis, a Gram-negative bacterium that causes febrile anemia in humans (Scherer et al., 1993). Noteworthily, analysis of the B. pinnipedialis data set returned an identical pattern of virulence factors (Figure 6a,b and Supporting Information: Table S6: https://doi.org/10.6084/m9.figshare.21251409.v1). Finally, a comparative analysis of virulence factors was performed against all available genomes from B. abortus, B. canis, B. melitensis, and B. suis. A non-zoonotic Brucella, we also included in our analysis the virulence factors profile of the only-available B. ovis genome. Results showed that B. ceti and B. pinnipedialis share the most virulence-related genes with zoonotic terrestrial species, mainly located in the core genome (Figure 6b). The gene BtpB, whose function is to inhibit the innate inflammatory response in alveolar macrophages through the TLR/nuclear factor kappa B (NF-kB)
pathway, and to suppress the formation of cellular reactive oxygen species (ROS) during Brucella infection (Li et al., 2022), is shared by B. ceti and B. pinnipedialis with B. abortus and B. ovis (Figure 6b). The gene RicA, which interacts with human Rab2 (de Barsy et al., 2011) and is also required by B. abortus for intracellular proliferation (Fugier et al., 2009), is exclusively shared by B. ceti and B. pinnipedialis with this terrestrial zoonotic Brucella (Figure 6b). Notably, despite all Brucella species being described as nonmotile organisms (Coloma-Rivero et al., 2021), we detected in all species the two genes flip and flfQ, type III secretion exporters belonging to the flagella pathway. Indeed, since flagellum is known to contribute to virulence, cell growth, and biofilm formation, it is deemed to provide Brucella with peculiar infection versatility (Coloma-Rivero et al., 2021). In addition, we found that the two genes are closely related to orthologs in species from the genus Bartonella, whose members are capable of wide-range interspecies transmission and long-lasting bacteremia (Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1, Supporting Information: Table S6: https://doi.org/10.6084/m9.figshare.21251409.v1). Virulence factors were also searched among pseudogenes (Supporting Information: Table S7: https://doi.org/10.6084/m9.figshare.21251397.v1) to investigate the possible lost role of pseudogenized loci. A total of 47 matches were found against VFDB and variably distributed within the data set. Of these, 10 were detected in all B. ceti and B. pinnipedialis samples, whereas 15 pseudogenes sporadically occurred each in one sample only. Conversely, some of the remaining matches showed a species-specific occurrence, with 12 pseudogenes related to virulence patterns observed in B. ceti samples only, and one pointing to a glucose/galactose transporter (gluP) exclusively detected in B. pinnipedialis. In addition, pseudogenes showed an ST-specific occurrence, with some putatively encoding for flagellar proteins (flhA, flgG, flfQ, and flaA) detected in the B. ceti ST26 samples only, and others putatively encoding for effectors of the type IV secretion system, such as the vceA protein, were detected only in the ST27 samples (Supporting Information: Table S7: https://doi.org/10.6084/m9.figshare.21251397.v1).

3.7 | B. ceti and B. pinnipedialis antimicrobial resistance (AMR) genes and prophage elements

The presence in our data set of genes encoding for products potentially involved in AMR was assessed by searching against the CARD (Alcock et al., 2020), Bacmet (Pal et al., 2014), and Resfinder (Bortolaia et al., 2020) databases. CARD database returned 2 hits as AMR candidates, which were present in all samples and whose genes are located in the B. ceti and B. pinnipedialis core genome. These are gyrA, which potentially confers resistance to the nalidixic acid (Khan et al., 2019), and mprF, which encodes for an integral membrane protein that confers resistance to cationic peptides (Ernst et al., 2009) (Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1, Supporting Information: Table S6: https://doi.org/10.6084/m9.figshare.21251409.v1). Among B. ceti gene products, only one match was returned by the Resfinder database, showing 100% identity to the tecC enzyme that confers resistance to tetracycline (Saenger et al., 2000). Notably, this gene product was detected in two samples only, tempting to speculate that B. ceti phenotypes may still display a substantial pan-susceptibility to antibiotics. By contrast, the tecC gene was not detected in any of the B. pinnipedialis samples (Supporting Information: Table S5: https://doi.org/10.6084/m9.figshare.21251406.v1, Supporting Information: Table S6: https://doi.org/10.6084/m9.figshare.21251409.v1). Comparison against the Bacmet database returned for both B. ceti and B. pinnipedialis samples the presence of five genes (namely, bepC, bepD, bepE, bepF, and bepG), all encoding for multi-drug efflux pumps and involved in the efflux of toxic and hydrophobic compounds, but also contributing to resistance to a drug such as a deoxycholate, sodium dodecyl sulfate and nalidixic acid (Martin et al., 2009; Posadas et al., 2007). Finally, the PHASTER web server (Arndt et al., 2016) was used to identify the B. ceti and B. pinnipedialis pan-genome prophage regions which can mobilize genetic determinants (von Wintersdorff et al., 2016). Although a substantial portion of the accessory genome of both species consisted of incomplete-prophage or questionable-prophage sequences—possibly representing signals of past infections—results showed no presence of intact prophages (Supporting Information: Table S8: https://doi.org/10.6084/m9.figshare.21251400.v1).

4 | DISCUSSION

The Brucella genus comprehends species such as B. melitensis, B. canis, B. abortus, and B. suis, which are all well-established zoonotic agents, but also species with peculiar host restrictions that – apart from sporadic cases of infection – hamper the spread of these bacteria across the human population (Moreno, 2014). Nevertheless, among these host-restricted species, the marine mammal-infecting B. ceti and B. pinnipedialis are emerging worldwide as pathogens of concern at the human-wildlife interface of coastal ecosystems (Guzmán-Verrí et al., 2012; Nymo et al., 2011). However, such interface is somehow limited as regards human interactions with marine mammals, and given the overall low prevalence of human brucellosis ascribable to B. ceti and B. pinnipedialis so far reported, a correct evaluation of the zoonotic potential of these organisms is of utmost importance (Głowacka et al., 2018). A key issue that needs to be addressed is whether the relative rarity of human infections with B. ceti and B. pinnipedialis is due to low levels of human exposure to the pathogen or rather to the poor ability of these bacteria to infect human hosts. On the one hand, evaluation of the level of exposure is not a trivial task, as it would require large epidemiological studies and extensive surveillance programs (Pereira et al., 2020). So far, most human cases of B. ceti infection have been attributed to the assumed consumption of seafood and, apart from cases that involved personnel working in aquatic parks, a clear correlation with a history of direct contact between the patient and infected marine mammals could not be made (Larsen et al., 2018; Maquart et al., 2008; Moreno, 2014; Nymo et al., 2016). On the other hand, thanks to a sufficient number
of *B. ceti* and *B. pinnipedialis* genomic sequences sampled and made available in databases, evaluation of the presence of genetic signatures of virulence offers a unique opportunity to decipher the molecular basis for their pathogenesis and to predict their impact on public health. In this work, we have undertaken a comprehensive characterization of all available *B. ceti* and *B. pinnipedialis* genomes. One main limitation of our study is the very few available data when compared to those obtainable for the related zoonotic species within the genus (i.e., *B. melitensis*, *B. canis*, *B. abortus*, and *B. suis*). Only about fifty *B. ceti* and a dozen of *B. pinnipedialis* samples were suitable for the analysis, with half of them coming from a single collection study (Costa Rica, 20 samples), one collected in an aquarium (Japan), and the remaining ones coming from marine mammals stranded mostly along European coasts. Noteworthily, when these samples were phylogenetically compared against all genomic assemblies within the *Brucella* genus, a further group of five samples, previously generically annotated in the NCBI assembly database as *B. spp* clustered in the aquatic *Brucella* clade. Therefore, although we were able to infer they belong to both *B. ceti* and *B. pinnipedialis*, these samples are representatives of the existing difficulties in subtyping such organisms. Moreover, albeit informative on the *B. ceti* and *B. pinnipedialis* zoonotic potential, the results obtained in this study cannot be yet representative of the overall population of the two pathogens presently circulating among free-ranging marine mammals worldwide. Notwithstanding the intrinsic limitations due to the number of samples and their geographic provenance, the genomic data set was however highly valuable in terms of temporal extension of samples collection (spanning over two-and-a-half decades) and the heterogeneity of the infected species, which included several odontocetes and mysticetes among cetaceans, and three ones among caniforms. In this regard, the metadata data set evidenced the absence of host restriction and an overall moderate tissue tropism. A paraphyletic relationship was established between *B. ceti* and *B. pinnipedialis* samples. Furthermore, intra-genus phylogeny revealed distribution over three *B. ceti* and two *B. pinnipedialis* different STs, with the 20 samples of the subgroup from Costa Rica showing substantial clonality. Analysis of the *B. ceti* and *B. pinnipedialis* pan-genome revealed the presence of an extended set of pathogenic molecular determinants, including genes for adherence, invasion, survival within host cells, and modulation of the immune response. Moreover, such a wide collection of pathogenic genes is markedly grouped in the core genome. This suggests that *B. ceti* and *B. pinnipedialis* pathogenic genes may account for the intrinsic genetic property of the two species, rather than being genetic features randomly acquired and placed within the accessory portion of their close genome. In addition, molecular determinants of virulence and pathogenesis showed high sequence similarity with their counterparts in *B. abortus*, *B. melitensis*, and *B. suis*/*B. canis*, thus enforcing the notion of *B. ceti* and *B. pinnipedialis* as potential zoonotic pathogens. This evidence is further supported by the fact that, as returned by the whole genome phylogeny analysis, *B. ceti* and *B. pinnipedialis* assemblies fall in a sister clade to that of *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* genomes. Given that these *Brucella* species were reported as capable of infecting human macrophages and epithelial cells (Larsen et al., 2013), as well as parasites of edible fish such as the Atlantic Cod (Gauds morhua) (Larsen et al., 2018; Nymo et al., 2016), investigations towards their zoonotic potential and capability of entering the human food chain should be intensified. Furthermore, we observed that the *B. ceti* and *B. pinnipedialis* genomes are pan-susceptible to all known antibiotics, and no evidence of recently acquired prophages was detected. Moreover, not all the assembled contigs were ascribable to known plasmids, which suggests that genetic determinants of virulence and pathogenesis cannot in principle be transmitted by conjugation. Nevertheless, we were able to identify antimicrobial genetic determinants in two *B. ceti* genomic samples from the Adriatic Sea, a Mediterranean region that is re-known for being subjected to high anthropogenic pressure (Basili et al., 2021; Bruschi et al., 2021). These aspects, combined with the genome closeness observed in our *B. ceti* and *B. pinnipedialis* genomic data set, lead to the hypothesis that these bacteria have a natural tendency to remain confined to their particular ecological niche with few opportunities for genomic exchange with other bacterial populations. However, it is tempting to speculate that, when exposed to anthropogenic pressures and high levels of microbial biodiversity, they may readily display capabilities for the lateral acquisition of antimicrobial resistance genes (Wattam et al., 2014). Altogether, these features should act as a warning in arguing that, under the convergence of specific ecological circumstances, *B. ceti* and *B. pinnipedialis* may represent a real threat to human health, thereby improving surveillance on all human activities that can raise the levels of exposure to these organisms a priority in the one-health agenda.

**AUTHOR CONTRIBUTIONS**

Massimiliano Orsini: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); project administration (lead); supervision (lead); validation (lead); writing—original draft (equal); writing—review & editing (equal).

Andrea Ianni: Investigation (equal); formal analysis (equal); validation (equal) project administration (equal); writing—review & editing (equal).

Luca Zinzula: Conceptualization (lead); data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); supervision (lead); validation (lead); writing—original draft (lead); writing—review & editing (lead).

**ACKNOWLEDGMENTS**

The authors want to thank Francesca Ellero for the fruitful discussion and the English language professional editing of the manuscript.

**CONFLICT OF INTEREST**

None declared.

**DATA AVAILABILITY STATEMENT**

All assemblies provided in this article are available under the NCBI BioProject PRJNA853936: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA853936. All data are provided in full in this paper except
for data in Supporting Information: Tables S1–S8, which are available in the figshare repository at https://doi.org/10.6084/m9.figshare.c.6226566
(Table S1: dataset metadata and assembly metrics. https://DOI.org/10.6084/m9.figshare.21251391.v1; Table S2: B. ceti and B. pinnipedialis pan-genome annotation. https://DOI.org/10.6084/m9.figshare.21251394.v1; Table S3: B. ceti pan-genome KEGG annotation. https://DOI.org/10.6084/m9.figshare.21251403.v1; Table S4: B. pinnipedialis pan-genome KEGG annotation. https://DOI.org/10.6084/m9.figshare.21251412.v1; Table S5: B. ceti virulence factors and resistance genes. https://DOI.org/10.6084/m9.figshare.21251406.v1; Table S6: B. pinnipedialis virulence factors and resistance genes. https://DOI.org/10.6084/m9.figshare.21251409.v1; Table S7: B. ceti and B. pinnipedialis pseudogenes putative matches against VFDB. https://DOI.org/10.6084/m9.figshare.21251397.v1; Table S8: B. ceti and B. pinnipedialis annotated prokaryotic sequences according to the PHASTER web server. https://DOI.org/10.6084/m9.figshare.21251400.v1).

ETHICS STATEMENT
None required.

ORCID
Massimiliano Orsini http://orcid.org/0000-0003-0087-3349
Luca Zinzulo http://orcid.org/0000-0001-6489-7070

REFERENCES
Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edelatmand, A., Huynh, W., Nguyen, A. L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfali, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., ... McArthur, A. G. (2019). CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research, 48(D1), D517–D525. https://DOI.org/10.1093/nar/gkz935
Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215, 403–410. https://DOI.org/10.1016/0022-2836(90)90360-2
Arellano-Reynoso, B., Lapaque, N., Salcedo, S., Briones, G., Ciocchini, A. E., Edalatmand, A., Huynh, W., Nguyen, A. L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfali, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., ... McArthur, A. G. (2019). CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research, 48(D1), D517–D525. https://DOI.org/10.1093/nar/gkz935
Audic, S., Lescot, M., Claverie, J. M., Cloeckaert, A., & Zygmont, M. S. (2011). The genome sequence of Brucella pinnipedialis B2/94 sheds light on the evolutionary history of the genus Brucella. BMC Evolutionary Biology, 11, 200. https://DOI.org/10.1186/1471-2148-11-200
Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. A., Lesin, V. M., Nikolenko, S. I., Pham, S., Pyshkin, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, 19, 455–477. https://DOI.org/10.1089/cmb.2012.0021
de Barys, M., Jamet, A., Filipon, D., Nicolas, C., Laloux, G., Rual, J. F., Muller, A., Twizere, J. C., Nkengfack, B., Vandenhaute, J., Hill, D. E., Salcedo, S. P., Gorvel, J. P., Letesson, J. J., & De Bolle, X. (2011). Identification of a Brucella spp. secreted effector specifically interacting with human small GTPase Rab2. Cellular Microbiology, 13, 1044–1058. https://DOI.org/10.1111/j.1462-5822.2011.01601.x
Basili, M., Campanelli, A., Frapiccini, E., Luna, G. M., & Quero, G. M. (2021). Occurrence and distribution of microbial pollutants in coastal areas of the Adriatic Sea influenced by river discharge. Environmental Pollution, 285, 117652. https://DOI.org/10.1016/j.envpol.2021.117672
Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R. L., Rebelo, A. R., Florensa, A. F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J. K., Stingl, K., Nguyen, M., Coppins, J., Xavier, B. B., ... Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy, 75, 3491–3500. https://DOI.org/10.1093/jac/dkaa345
Boschirioli, M. L., Ouahran-Bettache, S., Foulounge, V., Michaux-Charachon, S., Bourg, G., Allardet-Servent, A., Cazevieille, C., Liautard, J. P., Ramuz, M., & O’Callaghan, D. (2002). The Brucella suis virB operon is induced intracellularly in macrophages. Proceedings of the National Academy of Sciences, 99, 1544–1549. https://DOI.org/10.1073/pnas.032514299
Van Bressem, M., Raga, A., Di Guardo, G., Jeopson, P., Duignan, P., Siebert, U., Barrett, T., Santos, M., Moreno, I., Siciliano, S., Aguilar, A., & Van Waerebeek, K. (2009). Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Diseases of Aquatic Organisms, 86, 143–157. https://DOI.org/10.3354/dao02101
Brew, S. D., Perrett, L. L., Stack, J. A., MacMillan, A. P., & Staunton, N. J. (1999). Human exposure to Brucella recovered from a sea mammal. The Veterinary Record, 144, 483.
Brockhurst, M. A., Harrison, E., Hall, J. P. J., Richards, T., McNally, A., & MacLean, C. (2019). The Ecology and evolution of pangenomes. Current Biology, 29, R1094–R1103. https://DOI.org/10.1016/j.cub.2019.08.012
Bruschi, A., Lisi, I., De Angelis, R., Querini, S., Cossarini, G., Di Biagio, V., Salen, S., Soliddoro, C., Fassina, D., Ancona, S., & Silvestri, C. (2021). Indexes for the assessment of bacterial pollution in bathing waters from point sources: The northern Adriatic Sea CADEAU service. Journal of Environmental Management, 293, 112878. https://DOI.org/10.1016/j.jenvman.2021.112878
Buckley, K., Roe, W. D., Howe, L., Michael, S., Duignan, P. J., Burrows, E., Ha, H. J., Humphrey, S., & McDonald, W. L. (2017). Brucellosis in Endangered Hector’s dolphins (Cephalorhynchus hectori). Veterinary Pathology, 54, 838–845. https://DOI.org/10.1177/0300985817707023
Burgess, T. L., Johnson, C. K., Burdin, A., Gill, V. A., Doroff, A. M., Tuomi, P., Smith, W. A., & Goldstein, T. (2017). Brucella Infection in Asian Sea Otters (Enhydra lutris lutris) on Bering Island, Russia. Journal of Wildlife Diseases, 53, 864–868. https://DOI.org/10.7589/2016-09-220
Chen, L. (2004). VFDB: A reference database for bacterial virulence factors. Nucleic Acids Research, 33(Database issue), D325–D328. https://DOI.org/10.1093/nar/gki008
Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fstaf: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34, i884–i890. https://DOI.org/10.1093/bioinformatics/bty560
Cloeckaert, A., Zygmont, M. S., Scholz, H. C., Vizcaino, N., & Whatmore, A. M. (2021). Editorial: Pathogenomics of the Genus Brucella and Beyond. Frontiers in Microbiology, 12, 700734. https://DOI.org/10.3389/fmicb.2021.700734
Coloma-Rivero, R. F., Flores-Concha, M., Molina, R. E., Soto-Shara, R., Cartes, Á., & Oñate, Á. A. (2021). Brucella and its hidden flagellar
system. Microorganisms, 10(1), 83. https://doi.org/10.3390/microorganisms10010083

Cournil, P. (2006). Vancomycin resistance in gram-positive cocci. Clinical Infectious Diseases, 42(Suppl 1), S25–S34. https://doi.org/10.1086/491711

Curtiss, J, Colegrove, K, Dianis, A, Kinsel, M, Ahmed, N, Fauquier, D, Rowles, T, Niemeyer, M, Rotstein, D, Maddox, C, & Terio, K (2022). Brucella ceti sequence type 23, 26, and 27 infections in North American cetaceans. Diseases of Aquatic Organisms, 148, 57–72. https://doi.org/10.3354/dao03644

Damiano, M. A., Bastianelli, D., Al Dahouk, S., Köhler, S., Cloeckaert, A., Duncan, C. G., Tiller, R., Mathis, D., Stoddard, R., Kersh, G. J., Davison, N. J., Brownlow, A., Doeschate, M. T., Dale, E. J., Foster, G., and others (2018). First isolation of Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. International Journal of Systematic and Evolutionary Microbiology, 58, 2688–2693. https://doi.org/10.1099/ijs.6.05269-0

Fugier, E., Salcedo, S. P., de Chastellier, C., Pophillat, M., Muller, A., Arce-Genov, V., Fourquet, P., & Gorvel, J. P. (2009). The glycerolaldehyde-3-phosphate dehydrogenase and the small GTPase Rab2 are crucial for Brucella replication. PLoS Pathogens, 5(6), e1000487. https://doi.org/10.1371/journal.ppat.1000487

Gardner, S. N., Slezk, T., & Hall, B. G. (2015). kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. Bioinformatics, 31, 2877–2878. https://doi.org/10.1093/bioinformatics/btv271

Garofolo, G., Petrella, A., Lucifora, G., Di Francesco, G., Di Guardo, G., Pautasso, A., Iulini, B., Varello, K., Giorda, F., Goria, M., Dondo, A., Zoppi, S., Di Francesco, C. E., Giglio, S., Ferringo, F., Serrechìa, L., Ferrantino, M. A. R., Zilli, K., Janowicz, A., & Grattarola, C. (2020). Occurrence of Brucella ceti in striped dolphins from Italian Seas. PLoS One, 15(10), e0240178. https://doi.org/10.1371/journal.pone.0240178

Glowacka, P., Żakowska, D., Naylor, K, Niemcewicz, M., & Bielsawski-Drozd, A. (2018). Brucella—Virulence factors, pathogenesis and treatment. Polish Journal of Microbiology, 67, 151–161. https://doi.org/10.21307/pjm-2018-029

González Carreró, M. I., Sangari, F. J., Agüero, J., & García Lobo, J. M. (2002). Brucella abortus strain 2308 produces brucebactin, a highly efficient catecholic siderophore. Microbiology, 148, 353–360. https://doi.org/10.1099/00221287-148-2-353

Gruenheid, S., & Moul, H. (2012). Resistance to antimicrobial peptides in Gram-negative bacteria. FEMS Microbiology Letters, 330, 81–89. https://doi.org/10.1007/s00290-012-3018-x

Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). QUAST: Quality assessment tool for genome assemblies. Bioinformatics, 29, 872–875. https://doi.org/10.1093/bioinformatics/btt086

Guzmán-Verri, C., González-Barrientos, R., Hernández-Mora, G., Morales, J. A., Baquero-Calvo, E., Chaves-Olarte, E., & Moreno, E. (2012). Brucella ceti and brucellosis in cetaceans. Frontiers in Cellular and Infection Microbiology, 2, 3. https://doi.org/10.3389/fcimb.2012.00003

Guzmán-Verri, C., Manterola, L., Sola-Landa, A., Parra, A., Cloecckaert, A., Garin, J., Gorvel, J. P., Moriyón, I., Moreno, E., & López-Goñi, I. (2002). The two-component system BvrR/BvrS essential for Brucella abortus virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. Proceedings of the National Academy of Sciences, 99, 12375–12380. https://doi.org/10.1073/pnas.19243999

Hirvelä-Łosk, V., Nylund, M., Skrzypczak, T., Heikkinen, P., Kaulah, K., Jay, M., & Isomursu, M. (2017). Isolation of Brucella pinnipedialis from Grey seals (Halichoerus grypus) in the Baltic Sea. Journal of Wildlife Diseases, 53, 850–853. https://doi.org/10.7589/2016-06-144

Jensen, S., Nymo, I., Forcada, J., Hall, A., & Godfroid, J. (2013). Brucella antibody seroprevalence in Antarctic seals (Arctocephalus gazella, Leptonychotes weddellii and Mirounga leonina), Diseases of Aquatic Organisms, 105, 175–181. https://doi.org/10.3354/dao02633

Jolley, K. A., & Maiden, M. C. (2010). BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics, 11, 595. https://doi.org/10.1186/1471-2105-11-595

de Jong, M. F., Sun, Y. H., den Hartigh, A. B., van Dijl, J. M., & Tolsí, R. M. (2008). Identification of VceA and VceC, two members of the VjbR regulon that are translocated into macrophages by the Brucella type IV secretion system. Molecular Microbiology, 70, 1378–1396. https://doi.org/10.1111/j.1365-2958.2008.06487.x
Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). KEGG: Integrating viruses and cellular organisms. *Nucleic Acids Research*, 49(D1), D545–D551. https://doi.org/10.1093/nar/gkaa970

Keller, A., & Ankenbrand, M. J. (2021). Inferring core genome phylogenies for bacteria. Methods in *Molecular Biology*, 2242, 59–68. https://doi.org/10.1007/978-1-0716-1099-2_4

Khan, A. U., Shell, W. S., Melzer, F., Sayour, A. E., Ramadan, E. S., Eslchen, M. C., Moawad, A. A., Roessler, U., Neubauer, H., & El-Adawy, H. (2019). Identification, genotyping and antimicrobial susceptibility testing of Brucella spp. isolated from livestock in Egypt. *Microorganisms*, 7(12), 603. https://doi.org/10.3390/microorganisms7120603

Kitts, P. A., Church, D. M., Elschner, M. C., Moawad, A. A., Roesler, U., Neubauer, H., & El-Adawy, H. (2019). Brucella infection in marine mammals, with special emphasis on marine Brucella from a southern sea otter (Enhydra lutris nereis), California, USA. *Journal of Wildlife Diseases*, 55, 215–224. https://doi.org/10.7589/2015-12-326

Koerner, S., Stecher, G., Li, M., Knazy, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. https://doi.org/10.1093/molbev/msy096

Lambourn, D. M., Garner, M., Ewalt, D., Raverty, S., Sidor, I., Jeffries, S. J., Ryan, J., & Gaydos, J. K. (2013). Brucella pinnipedialis infections in Pacific harbor seals (Phoca vitulina richardsi) from Washington State, USA. *Journal of Wildlife Diseases*, 49, 802–815. https://doi.org/10.7589/2012-05-137

Larsen, A. K., Nymo, I. H., Briquemont, B., Sørensen, K. K., & Godfroid, J. (2013). Entrance and survival of Brucella pinnipedialis hooded seal strain in human macrophages and epithelial cells. *PLoS One*, 8(12), e84861. https://doi.org/10.1371/journal.pone.0084861

Larsen, A. K., Nymo, I. H., Sørensen, K. K., Seppola, M., Radven, R., Jiménez de Bagüés, M. P., Al Dahouk, S., & Godfroid, J. (2018). Concomitant temperature stress and immune activation may increase mortality despite efficient clearance of an intracellular bacterial infection in Atlantic Cod. *Frontiers in Microbiology*, 9. 2963. https://doi.org/10.3389/fmicb.2018.02963

Leinonen, R., Sugawara, H., & Shumway, M. (2011). The sequence read archive. *Nucleic Acids Research*, 39(Database issue), D19–D21. https://doi.org/10.1093/nar/gkr1019

Li, J., Zhang, G., Zhi, F., Zhai, Y., Zhou, D., Chen, H., Lin, P., Tang, K., Liu, W., Jin, Y., & Wang, A. (2022). BtpB inhibits innate inflammatory responses in goat alveolar macrophages through the TLR/NF-κB pathway and NLRP3 inflammasome during Brucella infection. *Microbial Pathogenesis*, 166, 105536. https://doi.org/10.1016/j.micpath.2022.105536

Low, A. J., Koziol, A. G., Manninger, P. A., Blais, B., & Carrillo, C. D. (2019). ConFindr: Rapid detection of intraspecies and cross-species contamination in bacterial whole-genome sequence data. *PeerJ*, 7, e6995. https://doi.org/10.7717/peerj.6995

Maqcart, M., Fardini, Y., Zygmont, M. S., & Cloeckaert, A. (2008). Identification of novel DNA fragments and partial sequence of a genomic island specific of *Brucella pinnipedialis*. *Veterinary Microbiology*, 132, 181–189. https://doi.org/10.1016/j.jvmicro.2008.04.015

Martin, F. A., Posadas, D. M., Carrica, M. C., Cravero, S. L., O’Callaghan, D., & Zorreguieta, A. (2009). Interplay between two RND systems mediating antimicrobial resistance in *Brucella suis*. *Journal of Bacteriology*, 191, 2530–2540. https://doi.org/10.1128/JB.00118-08

Mauroo, N. F., Poon, R. W. S., Beh, P. S. L., & Woo, P. C. Y. (2020). Detection of *Brucella* ceti in two Indio-Pacific Finless porpoises (*Neophocaena phocaenoides*) stranded in Hong Kong. *Journal of Wildlife Diseases*, 56, 698–701. https://doi.org/10.7589/2019-05-113

McDonald, W. L., Jamaludin, R., Mackereth, G., Hansen, M., Humphrey, S., Short, P., Taylor, T., Swingler, J., Dawson, C. E., Whatmore, A. M., Stobbenderf, E., Perrett, L. L., & Simmons, G. (2006). Characterization of a Brucella sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *Journal of Clinical Microbiology*, 44, 4363–4370. https://doi.org/10.1128/JCM.00680-06

Miller, M. A., Burgess, T. L., Dodd, E. M., Rhyain, J. C., Jang, S. S., Byrne, B. A., Guiland, F. M. D., Murray, M. J., Toy-Choutka, S., Conrad, P. A., Field, C. L., Sidor, I. F., & Smith, W. A. (2017). Isolation and characterization of marine Brucella from a southern sea otter (Enhydra lutris nereis), California, USA. *Journal of Wildlife Diseases*, 53, 215–224. https://doi.org/10.7589/2015-12-326

Moreno, E. (2014). Retrospective and prospective perspectives on zoonotic brucellosis. *Frontiers in Microbiology*, 5, 213. https://doi.org/10.3389/fmicb.2014.00213

Nagy, I., Sun, N., Varga, S., Boicu, M., Zinula, L., & Kukolya, J. (2019). Proteomics analysis of thermoplasma quinone droplets. *Proteomics*, 19(3), e1800317. https://doi.org/10.1002/pmc.201800317

Nymo, I. H., Radven, R., Beckmen, K., Larsen, A. K., Tryland, M., Quakenbush, L., & Godfroid, J. (2018). Brucella antibodies in Alaskan True seals and Eared seals-two different stories. *Frontiers in Veterinary Science*, 5, 8. https://doi.org/10.3389/fvs.2018.00008

Nymo, I. H., Seppola, M., Al Dahouk, S., Bakkemo, K. R., Jiménez de Bagüés, M. P., Godfroid, J., & Larsen, A. K. (2016). Experimental challenge of Atlantic Cod (Gadus morhua) with a Brucella pinnipedialis strain from Hooded seal (Cystophora cristata). *PLoS One*, 11(7), e0159272. https://doi.org/10.1371/journal.pone.0159272

Nymo, I. H., Tryland, M., & Godfroid, J. (2011). A review of Brucella infection in marine mammals, with special emphasis on Brucella pinnipedialis in the hooded seal (Cystophora cristata). *Veterinary Research*, 42(1). 93. https://doi.org/10.1186/1297-9716-42-93

Ocampo-Sosa, A. A., & García-Lobo, J. M. (2008). Demonstration of IS711 transposition in Brucella ovis and Brucella pinnipedialis. *BMC Microbiology*, 8, 17. https://doi.org/10.1186/1471-2180-8-17

Ohishi, K., Bando, T., Abe, E., Kawai, Y., Fujise, Y., & Maruyama, T. (2016). Long-term and large-scale epidemiology of Brucella infection in baleen whales and sperm whales in the western North Pacific and Antarctic Oceans. *Journal of Veterinary Medical Science*, 78, 1457–1464. https://doi.org/10.1292/jvms.16-0076

Ondov, B. D., Treangen, T. J., Melsted, P., Mallonee, A. B., Bergman, N. H., Pal, C., Bengtsson-Palme, J., Rensch, C., Kristiansen, E., & Larsson, D. G. J. (2014). BacMet: Antibacterial biocide and metal resistance genes database. *Nucleic Acids Research*, 42(Database issue), D737–D743. https://doi.org/10.1093/nar/gkt1252

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, 25, 1043–1055. https://doi.org/10.1101/gr.186072.114
Pereira, C. R., Cotrim de Almeida, J. V. F., Cardoso de Oliveira, I. R., Faria de Oliveira, L., Pereira, I. J., Zangherínimo, M. G., Lage, A. P., & Dorneles, E. M. S. (2020). Occupational exposure to Brucella spp.: A systematic review and meta-analysis. PLoS Neglected Tropical Diseases, 14(5), e0008164. https://doi.org/10.1371/journal.pntd.0008164

Posadas, D. M., Martín, F. A., Sabio y García, J. V., Spera, J. M., Delpino, M. V., Baldi, P., Campos, E., Cravero, S. L., & Zorreguieta, A. (2007). The TolC homologue of Brucella suis is involved in resistance to antimicrobial compounds and virulence. Infection and Immunity, 75, 379–389. https://doi.org/10.1128/IAI.01349-06

Ross, H., Foster, G., Reid, R., Jahans, K., & MacMillan, A. (1994). Brucella species infection in sea-mammals. Veterinary Record, 134, 359. https://doi.org/10.1136/vr.134.14.359-b

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. BMC Bioinformatics, 15, 206–2069. https://doi.org/10.1007/bioinfor matics/btu153

Seemann, T. (2022). mlst GitHub. https://github.com/seemann/mlst

Snipe, L., & Liland, K. H. (2015). micropan: An R-package for microbial pan-genomics. BMC Bioinformatics, 16, 79. https://doi.org/10.1186/s12859-015-0517-0

Sohn, A. H., Probert, W. S., Glaser, C. A., Gupta, N., Bollen, A. W., Wong, J. D., Grace, E. M., & McDonald, W. C. (2003). Human neuroimmunomodulation of Bartonella bacilliformis flagella and effect of antiflagellin operator system. Nature Structural Biology, 10, 4971. https://doi.org/10.1038/nsb.1210

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis of large phylogenies. Bioinformatics, 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu333

Suárez-Esquivel, M., Baker, K. S., Ruiz-Villalobos, N., Hernández-Mora, G., Barquero-Calvo, E., González-Barrientos, R., Castillo-Zelédon, A., Jiménez-Rojas, C., Chacón-Díaz, C.,Cloeckaert, A., Chaves-Olarte, E., Thomson, N. R., Moreno, E., & Guzmán-Verri, C. (2017). Brucella genetic variability in wildlife marine mammals populations relates to host preference and ocean distribution. Genome Biology and Evolution, 9, 1901–1912. https://doi.org/10.1093/gbe/evx137

Syberg-Olsen, M. J., Garber, A. I., Keeling, P. J., McCutcheon, J. P., & Husnik, F. (2022). Pseudofinder: Detection of pseudogenes in prokaryotic genomes. Molecular Biology and Evolution, 39(7), msac153. https://doi.org/10.1093/molbev/msac153

Tatusov, R. L. (2000). The COG database: A tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Research, 28, 33–36. https://doi.org/10.1093/nar/28.1.33

Tettelin, H., Riley, D., Cattuto, C., & Medini, D. (2008). Comparative genomics: The bacterial pan-genome. Current Opinion in Microbiology, 11, 472–477. https://doi.org/10.1016/j.mib.2008.09.006

Tian, G. Z., Lu, D. Y., Piao, D. R., Zhao, H. Y., Yang, X. W., & Jiang, H. (2019). Epidemiological characteristics of Brucella species isolated from different regions of the world using the MLVA genotyping. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi, 40, 676–681. (Chinese) https://doi.org/10.3760/cma.j.issn.0254-6450.2019.06.014

Tonkin-Hill, G., MacAlasdair, N., Ruis, C., Weimann, A., Horesh, G., Lees, J. A., Gladstone, R. A., Lo, S., Beaudoin, C., Floto, R. A., Frost, S. D. W., Corander, J., Bentley, S. D., & Parkhill, J. (2020). Producing polished prokaryotic pan-genomes with the Panaroo pipeline. Genome Biology, 21(1), 180. https://doi.org/10.1186/s13059-020-02904-0

Ueno, Y., Kamagai, M., Kanamori, H., Yanagisawa, M., Kino, S., Shigeno, S., Osaki, M., Takamatsu, D., Katsuda, K., Maruyama, T., & Ohishi, K. (2020). Draft genome sequence of Brucella ceti isolated in the Western Pacific Ocean. Microbiology Resource Announcements, 9(39), e00925-20. https://doi.org/10.1128/MRA.00925-20

Ueno, Y., Yanagisawa, M., Kino, S., Shigeno, S., Osaki, M., Takamatsu, D., Katsuda, K., Maruyama, T., & Ohishi, K. (2020). Molecular characterization of Brucella ceti from a bottlenose dolphin (Tursiops truncatus) with osteomyelitis in the western Pacific. Journal of Veterinary Medical Science, 82, 754–758. https://doi.org/10.1292/jvms.20-0015

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sukhikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., & Earl, A. M. (2014). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One, 9(11), e112963. https://doi.org/10.1371/journal.pone.0112963

Wattam, A. R., Foster, J. T., Mane, S. P., Beckstrom-Stemberg, S. M., Beckstrom-Stemberg, J. M., Dickerman, A. W., Keim, P., Pearson, T., Shukla, M., Ward, D. V., Williams, K. P., Sobral, B. W., Tsolis, R. M., Whatmore, A. M., & O’Callaghan, D. (2014). Comparative phylogenomics and evolution of the Brucellae reveal a path to virulence. Journal of Bacteriology, 196, 920–930. https://doi.org/10.1128/JB.01091-13

Wattam, A. R., Inzana, T. J., Williams, K. P., Mane, S. P., Shukla, M., Almeida, N. F., Dickerman, A. W., Mason, S., Moriyón, I., O’Callaghan, D., Whatmore, A. M., Sobral, B. W., Tiller, R. V., Hoffmaster, A. R., Frace, M. A., De Castro, C., Molinaro, A., Boyle, S. M., De B., K., & Setubal, J. C. (2012). Comparative genomics of early-diverging Brucella strains reveals a novel lipopolysaccharide biosynthesis pathway. mBio, 3(5), e00246-12. https://doi.org/10.1128/mBio.00246-12

Wattam, A. R., Williams, K. P., Snyder, E. E., Almeida, N. F. Jr., Shukla, M., Dickerman, A. W., Crasta, O. R., Kenyon, R., Lu, J., Shallom, J. M., Yoo, H., Ficht, T. A., Tsolis, R. M., Munk, C., Tapia, R., Han, C. S., Detter, J. C., Bruce, D., Brettin, T. S., & Setubal, C. (2009). Analysis of ten Brucella genomes reveals evidence for horizontal gene transfer despite a preferred intracellular lifestyle. Journal of Bacteriology, 191, 3569–3579. https://doi.org/10.1128/JB.01767-08

Whatmore, A. M., Dawson, C., Groussaud, P., Koylass, M. S., King, A., Shankster, S. J., Sohn, A. H., Probert, W. S., & McDonald, W. L. (2008). Marine mammal Brucella genotype associated with zoonotic infection. Emerging Infectious Diseases, 14, 517–518. https://doi.org/10.3201/eid1403.070829

Whatmore, A. M., Dawson, C., Muchowski, J., Perrett, L. L., Stubberfield, E., Koylass, M., Foster, G., Davison, N. J., Quance, C., Sidor, I. F., Field, C. L., & St. Leger, J. (2017). Characterisation of North American Brucella isolates from marine mammals. PLoS One, 12(9), e0184758. https://doi.org/10.1371/journal.pone.0184758

von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., Savelkoul, P. H. M., & Welfs, P. F. G. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Frontiers in Microbiology, 7, 173. https://doi.org/10.3389/fmicb.2016.00173

Zinzula, L., Mazzariol, S., & Di Guardo, G. (2022). Molecular signatures in cetacean morbillivirus and host species proteomes: Unveiling
the evolutionary dynamics of an enigmatic pathogen? Microbiology and Immunology, 66, 52–58. https://doi.org/10.1111/1348-0421.12949

Zygmunt, M. S., Vergnaud, G., & Cloeckaert, A. (2021). Whole-genome sequence of a Brucella pinnipedialis sequence type 54 strain isolated from a Hooded seal (Cystophora cristata) from the North Atlantic Ocean, Norway. Microbiology Resource Announcements, 10(18), e00271-21. https://doi.org/10.1128/MRA.00271-21

How to cite this article: Orsini, M., Ianni, A., & Zinzula, L. (2022). Brucella ceti and Brucella pinnipedialis genome characterization unveils genetic features that highlight their zoonotic potential. MicrobiologyOpen, 11, e1329. https://doi.org/10.1002/mbo3.1329