



Whole-Genome Sequence of the First Sequence Type 27 *Brucella ceti* Strain Isolated from European Waters

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ABSTRACT *Brucella* spp. that cause marine brucellosis are becoming more important, as the disease appears to be more widespread than originally thought. Here, we report a whole and annotated genome sequence of *Brucella ceti* CRO350, a sequence type 27 strain isolated from a bottlenose dolphin carcass found in the Croatian part of the northern Adriatic Sea.

Brucella spp. are known as very important zoonosis-causing terrestrial pathogens, and for the last two decades they have been known to cause brucellosis in marine mammals. Marine *Brucella* spp. have been classified into two groups. Isolates from cetaceans are classified as *B. ceti*, while isolates from pinnipeds are classified as *B. pinnipedialis* (1, 2). However, the division is not perfect, and cross-species infections, as well as zoonotic transmission, are possible (3). Mediterranean *B. ceti* strains have been isolated from striped dolphins in the Tyrrhenian Sea (4) and Ionian Sea (5), as well as from two striped dolphins and one bottlenose dolphin in the Spanish Mediterranean (6). All of these *B. ceti* strains belong to sequence type 26 (ST26) based on multilocus sequence typing (MLST) (5).

Brucella ceti strain CRO350 was isolated from a bottlenose dolphin (*Tursiops truncatus*) carcass found in the Croatian part of the northern Adriatic Sea during the summer of 2015 (7). Available molecular techniques identified this strain as a marine *Brucella* sp.; multilocus variable-number tandem-repeat analysis (MLVA) genotyping identified this strain closer to *B. pinnipedialis*, and MLST identified it as *B. ceti* ST27. To our knowledge, this is the first evidence of an ST27 strain in the Adriatic Sea and in European waters in general. The first ST27 strain was isolated from an aborted bottlenose dolphin fetus at an aquarium in San Diego, California, USA (strain F5/99) (3), and, subsequently, in the fetuses and neonates of bottlenose dolphins in South Carolina, USA (8). So far, ST27 appears to be the only sequence type known to have the ability to infect humans naturally (3, 9).

Not many *B. ceti* whole-genome sequences are currently available. Only one ST27 *B. ceti* strain is currently available publicly (10). Here, we report a whole-genome sequence of *B. ceti* strain CRO350. Genomic DNA was extracted using a QIAamp DNA minikit on a QIAcube (Qiagen, Germany). DNA concentrations were determined using the Qubit double-stranded DNA (dsDNA) BR assay kit (Invitrogen, Thermo Fisher Scientific, USA). The genomic DNA was prepared for Illumina (Illumina, USA) paired-end sequencing using the Illumina NexteraXT guide (no. 150319425031942), following protocol revision C.

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Paired-end reads were trimmed for adapter sequences and low-quality bases using Cutadapt version 1.13 (11), and a minimum Phred quality score threshold of 20 was established. Reads were assembled using SPAdes version 3.9.1 (parameters, $-k$ 21, 33, 55, 77, and 99; $-careful$; and $-cov-cutoff$ 3) (12). The assembly yielded 76 contigs, which were reordered using Mauve Contig Mover implemented in Mauve version 2.4.0 (13). The length of the assembled nucleotides (nt) was 3,365,450 nt, the N_{50} value was 124,418 nt, and the G+C content was 57.2%. Average coverage was $30.6\times$. Prokka version 1.12 (14) was used for genome annotation; the genome consisted of 1 transfer-messenger RNA, 3,131 coding sequences, 3 rRNAs, and 47 tRNAs.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [NKHE00000000](https://doi.org/10.1093/nucleic-acids/gkz000). The version described in this paper is the first version, NKHE01000000.

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REFERENCES

- Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckaert A. 2007. *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. *Int J Syst Evol Microbiol* 57:2688–2693. <https://doi.org/10.1099/ijs.0.65269-0>.
- Maquart M, Le Flèche P, Foster G, Tryland M, Ramisse F, Dønne B, Al Dahouk S, Jacques I, Neubauer H, Walravens K, Godfroid J, Cloeckaert A, Vergnaud G. 2009. MLVA-16 typing of 295 marine mammal *Brucella* isolates from different animal and geographic origins identifies 7 major groups within *Brucella ceti* and *Brucella pinnipedialis*. *BMC Microbiol* 9:145. <https://doi.org/10.1186/1471-2180-9-145>.
- Whatmore AM. 2009. Current understanding of the genetic diversity of *Brucella*, an expanding genus of zoonotic pathogens. *Infect Genet Evol* 9:1168–1184. <https://doi.org/10.1016/j.meegid.2009.07.001>.
- Alba P, Terracciano G, Franco A, Lorenzetti S, Cocumelli C, Fichi G, Eleni C, Zygmunt MS, Cloeckaert A, Battisti A. 2013. The presence of *Brucella ceti* ST26 in a striped dolphin (*Stenella coeruleoalba*) with meningoencephalitis from the Mediterranean Sea. *Vet Microbiol* 164:158–163. <https://doi.org/10.1016/j.vetmic.2013.01.023>.
- Garofolo G, Zilli K, Troiano P, Petrella A, Marotta F, Di Serafino G, Ancora M, Di Giannatale E. 2014. *Brucella ceti* from two striped dolphins stranded on the Apulia coastline, Italy. *J Med Microbiol* 63:325–329. <https://doi.org/10.1099/jmm.0.065672-0>.
- Isidoro-Ayza M, Ruiz-Villalobos N, Pérez L, Guzmán-Verri C, Muñoz PM, Alegre F, Barberán M, Chacón-Díaz C, Chaves-Olarte E, González-Barrientos R, Moreno E, Blasco JM, Domingo M. 2014. *Brucella ceti* infection in dolphins from the western Mediterranean Sea. *BMC Vet Res* 10:206. <https://doi.org/10.1186/s12917-014-0206-7>.
- Cvetnić Ž, Duvnjak S, Đuras M, Gomerčić T, Reil I, Zdelar-Tuk M, Špičić S. 2016. Evidence of *Brucella* strain ST27 in bottlenose dolphin (*Tursiops truncatus*) in Europe. *Vet Microbiol* 196:93–97. <https://doi.org/10.1016/j.vetmic.2016.10.013>.
- Wu Q, McFee WE, Goldstein T, Tiller RV, Schwacke L. 2014. Real-time PCR assays for detection of *Brucella* spp. and the identification of genotype ST27 in bottlenose dolphins (*Tursiops truncatus*). *J Microbiol Methods* 100:99–104. <https://doi.org/10.1016/j.mimet.2014.03.001>.
- Cloeckaert A, Bernardet N, Koylass MS, Whatmore AM, Zygmunt MS. 2011. Novel IS711 chromosomal location useful for identification of marine mammal *Brucella* genotype ST27, which is associated with zoonotic infection. *J Clin Microbiol* 49:3954–3959. <https://doi.org/10.1128/JCM.05238-11>.
- Wattam AR, Williams KP, Snyder EE, Almeida NF, Jr, Shukla M, Dickerman AW, Crasta OR, Kenyon R, Lu J, Shallom JM, Yoo H, Ficht TA, Tsois RM, Munk C, Tapia R, Han CS, Detter JC, Bruce D, Brettin TS, Sobral BW, Boyle SM, Setubal JC. 2009. Analysis of ten *Brucella* genomes reveals evidence for horizontal gene transfer despite a preferred intracellular lifestyle. *J Bacteriol* 191:3569–3579. <https://doi.org/10.1128/JB.01767-08>.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics* 25:2071–2073. <https://doi.org/10.1093/bioinformatics/btp356>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.