

Brucella ceti infection in cetaceans from Italian Seas: associated lesions and epidemiological data

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INTRODUCTION - Since 2007, isolates of *Brucella* spp. from marine mammals have been classified into two species, *B. ceti* and *B. pinnipedialis* [1]. Based on the common infection patterns of meningitis and/or meningoencephalitis, resembling “human neurobrucellosis” [2, 3], a specific susceptibility has been suggested for the striped dolphin (*Stenella coeruleoalba*) [4, 5, 6, 7–10]. *B. ceti* was first reported in the Mediterranean Sea in 2009 [11] while no evidence of seropositivity was detected in cetaceans of Italian Seas until the beginning of 2015 [12, 13].

MATERIAL AND METHODS - In this study, we focused on the pathogenic role of *B. ceti* and the genetic constitution of the strains, in order to make a comparative genomic analysis involving different strains from cetaceans stranded in Italy up to 2021. Based on macroscopic and microscopic pathological findings and *Brucella* analytical data (microbiological, biomolecular and serological), along with ancillary test results of 23 *B. ceti* positive culture, the most probable cause of death has been evaluated. All *Brucella* strains were sequenced using Illumina NextSeq. Genomes were then assembled using SPAdes (version 3.11.1). Sequence type (ST) was assigned with MLST tool (T. Seeman) using *Brucella* PubMLST database and SNP analysis was performed with In Silico Genotyper (ISG) (version 0.16.10–3). Phylogeny was reconstructed with FastTree (version 2.1.7) using concatenated SNP alignments and visualised with IQTree (version 1.6.9).

AIM - In order to gain a deeper understanding about brucellosis in cetaceans in Italy, 23 cases of *B. ceti* infection in striped dolphins stranded along the Italian coastline from 2012 to 2021, were investigated thanks to the Italian surveillance and diagnostic activity on stranded cetaceans (C.Re.Di.Ma.).

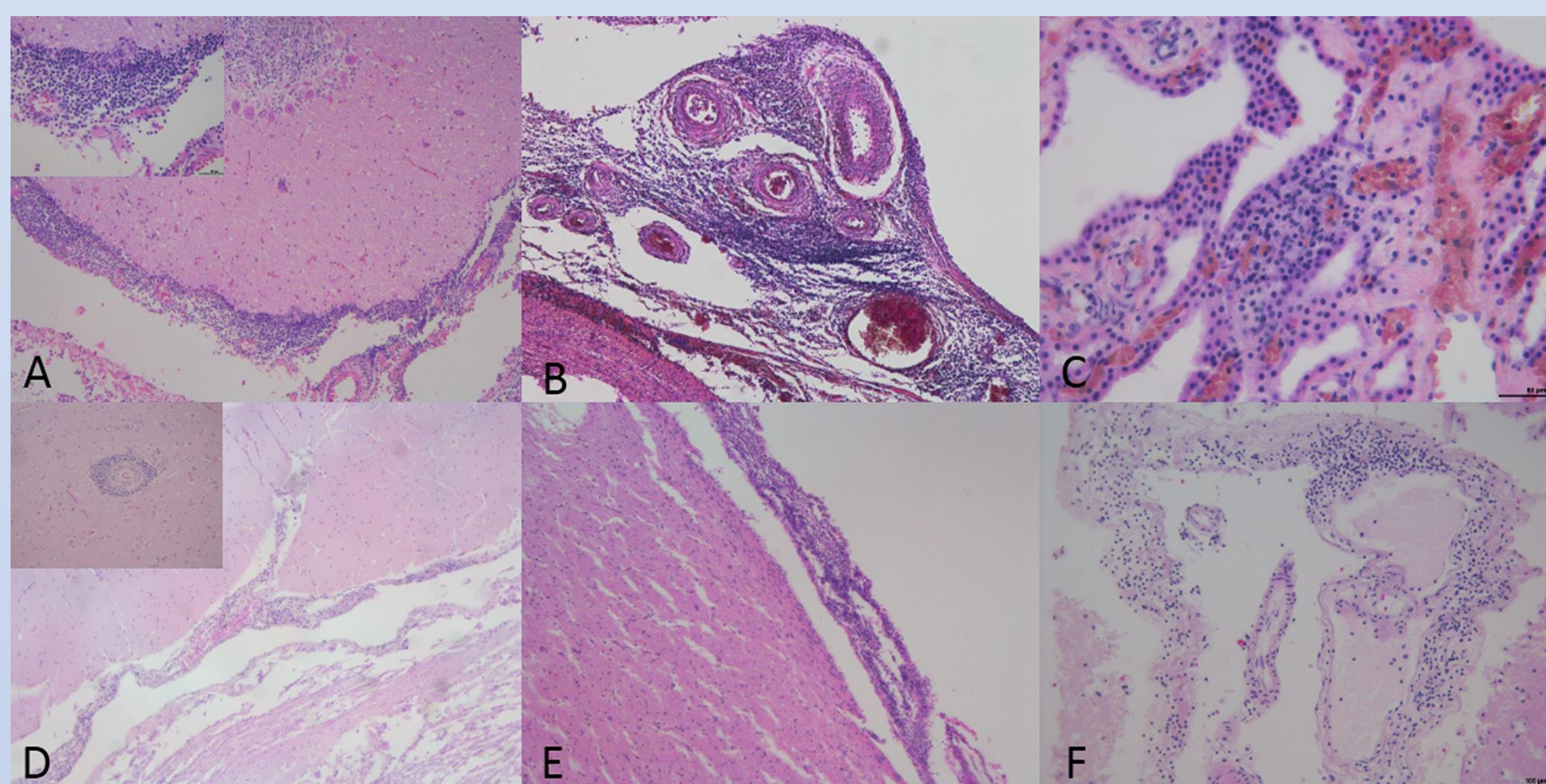
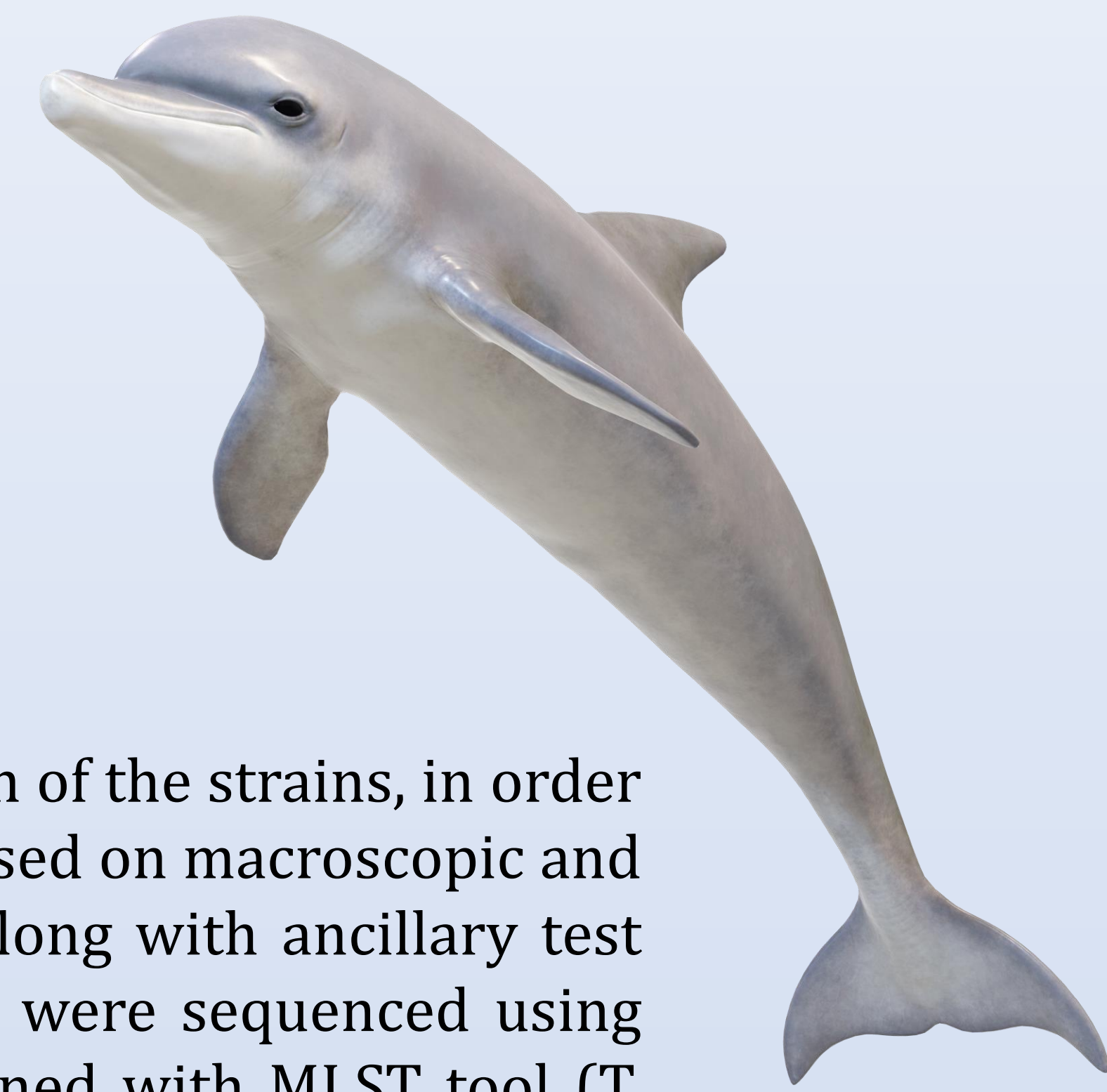


Fig. 1 - *B. ceti*-associated lesions in central nervous system of striped dolphins (*S. coeruleoalba*). (A) Severe non-suppurative meningitis. Cerebellar meninges are infiltrated by mononuclear cells (Case 2). 10x. H&E. Left upper inset: detail of the lympho-monocytic inflammatory infiltrate. 40x. H&E. (B) Severe non-suppurative meningitis. Meninges at the level of medulla oblongata are infiltrated by lympho-monocytic cells. (Case 5). 10x. H&E. (C) Mild non-suppurative meningoencephalitis. Choroid plexuses are infiltrated by lympho-monocytic cells. (Case 6). 40x. H&E. (D) Mild non-suppurative meningoencephalitis. Cerebellar meninges are infiltrated by mononuclear cells. (Case 7). 10x. H&E. Left upper inset: perivascular cuff characterized by the presence of lympho-monocytic cells. 20x. H&E. (E) Non-suppurative meningitis. Cerebral cortex meninges are infiltrated by mononuclear cells. (Case 8). 10x. H&E. (F) Non-suppurative meningitis. Meninges at the level of parietal cortex are infiltrated by mononuclear cells. (Case 9). 20x. H&E.

RESULTS - Pathological changes consistent with *B. ceti* infection were detected in the central nervous system (CNS) of 19/22 (86,3%) animals, showing non-suppurative meningoencephalitis; 5/19 showed *B. ceti*-associated pathological findings also in other tissues (endometritis, oophoritis, mastitis, pneumonia, myocarditis, hepatic necrosis, necrosis of lymphoid tissues). *B. ceti* was isolated from CNS of 19/23 animals investigated (82,6%).

Co-infections with other relevant pathogens, mainly involving Dolphin Morbillivirus (DMV) and *Toxoplasma gondii*, were detected in 13/23 cases (56,5%). The cause of stranding has been related to a severe cerebral impairment associated with *B. ceti* infection in 13/22 (59%) animals and a coinfection by *B. ceti* - DMV - *T. gondii* in 6/22 animals (27%).

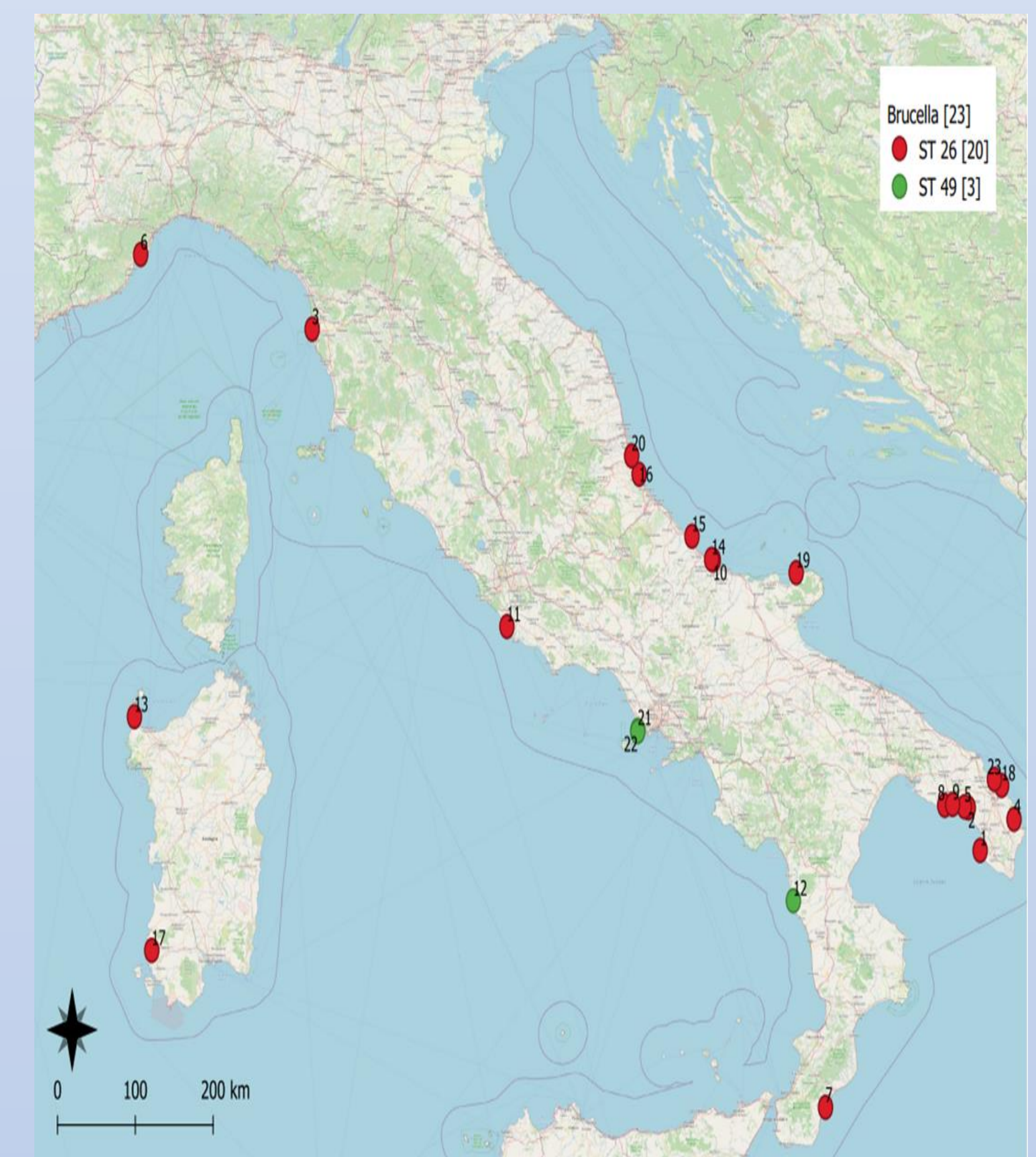


Fig. 2 - Geographical distribution of *B. ceti* strains in cetaceans stranded in Italy. MLST tool recognized ST 26 as dominant (N=20), with the identification of ST 49, only in 3 cases, in the Southern Tyrrhenian Sea.

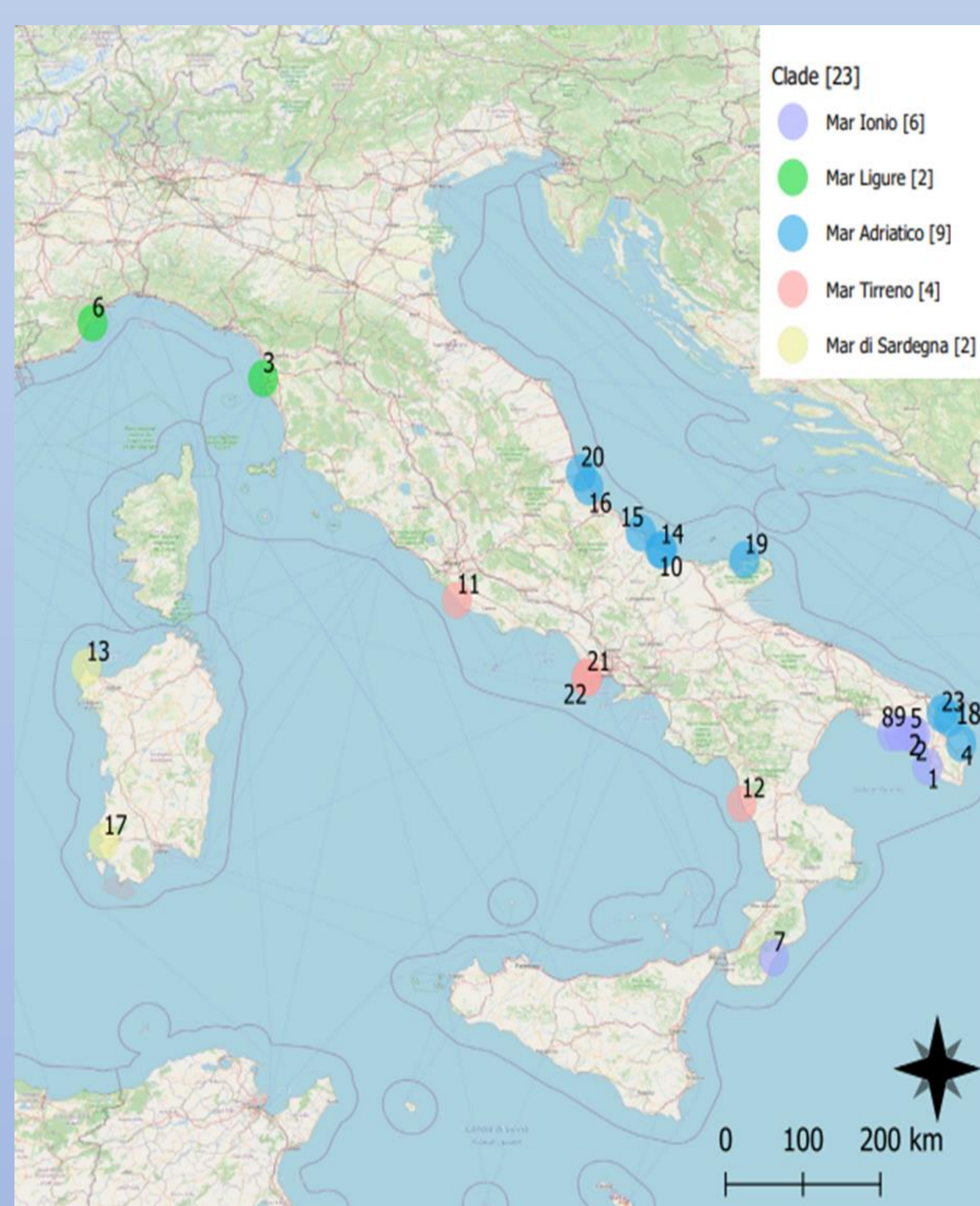


Fig. 3 - Whole genome SNP analysis showed that the 23 Italian strains clustered into five genetically distinct clades, associated with different sea sector of stranding event (Adriatic, Ionian, Ligurian, Sardinian, Tyrrhenian).

Whole genome SNP analysis showed two different sequence type (ST), ST 26 and ST 49 (Fig. 2); also, all the 23 Italian strains clustered into five genetically distinct clades: clade Mar Adriatico, clade Mar Ionio, clade Mar Ligure, clade Mar Tirreno and clade Mar di Sardegna (Fig. 3).

A clear separation between the clusters shows a link between phylogeny and geographical distribution of *B. ceti* strains in Italy. So, could the separation between clusters represent an «indication of separation» between the population of striped dolphins circulating in the Italian seas? A potential relationship between the separation of *B. ceti* clusters and a separation of striped dolphin groups could be suggested.

Therefore, since the circulation of *B. ceti* appears extended to almost all marine sectors (Fig. 2), especially in the central-southern Adriatic and Ionian seas, the infection due to *B. ceti* arouses a great concern for the zoonotic potential.

CONCLUSIONS - In this study, we observed a strong correlation between *B. ceti* infection and neuropathological findings in striped dolphins stranded in Italy. These findings further our understanding of *B. ceti* associated brain lesions (neurobrucellosis) and confirm his role as a primary neurotropic pathogen for striped dolphin in the Mediterranean Sea. Moreover, the data obtained with whole genome SNP analysis suggest an interesting relationship between phylogeny and geographical distribution of strains in Italy, that should be investigated with future strain characterization.

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