

# BLASTOCYSTIS IN THE MARINE ENVIRONMENT: NEW INSIGHTS INTO THE GENETIC DIVERSITY AND DISTRIBUTION IN CETACEANS AND MARINE TURTLES FROM THE MEDITERRANEAN SEA

R. Aco-Alburquerque<sup>1</sup>, S. Gabrielli<sup>1</sup>, M. Palomba<sup>1,2</sup>, F. Marcer<sup>3</sup>, E. Marchiori<sup>3</sup>, M. Santoro<sup>2</sup>, S. Mattiucci<sup>1</sup>

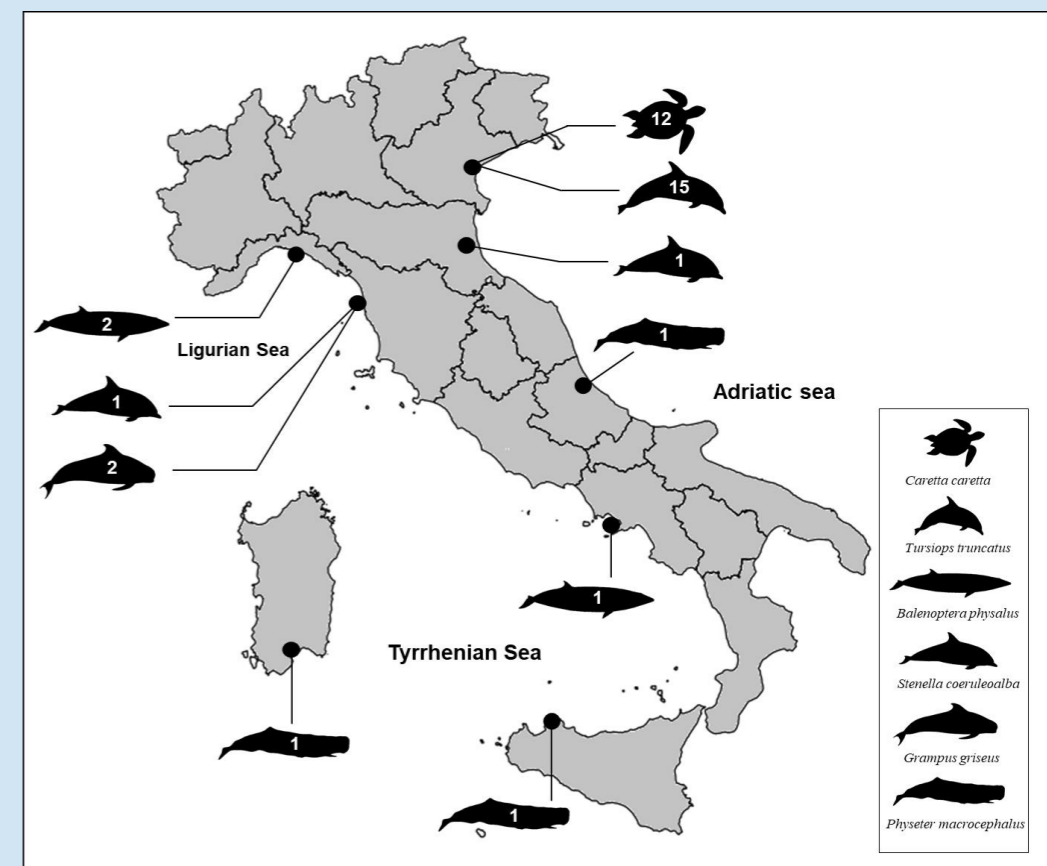
<sup>1</sup> Department of Public Health and Infectious Diseases, Section of Parasitology, "Sapienza University of Rome" and "Umberto I" Teaching Hospital, Rome, Italy; <sup>2</sup> Department of Integrative Marine Ecology, Anton Dohrn; <sup>3</sup> Department of Animal Medicine, Production and Health, University of Padua.

## INTRODUCTION

- Blastocystis* is widespread in several terrestrial animal hosts, including humans; a large genetic heterogeneity has been so far demonstrated with the characterization of distinct 25 subtypes (STs) (Maloney *et al.*, 2021, Parasitol Res, 120, 2219–2231).
- So far, a large-scale survey was conducted by screening fish caught in NorthEast Atlantic Ocean and marine mammals stranded on the coast of northern France for the presence of *Blastocystis* sp., showing a prevalence of 3.5% in fish and 13.8% in cetaceans. The most prevalent ST from cetaceans resulted to be ST2, ST3 and ST4 (Gantois *et al.*, 2020, Microorganisms, 8(3), 460).
- In the Mediterranean Sea, Marangi *et al.* (*Sci Rep* 11, 5822 (2021)) reported the presence of *Blastocystis* (ST3) in free-ranging fin whale and sperm whale.
- This study aimed to assess the presence of *Blastocystis* STs from 5 cetacean species (*Balaenoptera physalus*, *Physeter macrocephalus*, *Grampus griseus*, *Stenella coeruleoalba*, *Tursiops truncatus*) and the loggerhead sea turtle (*Caretta caretta*) stranded along the Italian Mediterranean Sea coast, during the years 2011-2021.

## MATERIALS AND METHODS

- A total of 25 frozen stool samples were examined from cetaceans and 12 from the loggerhead sea turtle in different locations of the Adriatic, Ligurian, and Tyrrhenian Seas.
- Genomic DNA was extracted from each fecal sample. A fragment of about 500 bp from the SSU rRNA gene was amplified using the primers proposed by Santín *et al.* (Parasitol. Res. 2011,119, 205-12). Positive samples were further amplified using the primers SSU-F1 and SSU-R1, for the full-length of the SSU rRNA and RD5 and BhrDr for the barcoding region, using the PCR-conditions described in Xiao *et al.* (Appl. Environ. Microbiol. 1999, 65, 1578-83) and Scicluna *et al.* (Protist. 2006, 157, 77-85) respectively.
- The sequences obtained were compared to those of *Blastocystis* STs so far deposited in GenBank by using the BLAST application.
- The Bayesian phylogenetic analysis (BI) was performed by using MrBayes3.2.



## RESULTS AND CONCLUSIONS

Host	species	n	Positive sequences (Gene region analysed)			STs identified
			Barcoding	Santin	Full-length SSU rRNA	
Fin whale	<i>Balaenoptera physalus</i>	3	2	2	1	Untypable
Sperm whale	<i>Physeter macrocephalus</i>	3	-	-	-	
Common bottlenose dolphin	<i>Tursiops truncatus</i>	15	-	-	-	
Risso's dolphin	<i>Grampus griseus</i>	2	-	-	-	
Striped dolphin	<i>Stenella coeruleoalba</i>	2	-	-	-	
Loggerhead sea turtle	<i>Caretta caretta</i>	12	3	3	-	Untypable
<b>Total</b>		<b>37</b>	<b>5</b>	<b>5</b>	<b>1</b>	

- The prevalence of the *Blastocystis* in the examined samples was about 13.5%, infecting the fin whale (*B. physalus*) and loggerhead sea turtle (*C. caretta*).
- Among the positive samples, n=5 were successfully sequenced by the barcoding and Santin primers and n=1 by the full-length SSU rRNA gene, showing a single infection with a specific ST.
- The BI analysis shows that the isolates from *B. physalus* and *C. caretta* correspond to potential new STs: they are forming distinct phylogenetic lineages with respect to those STs so far known and genetically characterized.
- Pairwise genetic distance values between *Blastocystis* subtypes here analyzed were estimated by the Kimura 2-parameter model (K2p). The lowest ST inter-subtype distance values were K2p=0.06 and K2p=0.07 observed between ST from *B. physalus* vs the sample from *C. caretta* 3 and vs ST2, respectively. The sample from *C. caretta* 3 showed K2p value = 0.07 vs ST2. Similar pairwise distance values have been observed between distinct STs, previously characterized.
- The findings seem to support the existence of distinct STs of *Blastocystis* adapted to marine mammals and turtles.
- The present survey represents the first data regarding *Blastocystis* distribution in marine turtles and new genetic insights on its occurrence in *B. physalus*.

