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Integrated study of dinoflagellate diversity in the Gulf of Naples

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Thesis submitted for the degree of Doctor in Philosophy
(PhD) in Life and Biomolecular Sciences

December 2017

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Abstract

Dinoflagellates are a diverse lineage of protists known as an essential component of marine planktonic communities. To study their seasonal diversity at the LTER-MC station in the Gulf of Naples, I used high throughput sequencing (HTS) metabarcoding of the V₄ region in the 18S rDNA. To taxonomically identify the metabarcode sequences, I established a database, called DinoREF, of taxonomically verified and nomenclaturally updated 18S reference barcodes with associated metadata. The reference sequences were organised into Superclades based on phylogenetics and higher taxonomic treatment. DinoREF contains 1,671 sequences for 422 species and covering 22% of the described species. The database revealed that the V₄ region alone cannot discriminate between some morphologically and genetically distinct species or genera. Moreover, many species and genera were collapsed together when clustered into 98% similarity OTUs. For the metabarcoding, dinoflagellate HTS V₄ reads were gathered from 48 environmental DNA samples taken over three years at LTER-MC. Results of a taxonomic cluster analysis showed three principal seasonal clusters, one with winter samples (16% of reads), one with mainly spring-summer samples (62%) and one with late summer-autumn samples (22%). Sorting reads into ribotypes and assigning them with DinoREF to taxa showed that reads belonging to the *Gyrodinium*, Gymnodiniales and Gonyaulacales Superclades were the most abundant. Winter samples were characterised by specific taxa thriving only in cold season. Results revealed 85 new records and detected 26 potentially toxic species for the Gulf of Naples. Many dinoflagellate genera such as *Tripes* are underrepresented in DinoREF because many species cannot be cultured. I applied single cell imaging, PCR and sequencing to gather 18S and 28S for 22 *Tripes* species. Using 18S V₄ region as barcode I assessed the seasonal abundances of these species. Some were common all year whereas others showed distinct seasonality, mainly occurring in winter.