



INTRODUCTION

The oceans are populated by an astronomical number of predominantly-uncultured microbes, which altogether guarantee ocean ecosystem function. Unicellular eukaryotic marine predators represent basal links in marine food webs and have so far been predominantly characterized as a functional group, despite they have different ecologies and evolutionary histories.

In order to better understand the eco-evolution of the ocean's smallest predators, we have investigated four species belonging to an uncultured cosmopolitan family: MAST-4. Using state-of-the-art single-cell genomics and meta-omics approaches, we aimed to highlight the importance of understanding the species-level ecology and genomics of tiny ocean predators.

RESULTS

- MAST-4 species show a very contrasting distribution, in fact species A show a mutual exclusion with species B and C, who co-occur in the same areas: sub-tropical vs tropical waters. (Figure 1).
- Temperature appears as the main driver of MAST-4 distribution, while MAST-4 abundance in the upper ocean is weakly coupled to bacterial distributions (Figure 2).
- Overall, none of the studied MAST-4 displayed any loss or gain of broad functional categories when compared to each other (Figure 3A), yet their glycoside hydrolases repertoire is different (Figure 3B&C) and present dissimilar expression patterns in the global surface ocean, even for co-existing species (Figure 4).

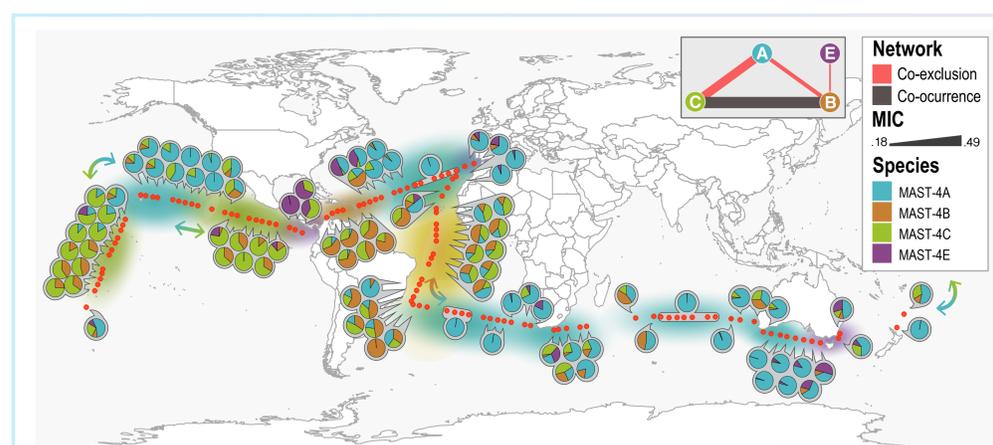


Figure 1. Distribution of MAST-4A/B/C/E species in the surface global ocean as inferred by ASVs based on the 18S rRNA gene (V4 region) from Malaspina. The top-right inset network shows the association patterns between each MAST-4. Background color shows the most abundant MAST-4 species in the region. Arrows point to areas with an important switch of the abundant species.

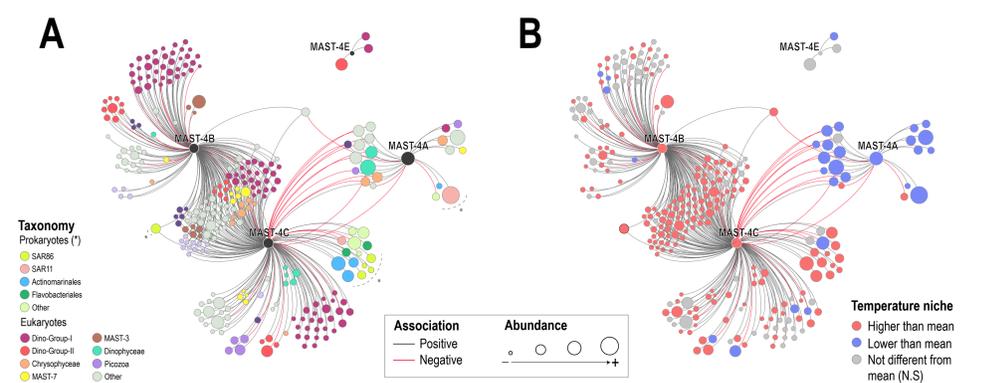


Figure 2. Association network including MAST-4 species, associated prokaryotes, and other pico-eukaryotes from the Malaspina expedition. In Panel A nodes are colored based on taxonomy while in Panel B node color indicates whether specific ASVs displayed weighted mean temperatures significantly lower or higher than the unweighted mean temperature (-24.5°C), pointing to species with temperature distributions that differ from chance.

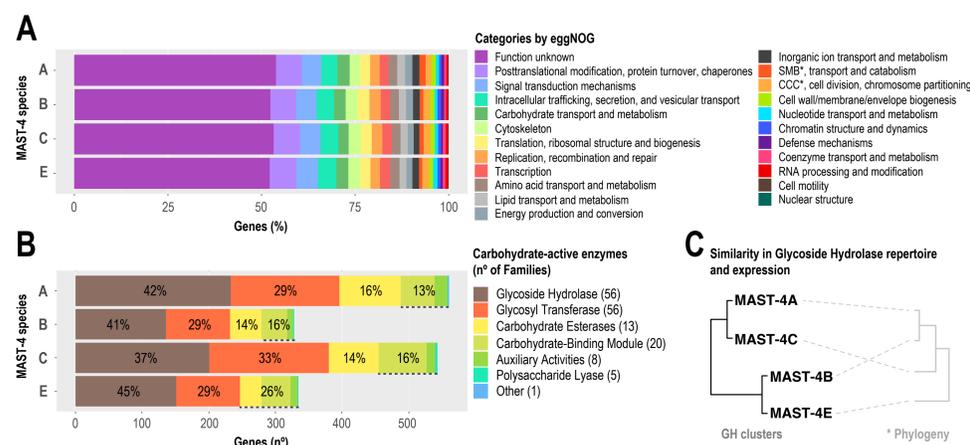


Figure 3. Functional profile of MAST-4 genes according to eggNOG and CAZy. Panel A: eggNOG annotations indicated as percentage of genes falling into functional categories. *SMB – Secondary Metabolites Biosynthesis, CCC – Cell Cycle Control. Panel B: Number of MAST-4 genes within CAZy categories and the corresponding percentage. Panel C: Clustering of MAST-4 species using Manhattan distances based on either their Glycoside Hydrolase (GH) composition. *Schematic representation of the phylogeny

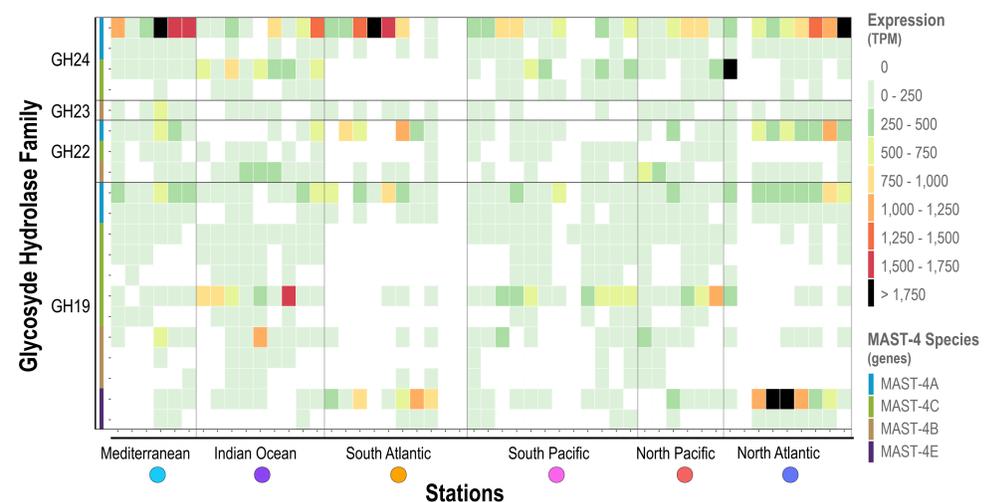


Figure 4. Expression and abundance of GHs in MAST-4 in the upper global ocean from Tara Oceans metatranscriptomes. Heatmap of the most expressed Glycoside Hydrolase families in MAST-4.

CONCLUSIONS

Altogether, our results suggest that the evolutionary diversification of MAST-4 was promoted by divergent adaptive evolution towards different temperature and/or diet niches possibly to avoid competition. The previous possibly led to the emergence of the species associated to tropical, subtropical-temperate and subpolar-polar waters. Our work represents a significant contribution to understand the evolution, diversity, biogeography and function of the smallest predators in the ocean.

METHODS

- Biogeographic distribution and habitat preference of MAST-4 species A/B/C/E using Amplicon Sequence Variants (ASVs) from Malaspina 2010 Expedition (1).
- Genome assembly and gene prediction of 69 Single Amplified Genomes (SAGs) from Tara Oceans expedition (2) co-assembled into four genomes.
- Genomic annotation with eggNOG and CAZy database.
- Detection of Ecological Relevant Genes (ERGs) related to food digestion analysing and their expression in the surface global ocean using Tara Oceans Metatranscriptomes.

References

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2. Sunagawa S, et al., Structure and function of the global ocean microbiome. *Science*. Vol. 348, 6237 (2015).

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