

Genetic and microbial community analyses in two physiological states and color morphs of the colonial ascidian *Polyclinum constellatum*

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Rationale:

- This study investigated the genetic similarity and microbial communities associated with red and green morphs of the colonial ascidian *P. constellatum* in actively filtering and resting (non-filtering) states.



Methods:

- Sample collection in Puerto Rico (2019 and 2020).
- DNA extraction and sequencing of host COI mtDNA ($n=56$) and 18S rRNA ($n=37$) gene fragments.
- Sequences aligned with Geneious¹ and consensus used to create NJ & ML phylogenetic trees with MEGA².
- 16S rRNA gene fragments were used to characterize microbial communities ($n=17$) from the ascidian tunic.
- Quality filtering of microbial sequences, OTU clustering, and taxonomic assignment using Mothur³.
- Alpha diversity metrics were conducted on microbial communities to estimate richness (S , d , $1/D$, $E_{1/D}$) and beta diversity analyses based on Bray-Curtis to identify differences in the overall communities.

Results:

- COI & 18S rRNA phylogenetic analyses placed all color morphs within the same genetic clade (Fig. 1).
- Similar microbial communities in each color morph.
- No richness change in microbial communities from different colors or physiological states, but there was a significant shift in microbial community structure between actively filtering and resting colonies (Fig. 2).
- Two bacterial symbiont taxa (genus *Kiloniella* & *Ruegeria*) were the main drivers of microbial community dissimilarity.

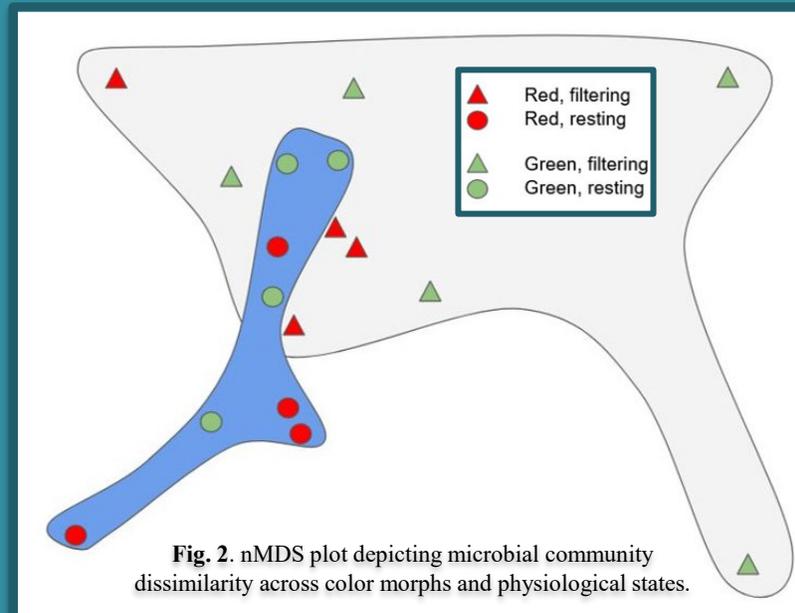


Fig. 2. nMDS plot depicting microbial community dissimilarity across color morphs and physiological states.

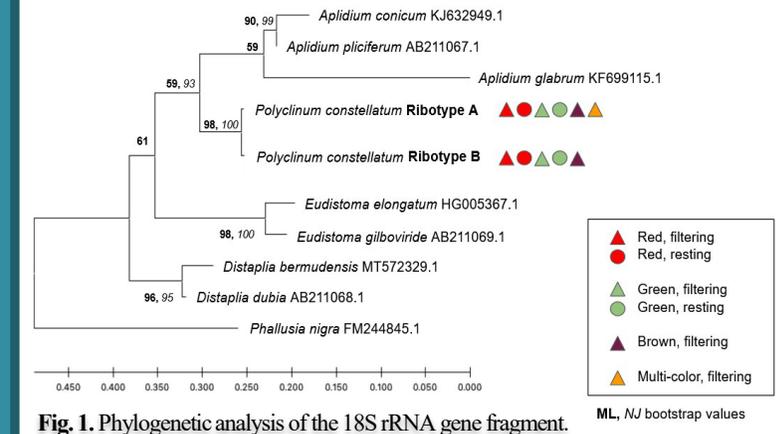


Fig. 1. Phylogenetic analysis of the 18S rRNA gene fragment.

Discussion & Implications:

- Color variability in *P. constellatum* is due to phenotypic plasticity (intra-species variation) and results in similar microbial communities, whereas distinct genetic lineages (inter-species variation) correspond to different microbial communities⁴.
- The physiological state of the host significantly alters microbiome structure, but not richness.
- Resting states are associated with variable oxygen concentrations and the presence of anaerobic⁵ and facultatively anaerobic (f.i. *Kiloniella*) bacteria.

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