

The Organelle in the Ointment: cryptic mitochondria bias cross-species microbiome comparisons

Dylan Sonett¹, Tanya Brown¹, Johan Bengtsson-Palme^{2,3}, Jacqueline L. Padilla-Gamiño⁴ and Jesse R. Zaneveld*¹

¹ Division of Biological Sciences, School of STEM, University of Washington Bothell, Bothell, WA, USA.

² Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

³ Centre for Antibiotic Resistance Research (CARE) at University of Gothenburg, Gothenburg, Sweden.

⁴ School of Aquatic and Fisheries Sciences, University of Washington, Seattle, WA, USA.

Abstract

The microbiomes of tropical corals are actively studied using 16S rRNA gene amplicons to understand microbial roles in coral health, metabolism, and disease resistance. However, due to the prokaryotic origins of mitochondria, primers targeting bacterial and archaeal 16S rRNA genes may also amplify homologous 12S mitochondrial rRNA genes from the host coral, associated microbial eukaryotes, and encrusting organisms. Standard microbial bioinformatics pipelines attempt to identify and remove these sequences by comparing them to reference taxonomies. However, commonly used tools severely under-annotate mitochondrial sequences in 1440 coral microbiomes from the Global Coral Microbiome Project, preventing annotation of over 95% of reads in some samples. This issue persists when using Greengenes or SILVA prokaryotic reference taxonomies, and in other hosts, including 16S studies of vertebrates, and of marine sponges. Worse, mitochondrial under-annotation varies between coral families and across coral compartments, biasing comparisons of α - and β -diversity. By supplementing existing reference taxonomies with over 3,000 animal mitochondrial rRNA gene sequences, we resolve roughly 97% of unique unclassified sequences as mitochondrial. These additional sequences do not cause a false elevation in mitochondrial annotations in mock communities with known compositions. We recommend using these extended taxonomies for coral microbiome analysis and whenever eukaryotic contamination may be a concern.

Keywords: Taxonomy, classification, mitochondria, 16S, contamination

***Corresponding author. Email: zaneveld@gmail.com**