UNIVERSITY of WASHINGTON | BOTHELL
SCHOOL OF SCIENCE, TECHNOLOGY, ENGINEERING AND MATHEMATICS

Dear Editor: February 23, 2021

Please find enclosed our manuscript entitled **"The Organelle in the Room: Under-annotated Mitochondrial Reads Bias Coral Microbiome Analysis"** by Dylan Sonett, Tanya Brown, Johan Bengtsson-Palme, Jacqueline L. Padilla-Gamiño and Jesse R. Zaneveld for submission to the International Society for Microbial Ecology Journal (ISMEJ) as a Research Article.

Coral reefs form marine biodiversity hotspots of immense economic and ecological value. They rely on intimate symbioses between coral animals and complex communities of microorganisms. As such, coral's microbial symbionts have been extensively studied using a variety of molecular methods including 16S rRNA gene amplicons. In this manuscript we demonstrate the presence of cryptic 12S rRNA mitochondrial reads that are severely under-annotated in 16S rRNA libraries by standard taxonomic annotation workflows. These cryptic mitochondrial reads subsequently appear as 'Unknown' sequences and are not annotated even at the domain level. We show that under-annotated mitochondrial reads form up to 95% of the total sequences in some samples from the Global Coral Microbiome Project – a global dataset of 1455 coral 16S rRNA libraries sequenced through the Earth Microbiome Project. We find that under-annotation of mitochondrial sequences is not equal across coral species, and demonstrate that it can therefore severely bias cross-species comparisons of coral microbiome alpha and beta diversity. Fortunately, we find that supplementing either the Greengenes or SILVA databases with additional mitochondrial sequences largely resolves this problem, without inducing false-positive results in mock community trials.

We think the manuscript will be useful to the community as a clear reference to a problem that has been discussed informally among many coral microbiologists. The manuscript also provides a simple, extensible, well-tested solution to this issue, which has been lacking. While we do not address in this manuscript the possibility that the same problem applies in other systems, the expanded versions of standard taxonomic references (and open-source code to update them in the future) is not specific to corals, and will therefore likely be of interest to any readers of ISMEJ working on the microbiome of other non-model organisms (e.g. sponges, medicinal or agricultural mushrooms, etc). We greatly appreciate your consideration of this manuscript for publication in ISMEJ and can recommend the following reviewers:

Dr. Amy Apprill (aapprill@whoi.edu): Expert in marine microbial ecology
Dr. Raquel Peixoto (raquel.peixoto@kaust.edu.sa): Expert in marine biodiversity/conservation
Dr. Jeroen van de Water (jvdewater@centrescientifique.mc): Expert in coral microbial ecology

This manuscript represents original research, has not been previously published and has not been submitted for publication elsewhere.

Best Regards,

Dr. Jesse Zaneveld (on behalf of all authors)
University of Washington Bothell

Box 358500 18115 Campus Way NE, Bothell, WA 98011-8246

425.352.5000 fax 425.352.5455 uwbinfo@uw.edu www.uwb.edu