

Toward Understanding the Complexity of Bacterial Consortia in Corals: Ecological Inferences from 16S rRNA-Based Community Analysis

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Characterizing the distribution and composition patterns of healthy coral-associated microbiomes is critical for establishing baselines from which to detect differences associated with coral diseases and with the response to environmental stressors. In this study, we found that the bacterial consortia associated with the coral *Porites astreoides* is dominated in abundance by a few bacteria from the order Oceanospirillales. This consistent specific association might indicate a symbiotic mutualistic association with the adult coral partner. Furthermore, we also identified a highly diverse rare bacterial "biosphere" also living along with the dominant bacterial symbionts, which its assemblage shown to be significantly structured along the geographical scale. We further discovered that some of these rare bacterial members showed significant association with other members of the community reflecting the complexity of the networked consortia. From an evolutionary perspective, characterizing new symbiotic interaction among bacterial members living in association with coral hosts would provide new foundation for exploring the impact of these symbioses on the fitness and evolution of coral hosts.

Introduction

Coral animal form association both intracellularly and extracellularly with a large microbiota. This multipartite symbiosis between coral host and its intracellular eukaryotic algae symbionts and the diverse microorganisms (bacteria, archaea, fungi, and viruses) found in association with corals, has been termed the holobiont [1]. The functional role of the bacterial organisms in the reef ecosystem and their contribution to coral well-being remain, for the large part, unclear. There is increasing evidence, though, that coral microbiota seem crucial to at least two aspects of the physiology of the host: biogeochemical cycling and pathogen defense / resistance [2]. Furthermore, it has been postulated that when environmental conditions are altered the microbial biota may undergo changes on ecological time scales that potentially enable particular coral holobionts to survive under the new environmental changes [3]. Testing these hypotheses will depend on first characterizing in great depth the coral microbial community and its changes in diversity across coral species, geographical locations, and time.

Primary Aims

- 1) Explore the diversity of the microbial community associated with an ecologically important Caribbean scleractinian coral, *Porites astreoides*.
- 2) Determine geographical patterns in the coral-associated microbial community structure.
- 3) Resolve co-occurrence patterns among bacterial members using network analyses.



Fig. 1. Colony of the scleractinian coral *Porites astreoides* used in the present study for microbial assessment



Fig. 2. Curacao island in the Caribbean Sea. Sites 1-3= Water Factory, Site 4= Snake Bay, Site 5= Playa Jeremi

Materials and Methods

Samples from *Porites astreoides* (scleractinian coral) (Fig. 1) were collected from fringing reefs along the south coast of Curacao island (Fig. 2) between 5 and 10 m depth in June 2010. Total bacterial DNA was isolated from both coral mucus and tissue. High throughput sequencing of the V5-region of 16S rRNA region was performed using a HiSeq 2000 (Illumina) and data were generated for a total of 22 coral samples (4-5 corals per population) yielding an average number of sequence reads of 31,071 per sample. Sequences were clustered in Operational Taxonomic Units (OTUs) at 97% similarity level and taxonomically classified from domain to genus using the RPD Naïve Bayesian Classifier. To explore differences in the bacterial diversity among population sites, the Shannon-Weiner diversity index was estimated and ANOVA analyses were performed to test for statistical differences. Non-metric dimensional scaling (nMDS) was also performed to explore spatial differences in the coral-associated bacterial community structure. To investigate potential biotic interactions among members of the coral-associated bacterial community, and host affinities to bacterial taxa, co-occurrence pattern analysis was conducted using network inference based on significant Spearman's rank correlations between OTUs (p -values < 0.01).

Results and Discussion

A total of 744,809 high quality-sequencing reads were obtained for the V5 16S rRNA region from 23 samples (4-5 replicates per population site). The average sequencing length ranged from 150 to 180 base pairs. After sequencing clustering, based on a similarity level of 97%, a total of 735 OTUs were resolved. These OTUs were affiliated to bacterial sequences from 15 phyla, with the most abundant phylum represented by Proteobacteria with average abundance per location ranging from 86 to 98% (Fig. 3). Within this phylum, the vast majority of the bacterial consortia belong to the class gamma-proteobacteria (73-99%; Fig. 4), which is consistent with similar findings from a large number of studies that have been conducted on coral species [2]. Other important bacterial phyla associated with the corals aside Proteobacteria were Cyanobacteria (1-10%) and Bacteroidetes (0.3-2%) (Fig. 3).

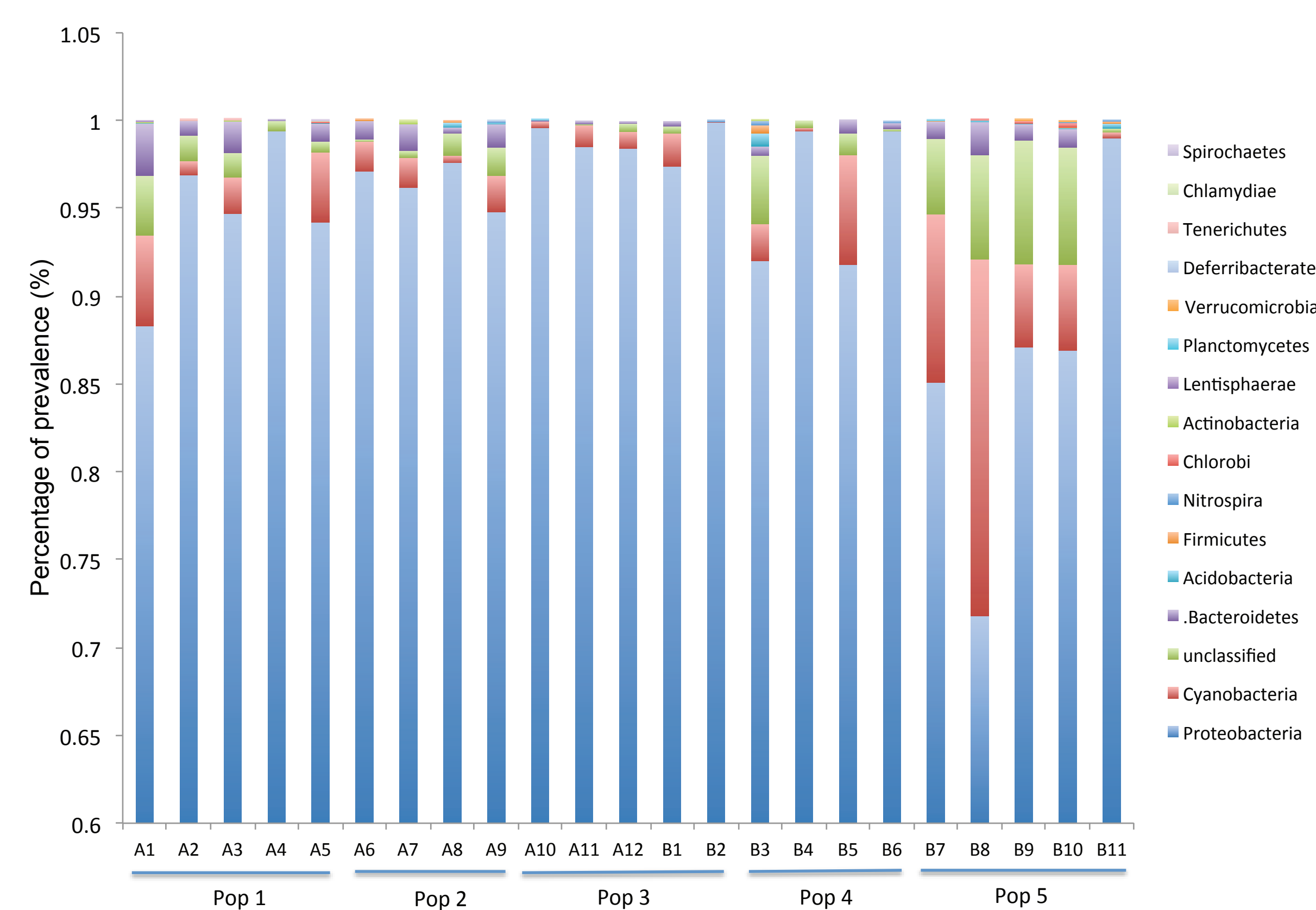


Fig. 3. Prevalence and distribution of bacterial phyla identified in the microbial community associated with the *P. astreoides* across all population sites. Pop 1= Water Factory 1; Pop 2= Water Factory 2; Pop 3= Water Factory 3; Pop 4= Snake Bay; Pop 5= Playa Jeremi. Taxonomic classification was done on a data set of 744,809 high quality sequencing reads using the RPD Naïve Bayesian Classifier.

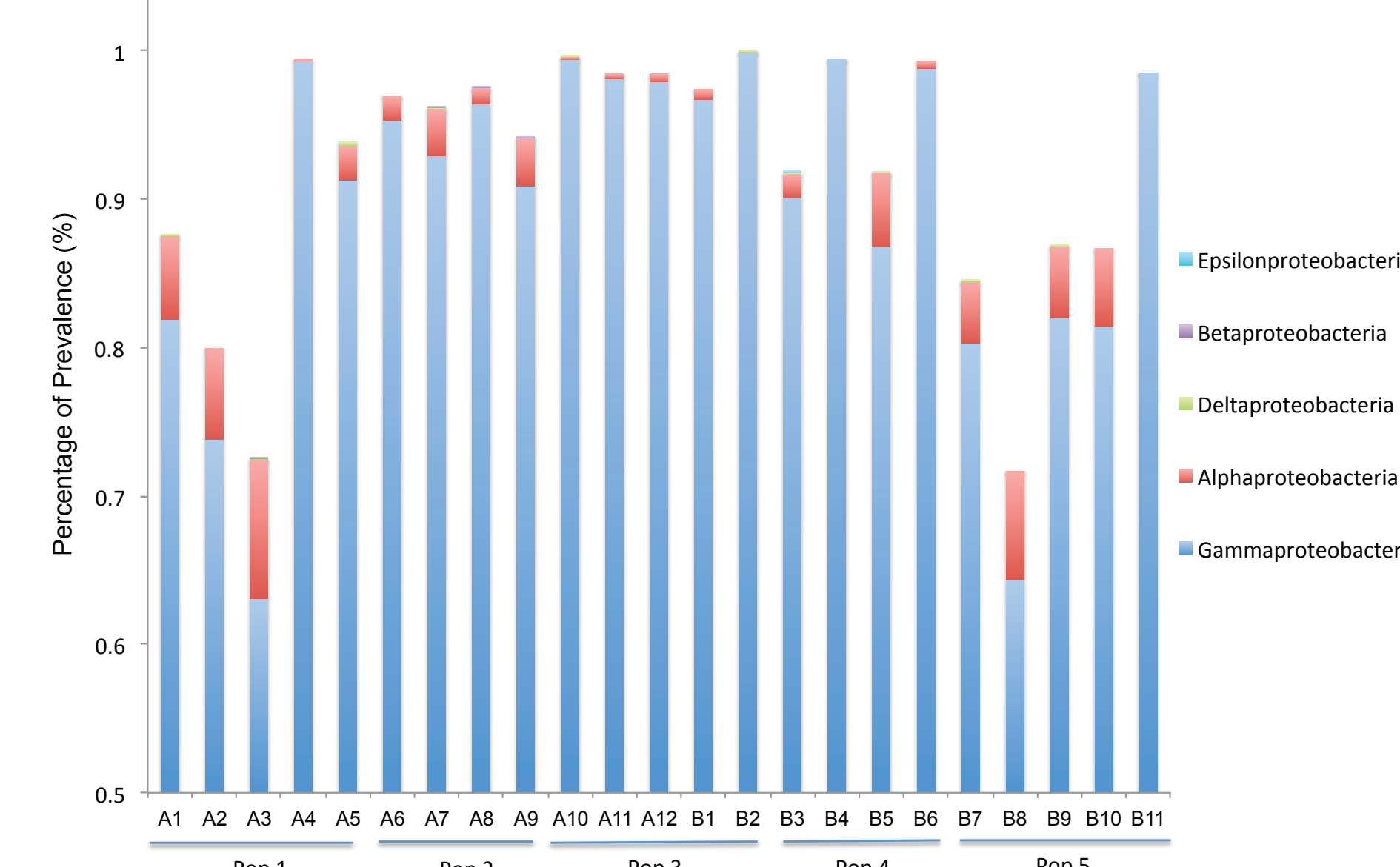


Fig. 4. Prevalence and distribution of bacterial classes within the Proteobacteria phylum identified in the microbial community associated with *P. astreoides* across all population sites. Pop 1= Water Factory 1; Pop 2= Water Factory 2; Pop 3= Water Factory 3; Pop 4= Snake Bay; Pop 5= Playa Jeremi. Taxonomic classification was done using the RPD Naïve Bayesian Classifier.

Furthermore, the taxonomic analysis of the resolved diversity showed consistent and dominant presence (~81.4% abundance, Fig. 5) by two OTUs affiliated to the order Oceanospirillales (within the gamma-proteobacteria), which corroborates a specific pattern of bacterial association emerging for this coral species and for many other corals within the same genus *Porites* [4]. Based on the sound effort of sequencing and biological replication used here, we argue that this specific association might indicate a symbiotic mutualistic association with the adult coral partner. Further studies are now needed to understand how the partners implicated in the coral/Oceanospirillales symbiosis are physiologically benefiting from the association. Also it is interesting to dissect the mechanism that drive the onset of these specific associations, since new findings suggests that Oceanospirillales are not the main bacteria transferred from the mother coral colony to the offspring upon the release of new larvae [5].

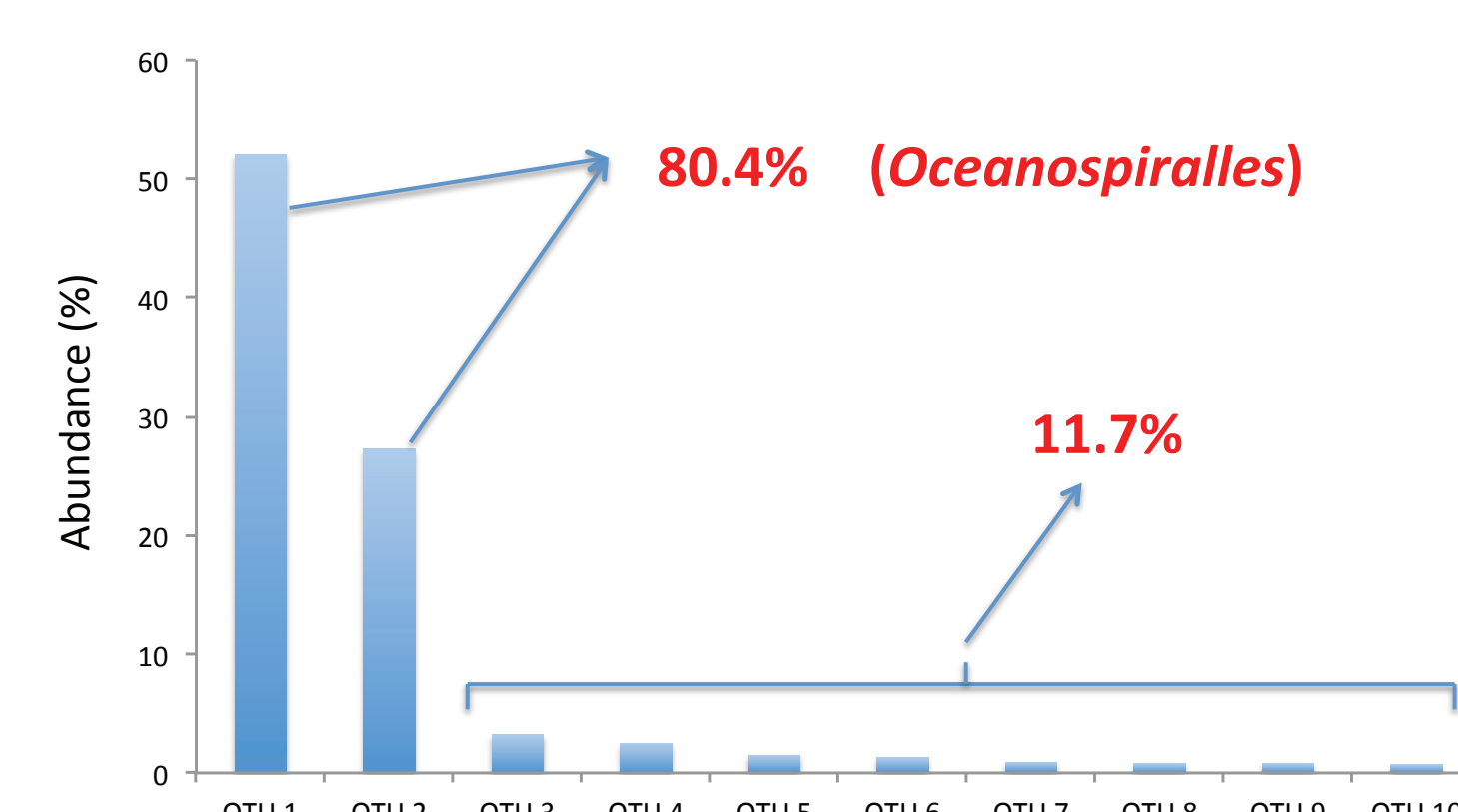


Fig. 5. Average abundance of the ten most common OTUs identified in association with *Porites astreoides*

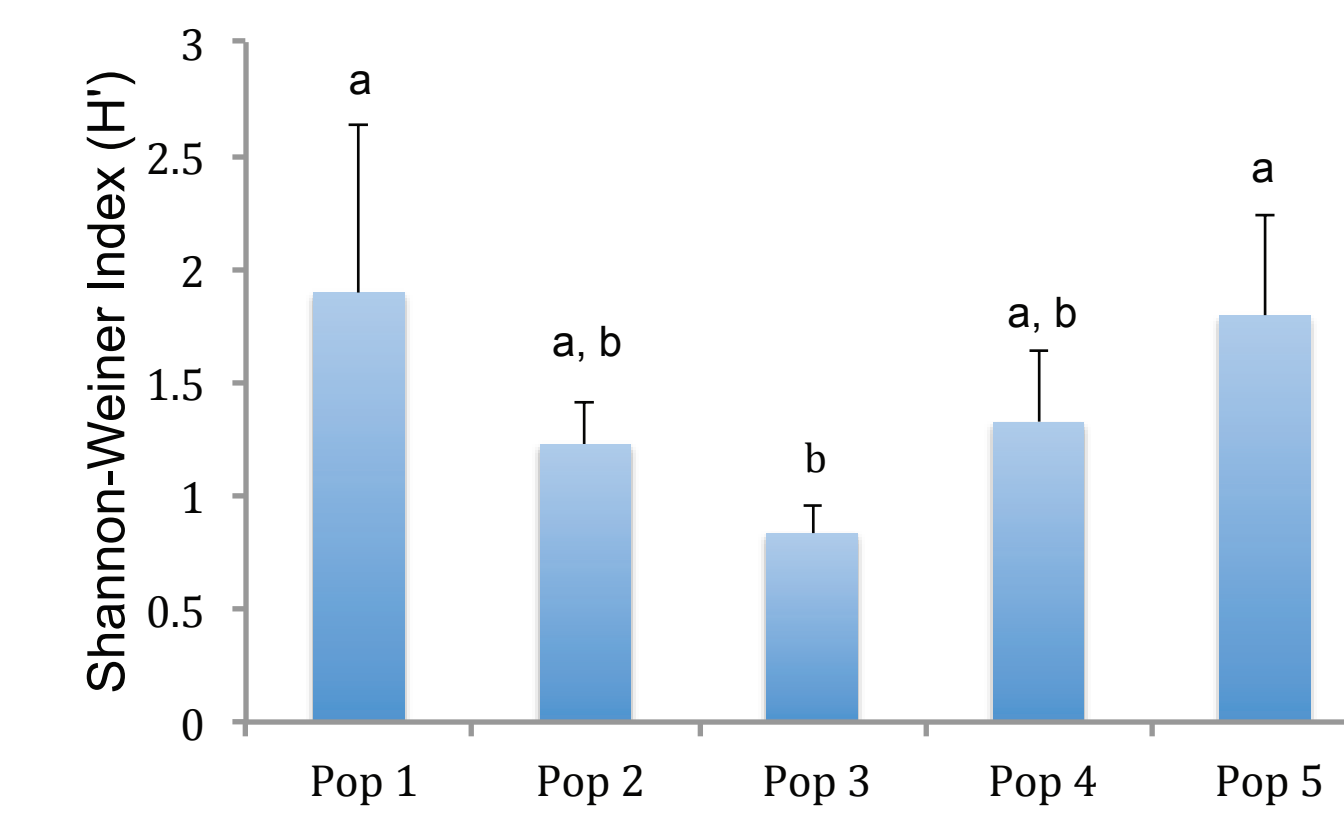


Fig. 6. Average Shannon-Weiner diversity index of the bacterial community associated with the coral *Porites astreoides* in each population site. Pop 1= Water Factory 1; Pop 2= Water Factory 2; Pop 3= Water Factory 3; Pop 4= Snake Bay; Pop 5= Playa Jeremi. N= 4-5. Bar= SD. Letters on top of the bars shown significance difference among population sites, $P < 0.05$.

While two main Oceanospirillales bacteria dominate the community assemblage, we also identified a highly diverse rare bacterial "biosphere" (~725 OTUs with abundances lower than 1%) living along with the dominant bacterial symbionts. Interestingly, the assemblage of this biosphere showed to be structured along the geographical scale. Significant differences in the Shannon-Weaver index (H') of diversity were detected across the spatial scales (Fig. 6; One-way Anova; $F=4.8215$, p -value=0.008).

Two-dimensional non-metric multidimensional scaling (nMDS) ordination of the coral-associated microbial assemblages from all collected sites showed also clear differences across the spatial scale (ANOSIM; $R=0.30$, p -value=0.001; Fig. 7). Samples from each population clustered close to each other, in particular from population site 2 (Water factory 2), 3 (Water factory 3) and 4 (Playa Jeremi). The samples from population site 1 (Water Factory 1) showed to be the most spread out, but still their cluster on the graph had little overlap with samples from other populations. Altogether, the significant community-structuring patterns indicate that the bulk of rare members of the bacterial community differ, both in composition and abundance, as a function of geographical.

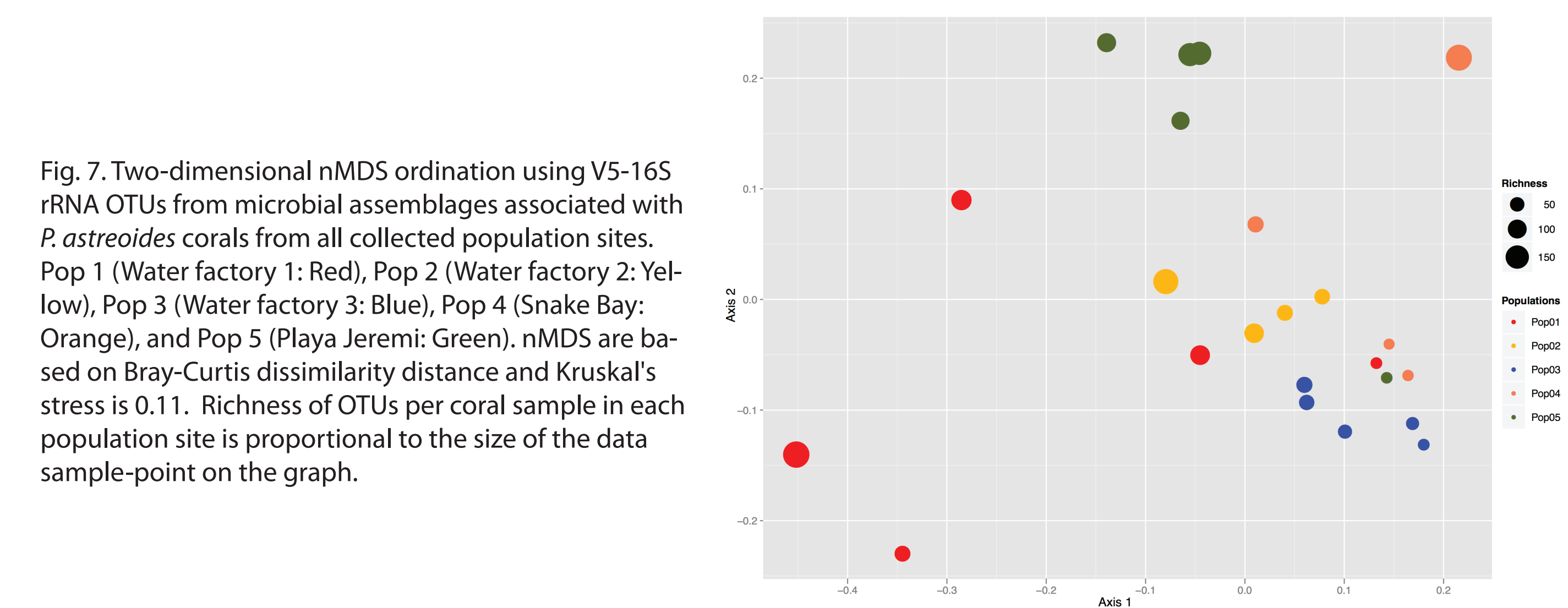


Fig. 7. Two-dimensional nMDS ordination using V5-16S rRNA OTUs from microbial assemblages associated with *P. astreoides* corals from all collected population sites. Pop 1 (Water factory 1: Red), Pop 2 (Water factory 2: Yellow), Pop 3 (Water factory 3: Blue), Pop 4 (Snake Bay: Orange), and Pop 5 (Playa Jeremi: Green). nMDS are based on Bray-Curtis dissimilarity distance and Kruskal's stress is 0.11. Richness of OTUs per coral sample in each population site is proportional to the size of the data sample-point on the graph.

In order to identify co-occurrence patterns of the microorganisms associated with the coral *P. astreoides*, we resolved the network of interconnection among the 739 identified OTUs. Overall, the *P. astreoides* microbial network was comprised of highly connected OTUs structured within a major cluster of nodes (59 nodes and 139 links ~ degree connectivity= 4.71). However, a small but also clearly structured cluster of nodes was comprised of mainly Oceanospirillales members (Fig. 8). The structural analysis also showed that the most common OTU 1, affiliated to Oceanospirillales, is not connected to the cluster of nodes represented by mainly Oceanospirillales members. Instead, OTU1 showed a co-occurrence patterns with two OTUs (OUT 9 and 12) affiliated with the order Rhodobacterales; one of them from the genus *Leisingera* (OTU 9). With regard to the other cluster dominated by networked Oceanospirillales OTUs, a clear co-occurrence among all these four OTUs was shown with the Alteromonadales member from the genus *Paramoritella* (OUT 11).

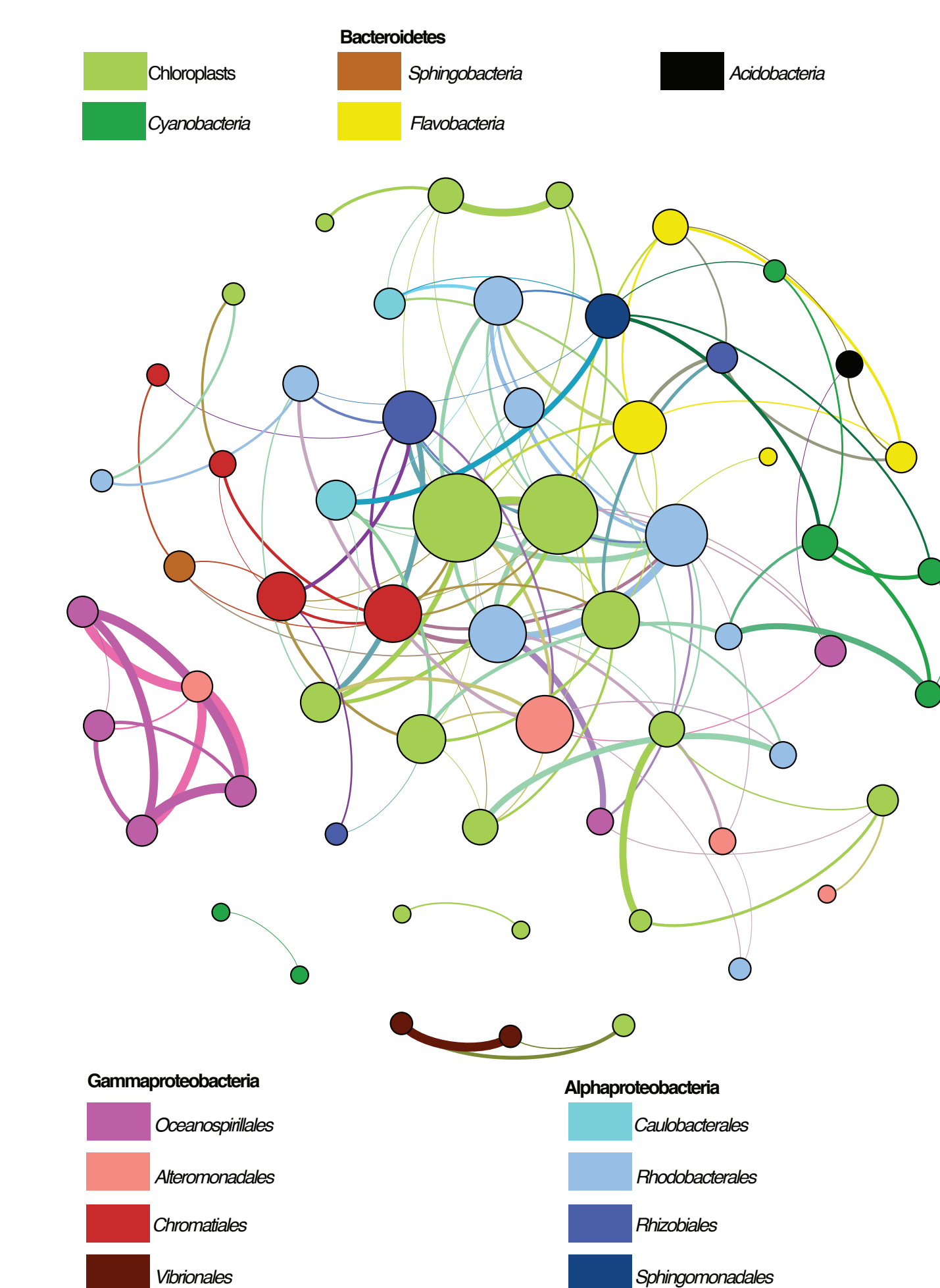


Fig. 8 Network of co-occurring 97% cutoff OTUs based on correlation analysis. A connection stands for a strong (Spearman's $\rho > 0.6$) and significant (P -value < 0.01). The size of the node is proportional to the number of connections.