

Complex and varying lichen microbiomes according to the vertical positions of thalli in *Cladonia* sp. from King George Island, Antarctica

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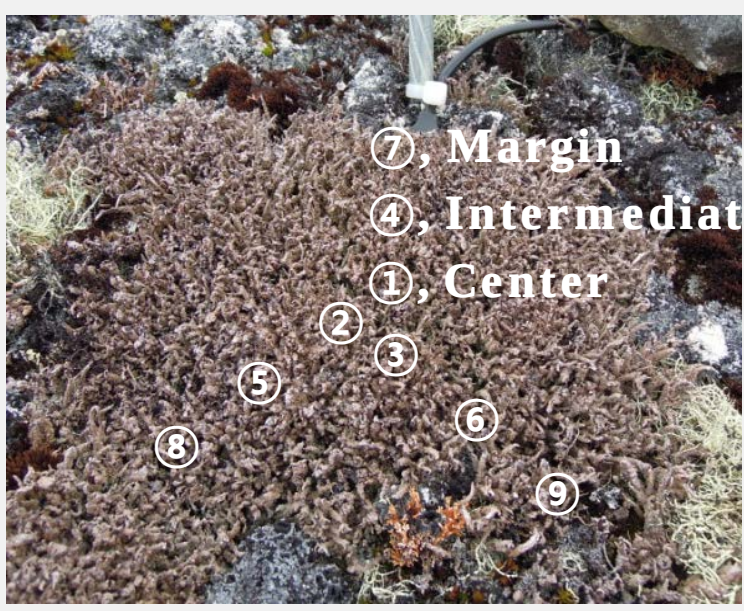
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Lichens are symbiotic organisms that are majorly composed of lichenized fungi (mycobiont) and green algae/or cyanobacteria (photobiont). However, lichen thalli also contain highly diverse microbes such as bacteria, archaea and microfungi (microbionts) as well as mycobiont and photobiont. Although research interests on ecological functions of microbionts have been raised greatly, very little knowledge on this topic has been gained, especially on microbionts of lichens from extreme environments. Microbial communities are usually affected by subtle changes of environments and different parts of lichen colonies, *Cladonia* sp., which forms lichen colonies composed of many slender individual thalli, may provide different micro-environments to the microbial communities of *Cladoia* sp. from King George Island, Antarctica. To reveal the microbial communities of different parts of the lichenized fungi, we sectioned thalli from center, intermediate, and marginal positions of colonies to apical, middle and basal parts. Bacterial 16S rRNA gene and eukaryotic nuclear large subunit rRNA gene (LSU) were analyzed by 454 pyrosequencing method. *Alphaproteobacteria* and *Acidobacteria* were the major bacterial phyla in all parts of the thalli with varying abundance. Apical parts of thalli contained relatively simple microbial communities that were majorly composed of *Acetobacteriaceae*. Basal parts contained much more complex microbial communities with diverse phyla and enriched with *Actinobacteria* and *Cyanobacteria*. LSU sequences were mostly composed of that of *Cladonia* sp., but also contained diverse fungal species including *Dithideomycetes*, *Lecanoromycetes*, *Leotiomycetes* and *Eurotiomycetes* with varying abundance depending on the vertical positions. Diverse algal species and different composition according to the vertical position provide insights on fungal selectivity to algal genotypes depending on the micro-environmental conditions. Since apical parts are usually more exposed to sun light and wind, and has lower humidity compared to basal parts, we considered this sectioning to represent different conditions.

Materials and Methods

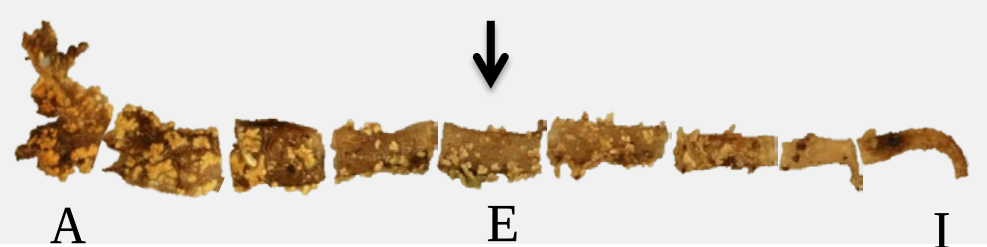
1. Site and Sampling

- Latitude : 62°14'24.84"S
- Longitude : 58°44'32.66"W
- Elevation : 25 m
- Mean annual temp. : -1.40 °C
- Mean annual RH : 94.25 %
- Mean annual PAR : 165.09 uE



2. Washing and Section

- Washing : 0.85 % NaCl
- Section : Apical(A), middle(E) and basal(I) part



3. DNA extraction, PCR and Sequencing

- GeneAll Soil SV mini kit
- Bacterial 16S rDNA (V1-V3)
- Eukaryotic LSU rDNA (D1-D2)
- 454 pyrosequencing, GS FLX Titanium

4. Microbiome analysis

- Trimming : Pyrotrimmer
- Clustering : Clustom, TBC
- Chimera check : Uchime
- Taxonomic assignment : EzTaxon, RDP
- Statistical analysis : Primer 6

Results

Lichen Forming Fungi

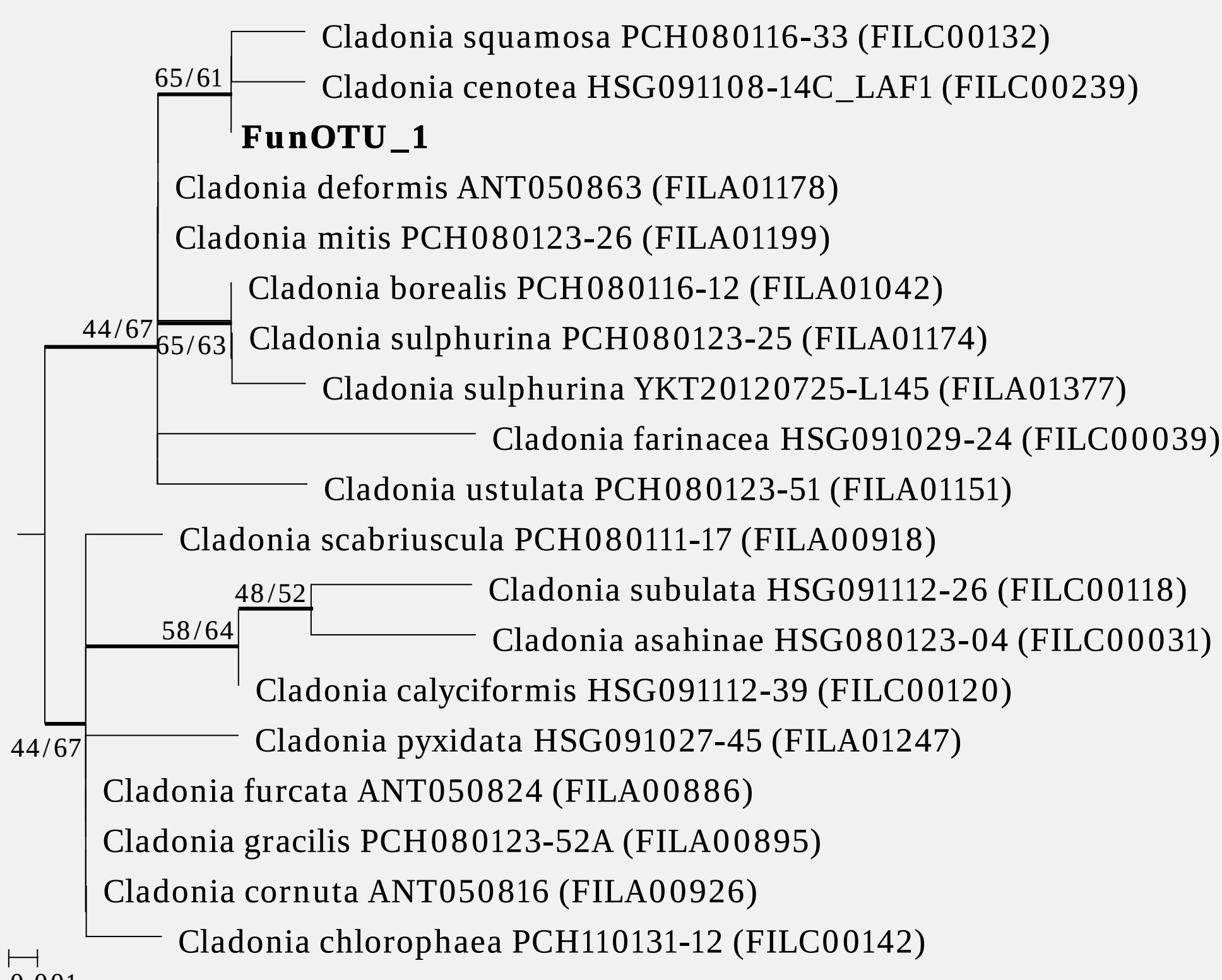
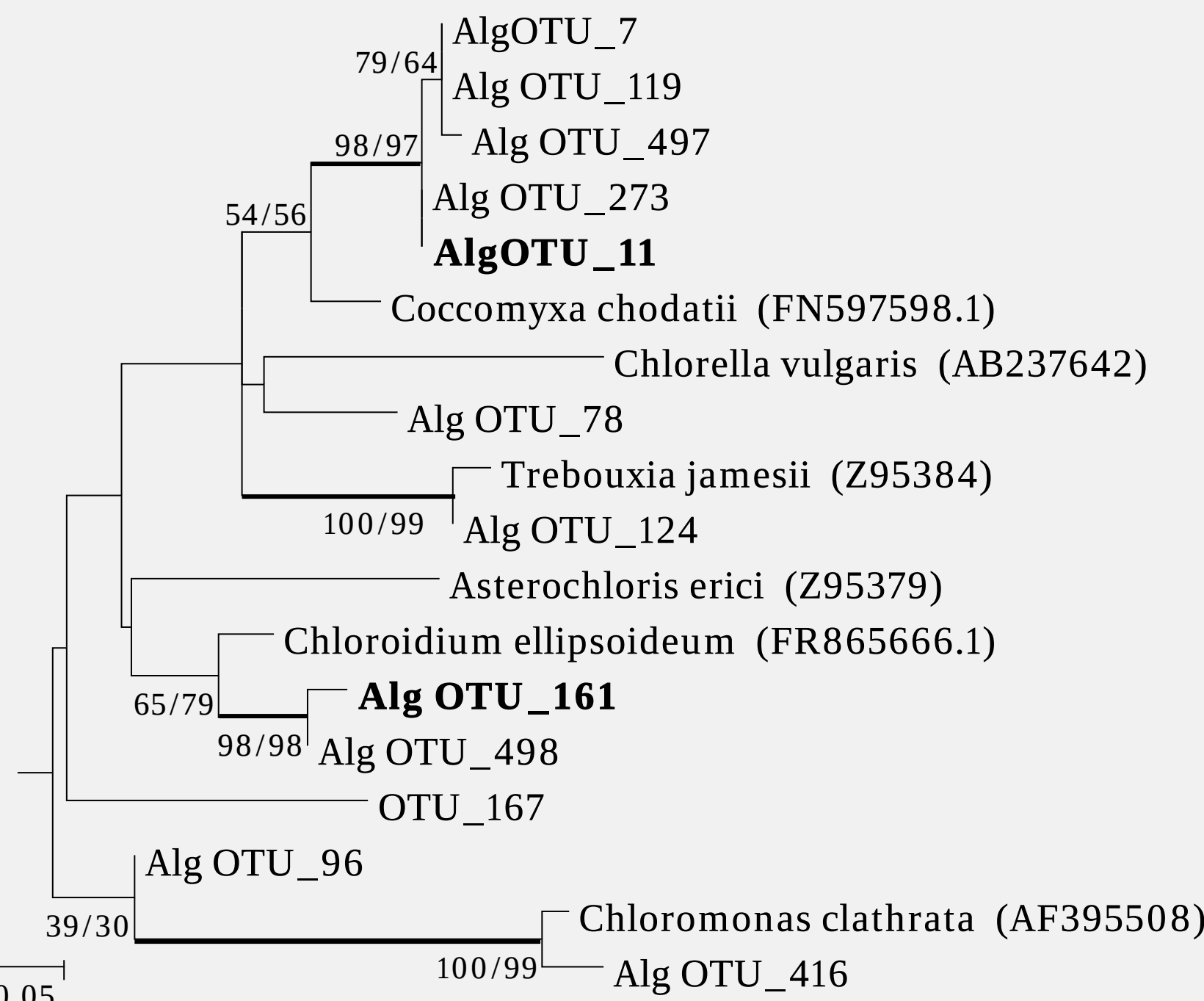


Fig 1. Maximum likelihood tree of LSU rDNA of mycobionts of *Cladonia* samples. Branches conserved in maximum parsimony and maximum likelihood were represented by thick lines(MP/ML).

Algae



OTU11	Center	Intermediate	margin	OTU161	Center	Intermediate	margin
Apical				Apical			
Middle				Middle			
Basal				Basal			

Fig 2. Phylogenetic tree using maximum likelihood method based on LSU rDNA of Viridiplantae. Branches conserved in maximum parsimony and maximum likelihood were represented by thick lines (MP/ML).

Lichen Associated Fungi

Class	OTU (Sequence reads)	Core			Intermediate			Margin			Total
		Apical 2014KGN-011A (64)	Middle 2014KGN-011E (126)	Basal 2014KGN-011I (64)	Apical 2014KGN-014A (11)	Middle 2014KGN-014E (23)	Basal 2014KGN-014I (17)	Apical 2014KGN-017A (31)	Middle 2014KGN-017E (99)	Basal 2014KGN-017I (34)	
Arthoniomycetes	FunOTU_17	7.7	0	2.6	7.7	8.0	0	11.4	5.8	0	3.9
Ascomycota	FunOTU_87	0	1.4	0	0	4.0	4.8	0	0	0	0.8
incertae sedis	FunOTU_371	0	0	0	0	20.0	0	0	0	0	1.0
Dothideomycetes	FunOTU_6	76.9	0.7	13.2	53.8	32.0	14.3	62.9	68.9	0	33.5
Eurotiomycetes	FunOTU_342	0	0	3.9	0	0	0	0	0	0	0.6
	FunOTU_148	0	0	3.9	0	0	14.3	0	0	0	1.2
	FunOTU_108	0	0.7	2.6	0	0	0	0	0	0	0.6
	FunOTU_102	0	2.9	0	0	0	0	0	0	0	0.8
Lecanoromycetes	FunOTU_110	0	3.6	0	0	4.0	0	0	1.0	27.0	3.3
	FunOTU_530	0	0	0	0	0	0	0	2.9	0	0.6
	FunOTU_38	0	15.8	0	0	0	0	0	2.9	0	4.9
	FunOTU_90	0	10.8	0	0	0	0	0	0	0	2.9
Leotiomycetes	FunOTU_45	0	22.3	0	0	0	0	0	0	5.4	6.4
	FunOTU_151	0	0	25.0	0	0	0	0	0	0	3.7
	FunOTU_59	1.5	23.0	10.5	0	4.0	38.1	0	1.9	21.6	11.7
	FunOTU_176	0	0	0	0	0	9.5	0	0	5.4	0.8
Cystobasidiomycetes	FunOTU_113	0	1.4	0	0	4.0	0	0	0	2.7	0.8
	FunOTU_39	0	1.4	0	0	0	0	0	1.0	8.1	1.2
	FunOTU_160	0	0.7	1.3	0	0	0	0	1.0	5.4	1.0
	FunOTU_94	0	3.6	1.3	0	0	0	0	0	5.4	1.6
Microbotryomycetes	FunOTU_130	0	1.4	3.9	0	0	0	0	0	0	1.0
	FunOTU_340	0	0	5.3	0	0	0	0	0	0	0.8
	FunOTU_335	0	0	5.3	0	0	0	0	0	0	0.8
	FunOTU_48	6.2	0	0	0	12.0	0	11.4	5.8	8.1	4.1
Tremellomycetes	FunOTU_66	1.5	0	0	7.7	0	0	2.9	1.9	0	1.0
	FunOTU_168	0	0	5.3	0	0	0	0	0	0	0.8
	FunOTU_41	4.6	0	0	15.4	4.0	0	0	2.9	2.7	1.9

Table 1. Relative abundance of major lichen-associated fungal OTUs (>0.5 %)

Bacteria

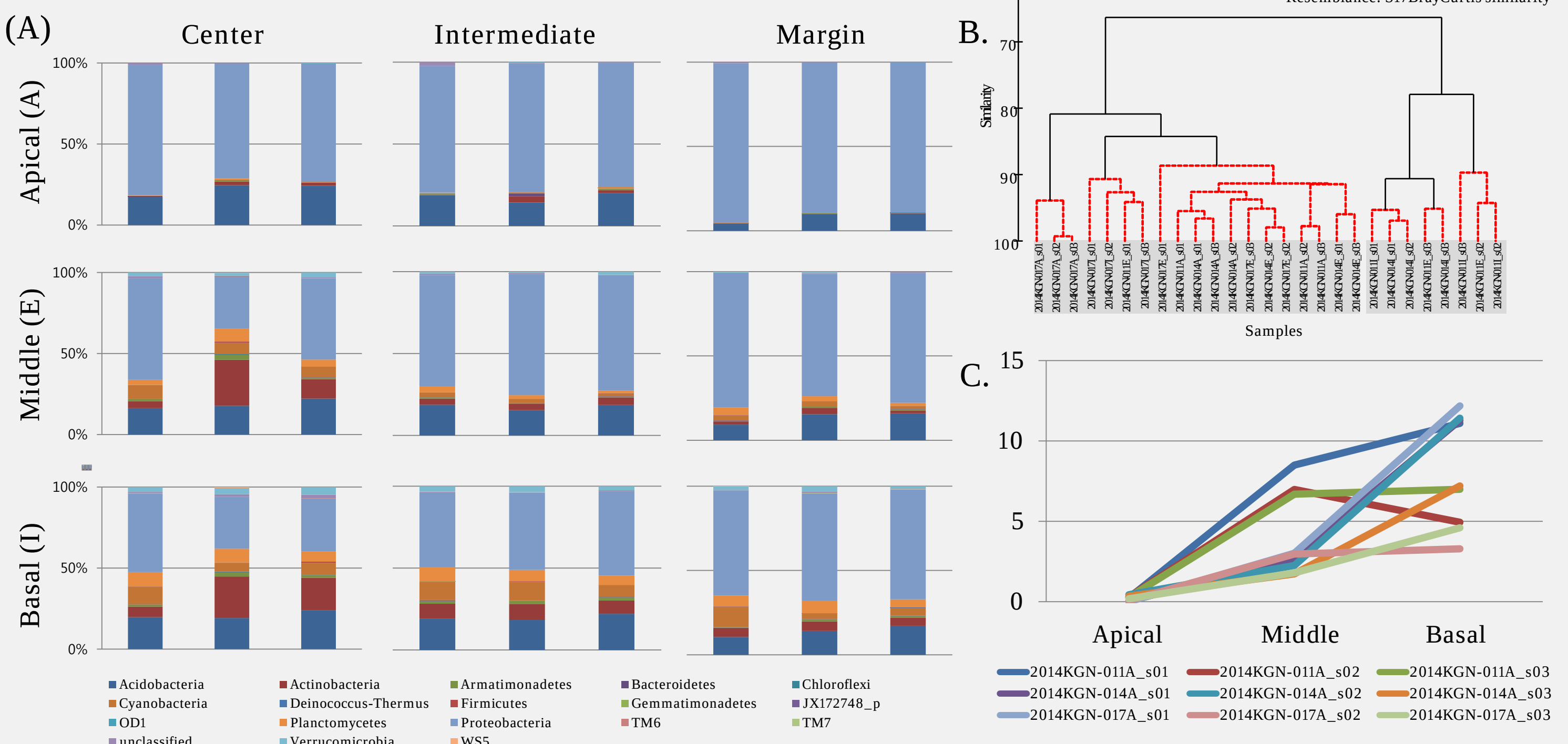


Fig 3. A. Bacterial phyla distribution B. Similarities of samples by bacterial composition C. Abundance of the Cyanobacterial OTUs

Conclusion

- Since apical parts are usually more exposed to sun light and wind, and has lower humidity compared to basal parts, we considered this sectioning to represent different conditions.
- The microbiome showed different pattern according to vertical position of *Cladonia* thalli.
- Especially, apical part and basal part presented distinct patterns.
- Diverse algal species and different composition according to the vertical position provide insights on fungal selectivity to algal genotypes depending on the micro-environmental conditions.