

# Microbial Communities of *Cladonia* Lichens and Their Biosynthetic Gene Clusters Potentially Encoding Natural Products

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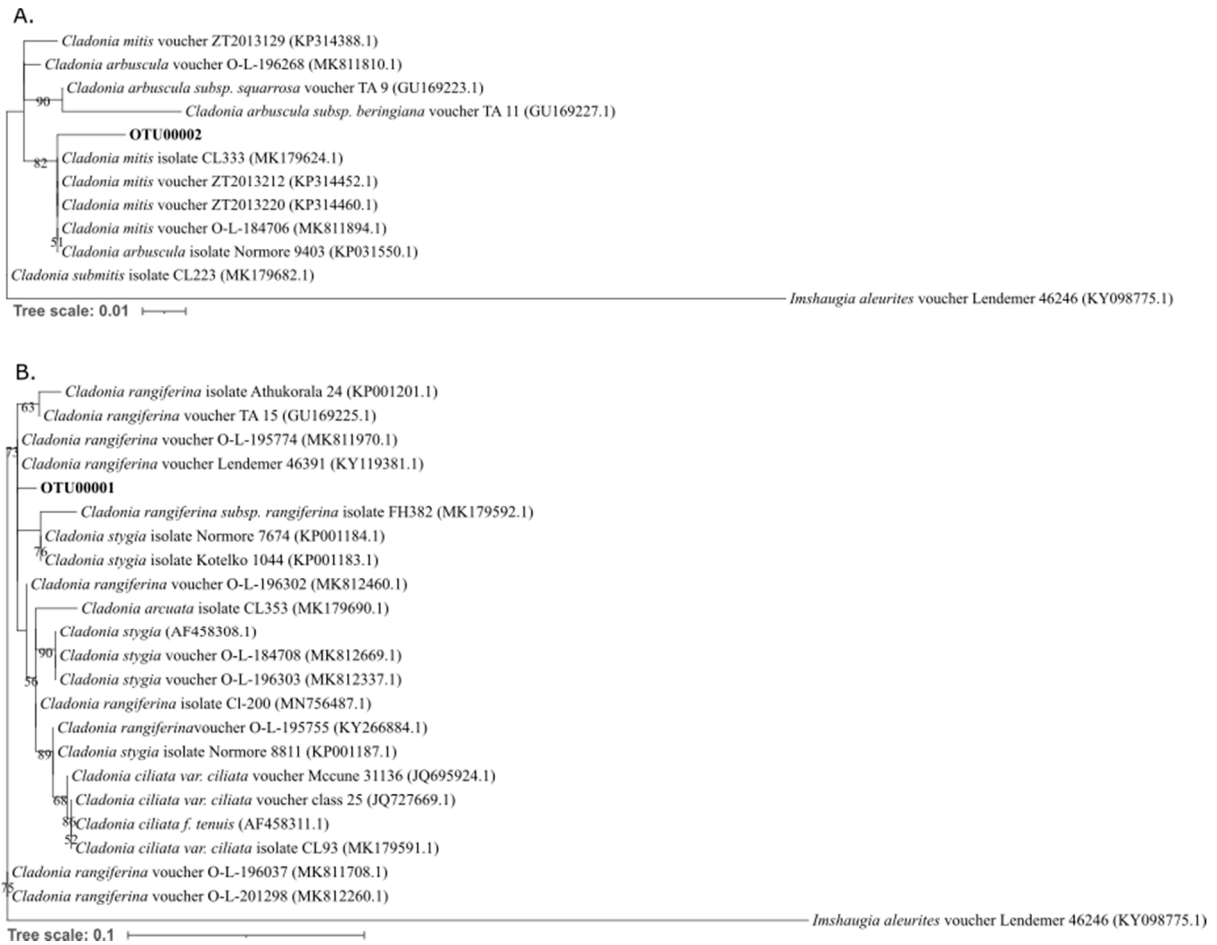
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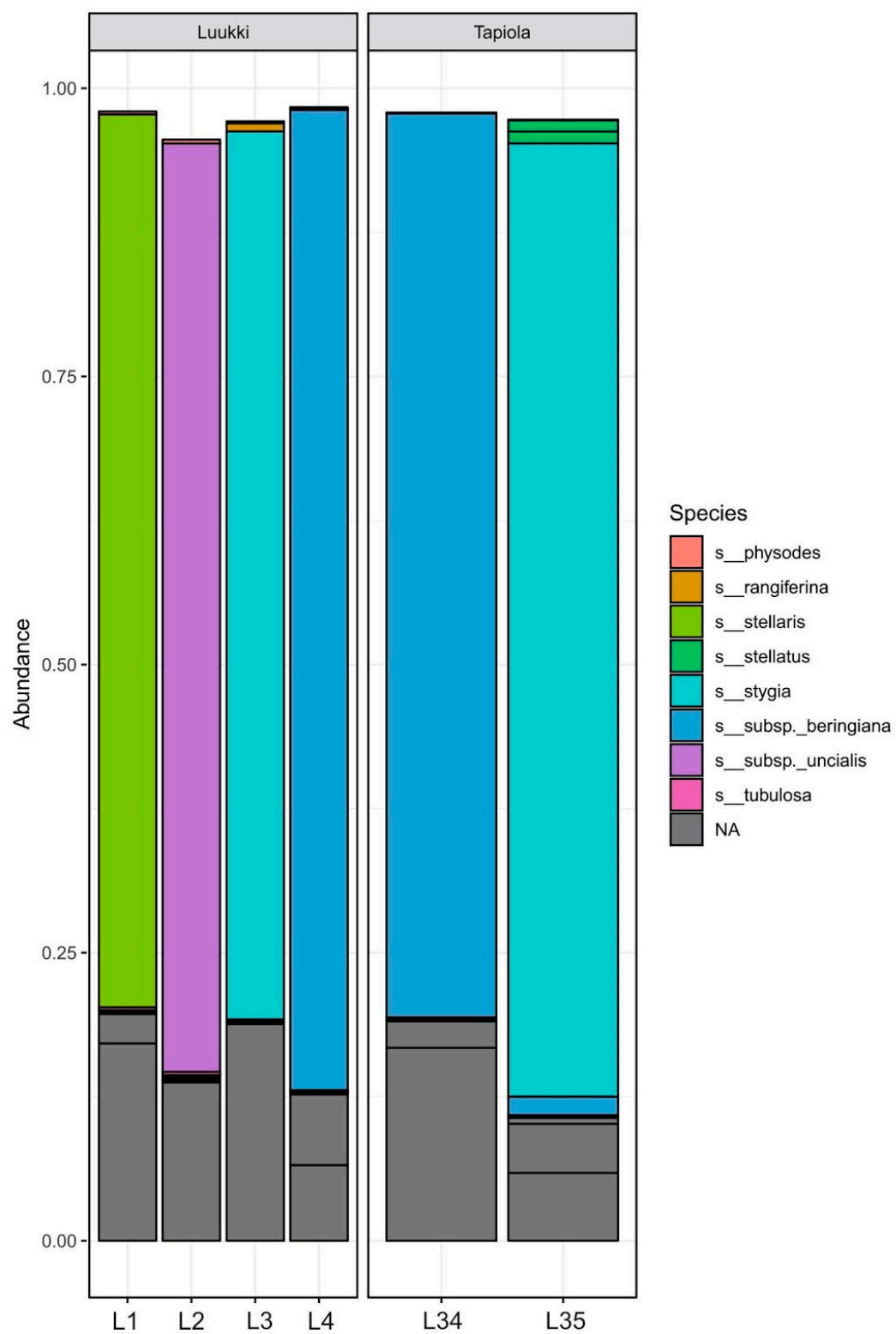
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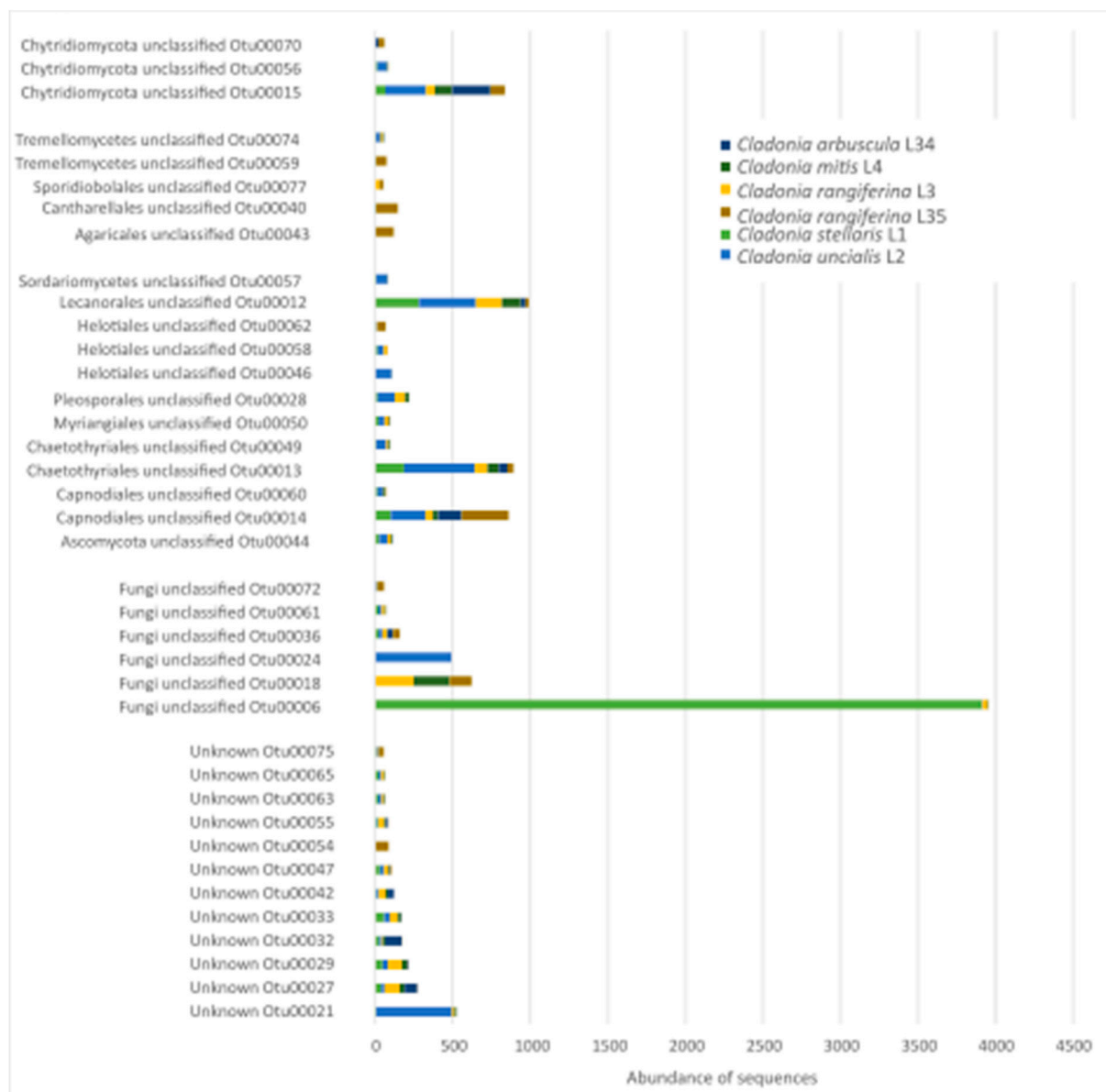
\* Correspondence: taniakeiko@gmail.com



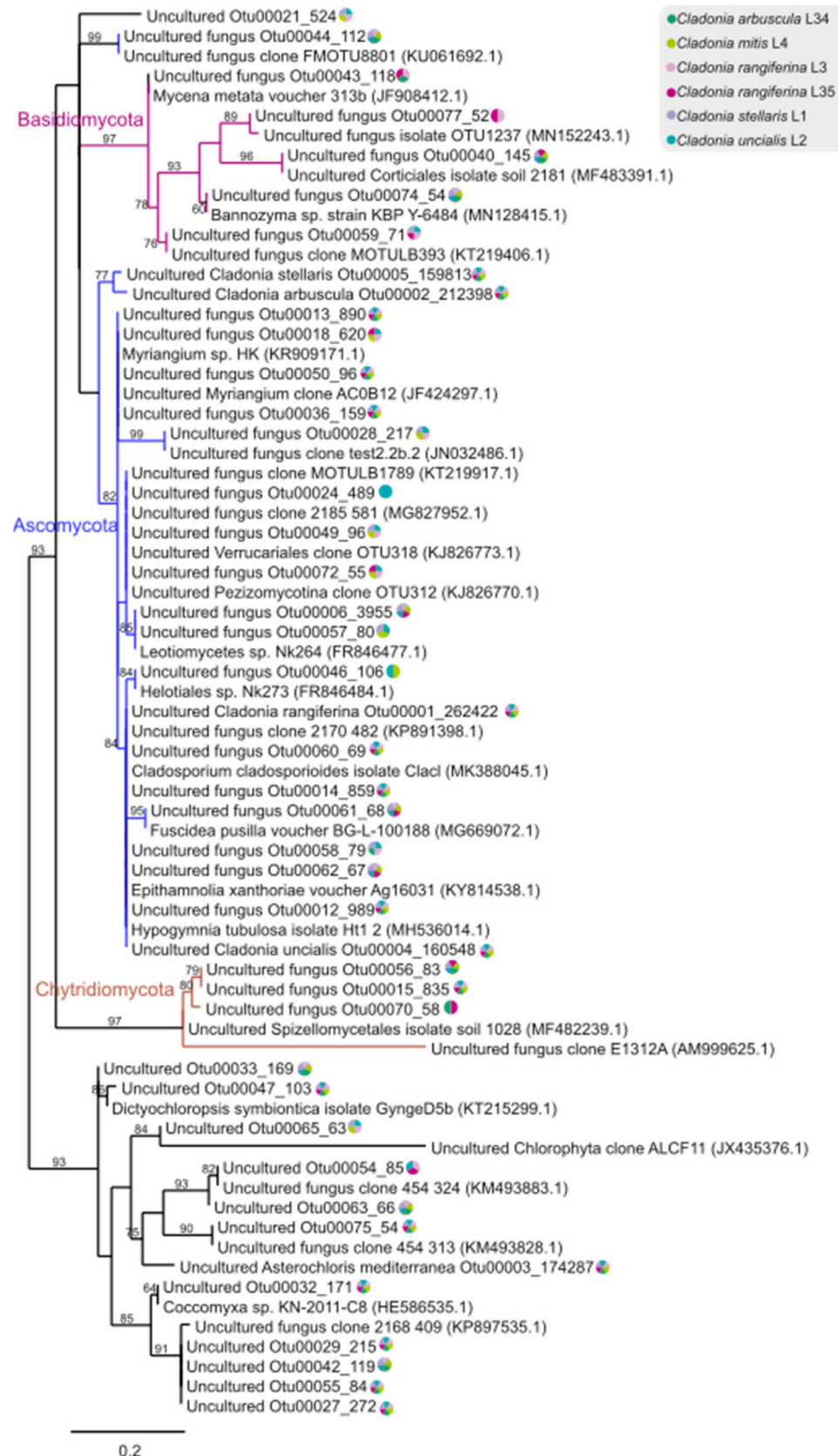
**Figure S1.** Maximum likelihood phylogenetic tree constructed using representative ITS2 amplicon sequences of two operational taxonomic units OTU 00002 (A) and OTU00001 (B) obtained from mothur analysis. Values above 50 % for the support of each node after 1000 bootstraps are shown.



**Figure S2.** Microbial community at species taxonomic level inferred by DADA2 based on amplicon sequence variants (ASVs) from samples collected from a boreal forest (Luukki) and urban area (Tapiola) sites.



**Figure S3.** Number of unclassified ITS2 sequences at family taxonomic level (n > 50) per sample.



**Figure S4.** Evolutionary history based on ITS2 unclassified operational taxonomic units (OTUs with above 50 sequences). The legends indicate the samples which contained the OTUs. Otu0006 mentioned in the main text is shown in bold.

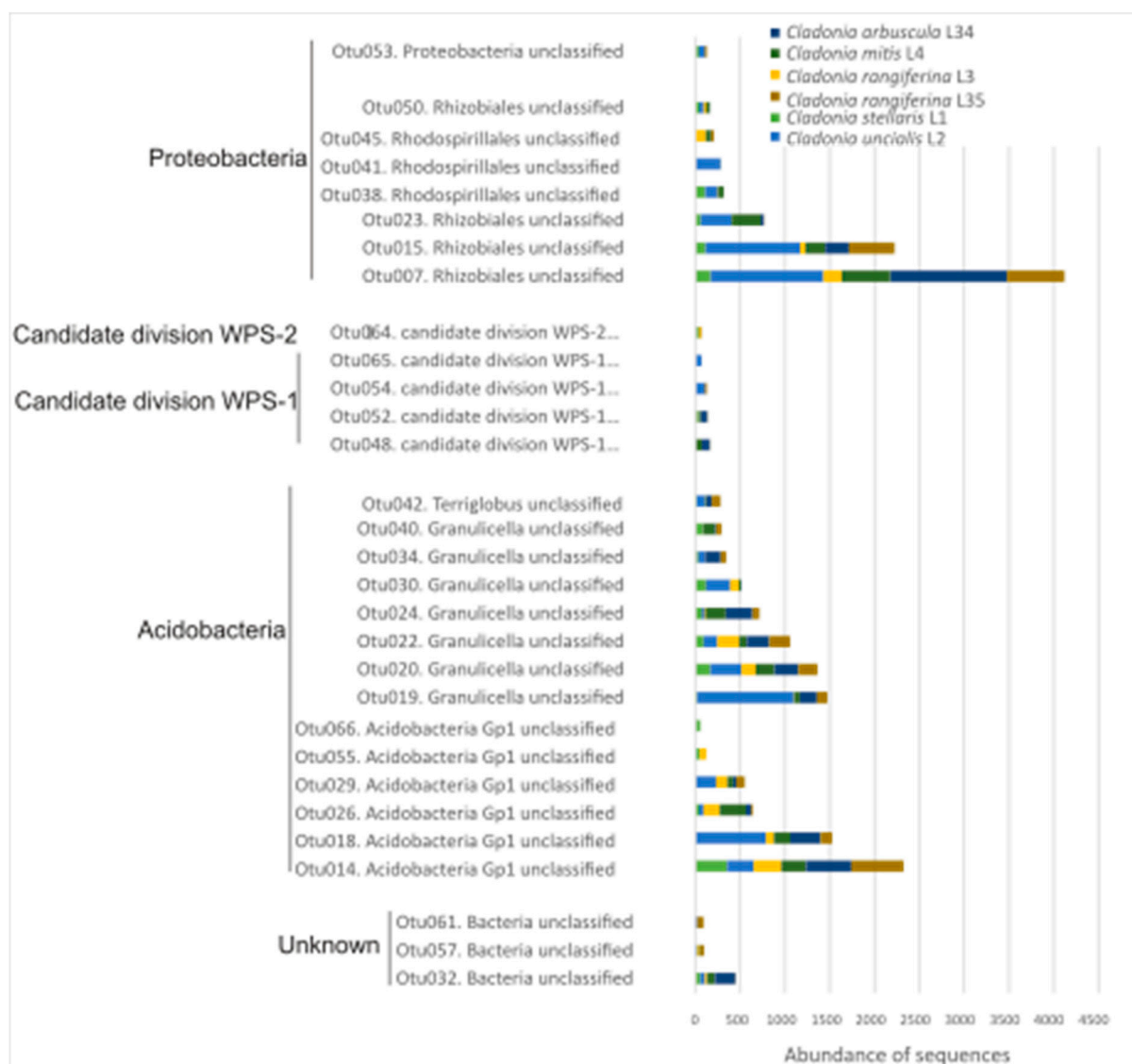
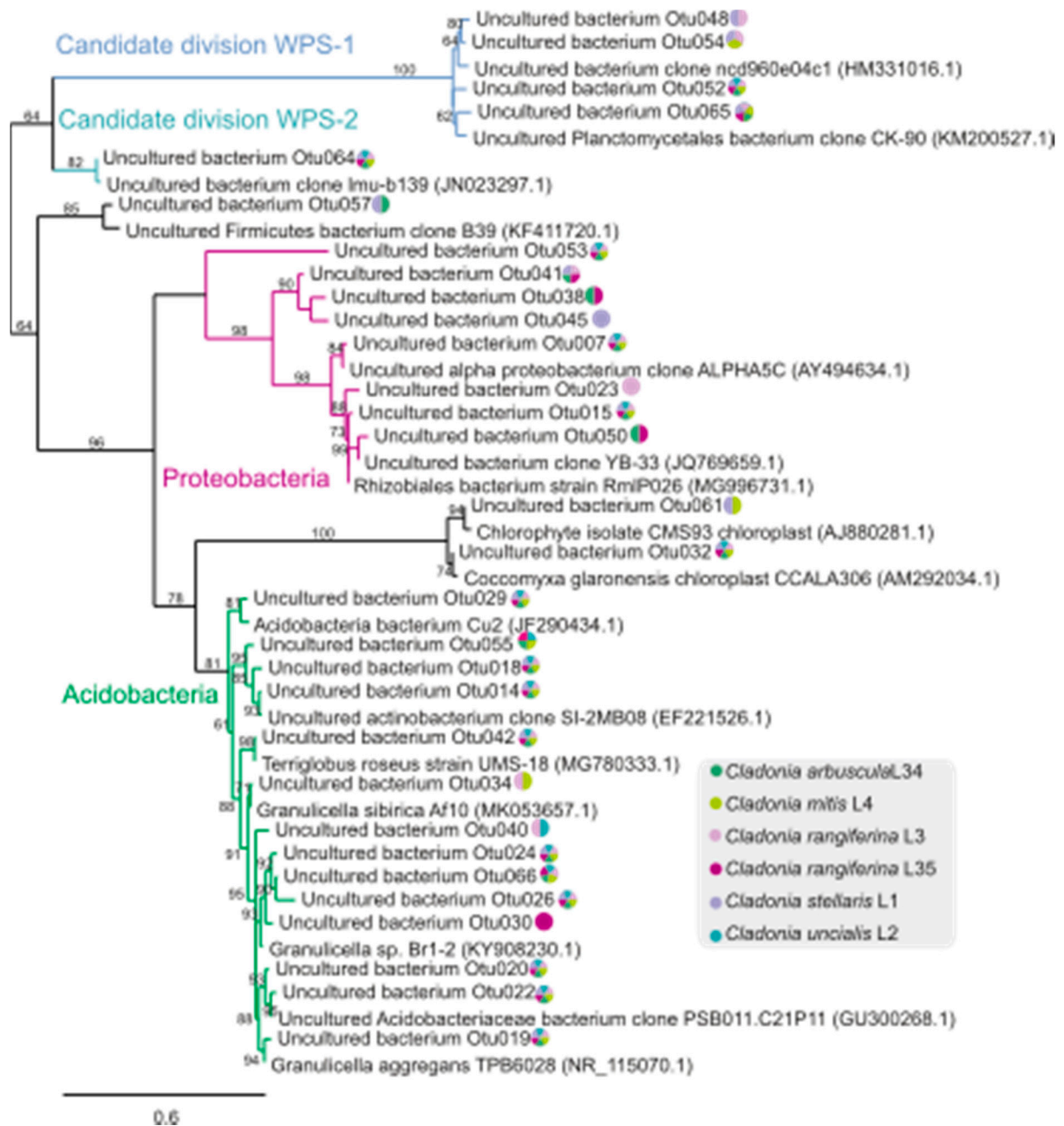
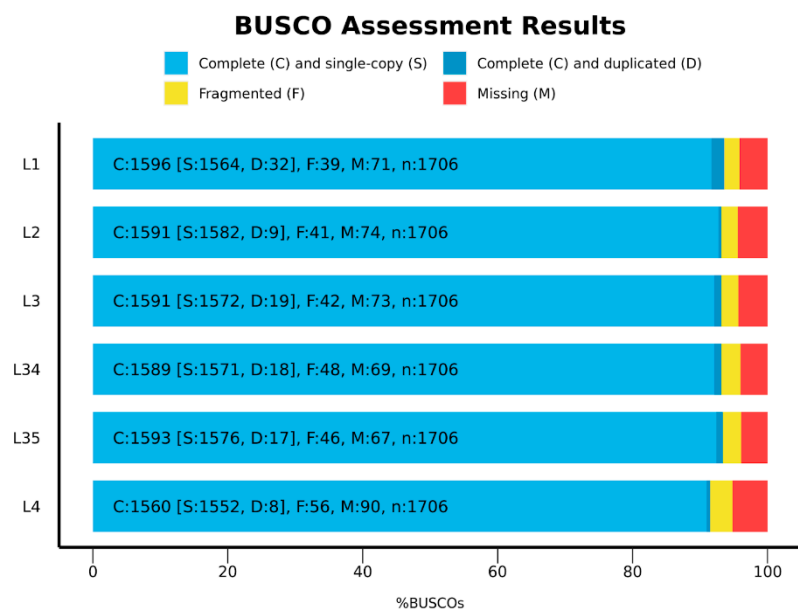


Figure S5. Number of unclassified 16S OTUs at predicted family level obtained per lichen sample.

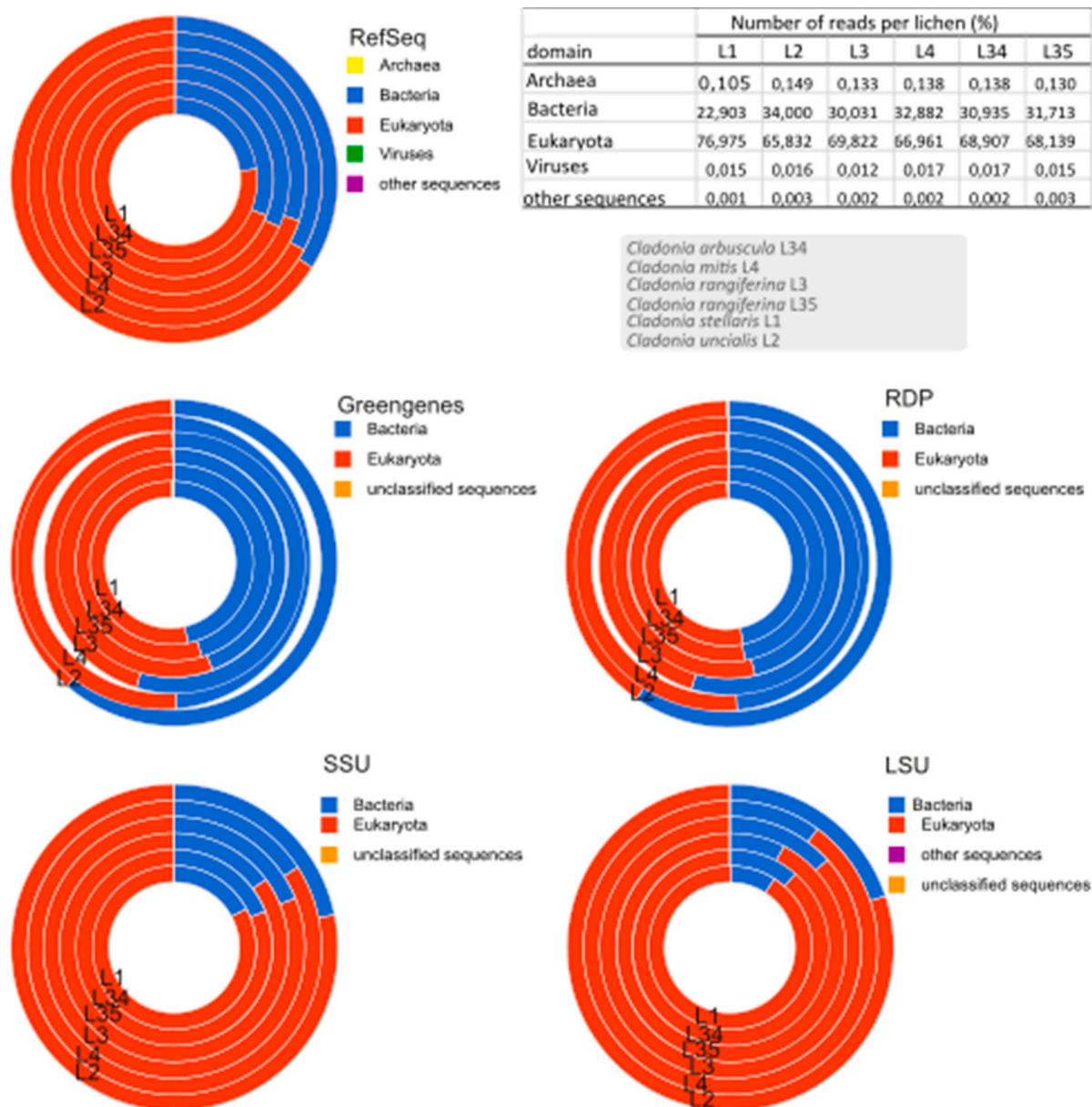


**Figure S6.** Evolutionary history based on 16S rRNA gene V3-V4 region unclassified sequences of representative operational taxonomic units (OTUs) above n = 50. The legends indicate the lichen samples which contained the OTUs.

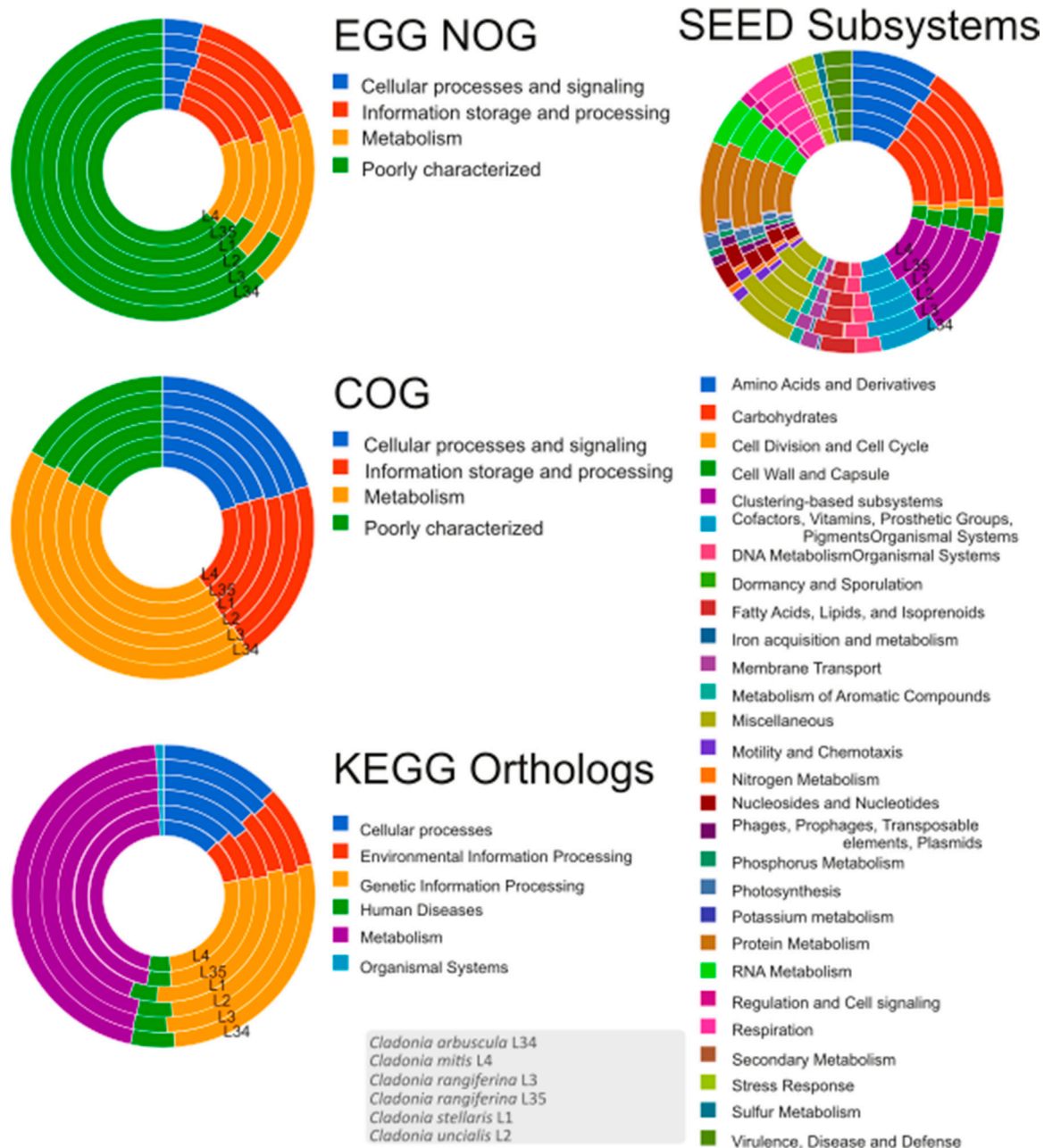


**Figure S7.** Completeness assignment based on 1706 expected gene content using BUSCO.





**Figure S8.** Taxonomic assignment of shotgun sequences obtained using different databases in MG-RAST. The inserted table indicates the percentage of reads assigned to different taxonomic taxa.

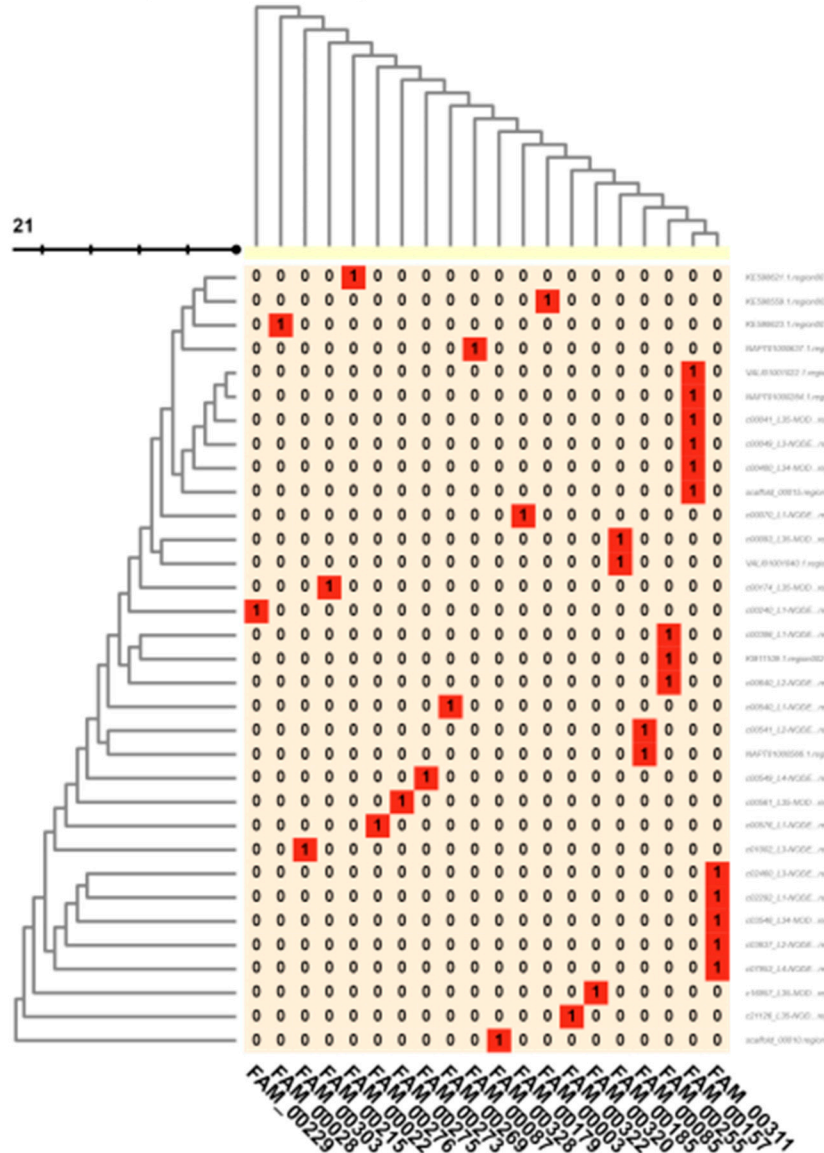


**Figure S9.** Functional assignment identified at the highest level using EGG NOG, COG, KEGG Orthologs and SEED sub-systems in the MG-RAST server. Sequences with assigned function represented 44 to 49% (L34 -44.22%, L35 -44.68%, L1 -46.21%, L2 -45.19%, L3 -49.21%, L4 -43.69%) of the total raw sequences submitted to MG-RAST.

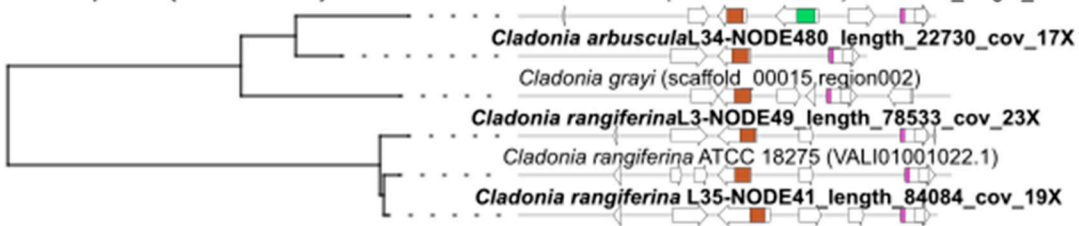
Phylogenetic tree and genomic maps of *Cladonia* species. The tree on the left shows relationships between *Cladonia metacoralifera*, *Cladonia arbuscula*, *Cladonia mitis*, *Cladonia uncialis*, *Cladonia rangiferina*, and *Cladonia stellaris*. To the right, genomic maps for each species are shown, including node numbers, lengths, and coverage. For example, *Cladonia arbuscula* L34-NODE34 has a length of 83264 and coverage of 20X. *Cladonia rangiferina* L35-NODE281 has a length of 35525 and coverage of 21X. *Cladonia stellaris* L1-NODE189 has a length of 41944 and coverage of 20X.

**Figure S10.** Hybrids of biosynthetic gene clusters containing both NRPS and PKS biosynthetic genes. A. Heatmap based on presence/absence of the 20 largest NRPS-PKS hybrid gene cluster families from lichen metagenomes obtained in this study and in sequenced genomes of *Cladonia* fungi. B. Phylogenetic history of hybrid NRPS and PKS biosynthetic gene cluster (FAM\_00220) obtained during BiG-SCAPE/CORASON analysis. All metagenomes (L1-L34, in bold) and *Cladonia* genomes have similar core biosynthetic protein with the same order of domains and same substrate prediction for the adenylation (phenylalanine) and acyltransferase (malonyl CoA) domains.

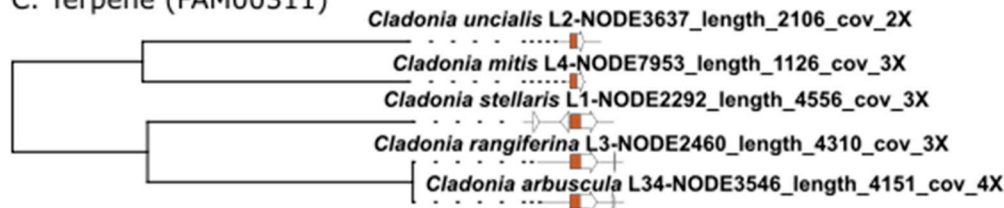
### A. Presence/absence of Terpene



### B. Terpene (FAM00157) *Cladonia uncialis* Normore 8774 (NAPT01000284.1) NODE284\_length\_34689\_cov\_68X



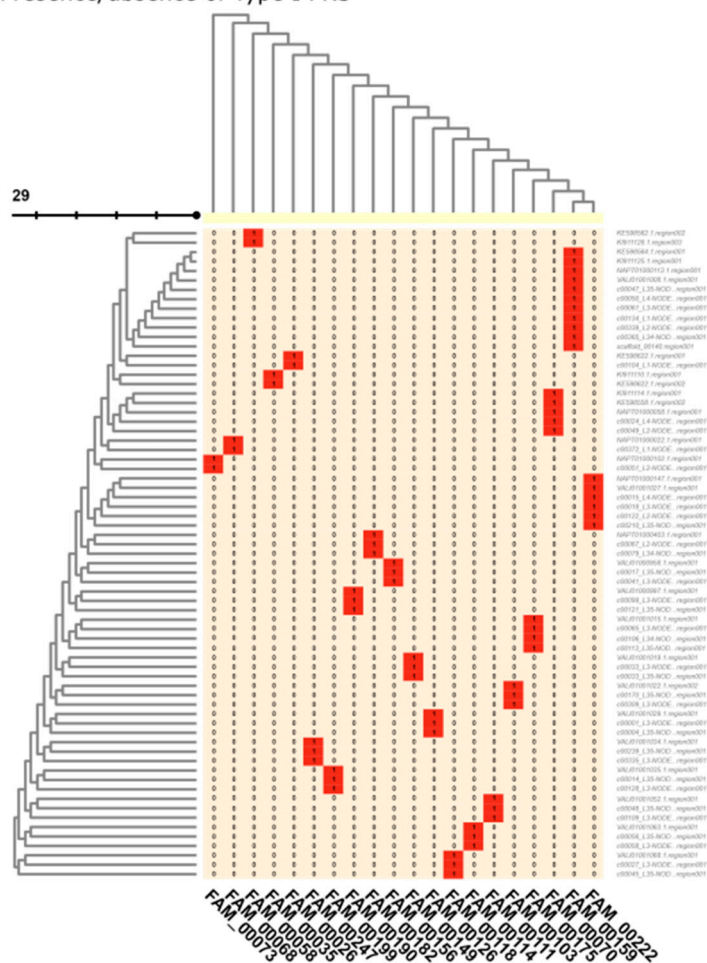
### C. Terpene (FAM00311)



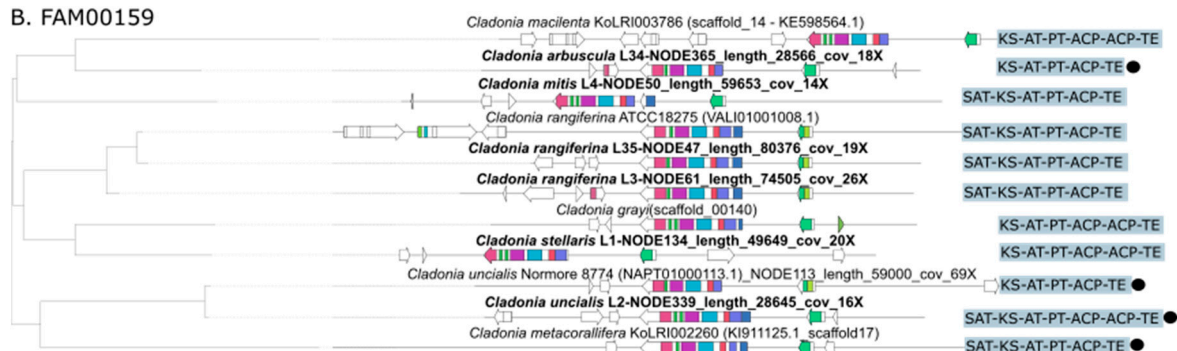
**Figure S11.** Biosynthetic gene clusters containing terpenes biosynthetic genes from metagenomes of studied strains and genomes of isolated *Cladonia* fungi. A. Heatmap based on presence/absence of the 20 largest Terpene families detected in the lichen metagenomes obtained in this study and in sequenced genomes of *Cladonia* species. Gene clusters families FAM00157 (B) and FAM00311 (C) detected in different *Cladonia* genomes/metagenomes using fungiSMASH and BiG-SCAPE/CORASON. Studied strains (data obtained from metagenomes) are highlighted in bold.



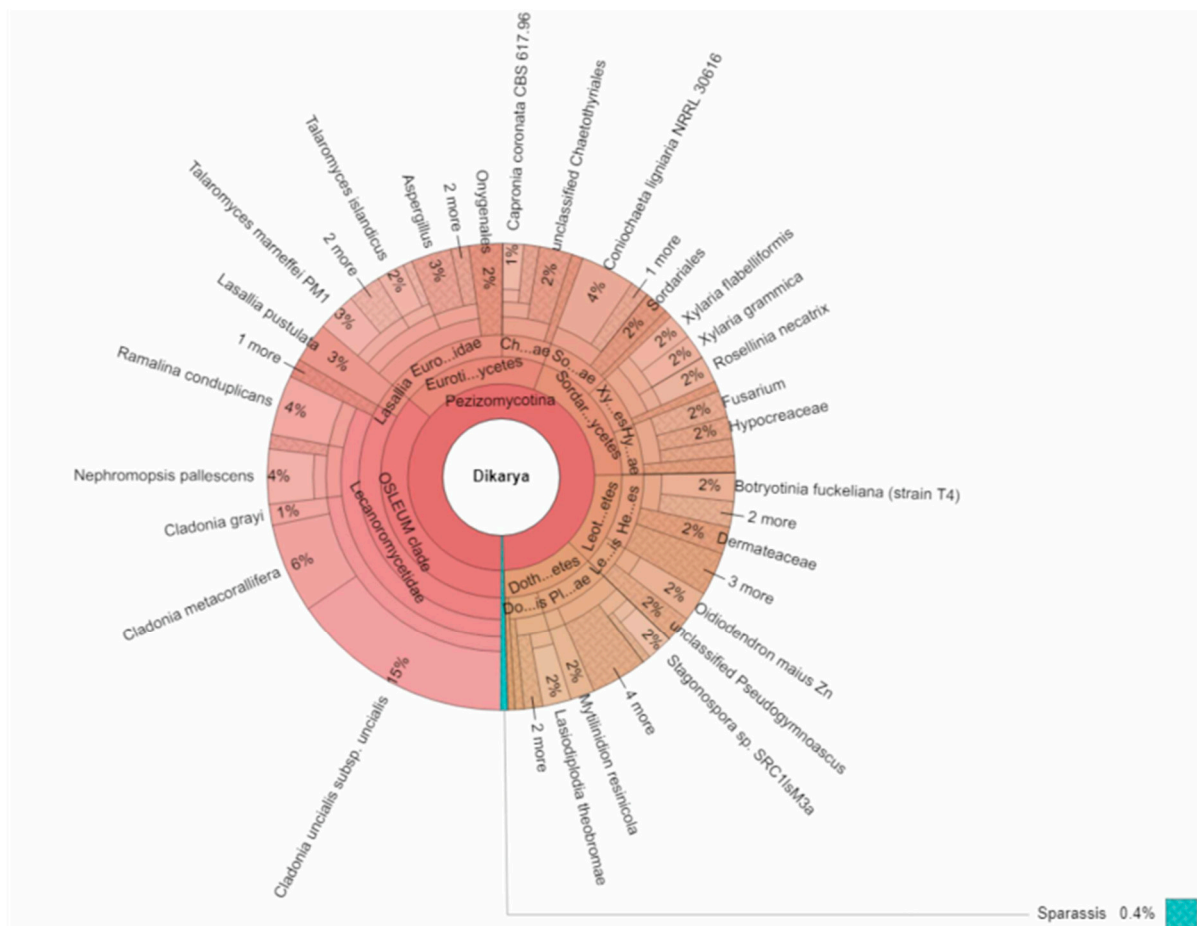
## A. Presence/absence of Type I PKS



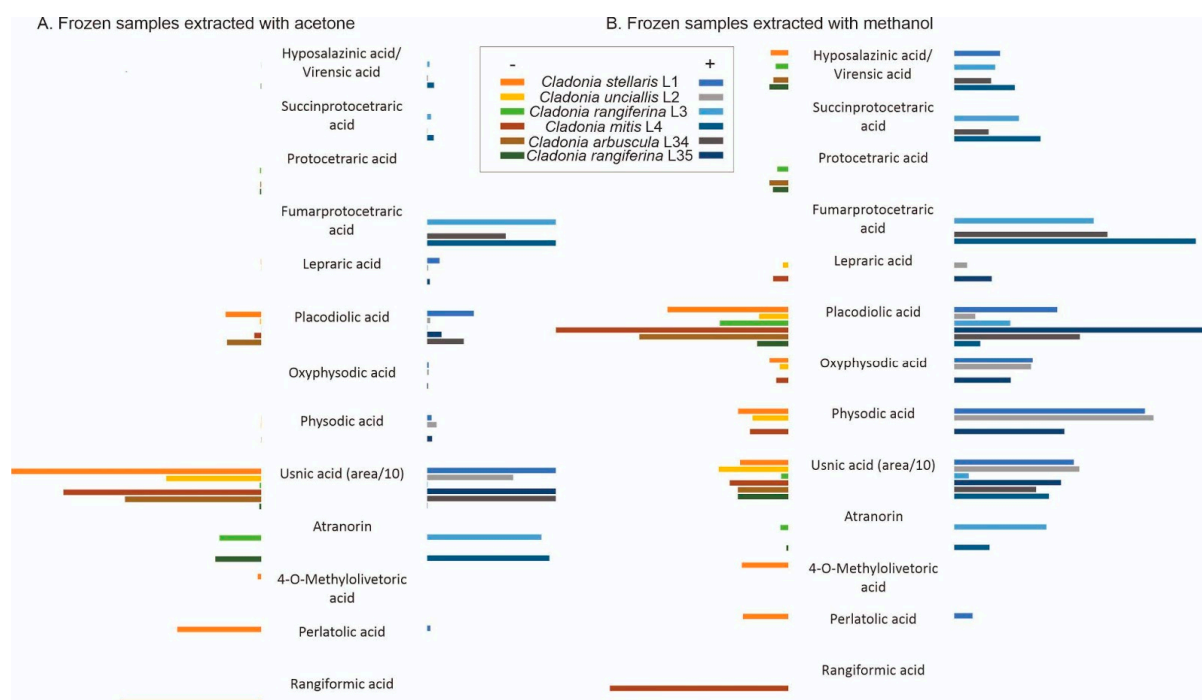
## B. FAM00159



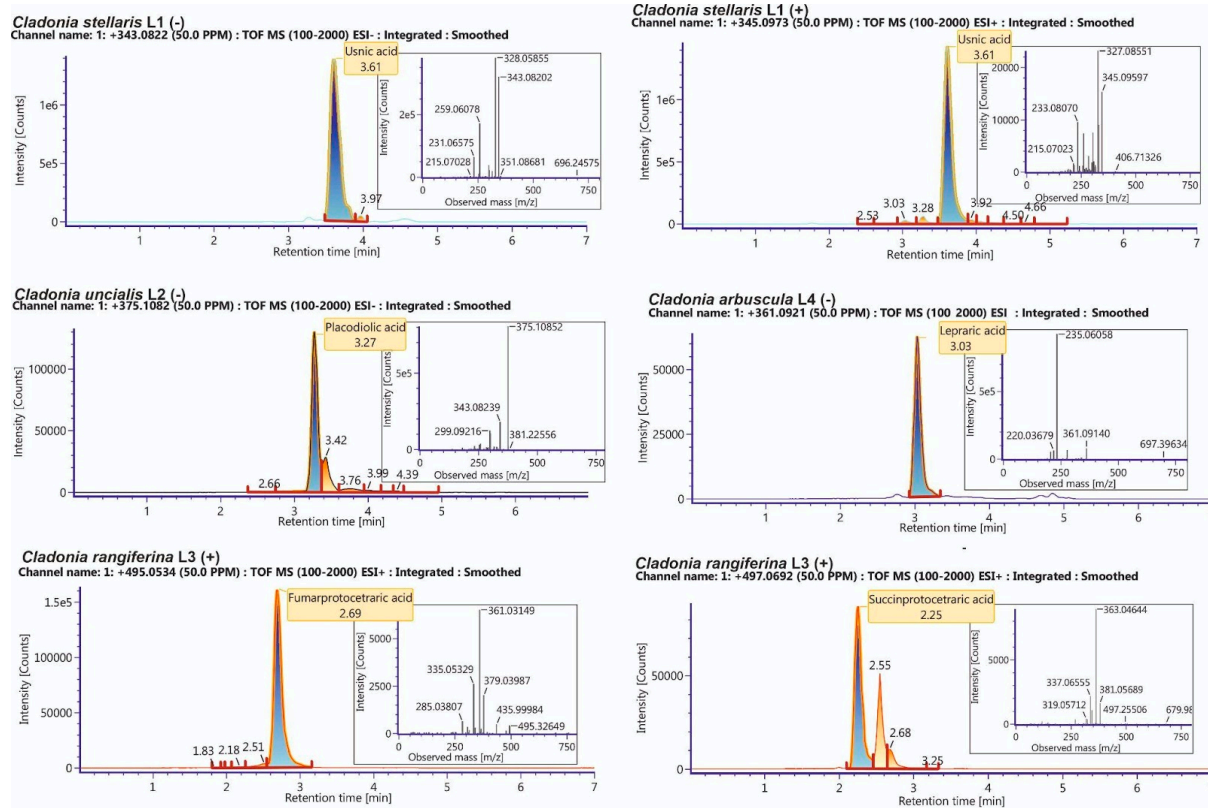
**Figure S12.** Type I PKS biosynthetic gene clusters. A. Heatmap based on presence/absence of the 20 largest Type I PKS families detected in the lichen metagenomes obtained in this study and in sequenced genomes of *Cladonia* fungi. B. Biosynthetic gene clusters containing PKS type I biosynthetic genes (FAM00159) detected in different *Cladonia* genomes/metagenomes. Studied strains (data obtained from metagenomes) are highlighted in bold. The prediction by fungiSMASH of *Cladonia* biosynthetic genes to be 100% similar to 1,3,6,8-tetrahydroxynaphthalene (MIBiG: BGC0001258) are indicated with a circle. Other samples returned with a similarity of 22% to naphthalene biosynthetic pathway (MIBiG: BGC0001906).



**Figure S13.** Taxonomic assignment based on Average Amino-acid Identity (AAI) values comparing 129 protein sequences predicted to be involved in the synthesis of PKS type I detected by fungiSMASH.



**Figure S14.** Relative amounts of the lichen secondary metabolites identified by ESI-Q-TOF mass spectrometric detection. Data were analyzed in positive and negative ion modes after compound separation by UPLC from frozen lichen samples extracted using either acetone (A) or methanol (B). Visualized data are derived from Supplementary Table S8.



**Figure S15.** Examples of chromatograms and spectra indicating the lichen compounds detected in the studied samples. Mass spectrometric data was analyzed in positive (+) and negative (-) ion mode.



**Table S1.** Number of sequences assigned to green algae per sample.

<b>Species</b>	<b>OTU</b>	<b>L1</b>	<b>L2</b>	<b>L3</b>	<b>L4</b>	<b>L34</b>	<b>L35</b>	<b>Total</b>
<i>Asterochloris mediterranea</i>	Otu00003	34797	33283	32291	20075	29518	24318	174282
<i>Trebouxia decolorans</i>	Otu00008	371	113	783	118	43	3	1431
<i>Trebouxia jamesii</i>	Otu00010	32	16	19	21	296	969	1353
<i>Trebouxia jamesii</i>	Otu00011	293	483	172	114	27	23	1112
<i>Trebouxia jamesii</i>	Otu00016	14	7	7	10	171	429	638
<i>Trebouxia jamesii</i>	Otu00017	81	124	44	57	317	10	633
<i>Trebouxia decolorans</i>	Otu00019	275	106	57	133	19	1	591
<i>Trebouxia decolorans</i>	Otu00030	48	51	38	39	31	2	209
<i>Trebouxia jamesii</i>	Otu00031	80	62	38	21	0	0	201
<i>Trebouxia jamesii</i>	Otu00034	35	47	47	28	5	5	167
<i>Trebouxia jamesii</i>	Otu00051	20	13	14	16	28	0	91
<i>Trebouxia jamesii</i>	Otu00064	23	23	12	7	0	0	65
<i>Trebouxia decolorans</i>	Otu00068	27	14	8	8	1	1	59

**Table S2.** Relative amount of reads from ITS2 amplicon OTU analysis which were classified to species of *Cladonia* and other classifications. Relative abundance of reads above 50 % for each lichen specimen are highlighted in the table.

Relative amount (%)	L1	L2	L3	L4	L34	L35
<i>Cladonia rangiferina</i> subsp <i>rangiferina</i> OTU00001	0,00	0,00	75,07	0,15	0,00	81,23
<i>Cladonia arbuscula</i> OTU00002	0,00	0,00	0,31	82,91	75,17	1,64
<i>Cladonia uncialis</i> subsp <i>biuncialis</i> OTU00004	0,00	79,70	0,00	0,09	0,00	0,00
<i>Cladonia stellaris</i> OTU00005	78,94	0,00	0,00	0,01	0,00	0,00
Unclassified	2,49	1,66	0,89	0,67	0,63	0,67
Other	18,27	17,98	23,36	15,91	23,81	16,00
Rare OTU (<50 reads per OTU)	0,29	0,64	0,36	0,26	0,38	0,45

**Table S3.** Summary of the quality assessment of the studied sequences calculated using QUAST using the genome of *Cladonia rangiferina* ATCC 18275 (ASM614605v1) as reference.

	new.contigs_L1	new.contigs_L2	new.contigs_L3	new.contigs_L4	new.contigs_L34	new.contigs_L35
# contigs (>= 0 bp)	100526	131864	126771	116310	153379	159796
# contigs (>= 1000 bp)	20102	16513	20835	10331	20597	21226
# contigs (>= 5000 bp)	1968	1289	1892	1459	2534	2192
# contigs (>= 10000 bp)	921	924	890	1014	985	887
# contigs (>= 25000 bp)	413	405	438	390	427	430
# contigs (>= 50000 bp)	130	138	159	87	144	157
Total length (>= 0 bp)	100838840	100203797	111939391	84030123	125533981	127848409
Total length (>= 1000 bp)	71152244	54508614	73472126	42995763	77586544	76471750
Total length (>= 5000 bp)	33916435	30312139	35307578	29837639	39581743	37290339
Total length (>= 10000 bp)	27102029	27685055	28803504	26549490	29341120	28766078
Total length (>= 25000 bp)	19023593	19147181	21458724	16345530	20380765	21469387
Total length (>= 50000 bp)	9033646	9960752	11703313	5846122	10439480	11771241
# contigs	35847	45212	40205	34739	43712	48298
Largest contig	138857	154269	158143	147081	176544	184648
Total length	82094879	74420864	86876711	59735367	93106593	94724226
Reference length	35673945	35673945	35673945	35673945	35673945	35673945
GC (%)	54.28	53.64	54.50	52.87	54.28	54.27
Reference GC (%)	45.44	45.44	45.44	45.44	45.44	45.44
N50	3501	2059	3362	4902	3774	3275
NG50	27372	27729	32474	22619	30651	33800
N75	1599	955	1460	920	1482	1293
NG75	10527	11564	14190	9725	13907	13440
L50	3705	3812	3918	1466	4147	4729
LG50	368	355	312	453	335	304
L75	12538	17852	13956	12215	14207	16434
LG75	888	838	718	1035	763	716
# N's per 100 kbp	0.00	0.00	0.00	0.00	0.00	0.00
NGA50	-	-	-	-	-	-

All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

**Table S4.** Summary of the quality assessment of the studied sequences calculated using MetaQUAST. The combined genome reference consisted of: *Acetobacter tropicalis*, *Acidiphilium multivorum* AIU301, *Acidocella* sp. MX AZ02, *Algisphaera agarilytica*, *Anabaena variabilis* ATCC 29413, *Aquisphaera giovannonii*, *Bradyrhizobium japonicum*, *Brevundimonas nasdae*, *Caulobacter vibrioides*, *Chlorella sorokiniana*, *Chthoniobacter flavus*, *Gluconacetobacter diazotrophicus* PA1 5, *Gluconacetobacter liquefaciens*, *Gluconacetobacter takamatsuzukensis*, *Granulibacter bethesdensis* CGDNIH4, *Kamptanema formosum* PCC 6407, *Komagataeibacter kakiaceti*, *Kozakia baliensis*, *Methylobacterium soli*, *Methylocystis heyeri*, *Phormidium* sp. CENA135, *Rhodoblastus sphagnicola*, *Rhodospila globiformis*, *Rhodovastum atsumiense*, *Singulisphaera acidiphila*, *Talaromyces rugulosus*.

	new.contigs_L1	new.contigs_L2	new.contigs_L3	new.contigs_L4	new.contigs_L34	new.contigs_L35
# contigs (>= 0 bp)	100526	131864	126771	116310	153379	159796
# contigs (>= 1000 bp)	20102	16513	20835	10331	20597	21226
# contigs (>= 5000 bp)	1968	1289	1892	1459	2534	2192
# contigs (>= 10000 bp)	921	924	890	1014	985	887
# contigs (>= 25000 bp)	413	405	438	390	427	430
# contigs (>= 50000 bp)	130	138	159	87	144	157
Total length (>= 0 bp)	100838840	100203797	111939391	84030123	125533981	127848409
Total length (>= 1000 bp)	71152244	54508614	73472126	42995763	77586544	76471750
Total length (>= 5000 bp)	33916435	30312139	35307578	29837639	39581743	37290339
Total length (>= 10000 bp)	27102029	27685055	28803504	26549490	29341120	28766078
Total length (>= 25000 bp)	19023593	19147181	21458724	16345530	20380765	21469387
Total length (>= 50000 bp)	9033646	9960752	11703313	5846122	10439480	11771241
# contigs	35847	45212	40205	34739	43712	48298
Largest contig	138857	154269	158143	147081	176544	184648
Total length	82094879	74420864	86876711	59735367	93106593	94724226
Reference length	227932695	227932695	227932695	227932695	227932695	227932695
N50	3501	2059	3362	4902	3774	3275
N75	1599	955	1460	920	1482	1293
L50	3705	3812	3918	1466	4147	4729
L75	12538	17852	13956	12215	14207	16434
# N's per 100 kbp	0.00	0.00	0.00	0.00	0.00	0.00

All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

**Table S5.** Sequenced metagenomes generated in this study. \*Raw sequences are available under accession number PRJEB34718. \*\*Compounds described in Figure 4 and Supplementary Figure S14.

Species and strain	Sequencer	Assembler	Assembled meta-genome (Mbp)	Genome coverage	Number of contigs	Accession number	Metabolites
Metagenomes							
<i>Cladonia arbuscula</i> L34	Illumina NextSeq500	SPAdes 3.12.0	93	-	19649	*	**
<i>Cladonia mitis</i> L4	Illumina NextSeq500	SPAdes 3.12.0	59	-	11760	*	**
<i>Cladonia rangiferina</i> L3	Illumina NextSeq500	SPAdes 3.12.0	86	-	20256	*	**
<i>Cladonia rangiferina</i> L35	Illumina NextSeq500	SPAdes 3.12.0	94	-	20592	*	**
<i>Cladonia stellaris</i> L1	Illumina NextSeq500	SPAdes 3.12.0	82	-	19362	*	**
<i>Cladonia uncialis</i> L2	Illumina NextSeq500	SPAdes 3.12.0	74	-	17633	*	**

**Table S6.** Published genomes of *Cladonia* included in the bioinformatic analyses.

Genomes	Sequencer	Assembler	Assembled genome (Mbp)	Genome coverage	Number of contigs	Accession number	Metabolites	Reference
<i>Cladonia grayi</i>	454 and Solexa gDNA	Velvet and Newbler	34.62	15x	414	<i>Cladonia grayi</i> Cgr/DA2myc/ss v1.0	Grayanic acid, fumarprotocetraric acid	Armaleo et al. 2019, Calchera et al. 2019
<i>Cladonia macilenta</i> KoLRI003786	Illumina HiSeq2000	SOAPdenovo	37.11	540x	240	AUPP000000000	Thamnolic, barbatic, didymic, squamatic, usnic and rhodocladonic acids	Park et al. 2013, Calchera et al. 2019
<i>Cladonia metacorrallifera</i> KoLRI002260*	Illumina HiSeq2000	Allpaths-LG	36.68	1023x	30	AXCT000000000	Usnic, didymic, squamatic and rhodocladonic acids	Park et al. 2014, Calchera et al. 2019
<i>Cladonia rangiferina</i> ATCC 18275	Illumina MiSeq	ABYSS v. AUG-2016	35.6	1,567	8	ASM614605v1	-	ASM614605v1
<i>Cladonia uncialis</i> Normore8774	Illumina MiSeq	SPAdes v. 3.11	32.85	43x	2124	ASM292778v1	Usnic and squamatic acids	Abdel-Hameed et al. 2016, Calchera et al. 2019

**Table S7.** Summary of biosynthetic gene cluster types found using bacterial antiSMASH at standalone for metagenomic data (\*\_anti) and fungal antiSMASH at web (\*\_fungi).

Sample	T1PK S	T3PK S	Ter- pen e	NRP S	NRPS- T1PK S	T3PKS - T1PKS	In- dol e	hy- brid/T1PKS - indole/ter- pene/ other	Si- dero- phor e	Othe r	Ar- ylpoly ene/ lasso- pep- tide/ bacte- riocin/ thio- pep- tide/ beta- lac- tone	To tal
<b>Metagenomes</b>												
<i>Cladonia arbuscula</i> L34_fungi	20	2	3	7	3	0	2	1	0	0	0	38
<i>Cladonia arbuscula</i> L34_anti	16	3	5	12	4	0	3	1	0	1	1	46
<i>Cladonia mitis</i> L4_fungi	28	2	2	5	2	0	0	1	1	0	0	41
<i>Cladonia mitis</i> L4_anti	20	4	5	16	2	0	1	0	1	1	0	50
<i>Cladonia rangifer- ina</i> L3_fungi	19	1	4	6	3	1	0	0	0	0	0	34
<i>Cladonia rangifer- ina</i> L3_anti	18	4	6	14	1	0	0	1	1	1	1	47
<i>Cladonia rangifer- ina</i> L35_fungi	17	1	6	4	2	1	0	1	0	0	0	32
<i>Cladonia rangifer- ina</i> L35_anti	17	3	9	14	1	0	0	0	1	1	1	47
<i>Cladonia stellaris</i> L1_fungi	12	0	6	5	4	1	0	2	0	1	0	31
<i>Cladonia stellaris</i> L1_anti	16	4	6	12	3	0	1	1	0	2	1	46
<i>Cladonia uncialis</i> L2_fungi	15	0	4	5	3	1	0	0	0	0	0	28
<i>Cladonia uncialis</i> L2_anti	16	2	7	14	1	0	1	0	0	0	1	42
<b>Genomes</b>												
<i>Cladonia grayi</i> _fungi	12	0	2	9	1	0	0	0	0	1	0	25
<i>Cladonia grayi</i> _anti	8	3	2	11	1	0	1	1	0	1	0	28

<i>Cladonia macilenta</i> KoLRI003786_fung i	18	0	3	6	3	1	0	0	0	0	0	31
<i>Cladonia macilenta</i> KoLRI003786_anti	17	2	4	12	3	0	0	0	0	1	0	39
<i>Cladonia metacoral- lifera</i> KoLRI002260_fung i	19	0	5	8	1	1	0	1	0	1	0	36
<i>Cladonia metacoral- lifera</i> KoLRI002260_anti	15	2	5	12	2	0	0	1	0	0	0	37
<i>Cladonia rangifer- ina</i> ATCC 18275 _fungi	18	0	2	6	2	1	0	1	0	0	0	30
<i>Cladonia rangifer- ina</i> ATCC 18275 _anti	16	3	2	15	1	0	0	2	1	1	1	42
<i>Cladonia uncialis</i> Normore8774_fungi	14	0	3	2	3	1	0	0	0	0	0	23
<i>Cladonia uncialis</i> Normore8774_anti	18	2	4	11	1	0	1	0	0	1	0	38

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**Table S8.** Compounds detected in lichen acetone (Ac) or methanol (Me) extracts from samples kept at room temperature or frozen, List 1: Unicellular fungi and green algae sequences added to the UNITE library, List 2: Compounds screened using the software UNIFI scientific information system (Waters).

**List 1.** Unicellular fungi and green algae sequences added to the UNITE library.

KU948729

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948734

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948735

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;

KU948736

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948741

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;

KU948742

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948743

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948744

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948745 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948746

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;

KU948747

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;

KU948751

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948753

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948755

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948756

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948760

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948763

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948764

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;

KU948765

k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;  
 KU948768  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948772  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948774  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948775  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948776  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948778  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948779  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Uncultured\_Cystobasidiomycetes;f\_Uncultured\_Cystobasidiomycetes;g\_Uncultured\_Cystobasidiomycetes;s\_Uncultured\_Cystobasidiomycetes;  
 KU948780  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948784  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 KU948785  
 k\_Fungi;p\_Basidiomycota;c\_Cystobasidiomycetes;o\_Cyphobasidiales;f\_Cyphobasidiaceae;g\_Uncultured\_Cyphobasidiales;s\_Uncultured\_Cyphobasidiales;  
 AB627405  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_corticola;  
 AB627375  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_corticola;  
 JF831923  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_decolorans;  
 FJ705205  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_decolorans;  
 FJ705184  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_decolorans;  
 JX036160  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Uncultured\_Trebouxia;  
 KF907553  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_jamesii;  
 KT827678 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_jamesii;  
 KT827707  
 k\_Plantae;p\_Chlorophyta;c\_Trebouxiophyceae;o\_Trebouxiales;f\_Trebouxiaceae;g\_Trebouxia;s\_Trebouxia\_jamesii;  
 JX675737  
 k\_Plantae;p\_Chlorophyta;c\_Ulvophyceae;o\_Trentepohliales;f\_Trentepohliaceae;g\_Trentepohlia;s\_Trentepohlia\_iolithus;  
 JX675738  
 k\_Plantae;p\_Chlorophyta;c\_Ulvophyceae;o\_Trentepohliales;f\_Trentepohliaceae;g\_Trentepohlia;s\_Trentepohlia\_arborum;  
 JX675736  
 k\_Plantae;p\_Chlorophyta;c\_Ulvophyceae;o\_Trentepohliales;f\_Trentepohliaceae;g\_Trentepohlia;s\_Trentepohlia\_umbra;  
 JX675731  
 k\_Plantae;p\_Chlorophyta;c\_Ulvophyceae;o\_Trentepohliales;f\_Trentepohliaceae;g\_Trentepohlia;s\_Trentepohlia\_sp;  
 JQ617945  
 k\_Plantae;p\_Chlorophyta;c\_Ulvophyceae;o\_Trentepohliales;f\_Trentepohliaceae;g\_Trentepohlia;s\_Trentepohlia\_sp;



KC489149

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Ulvophyceae;o\_\_Trentepohliales;f\_\_Trentepohliaceae;g\_\_Printzina;s\_\_Printzina\_cf\_lagenifera;  
KC489150

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Ulvophyceae;o\_\_Trentepohliales;f\_\_Trentepohliaceae;g\_\_Printzina;s\_\_Printzina\_lagenifera;  
KC489134 k\_\_Plantae;p\_\_Chlorophyta;c\_\_Ulvophyceae;o\_\_Trentepohliales;f\_\_Trentepohl-  
iaceae;g\_\_Printzina;s\_\_Printzina\_sp;

KY559106

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochloris\_irregu-  
laris;

KP031547

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochlo-  
ris\_cf\_glomerata;

KP257384

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochloris\_medi-  
terranea;

KP318674

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochloris\_lobo-  
phora;

KP318671

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochloris\_echi-  
nata;

KP318670

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochlo-  
ris\_friedlii;

KP318669

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochlo-  
ris\_woessiae;

KP318664

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Asterochloris;s\_\_Asterochlo-  
ris\_gaertneri;

KY981701

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Myrmecia;s\_\_Myrmecia\_israelensis;

KY981700

k\_\_Plantae;p\_\_Chlorophyta;c\_\_Trebouxiophyceae;o\_\_Trebouxiales;f\_\_Trebouxiaceae;g\_\_Myrmecia;s\_\_Myrmecia\_israelensis;

**List 2.** Compounds screened using the software UNIFI scientific information system (Waters):

1,3-Dihydro-4,6-dihydroxy-3-oxo-5-isobenzofurancarboxaldehyde  
1,7-Dibenzofurandicarboxylic acid, 3,9-dimethoxy-, dimethyl ester  
11H-Dibenzo[b,e][1,4]dioxepin-7-carboxylic acid, 3,8-dimethoxy-11-oxo-6-pentyl-1-valeryl-, methyl ester  
1-O-Methylfragilin  
2,4,5-Trichloro-1,3-dihydroxy-6-methoxy-8-methyl-9H-xanthen-9-one  
2,4,5-Trichlorolichexanthone  
2,4,7-Trichloro-3,8-dihydroxy-6-methoxy-1-methyl-9H-xanthen-9-one  
2,4-Dichlorolichexanthone  
2,4-Dichloronorlichexanthone  
2,4'-Di-O-methylnorsekikaic acid  
2,5,7-Trichlorolichexanthone  
2,5-Dichloro-3,8-dihydroxy-6-methoxy-1-methyl-9H-xanthen-9-one  
2,5-Dichlorolichexanthone  
2,5-Dichloronorlichexanthone  
2,7-Dichlorolichexanthone  
2,7-Dichloronorlichexanthone  
2-Chloro-1-hydroxy-3,6-dimethoxy-8-methyl-9H-xanthen-9-one  
2-Chloro-8-hydroxy-3,6-dimethoxy-1-methyl-9H-xanthen-9-one  
2-Chloronorlichexanthone  
2-Hydroxy-3-[(1-oxohexadecyl)oxy]propyl 6-deoxy-6-sulfo- $\alpha$ -D-glucopyranoside  
2-Hydroxychrysophanol  
2'-O-Methylanziaic acid  
2-O-Methylconfluentic acid  
2-O-Methyldivaricatic acid  
2'-O-Methylevernic acid  
2-O-Methylhiascic acid  
2'-O-Methylhyperphyllinic acid A  
2'-O-Methylhyperphyllinic acid B  
2'-O-Methylimbricaric acid  
2'-O-Methylmicrophyllinic acid  
2'-O-Methylperlatolic acid  
2-O-Methylperlatolic acid  
2'-O-Methylphysodic acid  
2-O-Methylsekikaic acid  
2-O-Methylstenosporic acid  
2''-O-Methyltenuiorin  
2'-O-Methyltenuiorin  
3,5-Dichloro-2'-O-methylanziaic acid  
3,5-Dichloro-2'-O-methylnorstenosporic acid  
3,5-Dichloro-4-O-demethylplanaic acid  
3,7-Dimethoxy-1,9-dibenzofurandicarboxylic acid  
3-O-Methylasemone  
3-Chlorodivaricatic acid  
3-O-Methylthiophanic acid  
4,4'-Disolorinic acid  
4,5-Dichloronorlichexanthone  
4,5-Di-O-methylhiascic acid  
4,7-Dichloronorlichexanthone  
4-O-Demethylglomellic acid  
4-O-Demethylloxodellic acid  
4-Chloro-1,6-dihydroxy-3-methoxy-8-methyl-9H-xanthen-9-one  
4-Chloronorlichexanthone  
4-Dechlorothiomelin  
4-Methoxyphthalic acid  
4-O-Demethylbarbatic acid  
4-O-Demethyldiffractaic acid  
4-O-Demethylglomelliferic acid  
4-O-Demethylimbricaric acid  
4-O-Demethylmicrophyllinic acid  
4-O-Demethylplanaic acid

4'-O-Demethylsekikaic acid  
4-O-Demethylsphaerophorin  
4-O-Demethylstenosporic acid  
4-O-Methylcryptochlorophaeic acid  
4-O-Methylcryptochloropheic acid  
4-O-Methylgyrophoric acid  
4-O-Methylisocryptochlorophaeic acid  
4-O-Methylolivetic acid  
4-O-Methylphysodic acid  
4-O-Methylsuperolivetic acid  
5,7-Dichloronorlichexanthone  
5,7-Dideoxyquercetin  
5-Chloro-2'-O-methylanziaic acid  
5-Chloroemodin  
5-Chlorovirensic acid  
5-O-Methylhiassic acid  
6,8,1'-Tri-O-methyl averantin  
6-O-Methylthiophanic acid  
6-Heptyl-3,8-dihydroxy-1-methyl-11-oxo-11H-dibenzo[b,e][1,4]dioxepin-7-carboxylic acid  
6-O-Methylaverantin  
6-O-Methylthiophanic acid  
7,7'-Dichlorohypericin  
7-Chloro-1,3-dihydroxy-6-methoxy-8-methylxanthone  
7-Chloro-1,6-di-O-methylemodin  
7-Chloro-1-O-methylemodin  
7-Chloroemodin  
7-Chlorolichexanthone  
7-Chloronorlichexanthone  
7H-Isobenzofuro[4,5-b][1,4]benzodioxepin-3,7(1H)-dione, 4,10-dimethoxy-5,8,11-trimethyl-  
7-O-Methylemodic acid  
8-O-Methylaverythin  
8-O-Methylthiomelin  
Alatinone  
Alectorialic acid  
Alectorialin  
Alectoronic acid  
Alectosarmentin  
alpha Acetyl hypoconstictic acid  
Amidepsine D  
Anthraquinone, 2-chloro-1,6,8-trihydroxy-3-methyl-  
Anziaic acid  
Aphthosin  
Apigenin  
Argopsin  
Arthoniaic acid  
Arthonin  
Arthothelin  
Asemone  
Aspicilin  
Atranorin  
Averantin  
Averythrin  
Azaleatin  
Baeomycesic acid  
Baicalein  
Barbatic acid  
Barbatolic acid  
Barbatolin  
Benzoic acid, 2-  
Benzoic acid, 2'-heptyl-4',6'-dihydroxy-4-methoxy-6-methyl-2,3'-oxydi-,  $\delta$ -lactone  
Benzoic acid, 2-heptyl-6-hydroxy-4-methoxy-, 4-carboxy-3-heptyl-5-methoxyphenyl ester  
Benzoic acid, 3-

Benzoic acid, 3-chloro-2-hydroxy-4-methoxy-6-pentyl-, 4-carboxy-3-hydroxy-5-pentylphenyl ester  
Boninic acid  
Buellolide  
Caloploicin  
Calycin  
Canarione  
Capillarisin  
Chloroatranorin  
Colensoic acid  
Coneuplectin  
Confluent acid  
Congrayanic acid  
Conloxodin  
Connorstictic acid  
Conorlobaridone  
Consalazinic acid  
Constictic acid  
Constipatic acid  
Contortin  
Crustinic acid  
Cryptochlorophaeic acid  
Cryptostictic acid  
Deacetyloccellin  
Decarboxythamnolic acid  
Dechlorodiploicin  
Demethylchodatin  
Demethylpsoromic acid  
Deoxyerythrolaccin  
Diacetylgraciliformin  
Didymic acid  
Diffractaic acid  
Diploicin  
Diploschistesic acid  
Divaricatic acid  
Echinocarpic acid  
Emodic acid  
Emodin  
Endocrocin  
Epanorin  
Epiphorellic acid 1  
Epiphorellic acid 2  
Eriodermin  
Erythrin  
Erythroglaucin  
Ethyl everminate  
Ethyl haematommate  
Eugenitin  
Eugenitol  
Eumitrin A1  
Eupafolin  
Euplectin  
Evernic acid  
Evernin  
Everninic acid  
Fallacinal  
Ferulic acid  
Flavipin  
Fragilin  
Fumarprotocetraric acid  
Funiculosin  
Fusarubin  
Galangin

Galbinic acid  
Gangaleoidin  
Genistein  
Glaucophaeic acid  
Glomellic acid  
Glomelliferic acid  
Glomelliferonic acid  
Glomellonic acid  
Grayanic acid  
Gyrophoric acid  
Haematommone  
Hematommic acid  
Hiascic acid  
Homosekikaic acid  
Hydroxyemodin  
Hyperconfluentic acid  
Hyperhomosekikaic acid  
Hyperlatolic acid  
Hypoconstictic acid  
Hypoprotocetraric acid  
Hyposalazinic acid  
Hypostictic acid  
Hypothallin  
Hypothamnolic acid  
Imbricaric acid  
Insignin  
Isoarthothelin  
Isohematommic acid  
Isohyperlatolic acid  
Isonotatic acid  
Isoobtusatic acid  
Isophysodic acid  
Isorhamnetin  
Isosubpicrolichenic acid  
Isousnic acid  
Isovicanicin  
Lanosterol  
Lasallic acid  
Lecanoric acid  
Lecideoidin  
Leoidin  
Leprapinic acid  
Lepraric acid  
Leprolomin  
Lichexanthone  
Lividic acid  
Lobaric acid  
Loxodellic acid  
Loxodin  
Lucidin  
Lusitanic acid  
Malonprotocetraric acid  
Melacarpic acid  
Menegazziaic acid  
Merochlorophaeic acid  
Methyl 2-O-methyllecanorate  
Methyl 3,5-dichlorolecanorate  
Methyl 4-O-demethylbarbatate  
Methyl barbatate  
Methyl evernate  
Methyl gyrophorate  
Methyl haematommate

Methyl lecanorate  
Methyl olivetolate  
Methyl orsellinate  
Methyl  $\beta$ -orsellinate  
Miriquidic acid  
Montagnetol  
Moretenone  
Morindone  
m-Scrobiculin  
m-Scrobiculin  
Neosartorin  
Nephroarctin  
Neuropogonin A  
Norcolensoic acid  
Nordivaricatic acid  
Norgangaleoidin  
Norlichexanthone  
Norlobaric acid  
Norlobaridone  
Norlobariol  
Normiriquidic acid  
Nornotatic acid  
Norperistictic acid  
Norsolorinic acid  
Norstictic acid  
Norwogonin  
Notatic acid  
Obtusatic acid  
Olivetolic acid  
Olivetoric acid  
O-Methylhypoprotocetraric acid  
Orsellinic acid  
Ovoic acid  
Oxyphysodic acid  
Paludosic acid  
Pannaric acid  
Pannaric acid 2-methyl ester  
Pannaric acid 6-methyl ester  
Pannarin  
Parietin  
Parietinic acid  
Pedalitin  
Perlatolic acid  
Phenarctin  
Physodalic acid  
Physodic acid  
Picrolichenic acid  
Pinastric acid  
Placodiolic acid  
Planaic acid  
Porphyrilin  
Porphyrillic acid  
Protocetraric acid  
Protosiphulin  
Pseudocyphellarin A  
Pseudocyphellarin B  
Pseudoplacodiolic acid  
Psoromic acid  
Pulvinic acid  
Pulvinic dilactone  
Pulvinone  
Ramalic acid

Rangiformic acid  
Resokaempferol  
Rhabarberone  
Rhamnetin  
Rhizinonic acid  
Rhizocarpic acid  
Rhodocladonic acid  
Salizinic acid  
Scabrosin  
Schizopeltic acid  
Secalonic acid A  
Secalonic acid B  
Secalonic acid C  
Sekikaic acid  
Sexangularetin  
Siphulelic acid  
Siphulin  
Skyrin  
Solorinic acid  
Sordidone  
Sphaerophorin  
Squamatic acid  
Stenosporic acid  
Stenosporonic acid  
Stictic acid  
Stictinic acid  
Strepsilin  
Subdidymic acid  
Submerochlorophaeic acid  
Subpaludosic acid  
Subpicrolichenic acid  
Substictic acid  
Succinprotocetraric acid  
Sulphuretin  
Superconfluentic acid  
Superlatolic acid  
Superpicrolichenic acid  
Sydowinin B  
Tamaraxetin  
Teloschistin  
Tenuiorin  
Tetra-O-methylaverythrin  
Thamnolic acid  
Thiomelin  
Thiophanic acid  
Thiophaninic acid  
threo-Hex-2-enaric acid, 2-deoxy-2-(4-methoxyphenyl)-5-C-phenyl-, 1,4-lactone, 6-methyl ester  
Thunberginol A  
Thuringione  
Umbilicaric acid  
Usnic acid  
Valsarin  
Variolaric acid  
Wedelolactone  
Verrucigeric acid  
Vicanicin  
Vinetorin  
Virensic acid  
Vittatolic acid  
Wrightiin  
Vulpinic acid  
Xanthorin

Xerocomic acid  
 $\alpha$ -Collatolic acid