Evolution of Polyketide Synthase Genes in *Cladonia* species

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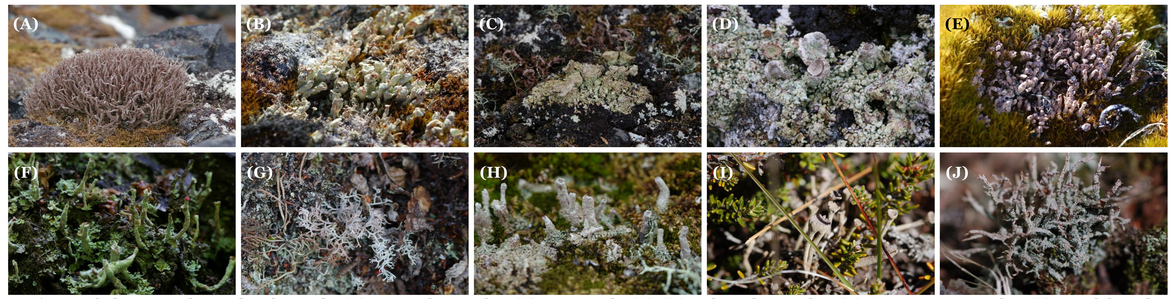
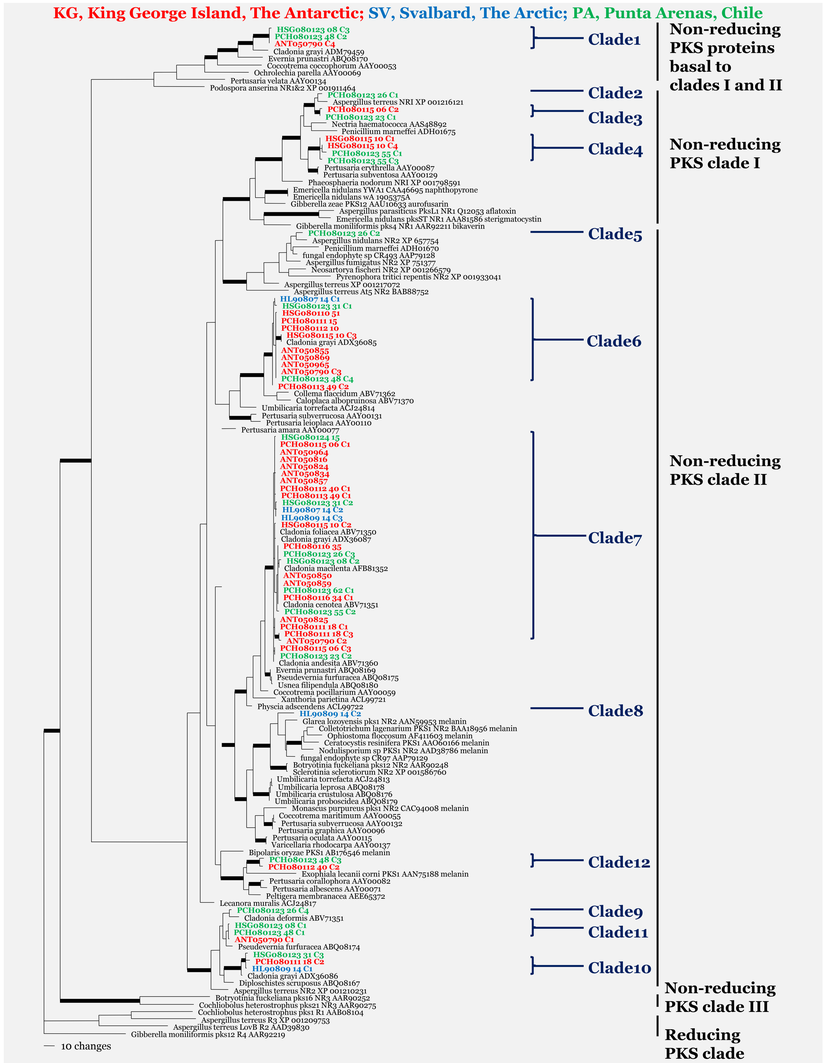
Lichens are well known for producing a great variety of secondary metabolites including polyketide chemicals. Polyketides are involved in stress responses such as drought, UV, microbial infection. Biosynthesis of polyketide chemicals are carried out by polyketide syntheses (PKS). Most of the lichens contain multiple copies of PKS genes and it is believed that each copy of the PKS gene is involved in biosyntheses of different polyketide chemicals. As it is regarded that each chemical has unique biological role in stress responses, it is important to study the evolution of the gene to understand its role in environmental adaptation of lichen species. In the current study, we amplified and sequenced KS domains of PKS genes from thirty two samples, which belonged to ten *Cladonia* species, collected from King George Island, Chile, and Svalbard.



▲**Fig . 1.** *Cladonia* samples used in this study. **(A)** *C. gracilis* complex; **(B)** *C. carneola*; **(C),(D)** *C. borealis* complex; **(D)** *C. squamosa* complex; **(E)** *C. polykactyla*; **(F)** *C. mitis*; **(G),(H)** *C. chlorophaea* complex; **(H)** *C. squamosa* complex



▲**Fig. 2.** Fig. Phylogenetic tree using Maximum parsimony method of deduced amino acid. sequences of 60 Clodonia PKS fragments and other known NR PKSs. Reducing PKS were used as the outgroup in the tree Branches conserved in Neighbor-joining and maximum parsimony were represented by thick lines. Percent bootstrap supports (>70%) were given at each node(NJ/MP).

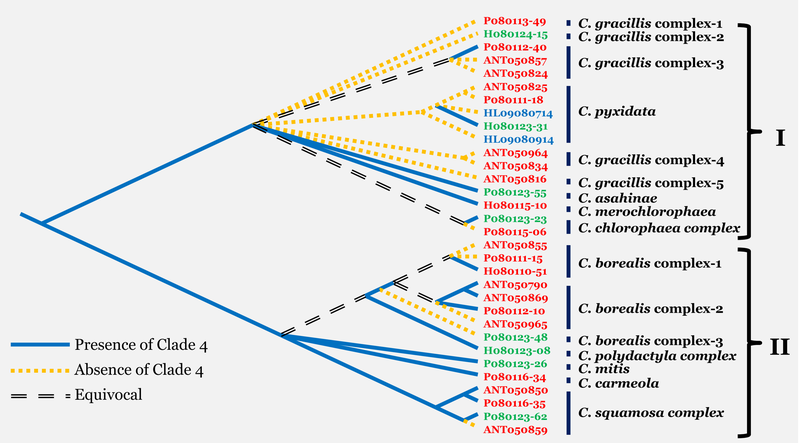
Sixty-two KS domain sequences were obtained by direct sequencing or cloning of the PCR amplicons. Phylogenetic analyses revealed that they belonged to non-reducing PKS gene. They were subgrouped into twelve clades by the criterion of monophyly and 90% similarity cut off.

▼**Table 1.** Occurrence of each clade of PKS genes

no.	Sample no.	rDNA clade	Species name	Locality	Clonig	Clade 1	Clade 2	Clade 3	**Clade 4**	Clade 5	**Clade 6**	**Clade 7**	Clade 8	Clade 9	Clade 10	Clade 11	Clade 12
1	PCH080112-40	A	*C. gracilis* complex	KG	O				+		+	*					+
2	ANT050824			KG					-		+	*					
3	ANT050857			KG					-		+	*					
4	HSG080124-15			PA					-		+	*					
5	PCH080113-49			KG	O				-		*	*					
6	ANT050964			KG					-		+	*					
7	ANT050834			KG					-		+	*					
8	ANT050816			KG					-		+	*					
9	PCH080123-55	*B*	*C. asahinae*	PA	O				*		+	*					
10	HL090807-14	C	*C. pyxidata* complex	SV	O				-		*	*					
11	HSG080123-31			PA	O				+		*	*			*		
12	PCH080111-18			KG	O				-		+	*			*		
13	ANT050825			KG					-		+	*					
14	HL090809-14			SV	O				-		+	*	*		*		
15	HSG080115-10	*D*	*C. merochlorophaea*	KG	O				*		*	*					
16	PCH080115-06	*E*	*C. chlorophaea complex*	KG	O			*	-		+	*					
17	PCH080123-23			PA	O			*	+		+	*					
18	PCH080112-10	F	*C. borealis* complex	KG					+		*	+					
19	ANT050790			KG	O	*			+		*	*				*	
20	ANT050869			KG					+		*	+					
21	ANT050965			KG					-		*	+					
22	HSG080110-51			KG					+		*	+					
23	PCH080111-15			KG					-		*	+					
24	ANT050855			KG					-		*	+					
25	PCH080123-48			PA	O	*			-		*	+				*	*
26	HSG080123-08	*G*	*C. Polydata* complex	PA	O	*			+		+	*				*	
27	PCH080116-34	*H*	*C. carneola*	KG					+		+	*					
28	PCH080123-26	*I*	*C. mitis*	PA	O		*		+	*	+	*	*				
29	PCH080116-35	J	*C. squamosa* complex	KG					+		+	*					
30	ANT050850			KG					+		+	*					
31	PCH080123-62			PA	O				+		-	*					
32	ANT050859			KG					-		-	+					

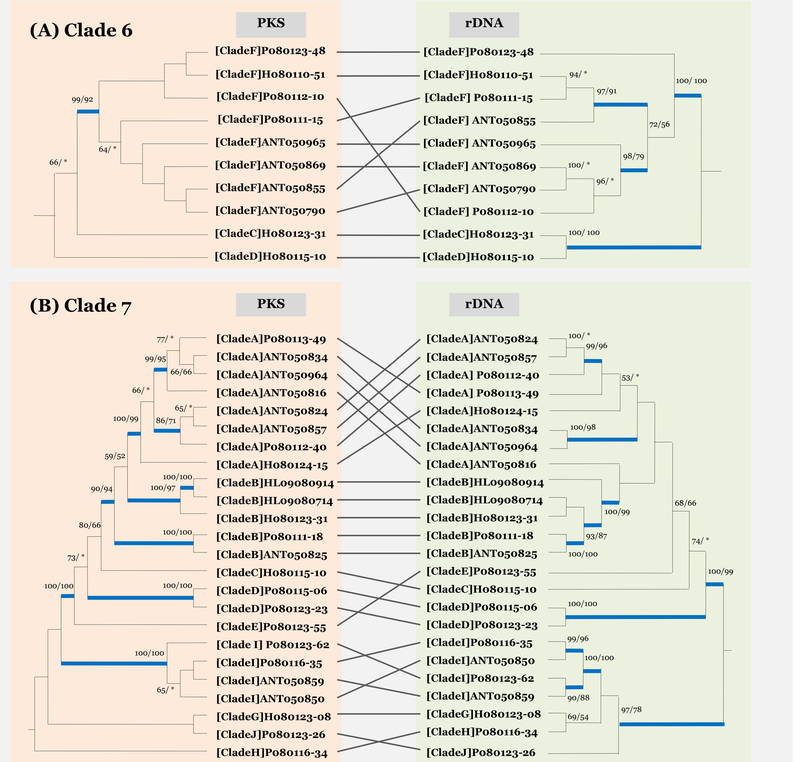
* , PKS genes detected by sequencing; +, PKS genes detected by specific PCR detection; -, no amplification by specific PCR detection

We examined presence of two of the most frequently found clades (clade 6 and clade 7) and one of the rare clades (clade 4) by specific amplification method. Clade 7 was detected from all of the samples, but clade 4 and clade 6 were detected only from fifteen and thirty samples, respectively.



▲**Fig. 3.** Trace back to the origin of Clade 4 in evolution of *Cladonia* spp.

Reconstruction of character change revealed that current distribution of clade 4 PKS genes can be explained by eight acquisition and loss events.



▲**Fig. 4.** Comparison of tree topologies between PKS and rDNA trees. Trees were constructed by NJ method. Percent bootstrap supports (>50%) were given at each node (NJ/MP),<50% were asterisked and 70% were represented by thick lines.

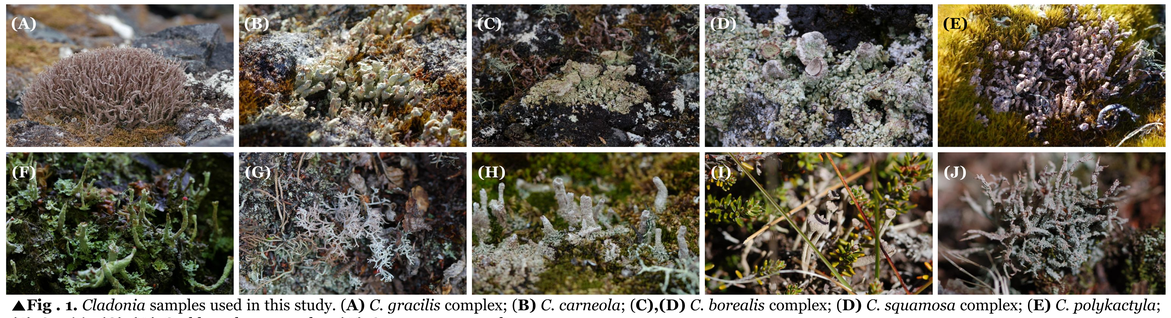
▼**Table 2.** Topology tests under varying constraints. Two hypothesis tests were conducted using Kishino - Hasegawa, Templeton and Winning-site tests.

Clade	Hypothesis†	P (Kishino-Hasegawa)	P (Templeton's)	P (Winning-sites)
Clade 6	H1	0.1575	0.1573	0.2891
	H2	1.000	1.000	1.000
Clade 7	H3	<0.0001-0.0003*	<0.0001-0.0001*	<0.0001-0.0005*
	H4	0.0046-0.0209*	0.0047-0.0209*	0.0078-0.0386*

†, H1, Phylogeny of PKS Clade 6 was compatible with rDNA dataset.; H2, Phylogeny of rDNA was compatible with PKS Clade 6 dataset.; H3, Phylogeny of PKS Clade 7 was compatible with rDNA dataset.; H4, Phylogeny of rDNA was compatible with PKS Clade 7 dataset.

* The null hypothesis of no difference between the best trees and constraint trees were rejected (P<0.05).

Comparison of PKS phylogeny and rDNA phylogeny revealed that clade 7 PKS genes evolved non-orthologously but we could not find concrete evidence to contradict orthologous evolution of clade 6 PKS genes.



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