Supplementary text, figures, and tables

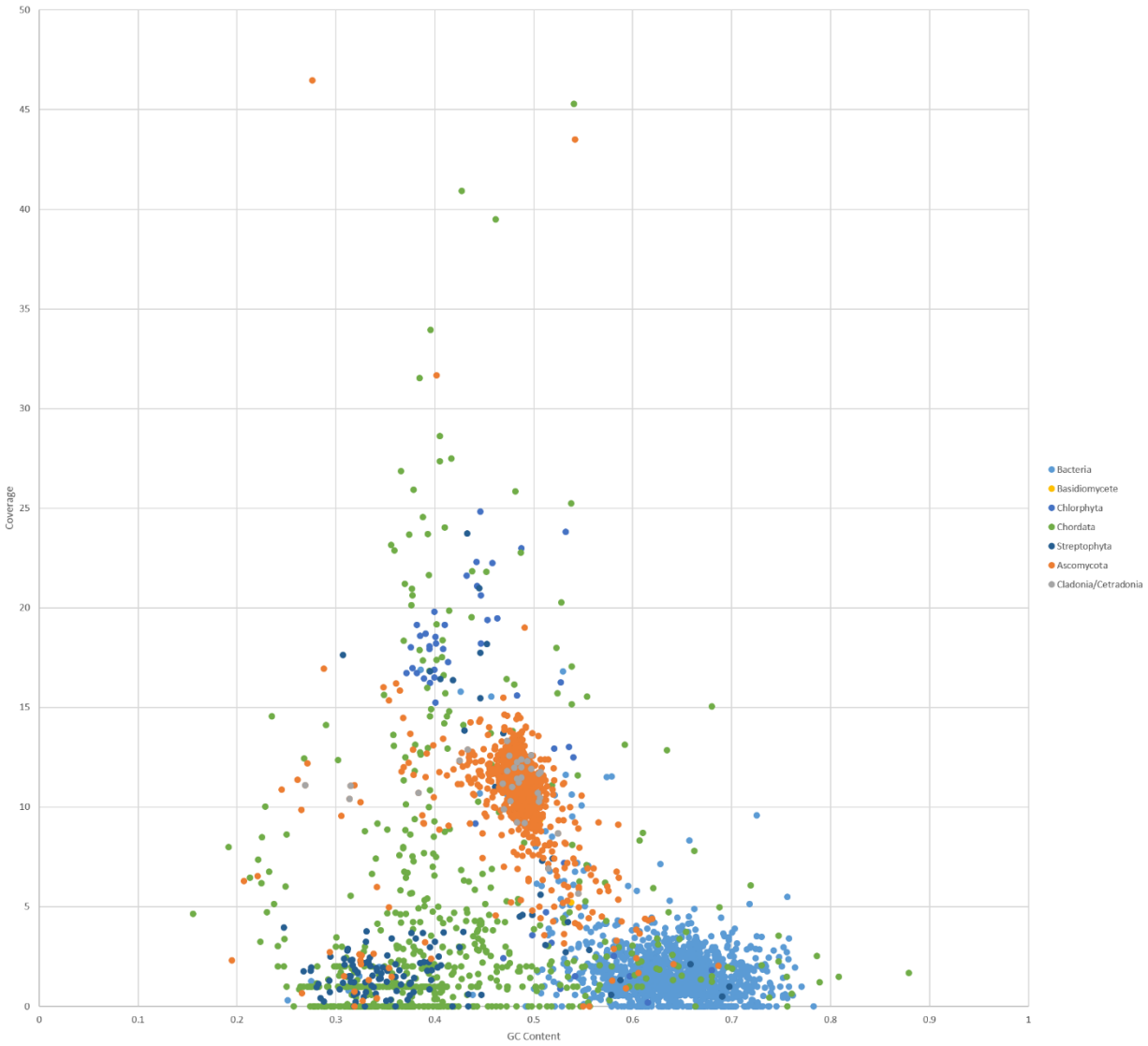
Determination of Fungal Mating System Methods

Recognition of suitable reproductive partners in ascomycetes is largely controlled by the mating-type locus (Dyer 2008). Most species are either homothallic, meaning all genes required for sexual reproduction are on the same chromosome and individuals are able to self-fertilize, or heterothallic, meaning the two mating types are in different individuals that must come together to recombine and produce sexual spores (Wilson *et al.* 2015). The two mating types are MAT 1-1-1 or the alpha domain, often referred to as MAT1-1, and MAT 1-2-1 or HMG-box, MAT1-2 for short (Scherrer *et al.* 2007). Mating-type loci evolve quickly and protein sequences are significantly divergent among individuals, making them difficult to locate and study (Lee *et al.* 2010). However, mating type genes have been sequenced in a few species of lichens (Seymour *et al.* 2005; Scherrer *et al.* 2007). Here we used previously published amino acid sequences to locate mating-type genes in *C. linearis*. First, a homology search was conducted between the published fragment of the MAT1-2 locus from *Cordyceps militaris* (BAC66500.1) and all amino acid sequences from the MAKER annotation using blastp, which is from the B224 sample (Altschul *et al.* 1990). We used the *C. militaris* amino acid sequence because it closely matched the *Cladonia galindezii* (AY634274.1) nucleotide sequence and was much longer than the fragment of the mating type gene from *Cladonia galendezii*. Two putative MAT1-2 genes were found among the amino acids sequences annotated in the *C. linearis* genome. These prospective genes were aligned using COBALT through the NCBI webserver with all available MAT1-2 amino acid sequences for ascomycetes and relationships among sequences were examined through a neighbor-joining tree (Papadopoulus & Agarwala 2007). The amino acid sequence that formed a monophyletic clade with *Cladonia galindezii* was treated as the *C. linearis* MAT1-2 gene and used for all subsequent analyses. A homology search was also conducted between MAT1-1 amino acid sequences from *Xanthoria* sp. (Scherrer *et al.* 2007) and the amino acid sequences from the MAKER output using blastp (Altschul *et al.* 1990). No matches were found between the two datasets. Next, we searched for both MAT loci in the remaining 31 read pools. To search for the MAT1-2 locus, reads were aligned to the contig that included the most likely putative MAT1-2gene using bowtie2 with the sensitive, local setting. The presence and depth of reads was examined at the MAT1-2 sequence site in the resulting alignments. The same process was used to search for MAT1-1 loci using sequences from *Xanthoria* sp., *Rhynchosporium secalis*, *Pyrenopeziza brassicae*, *Mycosphaerella graminicola*, and *Aspergillus nidulas* (Linde *et al.* 2003; Singh & Asby 1998; Waalwijk *et al*. 2002; Paoletti *et al.* 2007; Scherrer *et al.* 2007). No putative MAT1-1 gene was found in the annotated reference genome or any of the other read pools.

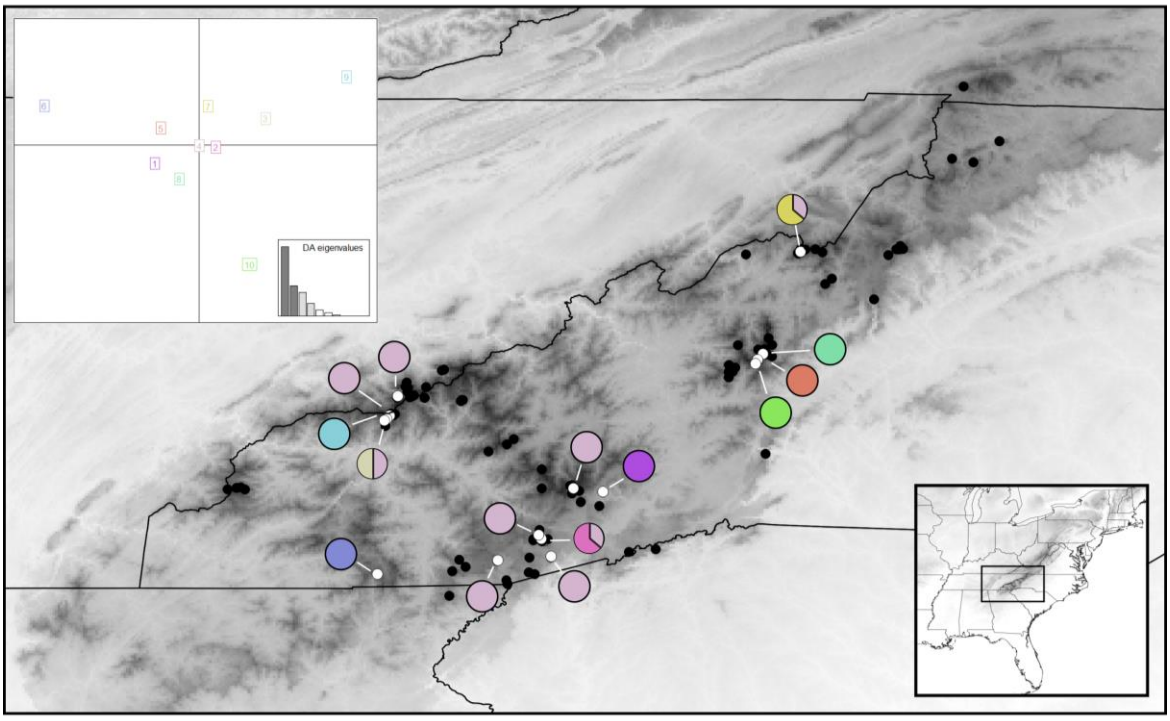
Mating System Results

The mating system of *Cetradonia linearis* was provisionally determined to be unisexual. Unisexuality is a particular type of homothallism where only one of the two mating type genes are present in a species (Wilson *et al.* 2015). Unisexual fungi are able to form ascospores through self-fertilization or outcrossing, usually resulting in the production of 8 spores/ascus, as was observed in fertile apothecia of *Cetradonia linearis* (Wilson et al. 2015). A MAT1-2 locus was identified in the reference genome, and in total the MAT1-2 was located in 14 of 32 total samples. The absence of the MAT1-2 gene from some individuals may either be due to the quantity of sequence data generated for those individuals, or to the presence of a MAT1-1 gene that was not detected. No trichogynes were observed in any specimen examination, but multiple ascospores were identified, and the presence of 8 spores/ascus was confirmed. The current data

are thus consistent with a unisexual mating system. However, further studies using single spore isolates and testing spore viability rates will be required to fully assess the mating system of this species and confirm unisexuality (Scherrer *et al.* 2007).



Supplementary Figure 1. Scatterplot showing results of Blobology pipeline used to identify contaminating reads.



Supplement Figure 2. Population genetic structure of *Cetradonia linearis*.) Spatial distribution of genetic clusters as recovered by DAPC. Inset: DAPC plot showing first two principal components with samples clustered into 10 distinct gene pools as identified by clustering algorithm.

Supplementary Table 1. Pairwise Fst between all sampled sites.

	AC	B	CD	FC	LG	MG	PK	PV	R	RP	SC	SH	SI	T	WS
AC	0														
B	0.568	0													
CD	0.515	0.588	0												
FC	0.621	0.662	0.528	0											
LG	0.523	0.409	0.629	0.646	0										
MG	0.507	0.634	0.621	0.687	0.611	0									
PK	0.447	0.618	0.602	0.679	0.582	0.505	0								
PV	0.510	0.478	0.487	0.579	0.410	0.571	0.560	0							
R	0.578	0.554	0.651	0.699	0.558	0.655	0.638	0.527	0						
RP	0.457	0.524	0.496	0.513	0.546	0.544	0.559	0.442	0.607	0					
SC	0.601	0.657	0.654	0.631	0.707	0.688	0.676	0.563	0.731	0.593	0				
SH	0.481	0.436	0.542	0.581	0.458	0.547	0.502	0.312	0.536	0.497	0.624	0			
SI	0.572	0.592	0.610	0.662	0.607	0.648	0.633	0.462	0.664	0.539	0.696	0.495	0		
T	0.565	0.553	0.628	0.667	0.573	0.641	0.624	0.344	0.644	0.569	0.717	0.381	0.558	0	
WS	0.497	0.431	0.581	0.601	0.484	0.571	0.551	0.346	0.543	0.513	0.655	0.409	0.503	0.425	0

Supplement Table 2. Results of partial Mantel test showing there is a significant relationship between genetic distance (Fst) and a geographic distance (km), but not between genetic distance and any environmental variables.

Variable	r	p
Geographic distance	*0.489*	*0.001*
Habitat (cliff vs. stream)	0.046	> 0.05
Mean temp. of wettest quarter	0.196	> 0.05
Mean temp. of warmest quarter	0.07	> 0.05
Annual precipitation	0.196	> 0.05