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Co-dispersal of symbionts in the lichen *Cladonia stellaris* inferred from genomic data

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ABSTRACT

We tested the congruence in dispersal patterns of the two main symbionts of the lichen *Cladonia stellaris* using genotyping-by-sequencing data. Based on 122 samples from eastern Canada, we recovered more than 21000 loci from the photobiont of *C. stellaris*. We described the population structure and estimate genetic diversity of the photobiont and identified the factors that contribute to explain genetic variation in both lichen partners. We also determined the identity of the dominant photobiont associated to *C. stellaris* using two molecular markers. Our results showed that *C. stellaris* is associated with *Asterochloris glomerata*, *A. irregularis*, and *A. pseudoirregularis*. Congruence in the genetic structure of photobiont and mycobiont were confirmed, suggesting co-dispersal of thallus fragments of *C. stellaris*. Genetic diversity of each symbiont was the factor that explained most of the genetic variation of the other symbiont, whereas geographical location and bioclimatic region seemed to have small or null explanatory power.

1. Introduction

Dispersal is an ecological process that involves the movement of an individual or multiple individuals away from one population to another location, or population, where they will settle and reproduce (Croteau, 2010). Dispersal is ubiquitous and essential for all organisms and of critical importance for population genetic dynamics. In lichens, symbionts can propagate independently or joined together. Sexual fungal spores are dispersed independently and thus, obtaining a compatible photobiont partner in the substratum where the spore germinates (horizontal transmission) is essential, whereas in asexual reproduction, mycobiont and photobiont are simultaneously dispersed within specialized propagules (e.g., isidia, soredia) or throughout thallus fragmentation (vertical transmission). Nevertheless, asexual reproduction does not always mean vertical transmission of the photobiont because the algae can be replaced by others available in the environment or from nearby lichen associations (algal switching) (Friedl, 1987; Ott, 1987). When symbionts are co-dispersed and algal switching does not occur, congruence patterns of population genetic structure between the two partners are expected (Werth and Sork, 2010).

One of the most crucial factors driving the lichen genetic structure is the reproductive strategy of the mycobiont. Vegetative propagules are often thought to have shorter dispersal ability than fungal spores (e.g., Gauslaa, 1997; Heinken, 1999; Alors et al., 2017; Ronnås et al., 2017); and therefore, asexually reproducing species are expected to exhibit spatial genetic structure (Werth, 2010). However, the lack of spatial structure has resulted in a recurrent pattern, also found in species with asexual reproduction (Myllys et al., 2003; Park et al., 2012; Pino-Bodas et al., 2020), meaning that vegetative propagules and thallus fragments can, indeed, have long-distance dispersal (LDD). Studies comparing differences and similarities in genetic patterns of these two lichen partners are common but based on few molecular markers (e.g., Robertson and Piercey-Normore, 2007; Fernández-Mendoza et al., 2011; Werth and Scheidegger, 2012; Steinová et al., 2019; Mark et al., 2020; Allen et al., 2021). Often, incongruence in genetic patterns (phylogeny and genetic structure) between the mycobiont and the photobiont have

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been revealed (e.g., Piercey-Normore, 2006; Yahr et al., 2006; Nelsen and Gargas, 2008; Dal Grande et al., 2012), suggesting that photobiont switching might be more common than expected (Piercey-Normore and DePriest, 2001; Wornik and Grube, 2009).

The green alga genus *Asterochloris* is one of the most common photobionts in lichen symbioses, and mainly restricted to species of the families Cladoniaceae and Sterocaulaceae (Nelsen and Gargas, 2008; Škaloud and Peksa, 2010). Recent phylogenetic analyses together with morphological examinations allowed description of an unexpected taxon diversity within this genus (e.g., Moya et al., 2015; Škaloud et al., 2015). So far, 61 *Asterochloris* lineages have been recovered based on a handful of molecular markers (Škaloud and Peksa, 2010; Peksa and Škaloud, 2011; Řídká et al., 2014; Vančurová et al., 2015; 2018; Steinová et al., 2019; Kim et al., 2020; Pino-Bodas and Stenroos, 2021), with *A. glomerata* being the most common photobiont lineage in species of the lichen genus *Cladonia* (Cladoniaceae) and the most frequent in North America (Pino-Bodas and Stenroos, 2021). Likewise, the degree of genetic variation within *Asterochloris* has been addressed, and several authors identified both biotic and abiotic factors explaining this variation (e.g., Yahr et al., 2006; Steinová et al., 2019; Kosecka et al., 2021; Pino-Bodas and Stenroos, 2021). Climatic variables (Nelsen and Gargas, 2009; Fernández-Mendoza et al., 2011; Magain et al., 2017; Dal Grande et al., 2018; Rolshausen et al., 2020) and identity of the mycobiont (Buckley et al., 2014; Vančurová et al., 2018; Jüriado et al., 2019) are recognized as the main drivers of the variation in other lichen photobionts. Elucidating the influence of factors such as geographical location, climatic conditions, or partners' identity in the genetic variation of *Asterochloris* is essential for understanding their distribution patterns, adaptation to the habitat, and evolution.

In a previous study, Alonso-García et al. (2021) inferred the population genetic structure and genetic diversity of *Cladonia stellaris* in eastern North America. The results suggested that mycobiont populations from southern Quebec were not spatially structured, southern populations were not genetically different from those of the north. The study revealed that the genetic structure of *C. stellaris* consists of four phylogenetic lineages, and it pointed to constant migration of individuals throughout the region (LDD). Taking advantage of the large genomic data at our disposal, in this study we aim to (i) determine the phylogenetic lineages of photobionts associated with *C. stellaris* in Quebec; (ii) describe the genetic structure and estimate genetic diversity of photobionts associated with the species; (iii) elucidate, from the genetic structure of mycobiont and photobiont, the main reproduction mechanisms of *C. stellaris*; and (iv) determine the factors that contribute to explain genetic variation in both lichen partners.

2. Material and methods

Genotyping-by-sequencing (GBS) data were previously generated by Alonso-García et al. (2021). A total of 122 samples of the lichen species *C. stellaris* from Quebec, eastern Canada, were included (Alonso-García et al. (2021), Supplementary Table 1). DNA extraction, and library preparation were described in Alonso-García et al. (2021). In summary, a double-digestion library using the enzymes PstI/MspI was performed and sequenced using Ion Torrent technology. Based on previous quality control and demultiplexed reads, we generated new genomic data for the photobiont. Likewise, new genetic data were generated to examine phylogenetic relationships among *C. stellaris* photobionts.

2.1. Systematics of Asterochloris

We used Sanger technology to identify the species of dominant photobiont associated with *C. stellaris.* Recent studies indicated that the photobiont identified by Sanger sequencing corresponds to the most abundant photobiont in a lichen sample (Molins et al., 2018; Paul et al., 2018). We used previously extracted DNA (Alonso-García et al., 2021) to amplify two loci, ITS rDNA and a fragment of actin type I locus containing an intron, in 19 samples of *C. stellaris*, selecting about 20% samples of each of the four mycobiont lineages. Locus selection was based on results from previous studies (Yahr et al., 2006; Škaloud and Peksa, 2010; Peksa and Škaloud, 2011; Škaloud et al., 2015; Pino-Bodas and Stenroos, 2021). The primers used were ITS1T/ITS4T (forward and reverse) (Kroken and Taylor, 2000) to amplify the ITS rDNA, and ActinF2 Astero/ActinR2 Astero (forward and reverse) (Škaloud and Peksa, 2010) to amplify Actin locus. Thermocycling conditions were: 94 °C 3 min; 35 cycles of 30 s at 94 °C, 30 s at 53 °C, 1 min at 72 °C; 7 min at 72 °C for ITS rDNA; and 94 °C 3 min; 35 cycles of 30 s at 94 °C, 1 min at 61 °C, 1 min at 72 °C; 10 min at 72 °C for actin. Finally, 3 μl of the amplification products were visualized on a 1.7% agarose gel. Successful amplifications were purified and sequenced at the *Plateforme d'analyses génomiques* (*Institut de Biologie Intégrative et des Systèmes*), at Laval University (Quebec City, QC, Canada).

The new ITS rDNA and actin sequences of *Asterochloris* were included in the global alignment of Pino-Bodas and Stenroos (2021) (Supplementary Table 2). The sequences of each region were aligned with MAFFT v. 7.0 (Katoh et al., 2019) with default parameters. Then, the alignments were checked and improved in BIOEDIT v. 7.0 (Hall, 1999). Ambiguous regions were delimited and removed using Gblocks v. 0.91b (Castresana, 2000). Maximum Likelihood (ML) analyses were implemented using RAxML v. 7.0.3 (Stamatakis, 2014) assuming the GTRGAMMA model for each alignment. The node support was estimated with rapid bootstrap algorithm, using 1000 pseudoreplicates. No incongruences were detected between the topology of ML trees of ITS rDNA and actin (verifying the clades with at least 75% bootstrap support) and the alignments were concatenated. The models TrNef + G for actin and SYM + G for ITS rDNA were selected by JModeltest (Posada, 2008) under Akaike Information Criterion (AIC) as the optimal substitution models. Four partitions were considered in the analyses of concatenated dataset, ITS rDNA and each three-codon position of actin. The concatenated dataset was analyzed by ML, with the same conditions as the single locus alignments, and Bayesian inference. The Bayesian analysis was run in MrBayes v. 3.2 (Ronquist and Sanmartín, 2011) in CIPRES portal (Miller et al., 2010) with two simultaneous runs with 20000000 generations, each starting with a random tree and employing four chains per run. Every 1000th tree was saved into a file. The first 5000000 generations (i.e., the first 5000 trees) were deleted as the 'burn-in' of the chain. The convergence of the chains was assessed with average standard deviation of split frequencies < 0.05 and plotting the likelihood versus generation number in Tracer v. 1.7 (Rambaut et al., 2018).

2.2. Locus construction and SNPs calling

We mapped our reads to the nuclear genome of *Asterochloris glomerata* (Armaleo et al., 2019). We used the software bwa v. 0.7 (Li and Durbin, 2009) configured as previously described by Alonso-García et al. (2021) (script 1). To build the loci, the *gstacks* module from Stacks v. 2.5 (Rochette et al., 2019) was run considering each individual a single population (script 2). The single nucleotide polymorphisms (SNPs) were filtered using the *population* module (script 3) from Stacks v. 2.5. First, we considered each individual as a single population, and we selected loci present in at least 50% (-R 0.5) and 75% (-R 0.75) of the individuals. In both cases, we called linked and unlinked (*–write-single-snp*) SNPs.

2.3. Population structure analyses

We used the unlinked SNPs filtered at 75% to perform a maximum likelihood (ML) phylogenetic analysis with RAxML v. 8.2.9 (Stamatakis, 2014), and to calculate the co-ancestry matrix with fineRADstructure (Malinsky et al., 2018), using the parameter configuration previously described by Alonso-García et al. (2021). Linked SNPs filtered at 75% were used to build a principal component analysis (PCA), and to estimated percentage of missing data with R package Adegenet v. 2.0.2

(Jombart et al., 2016). Secondly, we called SNPs considering phylogenetic relationships from the ML analysis (individuals grouped by phylogenetic clade), and selecting loci present in at least 75% (-r 75) of the individuals in a population (within each clade). The SNP database was then used to estimate genetic diversity using the population module.

A partial Mantel test was calculated between the genetic distance matrices of photobiont and mycobiont controlling the effect of geographical distances. This test was implemented in the vegan package (Oksanen et al., 2020) using 2000 random permutations. The Euclidean distances were calculated from the geographic coordinate matrix (Supplementary Table 1). The genetic distance of mycobiont and photobiont were based on linked SNPs matrix filtered at 75%.

2.4. Factors influencing symbiont's genetic diversity

Redundancy (RDA) and partial redundancy analyses (pRDA) were performed using the vegan package (Oksanen et al., 2020) to determine the relative contribution of three factors to the genetic variation of the mycobiont and the photobiont of *C. stellaris*. Three explanatory matrices were used in both cases, the identity of the symbiotic partner, the bioclimatic region, and geographical location. The bioclimatic region and geographical location were binary matrices containing the bioclimatic domain (forest tundra, arctic tundra, closed-crown forest, and lichen woodland), and geographical location (Supplementary Table 1). The third explanatory matrices representing the identity of the symbiotic partner were built with the first three components of the PCA. For the mycobiont, we used the PCA of the photobiont, and vice versa. As response matrices for the mycobiont and the photobiont, we also used the three first components of the PCA. The variation explained by each variable group was estimated using adjusted R^2, and the statistical significance was assessed using a permutation-based ANOVA test with 2000 permutations. Venn diagrams were generated in R, using the package Euler (Larsson, 2021). To avoid the overestimation of the variance caused by collinearity of variables (Blanchet et al., 2008), we selected the variables using *forward.sel* function of adespatial package (Drya et al., 2019) for R. RDA was calculated adding explanatory variables sequentially. The variables were rejected if adjusted R^2 decreased when they were included, or if P-value > 0.05.

3. Results

3.1. Three species of Asterochloris associated with C. stellaris from eastern Canada

We aligned 315 sequences of *Asterochloris*, 19 newly generated here (accession number and voucher information in Supplementary Table 2). The combined dataset (ITS rDNA and actin) contained 1209 characters, 567 of which were parsimony-informative positions. The ML analysis yielded a tree with a likelihood value of −Ln L = 13997.72; the arithmetic mean likelihood of the Bayesian tree sampling was −Ln L = 14568.12. Both loci recovered the same topology. The 50% consensus majority tree from Bayesian analysis is shown in Supplementary Fig. 1. The sequences of eight specimens belong to *A. irregularis* clade, five to *A. pseudoirregularis* and six to *A. glomerata*. These three species are closely related.

3.2. Reads mapped, loci generated, and SNPs called

Approximately 204 million sequences were previously generated for 122 samples of *C. stellaris* (Alonso-García et al., 2021). We recovered around 158 millions of filtered and demultiplexed reads to generate genomic data for the photobiont.

After mapping to the algal reference to filter out nonalgal sequences, the number of reads was reduced. The number of reads generated and mapped for the photobiont and the mycobiont (Alonso-García et al., 2021) of each sample can be found in Supplementary Fig. 2A and Supplementary Table 3. Supplementary Table 4 compiles detailed *gstacks* results for the two lichen partners showing the number of loci generated (Supplementary Fig. 2B) and mean depth coverage for each sample.

A total of 248175 loci were generated for the photobiont of *C. stellaris* and 21099 retained after filter (-R 0.75) (Supplementary Table 5). For the mycobiont, a total of 28046 loci were generated and 1377 retained using the same filter (Supplementary Table 5). The *populations* module identified 9043 and 709 SNPs (photobiont and mycobiont, respectively). A comparison of these values for the photobiont and the mycobiont, including filter of 50%, is displayed in Supplementary Table 5. Statistical results for each individual are compiled for both lichen partners in Supplementary Table 6, Supplementary Fig. 2C, and Supplementary Fig. 2D. They comprise number of private, variant, and polymorphic (SNPs) sites. In all cases, the number of loci and SNPs generated is always higher for the photobiont than for the mycobiont (Supplementary Fig. 2).

3.3. Description of population structure

The ML phylogenetic tree of the photobiont recovered four well supported clades (bootstrap values (BS) $\geq$ 90) (Fig. 1). Clade A and D included samples from the forest and the arctic tundra, the lichen woodlands in Kuujjuarapik and *Parc National des Grands Jardins* (PNGJ), and different localities within the closed-crown forest such as James Bay Road, Fjord Saguenay and Reserve Ashuapmushuan (Fig. 1, Supplementary Table 1); clade B contained samples from the lichen woodland in PNGJ and from the closed-crown forest (Fjord Saguenay, lac Madeleine, Montmorency forest and Reserve Ashuapmushuan) (Fig. 1, Supplementary Table 1); finally clade E grouped samples from the lichen woodlands in Kuujjuarapik, Radisson and PNGJ, as well as samples from the closed-crown forest (James Bay Road, Fjord Saguenay, lac Madeleine, Montmorency forest, and Reserve Ashuapmushuan) (Fig. 1, Supplementary Table 1). Next, we compare the phylogenetic relationships for the two lichen partners of *C. stellaris*. Mycobiont's results were shown in Fig. 4 from Alonso-García et al. (2021). Clade A, clade B and clade D clustered the same samples for the mycobiont and the photobiont. Clade E also clustered the same samples in both cases, but it was unsupported for the mycobiont. This unsupported clade E included a supplementary subclade (called clade C in Alonso-García et al., 2021). In the photobiont phylogeny, samples from clade C were split into and included in clade E (two samples) and clade B (three samples). The same pattern had already been shown in Alonso-García et al. (2021) using a different analysis approach (bowtie2-mapping) (Appendix S3; Alonso-García et al., 2021).

The co-ancestry of *Asterochloris* matrix (Supplementary Fig. 3) showed a pattern consistent with ML phylogenetic relationships, except for the sample Alonso_543. This sample was grouped in clade E in the coalescence tree (Supplementary Fig. 3) but not assigned to any clade in the ML phylogeny (Fig. 1). The analysis generated four clusters. Individuals from the same cluster shared more co-ancestry with each other than among clusters. Clade A exhibited the highest co-ancestry, followed by clades D, B and E. Regarding the co-ancestry matrix of *C. stellaris*, the additional clade C was also recognized in this analysis (Fig. 6, Alonso-García et al., 2021). Otherwise, results from the photobiont matched with the mycobiont, with levels of shared co-ancestry being lower for the mycobiont than for the photobiont (Alonso-García et al., 2021).

For the photobiont, the first two principal component axes explained 53.23% and 27.98% of variance (Fig. 2A). The PCA plotted four separated clusters, corresponding with ML phylogenetic clades. The PCA of the mycobiont (Fig. 2B) also plotted the phylogenetic clades of the mycobiont. Clades C and D are well defined, whereas clades A, B and E are somehow overlapped.

The Mantel test shows a significant positive correlation between the mycobiont and photobiont genetic distance ($R = 0.735$, P-value < 0.001).

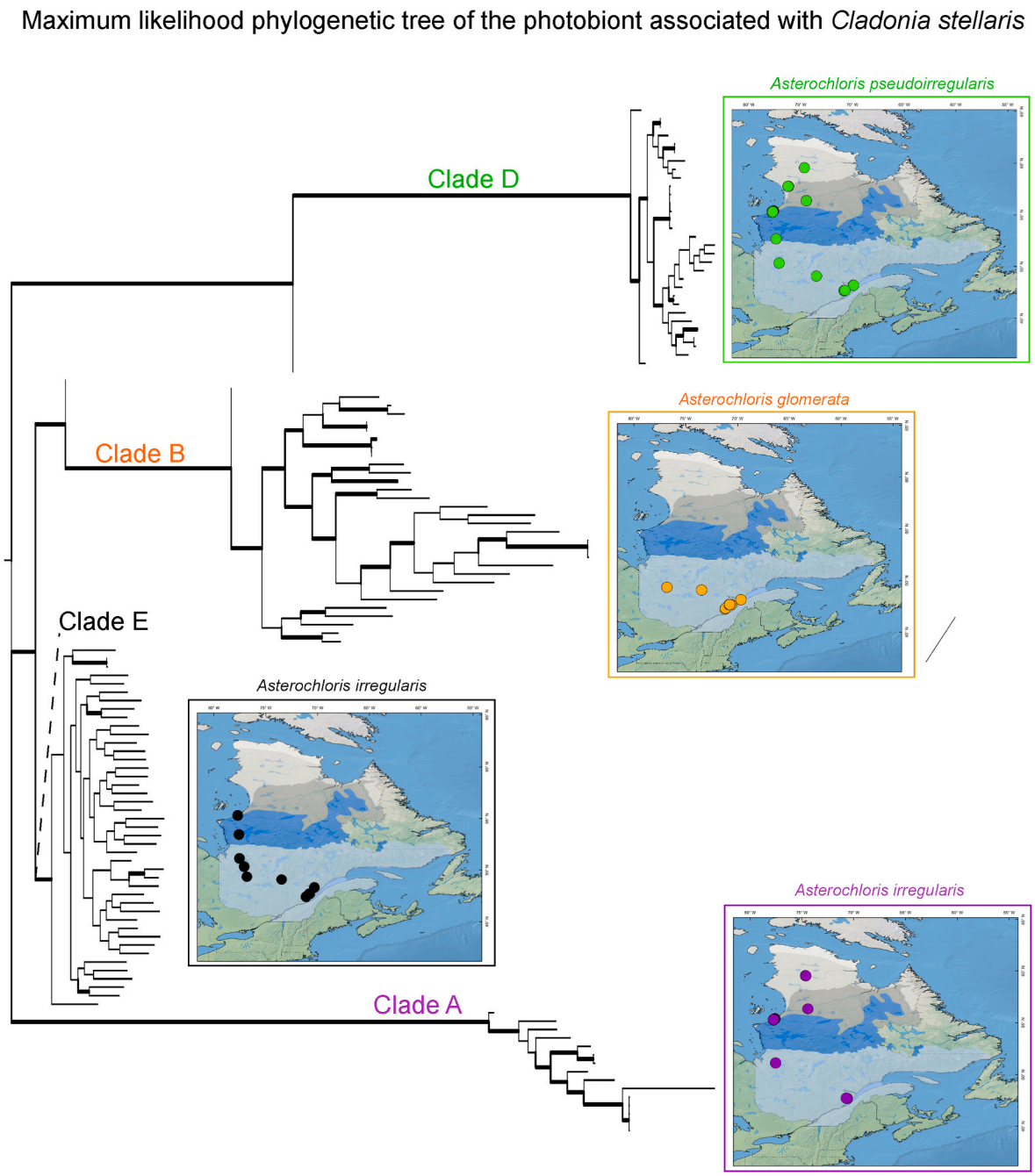

Fig. 1. Maximum likelihood phylogenetic tree of the green alga *Asterochloris* associated with the lichen *Cladonia stellaris* from eastern Canada based on GBS data. The tree was built from 6002 unlinked SNP dataset and midpoint rooted. Thicker branches represent well-supported bootstrap values (≥ 90). The identity of the algae species is based on loci ITS rDNA and actin type I (Fig. S1). The geographic distribution of samples according to their phylogenetic clades is plotted on eastern Canada maps.

3.4. Estimates of genetic diversity

Values of genetic diversity of the photobiont and the mycobiont within and among phylogenetic clades are shown in Table 1. The number of total, private, variant, and polymorphic sites was always higher for the photobiont than for the mycobiont. The mycobiont had higher values of nucleotide diversity. In both lichen partners, clade B had the greatest number of polymorphic (5520 and 914, photobiont and mycobiont, respectively) and private (3784 and 196, photobiont and mycobiont, respectively) sites. Clade E had the lowest values for the photobiont (1996 polymorphic and 1636 private sites) and clade C for the mycobiont (103 polymorphic and 20 private sites) (Table 1). When we exclude the clade C (just present in the mycobiont), the lowest values of polymorphic sites for the mycobiont were found in clade A (561) and D (597), but not in E (718).

3.5. Identification of factors influencing symbiont's genetic diversity

Seven and three variables fulfilled the conditions (Supplementary Table 7) and were selected for the RDA model of the photobiont and the mycobiont, respectively. No bioclimatic region was selected as predictor of genetic variation of the mycobiont. Fig. 3A shows RDA results to explain the genetic variation of the photobiont. The total variance explained for all factors studies was 0.82 (P-value = 0.001). The identity

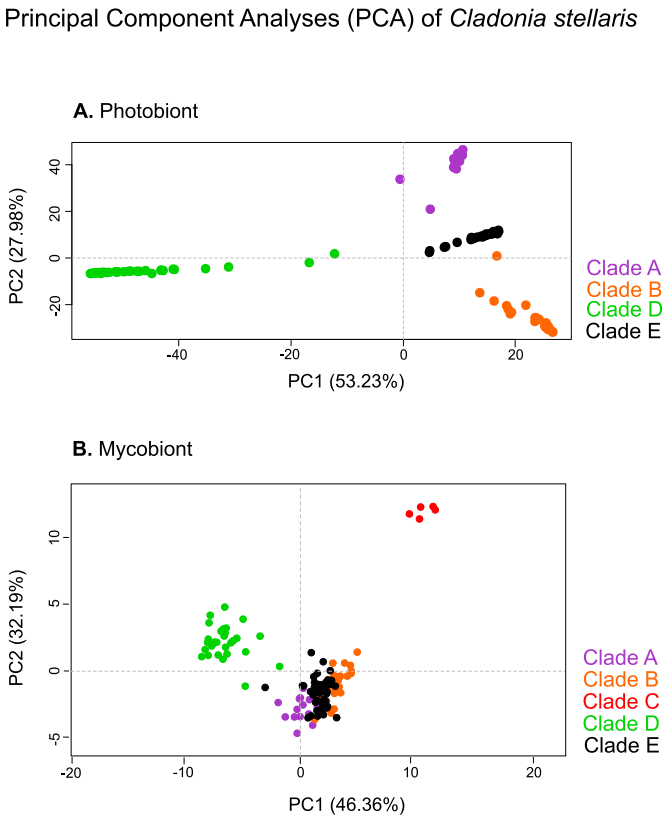

Fig. 2. Principal Component Analysis (PCA) of the mycobiont and the photobiont of *Cladonia stellaris* from eastern Canada based on GBS data. They were built using 1450 and 9043 SNPs, respectively. Samples are colored according to the maximum likelihood phylogenetic clades from Fig. 1.

of the mycobiont was the factor that explained most of the genetic variation of the photobiont (0.682, *P*-values = 0.001) while the geographical location (0.05, *P*-value = 0.001) and the bioclimatic region (0.004 *P*-value = 0.037) explained a small amount of the variation. Fig. 3B shows RDA results to explain the genetic diversity of the mycobiont. The total variance explained for all factors was 0.642 (*P*-value = 0.001). The identity of the photobiont was the factor that explained most of the genetic variation of the mycobiont 0.545 (*P*-value = 0.001).

4. Discussion

Cladonia stellaris is associated with three species of the green alga *Asterochloris* in eastern Canada: *A. glomerata*, *A. irregularis*, and *A. pseudoirregularis*. The photobiont and the mycobiont lack spatial genetic structure but consist of four identical phylogenetic lineages mirroring their distribution across eastern Canada. Our results suggest co-dispersal of both symbionts of *C. stellaris* and pointed out LDD of thallus fragments. The highest values of genetic diversity of *C. stellaris* were found in the lineage associated to the photobiont *A. glomerata*, distributed exclusively in southern Quebec. Genetic diversity of the photobiont explained most of the genetic variation of the mycobiont, and vice versa, providing further evidence of co-dispersal.

4.1. *Asterochloris glomerata, A. irregularis, and A. pseudoirregularis in C. stellaris*

As expected from other studies (Yahr et al., 2004, 2015; Steinová et al., 2019; Pino-Bodas and Stenroos, 2021), all the photobionts associated with *C. stellaris* belong to the genus *Asterochloris*. Only sporadically, other genera of photobionts have been reported in association with *Cladonia* (Peršoh et al., 2004; Vančurová et al., 2021). Specifically, the photobionts identified here belonged to three distinct species, *A. glomerata*, *A. irregularis* and *A. pseudoirregularis*. These three species are common photobionts in *Cladonia*, as well as in other genera, and establish symbioses with a wide range of species (Pino-Bodas and Stenroos, 2021). *Asterochloris glomerata* and *A. irregularis* were previously reported in symbiosis with *C. stellaris* (Piercey-Normore and DePriest, 2001), whereas *A. pseudoirregularis* is here for the first time reported in symbiosis with *C. stellaris*. In Arctic and Antarctic ecosystems, these *Asterochloris* species had been previously found associated with different species of *Cladonia* and *Stereocaulon* (Kim et al., 2020; Pino-Bodas and Stenroos, 2021). The ability of mycobionts to associate with a wide range of photobionts is considered an adaptive strategy that allows them to survive in a wide range of environmental conditions (Yahr et al., 2004, 2006; Fernández-Mendoza et al., 2011; Muggia et al., 2014). However, in this study we have not found bioclimatic region to be an important factor explaining the genetic variation of photobionts. This is probably because these three species of photobiont are well adapted to cold boreal climates (Vančurová et al., 2018; Pino-Bodas and Stenroos, 2021).

The phylogeny of *Asterochloris* based on GBS data shows that the specimens identified as *A. irregularis* form two clades, suggesting either subspecific status or a local strain of the species. This is the first study using GBS data to investigate the population structure in a photobiont of a lichen, and one of the first in Chlorophyta. Our results showed that the

Table 1
Summary population statistics for the phylogenetic clades of the two partners of *Cladonia stellaris* from Quebec. Results are filtered with *-r 0.75 –max-obs-het 0 –min-maf 0.05*. All positions (variant and fixed) are shown. Statistics include: Num ind. (total average number of individuals genotyped at each locus), private (number of unique sites to each clade), sites (total number of sites for each clade), variant (number of sites across all populations that are variant), polymorphic (number of sites inside each population that are variant, SNPs), and π (mean nucleotide diversity).

A. Photobiont						
Phylogenetic clades	**Num ind.**	**Private**	**Sites**	**Variant**	**Polymorphic**	**π**
A	13.92	2684	3254183	11697	2074	0.00020
B	29.50	3784	3684806	13908	5520	0.00046
D	28.52	3050	3303999	12159	2221	0.00011
E	37.52	1636	3291738	10893	1996	0.00019
B. Mycobiont						
Phylogenetic clades	**Num ind.**	**Private**	**Sites**	**Variant**	**Polymorphic**	**π**
A	14.2	73	173769	781	561	0.00090
B	26.0	196	198520	1054	914	0.00125
C	4.8	20	278762	1075	103	0.00019
D	28.9	33	168009	720	597	0.00082
E	39.7	50	173648	791	718	0.00099

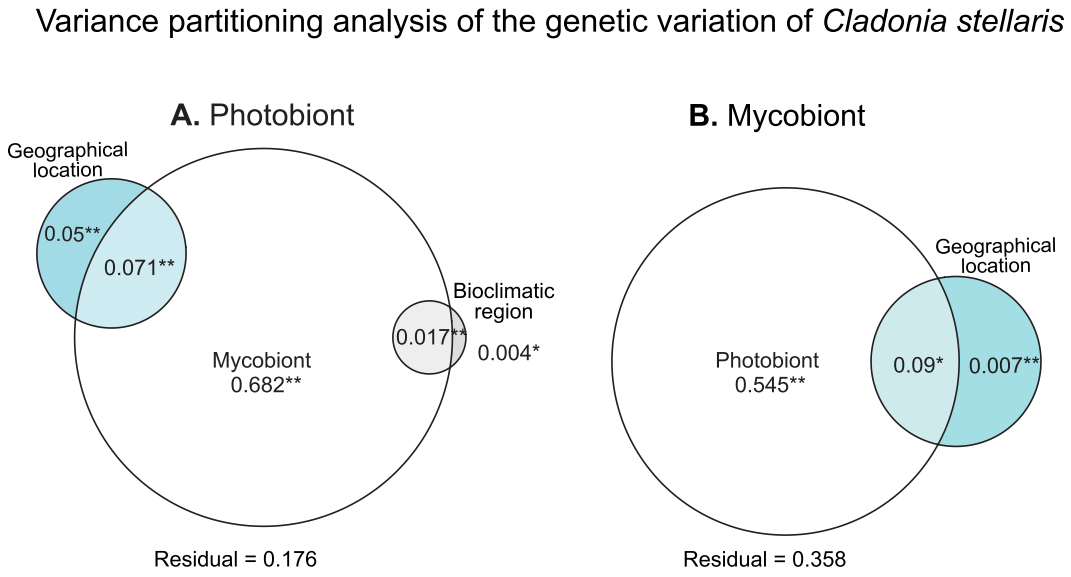

Fig. 3. Venn diagrams showing the variation partitioning of the genetic variation of photobiont (**A**) and mycobiont (**B**) explained by each group of explanatory variables (identity of the symbiotic partner, the bioclimatic region, and the geographical location) and the variation shared by groups of variables. The explained variation indicated is the adjusted R^2 values. The significance was tested with 2000 permutations, *P < 0.05; **P < 0.01.

restriction enzymes PstI/MspI, used to digest the genomic DNA, are suitable not only for the mycobiont but also for *Asterochloris*. In fact, the number of assembled loci is higher for the photobiont than for the mycobiont.

4.2. Identical genetic structure indicates co-dispersal of symbionts in *C. stellaris*

Alonso-García et al. (2021) reported the lack of a spatial genetic structure in *C. stellaris* and suggested LDD in the species. Whether LDD occurred via sexual spores or asexual propagules remains unknown. Here, we confirm that both symbionts of *C. stellaris* have the same population structure, phylogenetic relationships, and co-ancestry clusters. Additionally, a Mantel test showed significant correlation between the genetic distances of mycobiont and photobiont. Our results suggest co-dispersal of the two lichen partners in *C. stellaris.* Therefore, we confirm that asexual reproduction by thallus fragmentation and vertical transmission of the photobiont is dominant in this lichen species. To our knowledge, this is the first time that congruence in phylogeny and population structure of the two partners of *Cladonia* has been demonstrated. It is noteworthy that the association of *C. stellaris* with three different photobiont lineages could indicate that photobiont switching events have occurred sometime in the past. In other lichen groups with asexual reproduction and vertical transmission of symbionts, switch events have been documented (Nelsen and Gargas, 2008; Fernández-Mendoza et al., 2011; Dal Grande et al., 2012; Lindgren et al., 2020). The association of *C. stellaris* with different lineages of photobionts may have helped to expand its distribution range. However, understanding the complex patterns of mycobiont-photobiont association requires further study by dating the phylogenies of both symbionts, wider sampling and event-based analysis.

Co-dispersal of both symbionts is a strategy that ensures the maintenance of symbiosis. However, population genetic studies have often found association patterns that seemed to indicate an absence of co-dispersal (Yahr et al., 2006; Wornik and Grube, 2009). Studies comparing phylogenetic patterns between symbionts in other *Cladonia* species showed incongruence between the two partners of the lichen (Piercey-Normore and DePriest, 2001; Yahr et al., 2006; Beiggi and Piercey-Normore, 2007; Robertson and Piercey-Normore, 2007). However, co-dispersal is likely to be frequent, although after establishment there may be a switch to a locally adapted photobiont (Yahr et al., 2006). In fact, some authors have found different pools of photobionts in young and adult thalli (Molins et al., 2021), which corroborates this hypothesis. Our results suggest that in *C. stellaris* after the establishment of vegetative propagules and thallus formation, there would be no switch by locally adapted photobionts, probably because the three photobiont species found are well adapted to their corresponding habitats. Congruence was already reported in *Lobaria pulmornaria* by Werth and Scheidegger (2012). They compared the spatial genetic structure of *L. pulmonaria* and its photobiont, *Dictyochloropsis reticulata* using 18 microsatellite loci. They revealed co-dispersal and highly congruent genetic structures in the two partners of the lichen at a reduced spatial scale. They concluded that the reproductive mode of both species was clonal, and that vertical transmission of the photobiont occurred. This congruence of mycobiont-photobiont population structure in *L. pulmonaria* was later observed by other authors (Dal Grande et al., 2012; Nadyeina et al., 2014; Allen et al., 2021). In several species of lichens from New Zealand (Buckley et al., 2014), as well as in *Ramalina menziesii* from western North America (Chen et al., 2016), phylogenetic congruence was also highly significant, although in the *R. menziesii* congruence only occurred across ecoregions and not within them.

Long-distance dispersal of lichen vegetative propagules was believed to be limited (e.g., Heinken, 1999; Walser, 2004), but many authors demonstrated the opposite. For example, Werth et al. (2006) confirmed that clonal propagules of *L. pulmonaria* were dispersed over larger distances (i.e., > 200 m). Transoceanic migrations of soredia and thallus fragments have also been evidenced for other lichens (Harmata and Olech, 1991; Högberg et al., 2002). In species of *Cladonia* with asexual reproduction, this long-distance distribution pattern has been already reported. Myllys et al. (2003) suggested LDD via air currents as a possible reason for the disjunct distribution of the two closely related bipolar species, *Cladonia mitis* and *C. arbuscula*. In *Cladonia borealis*, LDD was proposed to explain the origin of the species in King George Island (Antarctica) (Park et al., 2012), and more recently, Pino-Bodas et al. (2020) showed gene flow in *Cladonia subturgida* among the different regions of the Mediterranean basin. Our results corresponded with these studies and indicated that *C. stellaris* exhibited LDD of thallus fragments, meaning a constant migration of individuals across Quebec. Further studies, including samples from Europe and the rest of North America, will be necessary to verify the role of this long-dispersal in *C. stellaris* at a global scale.

4.3. Symbiont's genetic diversity explains most of the variation

This is the first population study to estimate values of genetic diversity in two lichen partners using a large genomic dataset. Our analyses are based on almost 21100 loci for the photobiont and more than 1300 for the mycobiont. We found that clade B harbored the highest values of genetic diversity (private and polymorphic sites) for both partners of *C. stellaris*. Clade B is associated with the photobiont species *A. glomerata*, and it is exclusively distributed in southern Quebec.

When comparing values of genetic diversity between symbionts of *C. stellaris*, we detected higher values in the phylogenetic lineages of the photobiont than of the mycobiont. In several species of lichens, previous studies reported higher number of genotypes for the photobiont than for the mycobiont (Walser et al., 2003; Mansournia et al., 2012; Park et al., 2015), and they also highlighted that photobiont markers were more variable than mycobiont makers (Fernández-Mendoza et al., 2011; Domaschke et al., 2012). In contrast, a study on *C. arbuscula* displayed lower levels of genetic variation for the photobiont than for the mycobiont (Robertson and Piercey-Normore, 2007), and in *L. pulmonaria*, Allen et al. (2021) found no differences in values of genetic diversity between the two partners.

Several factors have been identified as main drivers of the photobiont genetic variation in *Cladonia* species. Geographic location and habitat seemed to be the main drivers in *C. subtenuis* (Yahr et al., 2006). In a complex of four red-fruited *Cladonia* species, geography and climate structured the photobiont, but the reproductive and dispersal strategies were revealed to have the greatest impact on photobionts' genetic variation (Steinová et al., 2019). In Bolivian *Cladonia* species, mycobiont identity explained 31% of the photobiont variability, whereas climate and geography represented less than 6% (Kosecka et al., 2021). A recent study including 172 species of *Cladonia* collected all over the world demonstrated that mycobiont identity and climate were the main drivers for the genetic variation of *Asterochloris*, beyond host phylogeny and geography (Pino-Bodas and Stenroos, 2021). Our results clearly displayed that a large proportion of variance in both photobiont and mycobiont was explained by partner genetic variation, a result expected due the high phylogenetic congruence of both partners. Geographical location explained 5% of the genetic variation for the photobiont, but it did not account for the mycobiont. The bioclimatic region was not a significant factor shaping the genetic variation in *C. stellaris* symbionts. Likely, this is because both partners are well adapted to living in regions ranging from the arctic tundra to the closed-crown forest. *Cladonia stellaris* is a circumpolar species with a distribution from arctic to northern temperate regions (Yarranton, 1975; Pino-Bodas and Stenroos, 2021). According to the niche estimation of Vančurová et al. (2018), *A. glomerata* and *A. irregularis* have preferences for habitats with low temperature, and Pino-Bodas and Stenroos (2021) confirmed that these species are common photobionts of *Cladonia* in cold areas of the Northern Hemisphere.

Finally, it should be noted that our study only considered the dominant photobiont, identified using a phylogenetic approach based on ITS rDNA and actin I loci. According to our methodological approach we cannot discard the presence of multiple photobionts in the thallus, as has been found in other lichen species (Piercey-Normore 2006; Dal Grande et al., 2014; Moya et al., 2017; Molins et al., 2018). However, most studies conclude that a single photobiont is present in most specimens (Dal Grande et al., 2018; Paul et al., 2018) and when multiple photobionts are present, a single photobiont is clearly dominant, accounting for about 90% of the diversity (Onuț-Brännström et al., 2018; Blázquez et al., 2021). Therefore, only one photobiont might be playing a relevant functional role in the thallus, while the remaining photobionts would constitute a photobiont reservoir, that in case of changing environmental conditions would allow a rapid adaptation to the new ones (Onuț-Brännström et al., 2018). Taking this into account and based on the high number of photobiont reads mapped against the reference genome (mean = 25%, range = 7–44% of total sequenced reads), we consider that our results would not change if the total diversity of photobionts present in the thalli were analyzed.

5. Conclusion

We undertook the first population genomic study of a lichen photobiont. We compared the genetic structure of both symbionts of *C. stellaris* to test for co-dispersal and to elucidate the main reproduction mechanisms in this lichen. We also estimated values of genetic diversity and investigated the factors that explain genetic variation. *Cladonia stellaris* from eastern Canada is associated with three closely related species of the green algae *Asterochloris*, *A. glomerata*, *A. irregularis*, and *A. pseudoirregularis*. The genetic structure of *Asterochloris* consists of four phylogenetic lineages lacking spatial structure. Congruence between the genetic structures of the photobiont and the mycobiont is corroborated, suggesting co-dispersal of both symbionts. The lack of spatial structure confirms the LDD ability of thallus fragments in *C. stellaris.* The lichen lineage associated with *A. glomerata* is distributed exclusively southern Quebec and harbors higher genetic diversity. The genetic variation of the photobiont was mainly explained by the genetic diversity of the mycobiont, and vice versa. Geographical location and bioclimatic region seemed to have small or null explanatory power.

Declaration of competing interest

All authors declare that they have no conflict of interest for the manuscript "Co-dispersal of symbionts in the lichen *Cladonia stellaris* inferred from genomic data" (FUNECO-D-22-00017).s.

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Appendix A. Supplementary data

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